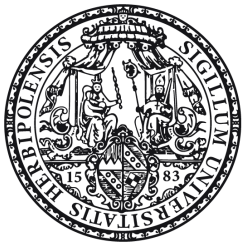
Novel $Ca_v1.2$ and PMCA4b interacting PDZ domain containing proteins

DISSERTATION ZUR ERLANGUNG DES
NATURWISSENSCHAFTLICHEN DOKTORGRADES
DER JULIUS-MAXIMILIANS-UNIVERSITÄT WÜRZBURG



vorgelegt von

Doreen Fetting

aus

Leipzig

Würzburg 2011

Eingereicht am: _____

bei der Fakultät für Biologie.

1. Gutachter: Prof. Dr. Kai Schuh

2. Gutachter: Prof. Dr. Charlotte Förster

der Dissertation.

1. Prüfer: Prof. Dr. Kai Schuh

2. Prüfer: Prof. Dr. Charlotte Förster

3. Vorsitzender:

des öffentlichen Promotionskolloquiums.

Tag des öffentlichen Promotionskolloquiums: _____

Doktorurkunde ausgehändigt am:

Die vorliegende Arbeit wurde auf Anregung und unter Anleitung von

Herrn Prof. Dr. Kai Schuh

am Physiologischen Institut

der Julius-Maximilians-Universität Würzburg angefertigt.

„Darin besteht das Wesen der Wissenschaft. Zuerst denkt man an etwas, das wahr sein könnte. Dann sieht man nach, ob es der Fall ist und im Allgemeinen ist es nicht der Fall.“

Bertrand Russell (1872-1970), brit. Philosoph u. Mathematiker

Teile der vorliegenden Dissertation wurden bereits an folgenden Stellen veröffentlicht:

Doreen Fetting, Priscilla Y. Tng, Vladimir Milenkovic, Nadine Reichhart, Olaf Strauss, Oliver Ritter, Peter M. Benz, and Kai Schuh (2010) Identification of novel L-type $Ca_v1.2$ Ca^{2+} channel and plasma membrane Ca^{2+} ATPase isoform 4b (PMCA4b) interacting PDZ (PSD95/DLG/ZO-1) domain protein. Submitted in JBC, November 2010, presently in Revision

Doreen Fetting, Priscilla Tng, Kai Schuh (2009) Novel $Ca_v1.2$ and PMCA4b interacting PDZ domain containing proteins. Deutsche Physiologische Gesellschaft in Gießen (Vortrag)

Doreen Fetting, Priscilla Tng, Kai Schuh (2009) Novel $Ca_v1.2$ interacting PDZ domain containing proteins. CBCS/ESC Summer School in Nizza (European Society of Cardiology) (Posterpräsentation)

Doreen Fetting, Ruth Freudinger, Kai Schuh (2008) Novel $Ca_v1.2$ interacting PDZ domain containing proteins. Deutsche Physiologische Gesellschaft in Köln (Posterpräsentation)

SUMMARY

The voltage –gated calcium channel, $Ca_v1.2$, and the plasma membrane calcium ATPase, PMCA4b, play important roles in excitable and non-excitable cells. The central function of $Ca_v1.2$ is to regulate the calcium entry into cells upon depolarization, while PMCA4b is responsible for calcium extrusion and has an influence on cellular calcium homeostasis. Both proteins control fundamental functions in the heart and brain, but the specific functions and the precise mechanisms are still investigated. In order to identify new interaction partners that may regulate the activities of the $Ca_v1.2$ and the PMCA4b, we used three independent assays and co-localization studies. The assays, which were used are PDZ domain arrays (testing 124 different PDZ domains), GST pull-downs, and conventional immunoprecipitation assays. In the PDZ arrays, strongest interactions with $Ca_v1.2$ and PMCA4b were found for the PDZ domains of MAST-205, MAGI-1, MAGI-2, MAGI-3, and ZO-1. Additionally, we established interactions between $Ca_v1.2$ and the PDZ domains of NHERF1/2, Mint-2, and CASK. PMCA4b was observed to interact with Mint-2, and its interactions with Chapsyn-110 and CASK were confirmed. Furthermore, we validated interaction of $Ca_v1.2$ and PMCA4b with NHERF1, CASK, MAST-205 and MAGI-3 via immunoprecipitation. We also demonstrated direct interaction of the C-terminus of $Ca_v1.2$ and the PDZ domain of nNOS. We assumed that nNOS overexpression would reduce Ca^{2+} influx through $Ca_v1.2$. To address this question, we measured Ca^{2+} currents in stably transfected HEK 293 cells expressing the $Ca_v1.2$ (α1b and β2a subunit of the smooth muscle L-type calcium channel) and nNOS. It has been shown that NO modulates ion channel activity by nitrosylation of sulfhydryl groups on the channel protein. So we propose that the interaction between the C-terminus of $Ca_v1.2$ and the PDZ domain of nNOS inhibits the currents by an S-nitrosylation of the channel protein. All these interactions connect both proteins to signaling networks involved in signal transmission, cell adhesion, and apoptosis, which may help provide new hints about the physiological functions of $Ca_v1.2$ and PMCA4b in intra- and intercellular signaling.

ZUSAMMENFASSUNG

Der spannungsabhängige Calcium-Kanal, $Ca_v1.2$, und die Plasmamembran Calcium ATPase, PMCA4b, spielen eine wichtige Rolle in erregbaren und nicht-erregbaren Zellen. Der $Ca_v1.2$ Kanal reguliert den Calciumeintritt in die Zelle nach einer Depolarisation, während die PMCA4b für den Calciumausstrom und für die Calcium-Homöostase verantwortlich ist. Beide Proteine haben einen grossen Einfluss auf die Funktionen von Herz und Gehirn, aber die genauen Aufgaben und spezifischen Mechanismen, sind noch nicht geklärt. In dieser Arbeit benutzten wir drei unabhängige Assays und Kolokalisationen, um Interaktionspartner von $Ca_v1.2$ und PMCA4b zu identifizieren, welche möglicherweise die Aktivitäten von $Ca_v1.2$ und PMCA4b regulieren. Die Assays, die wir benutzten waren PDZ Domain Arrays (getestet wurden 124 unterschiedliche PDZ Domänen), GST Pull Downs und konventionelle Immunopräzipitationen. Die Ergebnisse des PDZ Arrays zeigten, dass die PDZ Liganden $Ca_v1.2$ und PMCA4b stark mit den PDZ Domänen von MAST-205, MAGI-1, MAGI-2, MAGI-3 und ZO-1 interagierten. Zusätzlich, konnten wir Interaktionen zwischen $Ca_v1.2$ und den PDZ Domänen von NHERF1/2, Mint-2 und CASK nachweisen. Es wurde beobachtet, dass PMCA4b mit dem PDZ Protein Mint-2 ein starkes Signal auf der Membran zeigte. Andere Interaktionen von PMCA4b und PDZ Proteinen, konnten durch unseren PDZ Domain Array bestätigt werden (z.B. Chapsyn-110 und CASK). Weiterhin untersuchten wir die Interaktionspartner (NHERF1, CASK, MAST-205 und MAGI-3) von $Ca_v1.2$ und PMCA4b durch Immunopräzipitationen genauer. Ein sehr interessantes PDZ Protein, welches wir durch alle drei unabhängigen Assays bestätigen konnten, war nNOS. Schuh et al. konnte schon 2001 zeigen, dass die PDZ Domäne von nNOS mit der PMCA4b interagiert. In der vorliegenden Arbeit konnten wir eine direkte Interaktion des C-terminus von $Ca_v1.2$ und dem PDZ Protein nNOS nachweisen. Wir fomulierten eine Hypothese, die lautete, dass eine nNOS Überexpression den Calcium-Einstrom durch den $Ca_v1.2$ Kanal reduziert. Um diese Hypothese zu bestätigen wurden Calcium-Ströme in stabil transfizierten HEK 293 Zellen gemessen. Diese HEK 293 Zellen waren stabil transfiziert mit der α1b und β2a Untereinheit des L-type Calcium Kanals und mit nNOS. Es konnte in anderen Studien gezeigt werden, dass NO die Ionenkanal-Aktivität durch Nitrosylierung von Sulfhydryl-Gruppen an den Kanal-Proteinen moduliert. Wir denken, dass die Interaktion zwischen dem C-terminus von $Ca_v1.2$ und dem PDZ Protein nNOS, die Calcium-Ströme durch eine S-Nitrosylierung von $Ca_v1.2$ inhibiert.

Durch all diese Interaktionen wird klar, dass $Ca_v1.2$ und PMCA4b eine wichtige Rolle spielen im signalen Netzwerk, in der zellulären Erregung, in Zelladhäsion und Apoptose. Und

das wiederum gibt Aufschluss über die physiologischen Funktionen von $Ca_v1.2$ und PMCA4b in intra- und interzellulären Signalen.

Acknowledgement

I would like to thank Prof. Dr. Kai Schuh for the friendly acceptance in his research group and for the interesting project, for all his support, the patient mentoring and the excellent working conditions. Also, for the freedom to work independently and for the lunch breaks where we talked a lot about bicycles. It's just a bummer that there was no chance to ride a bike together. Maybe next time.

Thanks Prof. Dr. Charlotte Förster, a member of the Faculty of Biology, Department of Genetic, who was willing to present the project for the Faculty of Biology and for her considerate acceptance of giving a second opinion.

Thanks all the colleagues of the physiology, who supports me in the daily lab life, and for the possibility to work in such a nice atmosphere, especially my working group for the helpful discussions of experimental problems, the technical assistance, and for the life outside the lab. That means some wine events, our trip to the zoo, the canoe tour, and the really cold trip to the Frankenwarte, some evenings in cocktail bars …. In particular I thank Dr. Peter Benz for his consultation in bioinformatics and biochemistry topics, all the tips and tricks, and our master student Priscilla Tng, who did parts of the PDZ arrays and the $Ca_v1.2$ antibody purification.

Also, I would like to thank Vladimir Milencovic and Olaf Strauss from the University of Regensburg, who measured the calcium currents via patch clamp technique.

My best friends from Würzburg, Sara and Hilde for the funny girlie evenings.

My parents Eberhard and Andrea Fetting, my small brother Manuel and my grandmother for all encouragement and the positive pressure because they were asking me since 2009 every week "What do you think how long would it be take that you are a doctor?" Thanks for being there. And my boyfriend Markus for all his support and patience, for all the nice adventures and outside activities that we did together, and for your love.

Table of Contents

1 INTRODUCTION

Cytosolic calcium is an ubiquitous intracellular signal and is essential in various signal transduction pathways, controlling a wide range of cellular activities (Berridge, 2002; Carafoli et al., 2001). Calcium ions (Ca^{2+}) play a major role in controlling the function of all body cells by acting as carriers of intracellular signals. Cells obtain external signals through neurotransmitters and hormones, which bind to receptors on their surface. These signals are transferred to the inside of the cell either through the opening of channels in the cell membrane, allowing external Ca^{2+} ions to enter the cell, or by releasing Ca^{2+} ions from internal stores (endoplasmic reticulum, ER) into the cytoplasm. Ca^{2+} as a second messenger mediates cellular functions like muscle excitation-contraction coupling, neurotransmitter and hormone release, metabolism, cell division and differentiation (Berridge, 2002; Carafoli et al., 2001). Entry of Ca^{2+} is driven by the presence of a large electrochemical gradient across the plasma membrane. Much is known about the voltage gated L-(long-lasting), P/Q-(purkinje), N-(neural), R-(residual) and T-(transient) type channels (VGCC), and the ligand-gated calcium channels inositol triphosphate- (IP_3R), N-methyl-D-aspartate- (NMDA) and ryanodine-receptors (RyR). Calcium ATPases like the plasma membrane Ca^{2+} ATPase (PMCA) and the sarcoplasmatic reticulum (SR) Ca^{2+} ATPase (SERCA) also contribute to the transport of Ca^{2+} out of cells, and back into the SR, respectively. Our main focus is the voltage-gated L-type calcium channel $Ca_v1.2$ (LTCC) and the plasma membrane Ca^{2+} ATPase 4b (PMCA4b).

1.1.1. Release of Ca^{2+} from internal stores

Ca^{2+} is stored intracellularly in specialized compartments such as the endoplasmic reticulum (ER) or the sarcoplasmic reticulum (SR). In principle ER = SR, in every cell it is called ER only in muscle cells it is called SR. The binding of several hormones and growth factors to particular receptors on the plasma membrane leads to the activation of phospholipase C (PLC), which catalyzes the hydrolysis of phosphatidylinositol 4,5-biphosphate (PIP_2) to generate the intracellular messengers inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG) (Bootman et al., 2001a; Bootman et al., 2001b). IP_3 disseminates into the cell interior and binds to specific IP_3 receptors (IP_3Rs) in the ER/SR. After conformational change, the receptors open, allowing the Ca^{2+} that is stored at high concentrations in the ER/SR to enter the cytoplasm. Besides IP_3, a variety of established intracellular messengers exist, which increase intracellular Ca^{2+} concentration: cyclic adenosine 5'-diphosphoribose (cADPR), which stimulates ryanodine receptors (RyRs), nitric oxide (NO), diacylglycerol (DAG), sphingolipids and Ca^{2+} itself (Bootman et al., 2001b). Analogous to the IP_3R are the ryanodine receptors (RyRs), a class of intracellular Ca^{2+} release channels found in excitable tissues like neurons and muscles. These receptors are named after the plant alkaloid ryanodine that binds to the channel with high affinity. RyRs mediate the calcium release from internal Ca^{2+} stores, which is an essential step in muscle contraction. In cardiac muscles, channel activation occurs via intracellular Ca^{2+} that are amplified by Ca^{2+} release from ryanodine-sensitive Ca^{2+} stores (Fabiato, 1983).

1.1.2. Ca^{2+} influx through voltage gated calcium channels (VGCCs)

Voltage-gated Ca^{2+} channels are protein complexes that control Ca^{2+} currents in cells. This group of channels are transmembrane channels and they are the fastest Ca^{2+} signal molecules. In one second, over one million Ca^{2+} ions pass through these channels and can increase the $[Ca^{2+}]_{inside}$ by a factor upto 20.000 (Bootman and Berridge, 1995; Clapham, 2007). There are two groups of VGCCs: the **H**igh **V**oltage **A**ctivated channels (HVA) and the **L**ow **V**oltage **A**ctivated (LVA) (Yaari et al., 1987). Biophysical and pharmacological characteristics separate the channels in different subtypes (Table 1.1.). L-type calcium channels (LTCCs) have a **L**arge conductance, and a **L**ong lasting opening, with barium as carrier. The channel is only active when a **L**arge depolarisation at the cell membrane is modulated. The T-type calcium channel has a **T**iny conductance, a **T**ransient opening, and is active when the

membrane potential is negative (Cribbs et al., 1998; Klugbauer et al., 1999; Perez-Reyes et al., 1998). Additionally the channels are divided in N-type channels, which are mainly which are mainly found in neurons, and the P/Q- and R-type channels. For their activation, they require a strong depolarisation at the membrane (Llinas et al., 1989; Randall and Tsien, 1995). Members of the VGCC Ca_v1 and Ca_v2 families consist of a pore-forming α1-subunit (190-250 kDa), which has four domains (I-IV), each containing six transmembrane segments (S1-S6). The α1-subunit is associated with an intracellular β-subunit and an α2-subunit, which is completely extracellular and is linked to the membrane by disulphide bonds to a transmembrane δ-subunit. In several channels, the complex is completed by a γ-subunit, which is only expressed in some tissues (Bers, 2002; Bodi et al., 2005; Catterall, 2000; Kamp and Hell, 2000; Leung et al., 1988; Striessnig et al., 1986; Witcher et al., 1993) (Figure 1.1.). Members of the Ca_v3 family might contain only a single α1-subunit, but the exact subunit composition of these channels is not clear.

Table 1.1.: Classification of VGCCs

Activation profile	Native current	α1-subunit subtypes	localization	inhibitors	literature
HVA	P/Q-type	α_{1A} ($Ca_v2.1$)	neurons, neuroendocrine cells	ω-Agatoxin IVA	Mori et al., 1991 Starr et al., 1991
HVA	N-type	α_{1B} ($Ca_v2.2$)	neurons, neuroendocrine cells	ω-Conotoxin GVIA SNX-111	Dubel et al., 1992 Williams et al., 1992
HVA	L-type	α_{1C} ($Ca_v1.2$)	heart, smooth muscles, brain, pancreas	DHP PAA BTZ	Biel et al., 1990 Mikami et al., 1989 Snutch et al., 1991
		α_{1D} ($Ca_v1.3$)	brain, pancreas, kidney, ovar, cochlea	DHP PAA BTZ	Seino et al., 1992
		α_{1F} ($Ca_v1.4$)	retina	DHP D-cis-Diltiazem	Strom et al., 1998
		α_{1S} ($Ca_v1.1$)	sceletal muscle transverse tubules	DHP PAA BTZ	Tanabe et al., 1987
HVA	R-type	α_{1E} ($Ca_v2.3$)	brain, cochlea, retina, heart	SNX-482	Niidome et al., 1992
LVA	T-type	α_{1G} ($Ca_v3.1$)	brain, heart	Kurtoxin Mibefradil	Perez-Reyes, 1998
		α_{1H} ($Ca_v3.2$)	brain, heart	Kurtoxin Mibefradil	Cribbs et al., 1998
		α_{1I} ($Ca_v3.3$)	brain	Kurtoxin Mibefradil	Lee et al., 1999

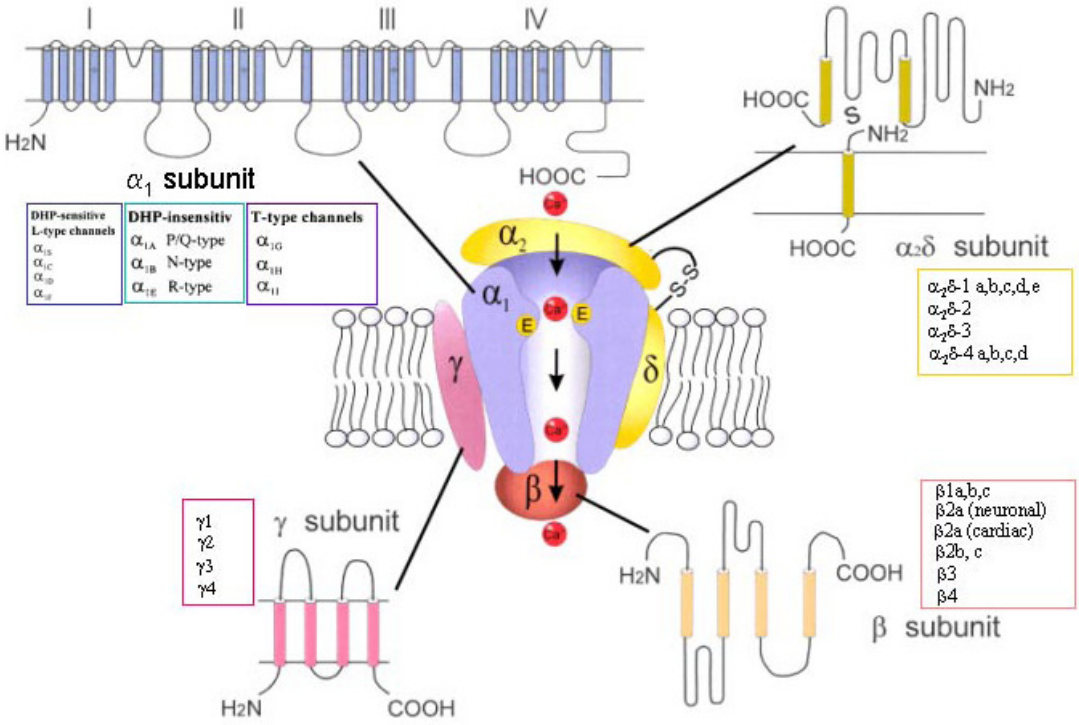
The table shows an overview about the classification of the voltage gated calcium channels, their localization, and their inhibitors. DHP = Dihydropyridines; PAA = Phenylalkylamines; BTZ = Benzothiazepines

1.1.3. α1-, β-, $\alpha_2\delta$-, and γ-subunit

Ten α1 isoforms are well-known and these can be classified into three families: Ca_v1, Ca_v2, and Ca_v3 (Ertel et al., 2000). The Ca_v1 group ($Ca_v1.1$-1.4) consists of subunits of channels that mediate L-type Ca^{2+} currents, which are α_1S, α_1C, α_1D, and α_1F. P/Q-, R- and N-type channels, comprising α_1A, α_1B, and α_1E, are listed under the Ca_v2 family ($Ca_v2.1$-2.3). T-type channels, α_1G, α_1H, and α_1I, are $Ca_v3.1$-3.3 (Table 1.1.). Figure 1.1 shows the structure of the α1-subunit from the L-type Ca^{2+} channel. The segment 4 (S4) works as a voltage sensor and

the ion selectivity filter is built from the intracellular loop between S5 and S6. The interaction motif for the β-subunit is located between the I-II linker (Hofmann et al., 1999). In the intracellular C-terminal tail (CT), an isoleucin-glutamine (IQ) motif, which binds calmodulin (CaM) is located. CaM is a 17 kDa protein and acts as a calcium sensor. The CaM-binding on the IQ motif is important for the autoregulation of the L-type Ca^{2+} channel (Zuhlke et al., 2000). The EF-hand region of CT is also involved in Ca^{2+}-dependent inactivation (CDI) (Budde et al., 2002; Peterson et al., 1999; Striessnig, 1999). CDI is an important feedback mechanism that prevents excessive influx of Ca^{2+}, which would be potentially toxic for the cell. The mechanisms that underlie this feedback inhibitions have been uncovered only recently. It was found that permeating Ca^{2+} inhibits LTCCs by interacting with calmodulin that is tightly bound to specific regions in the C-terminus of the channel (for detail review of CDI see (Budde et al., 2002). The C-terminal tail of $Ca_v1.2$ (α_1C) contains an unique class I PDZ [postsynaptic density-95 (PSD-95)/Disc large/Zonula occludens-1 (ZO-1)] interaction sequence, that has been shown to associate with synapse specific scaffolding proteins, MAGUK proteins, and nucleotid exchange factors that contain PDZ domains (Kurschner et al., 1998). Associations with PDZ proteins play an important role in coupling L-type VGCCs. Summarized, the function of α1-subunits are voltage sensing, ion selection and passage through a conserved pore lined by S6, autoregulation, and drug binding (Bodi et al., 2005; Carafoli et al., 2001; Catterall, 2000; Striessnig, 1999). The $\alpha_2\delta$-subunit is directly associated with the α_1 subunit by surface interaction. The $\alpha_2\delta$-subunit is a glycosylated protein, which is highly conserved in most tissues, while the transmembrane δ-subunit anchors the extracellular α_2 protein by disulfide bridges to the plasma membrane (Hofmann et al., 1994). The δ subunit is sufficient to stabilize the gating properties to the channels, whereas α_2 is essential to stabilize DHP binding to the α_1-subunit (Gurnett et al., 1996). The intracellular β-subunit (55-75 kDa) is the most important subunit for fine-tuning of L-type VGCC activity. It also stabilizes the pore region and facilitates conformational changes, which open the channel once the voltage sensor movement is completed. Furthermore, all different isoforms of the β-subunit (Figure 1.1.) enhance L-VGCC membrane density by modulating α_1-subunit expression (Neely et al., 1993). The γ-subunit was originally found only in skeletal muscle calcium channels. However, a neuronal γ-subunit isoform has been identified recently (Striessnig, 1999). Although these auxiliary subunits change the properties of the channel complex, the pharmacological and electrophysiological diversity of calcium channels arises mostly from the existence of multiple α_1-subunits.

Figure 1.1.: Subunit composition of voltage-dependent calcium channels

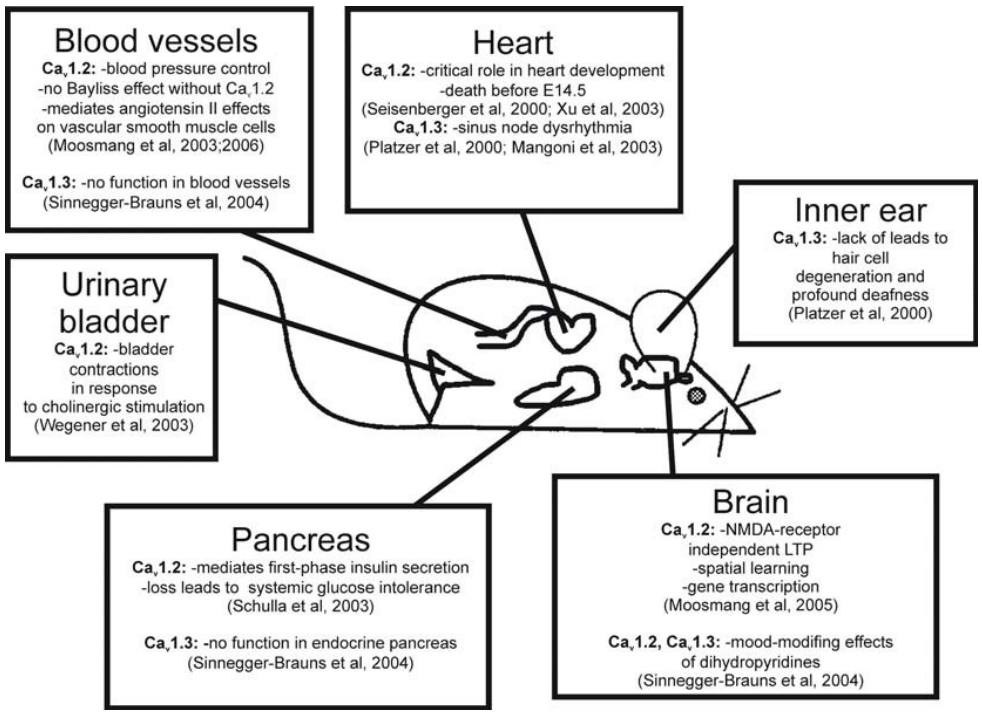


Folding structures of the diverse subunits. There are several genes and splice variants for each calcium channel subunit (see boxes). Adapted from Klugbauer et al., 2002, Hofmann et al., 1999.

1.1.4. $Ca_v1.2$ calcium channel

The Ca_v1 calcium channel family includes four members, $Ca_v1.1$ (α1S), $Ca_v1.2$ (α1C), $Ca_v1.3$ (α1D) and $Ca_v1.4$ (α1F). 75 % of the sequences of these proteins are identical to one another. $Ca_v1.1$ is mainly expressed in skeletal muscle where it is a key element of the excitation-contraction coupling (Tanabe et al., 1988). $Ca_v1.2$ is the most widely distributed member of the Ca_v1 family. For example, the $Ca_v1.2a$ isoform is expressed in cardiomyocytes (Mikami et al., 1989), while the $Ca_v1.2b$ isoform is predominantly found in smooth muscle cells (Biel et al., 1990). $Ca_v1.2$ transcripts are also expressed in many types of neurons as well as in endocrine cells. $Ca_v1.2$ is involved in the control of essential physiological functions including smooth muscle tone (Moosmang et al., 2003), heart contractility (Reuter, 1979), secretion of hormones (Milani et al., 1990), and integration of synaptic inputs (Bean, 1989). Furthermore, mutations in the CACNA1C gene (human), which codes for the $Ca_v1.2$ subunit, are causative for the Timothy syndrome. This disease is characterized by a multiorgan disorder with serious cardiac defects, sudden death, and other comorbidities (Splawski et al., 2005; Splawski et al., 2004). The $Ca_v1.3$ channel was first cloned from neuronal and endocrine tissue and is possibly involved in the control of hormone secretion (Seino et al., 1992; Williams et al., 1992). The recent studies indicate that the channel is also expressed in myocardial tissue (Platzer et al., 2000). The $Ca_v1.4$ gene is the only calcium channel gene localized on the X-chromosome (Xp11.23) (Striessnig et al., 2010), and is specifically expressed in retinal photoreceptors and bipolar cells (Striessnig et al., 2010).

Figure 1.2.: Expression and function of $Ca_v1.2$ in mice



This figure shows the expression and the function of the calcium channel $Ca_v1.2$ in mouse. The scheme is adapted from (Moosmang et al., 2005).

1.1.5. Mouse knockout models of L-type calcium channel (LTCC)

Cloning of L-type calcium channels and their auxiliary subunits in different studies have demonstrated a large understanding about the function and regulation of these channels. The strategy to study calcium channels by knocking out genes is an important model to clarify and confirm heterologous expression studies and central *in vivo* functions of the calcium channels. Efforts to identify the native role of the diverse L-type calcium channel subunits, have produced a varity of knockout mice (Table 1.2.). VGCCs control two key processes required for normal heart function. First, Ca^{2+} influx through calcium channels is a prerequisite for excitation-contraction coupling in cardiomyocytes, and hence for heart contraction. Secondly, Ca^{2+} influx contributes to the generation of pacemaker potentials in cardiac conduction tissue, repeat of hence, is involved in the regulation of heart rhythmicity see review (Hofmann et al., 1999; Stieber et al., 2003). A typical heart cell contains both L- and T-type currents, but the L-type channels at the transverse tubules are more interesting. During the heart systole (plateau of cardiac action potential), the membrane is depolarized over 100 ms. As a result,

Ca^{2+} streams along the concentration gradient over the $Ca_v1.2$ in the cell. This Ca^{2+} influx triggers an intracellular Ca^{2+} release from SR over the ryanodine receptor. The increase of $[Ca^{2+}]_{inside}$; (from 100 nM to 1 mM) cause cell contraction. During the diastolic relaxation, the Ca^{2+} goes the way from cytosol over Na^+/Ca^{2+} exchanger (NCX) to the outside of the cell and through the Ca^{2+} ATPase of SR (SERCA) back to the SR (Bers, 2002). Mice lacking the $Ca_v1.2$ L-type calcium channel die *in utero* before day 15 postcoitum (p.c.), indicating that this channel is necessary for normal embryonic development (Seisenberger et al., 2000) and is indispensible during heart development (Seisenberger et al., 2000; Xu et al., 2003b).

Mice deficient for $Ca_v1.3$ are viable and have a natural life span (Platzer et al., 2000). While the deletion of $Ca_v1.3$ has no obvious consequence on embryonic development, $Ca_v1.3$ knockout mice reveal sinoatrial node dysfunction resulting in bradycardia and arrhythmia. Therefore, $Ca_v1.3$ is likely to be involved in the generation of pacemaker potentials in the sinus node region. Further analysis of the phenotype of $Ca_v1.3$ null mice revealed that these mice are deaf (Martinez-Dunst et al., 1997).

β1 knockout mice are unable to move and die at birth from asphyxiation (Gregg et al., 1996). The β1 knockout mice show a reduction in muscle mass with disorganization of thick and thin filaments of skeletal muscle. The early death of the homozygous animals has not permitted a close examination of the role of the β1 subunit in brain and heart.

The inactivation of the cardiac β2 subunit of VGCCs results in low cardiac calcium current densities and in embryonic death at embryonic day 9.5.

In β3 knockout mice electrophysiological analyses indicated a 30% reduction in Ca^{2+} channel current density, a slower inactivation rate, and a decreased dihydropyridine-sensitive current (Namkung et al., 1998). Despite the reduction in L-type calcium channel density, β3 null mice showed normal blood pressure.

The γ1 knockout mice are viable and show no distinguished phenotype from wild type. The features of γ1 knockout mice are the increased L-type current amplitude, the deceleration of the inactivation and shifts in the steady state inactivation to more positive potentials (Freise et al., 2000).

Table 1.2.: Deletion of L-type calcium channel subunits in mice

deleted subunit gene	tissue	phenotype	reference
$Ca_v1.2$	heart, smooth muscle, brain	embryonic lethal < 14,5	Seisenberger et al., 2000
$Ca_v1.3$	endocrine, smooth muscle, heart, brain	deaf, arrhythmia, bradycardia	Platzer et al., 2000
β1	skeletal muscle, heart, brain	decreased L-type calcium current, death at birth	Gregg et al., 1996
β2	heart, smooth muscle, brain	embryonic lethal < 9,5, decreased cell surface expression of calcium channel	Weisgerber et al., 2003
β3	heart, brain, aorta	decreased L- and N-type current	Scott et al., 1996 Namkung et al., 1998
γ1	skeletal muscle	increased L-type calcium current amplitude	Freise et al., 2000

1.2. Plasma membrane calcium ATPase (PMCA)

1.2.1. Localization and Function of PMCA

The PMCA was described first in erythrocytes (Schatzmann, 1966). It is responsible for the calcium transport against a concentration gradient in the extracellular room or into the SR, and is expressed ubiquitously in all eukaryotic cells. PMCA is an important enzyme for Ca^{2+} homeostasis (Cartwright et al., 2007, 2009; Strehler et al., 2007a; Strehler et al., 2007b). The P-type ATPase PMCA is a transmembrane protein and has a molecular weight between 130-150 kDa, depending on the isoform and splice variant, respectively. Four major isoforms PMCA1-4 (Table 1.3.) and over twenty splice variants have been described, so far (Cartwright et al., 2009). They are expressed developmental-, tissue- and cell-specifically but the PMCA1 and 4 are housekeeping forms and expressed ubiquitously (Strehler, 1991; Strehler et al., 1991). All four isoforms occur in excitable cells like neuron cells, skeletal cells, and cardiomyocytes (Carafoli and Stauffer, 1994; Hammes et al., 1994). PMCA has a greater role in spatial Ca^{2+} signaling within the cell than previously thought (Cartwright et al., 2007, 2009; Strehler et al., 2007a). This type of ATPase is also localized in caveolae (Fujimoto, 1993), which are rich in lipids, receptors, signal transducers and effectors, and involved in signal transduction organisation (Kurzchalia and Parton, 1999; Maxfield, 2002). Caveolae are plasmamembrane invaginations and have a size from 50 to 100 nm. Important

structural proteins are the 20-22 kDa caveolins 1-3 (Rothberg et al., 1992). Some interaction partners of caveolins were described (Segal et al., 1999; Venema et al., 1997). The localization of numerous signaling proteins in caveolae suggested that these invaginations are crucial for signal transduction. Such proteins for example are receptors for Atrial Natriuretic Peptide (ANP), Muscarin m2, Bradycinin B2, Platelet Derived Growth Factor (PDGF), Insulin, Endothelin, protein kinases (Ras, Src, Raf), endothelial NO-Synthase (eNOS), and neuronal NO-Synthase (nNOS) (Anderson, 1998).

Table 1.3.: PMCA isoforms and gene nomenclature of the Human Genome Organisation (HUGO) and the exactly gene locus.

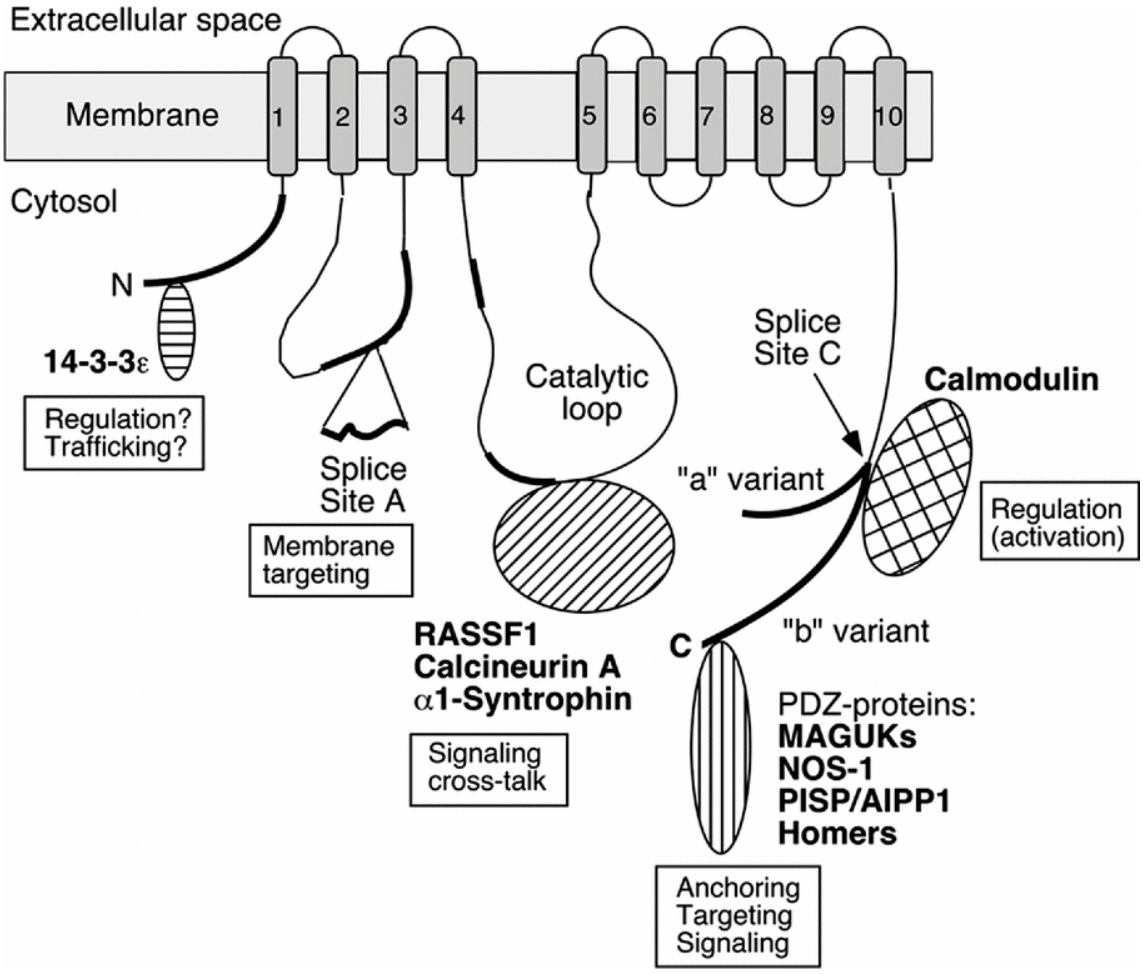
isoform	HUGO nomen clature	gen localization
PMCA1	ATP2B1	12q21 - 12q23
PMCA2	ATP2B2	3p26 - 3p25
PMCA3	ATP2B3	Xq28 - Xq28
PMCA4	ATP2B4	1q25 - 1q32

1.2.2. Structure of the PMCA

PMCAs have ten transmembrane domains (TM1-10), four cytosolic linkers (TM2-TM3, TM4-TM5, TM6-TM7 and TM8-TM9), and cytosolic N- and C-terminal tails (Brini, 2009; Cartwright et al., 2009; Di Leva et al., 2008). A 14-3-3 protein binding site has been described to be located in the N-terminal tail, and association with this protein effects the inhibition of pump activity (Rimessi et al., 2005). The C-terminal tail includes sites that control pump activity, protein kinase C (PKC) and protein kinase A (PKA) phosphorylation sites (Monteith and Roufogalis, 1995; Penniston and Enyedi, 1998; Strehler and Zacharias, 2001), and an autoinhibitory calmodulin binding domain (CaM-BD) (Carafoli et al., 1992; James et al., 1988; Vorherr et al., 1992; Vorherr et al., 1990), where the inhibition is lifted upon binding of Ca^{2+}-bound calmodulin (Ca^{2+}/CaM). At the C-terminal tail an alternative splice site (Strehler and Zacharias, 2001), a positive modulatory homodimerization site (Brini, 2009), and a PDZ binding domain is located (DeMarco and Strehler, 2001; Kim et al., 1998; Schuh et al., 2003). The TM2-TM3 loop harbours a phospholipid sensitive region (Brodin et al., 1992; Zvaritch et al., 1990), and a splice A site (Chicka and Strehler, 2003; Hill et al., 2006; Strehler and Zacharias, 2001). TM4-TM5 is the longest cytoplasmic linker and it is where the ATP-binding site and the catalytic domain are situated. This loop also interacts with the CaM-BD and is therefore implicated in autoinhibition (Falchetto et al., 1991), similar

to TM2-TM3 (Fig.1.3.). Active PMCA has two conformational states (Krebs et al., 1987): the PMCA Ca^{2+} binding change, leading to the E2 state, where the bound Ca^{2+} is released extracellulary due to the decline in Ca^{2+} affinity of the binding site. The enzyme then returns to the E1 conformation when the phosphate is cleaved from it.

Figure 1.3.: Scheme of the PMCA and their regions of structural diversity among isoforms and sites of protein–protein interactions



The N- and C-terminal endings are marketed, and the location of the catalytic loop is indicated. Regions of significant sequence divergence among isoforms are illustrated as bulky black lines. 'Splice Site A' and 'Splice Site C' characterize the regions by alternative splicing. Site A, the insertion of a peptide segment encoded by alternatively spliced exon is indicated; at site C, the two key splice variants 'a' and 'b' are shown with split tails. A choice of PMCA-interacting proteins are demonstrate close to the domain of the PMCA where they bind, and their identified or expected roles in providing functional diversity are indicated. The PMCA is represented in its activated state with CaM bound to the C-tail. AIPP, ATPase-interacting PDZ protein; MAGUK, membrane-associated guanylate kinase; NOS-1, nitric oxide synthase-1; PISP, PMCA-interacting single-PDZ protein; RASSF1, Ras association domain family-1. Adapted from (Strehler et al., 2007b).

1.2.3. PMCA4b

Mammalian PMCAs are products of four genes (ATP2B1 - ATP2B4) (Table 1.3.), which share 80-90% sequence homology at the amino acid level in human, rat and mouse (Strehler and Zacharias, 2001). Differential splicing of PMCA RNA transcripts results in different subtypes of these isoforms. More than 20 splice variants have been identified (Strehler and Zacharias, 2001). The C-termini of the b-splice variants of all PMCA isoforms is supposed to bind preferentially type 1 PDZ domains as the consensus sequence is E-T/S-X-L/V (where X stands for any amino acid). The human ETSV* motif (* = stop, possess different C-terminal ends) of the PMCA4b interacts with members of the membrane-associated guanylate kinase (MAGUK) family (DeMarco and Strehler, 2001; Strehler and Zacharias, 2001), such as postsynaptic density protein-95/synapse-associated protein 90 (PSD-95/SAP90), synapse-associated protein 97 (SAP97/hDlg), synapse-associated protein 102 (SAP-102), postsynaptic density protein-93/Channel associated protein of synapse-110 (PSD-93/Chapsyn-110) (DeMarco and Strehler, 2001) and calcium/calmodulin-dependent serine protein kinase (CASK) (Schuh et al., 2003). In addition PMCA4b interacts also with PMCA-interacting single PDZ protein (PISP) (Goellner et al., 2003), Na^+/H^+ exchanger regulatory factor 2 (NHERF2) (DeMarco et al., 2002) and neuronal nitric oxide synthase (nNOS), which regulates its activity (Schuh et al., 2001). PMCA4b can also interact other proteins via other domains, for example via second intracellular loop with the tumor suppressor Ras-associated factor 1, calcineurin and α1 synthrophin (Armesilla et al., 2004; Buch et al., 2005; Williams et al., 2006b).

1.2.4. Mouse knockout models of PMCA

Knockout mice have been designed and the phenotypes analyzed for each isoforms of the PMCA pumps, except the PMCA3. That isoform is typically expressed in tissues of developing embryos, its function maybe essential for normal growth of gestation. PMCA1 was interrupted by targeting the catalytic phosphorylation site, but homozygous knockout mice resulted in embryonic lethality. Null mutant embryos were recognized up to day 3 of gestation but not for the stage of organogenesis. The lack of ability to breed fully developed life animals emphasizes the necessary role of this housekeeping isoform from the earliest ages of development. On the other hand, heterozygous mutants did not present a pathological phenotype, even if the smooth muscle of blood vessels appeared apoptotic. While this smooth muscle does not express the other omnipresent isoform PMCA4, the absence of PMCA1 gene on one allele was obviously inefficiently compensated (Krebs, 1996). Mice subjected to the

targeted ablation of the PMCA4 survived and seemed healthy at first sight. Histological investigations of organs presented no major tissue alterations or in vivo cell death. Despite is ubiquitous expression, PMCA4 appears to be less critical than PMCA1 in the maintenance of Ca^{2+} homeostasis. A major phenotype alteration was, on the other hand, detected, and this was male infertility. Sperm were unable to get efficient hyperactivated motility and was unable to contact and fertilize the egg (Okunade et al., 2004). This was evidently due to the fact that isoform 4 represents 90% of all PMCA pumps expressed in testis cells (Schuh et al., 2004). The investigation of the phenotypes of PMCA2 knockout mouse has exposed interesting characteristics. While the animals appeared quite normal at birth, they started to present balance impairment around day 10 (Furuta et al., 1998). Recording of the auditory brain response have shown that they were deaf, and the study of the vestibular inner ear explained the absence of otoconia (Kozel et al., 1998). It was also observed that sensory hair cells started to degenerate after day 10. The most severely affected animals also presented partial loss of nerve cells (Furuta et al., 1998; Garcia and Strehler, 1999; Strehler and Zacharias, 2001).

1.3. PDZ Domains

Different biological activities are regulated through interactions of modular protein domains, like WW domain (protein domain with two highly conserved tryptophans that binds proline-rich peptide motifs), Sre homology 3 (SH3) and PDZ domains, and their corresponding binding partners (Pawson, 2007). The name PDZ comes from the first three proteins in which these domains were identified: **P**SD-95 (a 95 kDa protein, post-synaptic density protein), **D**lg (the *Drosophila melanogaster* Discs Large protein) and **Z**O-1 (the zonula occludens 1 protein involved in maintenance of epithelial polarity) (Cho et al., 1992; Kim et al., 1995; Woods and Bryant, 1993). These protein-protein interactions can offer important views into biological processes such as cell proliferation and cell polarity (Pawson, 2007; Pawson and Nash, 2003). In various species there are PDZ domains, for example in *Caenorhabditis elegans*, *D. melanogaster*, and *Homo sapiens* (Doyle et al., 1996; Kennedy, 1995; Morais Cabral et al., 1996). In the mouse genome, for example, 928 PDZ domains have been recognized in 328 proteins, which are present in single or multiple copies or in combination with other interaction modules (Spaller, 2006). PDZ domains are absent in yeast, but numerous PDZ-like domains are present in bacteria and plants (Pallen and Ponting, 1997). From the abundance and variety of PDZ domains in cells, it is obvious that many cellular and biological functions, especially those involving signal transduction complexes are mediated by PDZ-mediated

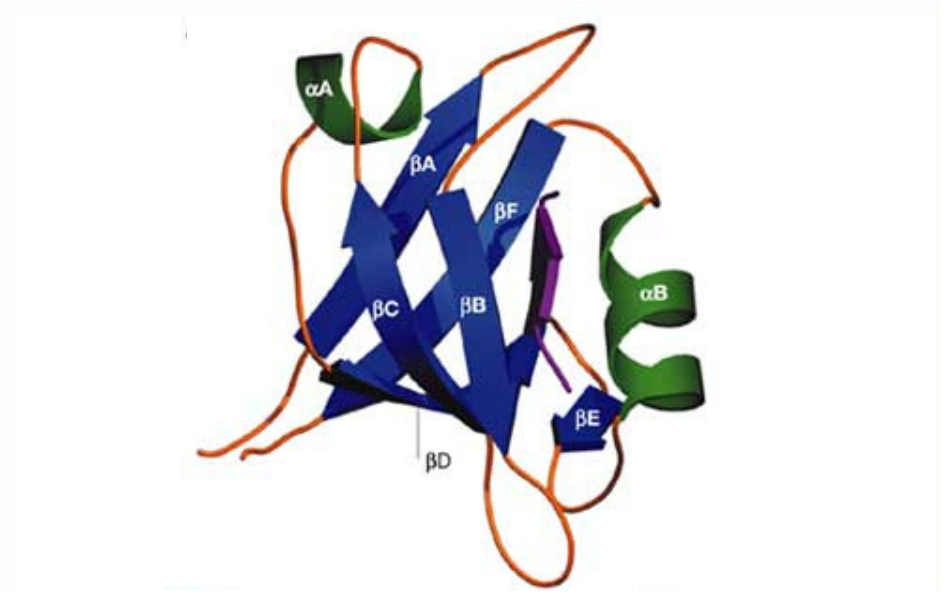
interactions (Bezprozvanny and Maximov, 2001; Brone and Eggermont, 2005; Fan and Zhang, 2002; Garner et al., 2000; Harris and Lim, 2001; Hung and Sheng, 2002; Kim and Kim, 2005; Petit et al., 2009; Sheng and Sala, 2001; Zhang and Wang, 2003).

1.3.1. Structural characteristics of PDZ domains

PDZ domains are relatively small (≥90 amino acids), fold into a compact structure and have N- and C-termini that are in close proximity in the folded structure.

PDZ domains are modular items consisting of 5 or 6 β-stranded (βA-βF) and 2 or 3 α-helical structures (αA-αC) Figure 1.4. (Fanning and Anderson, 1996; Kim and Sheng, 2004; Long et al., 2003). PDZ domains characteristically recognize the C-termini of target proteins (Saras and Heldin, 1996) but a few also bind the internal sequence motif of target proteins through a single binding site on the domains (Cowburn, 1997; Giallourakis et al., 2006; Wang et al., 2008). The nomenclature for residues within the PDZ-binding motif is as follows: the C-terminal residue is referred to as the P_0 residue; subsequent residues towards the N-terminus are termed P_{-1}, P_{-2}, P_{-3}, etc. Studies show that PDZ domains can be divided into three main classes: class I PDZ domains recognize the motif S/T-X-Φ-COOH (Φ is a hydrophobic amino acid and X is any amino acid), class II PDZ domains identify the motif Φ-X-Φ-COOH; and class III PDZ domains recognize the motif X-X-C-COOH. There are few other PDZ domains that do not fall into any of these classes (Table 1.4.) (Schultz et al., 1998; Songyang et al., 1997).

Figure 1.4.: Three-dimensional structure of PDZ domains



Structure of the third PDZ domain of PSD-95 (α-helices in green, β-strands in blue) coordinated with its target C-terminal peptide (purple) (Kim and Sheng, 2004).

Table 1.4.: Examples of PDZ ligands (Harris and Lim, 2001)

PDZ domain	Consensus binding sequence*					Ligand protein	Reference
	P_{-3}	P_{-2}	P_{-1}	P_0			
Class I		S/T	X[¤]	Φ[§]	-COOH		
Syntrophin	E	S	L	V	-COOH	voltage-gated Na^+ channel	Schultz et al., 1998a
PSD-95 D1;2	E	T	D	V	-COOH	Shaker-type K^+ channel	Kim et al., 1995
NHERF		T	X	L	-COOH	β2-adrenergic receptor	Hall et al. 1998
Class II		Φ	X	Φ	-COOH		
hCASK	E	Y	Y	V	-COOH	Neurexin	Songyang et al., 1997
Class III		X	X	C	-COOH		
Mint-1	D	H	W	C	-COOH	N-type Ca^{2+} channel	Maximov et al., 1999
Other							
nNOS	G	D	X	V	-COOH	PMCA4b	Schuh et al., 2001
MAGI		S/T	W	V	-COOH	PTEN	Wu et al., 2000

* P_0 is the C-terminal residue, P_{-1} is one residue N-terminal to it etc.
¤X denotes any amino acid
§Φ denotes a hydrophobic amino acid, usually V, I or L

1.3.2. Higher-order organization of PDZ domain containing proteins

Multi-PDZ domain containing proteins

A remarkable aspect of PDZ domains is the frequency with which multiple domains occur within the same polypeptide. In humans 18% of the PDZ domain containing proteins have three or more PDZ domains within the same polypeptide. These are, for example, MUPP, in which the protein consists of 13 PDZ motifs (Ullmer et al., 1998). Other multi-PDZ domain containing proteins include INAD and NHERF (Fig. 1.5.). INAD have 5 PDZ motifs, NHERF 2 PDZ domains.

MAGUK proteins

Another large family of PDZ domain containing proteins is the MAGUK (**m**embrane-**a**ssociated **gu**anylate **k**inase) family. This subgroup contains between one and three PDZ domains, an SH3 domain and a guanylate kinase homology (GuK) domain (Gomperts, 1996) (Fig. 1.5.). There is no confirmation that this domain has enzymatic activity. Several of these proteins, including Dlg, ZO-proteins, and the MAGI (membrane-associated guanylate kinases with inverted orientation) proteins, are associated with the tight junctions (TJ) of various cell types and are seemingly implicated in assembly of these main structures.

PDZ domain containing proteins with other sequence motifs

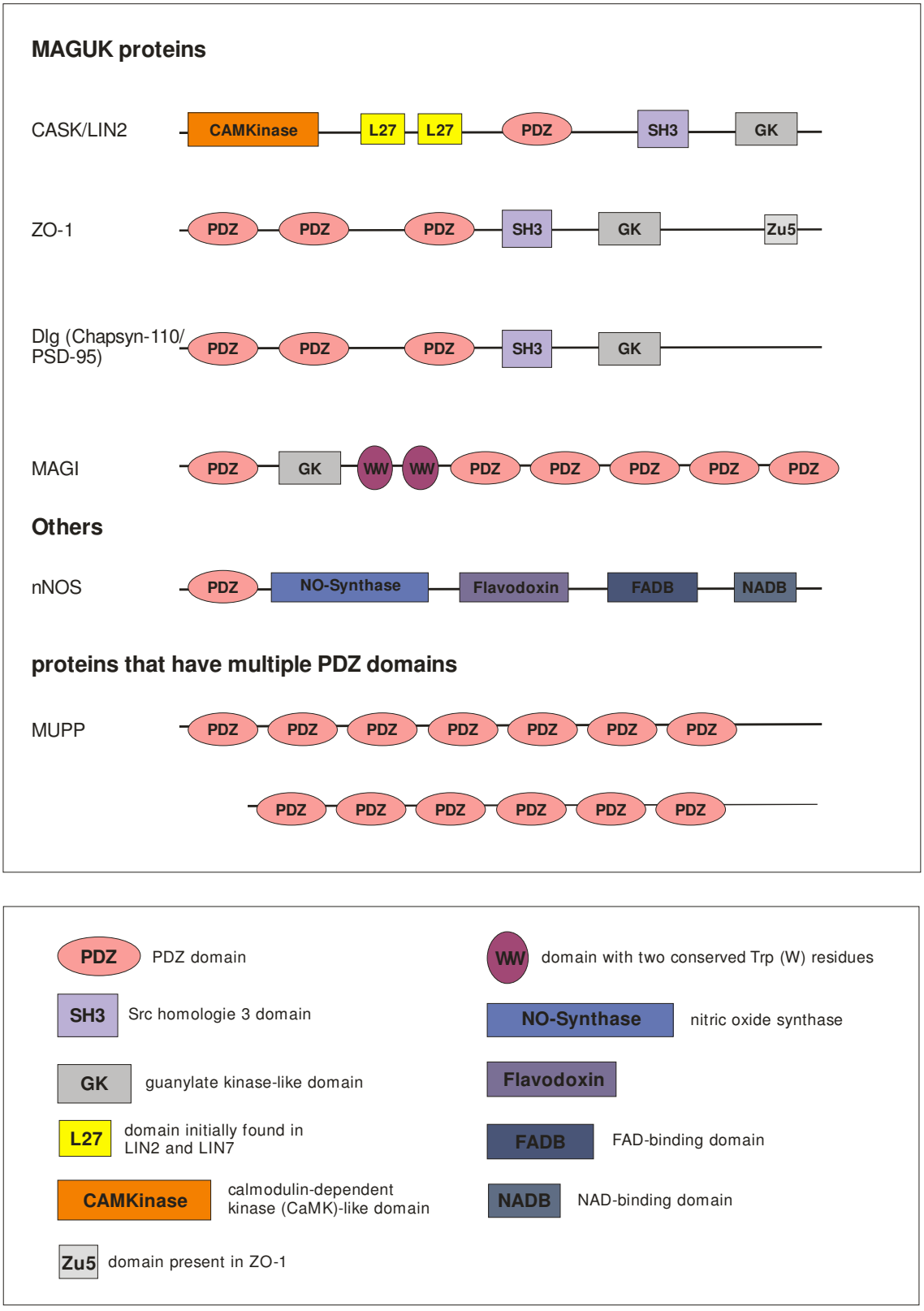
A third large group of PDZ proteins contain a variety of other sequence motifs (but not guanylate kinase-like domain) with one or more PDZ domains. In that family proteins containing leucine-rich repeats (LAP proteins), LIM or crib motifs (Lasky, 2005).

1.3.3. The PDZ domain mechanism of recognition

At the ending of the peptide-binding groove is the carboxylate-binding loop. This loop contains the sequence motif GLGF (Gly-Leu-Gly-Phe) and is located between βB and αB. The first Gly residue in this motif is not strictly conserved in canonical PDZ domains, and can be changed by a Ser, Thr, or Phe residue (Laskowski et al., 2005). The second and the fourth residues are hydrophobic (e.g. Val, Ile, Leu, or Phe). The side chains of the P_0 and P_{-2} ligand residues point directly into the base of the peptide-binding pocket. It can be suggested that ligand positions 0 and -2 are crucial for recognition and binding to target proteins. The importance of these two positions also lead to the general classification of PDZ domains into three classes (see Table 1.4.) (Bezprozvanny and Maximov, 2001; Doyle et al., 1996; Harrison, 1996; Song et al., 2006). Numerous examples demonstrate that some PDZ domains can also recognize internal peptide motifs, lipids and other PDZ domains. The best characterized example of an internal-motif-mediated PDZ interaction is the PDZ domain of nNOS and the PDZ domain of either syntrophin or PSD-95. The domains interact in a remarkable linear head-to-tail arrangement (Brenman et al., 1996; Gee et al., 1998; Hillier et al., 1999). The 30-residue extension to the nNOS PDZ domain adopts an extended β-hairpin fold (called β-finger) (Christopherson et al., 1999). This nNOS β-hairpin pockets in the binding site of syntrophin protein, which mimicks a peptide ligand through its P_0 and P_{-2} pocket interaction.

PDZ domain containing proteins play key roles in organizing polar sites of cell-cell communication. They assemble receptors and their downstream effectors. PDZ domain containing proteins crosslink many different polypeptides by binding to C-terminal sequences. Internal motif recognition is not an exception to the rules of PDZ recognition but another way to satisfy the same energetic requirements. A complete understanding of the regulatory mechanisms of PDZ-mediated interactions will enhance our knowledge of many cellular and biological processes.

Figure 1.5.: Schematic diagram of PDZ domaining proteins

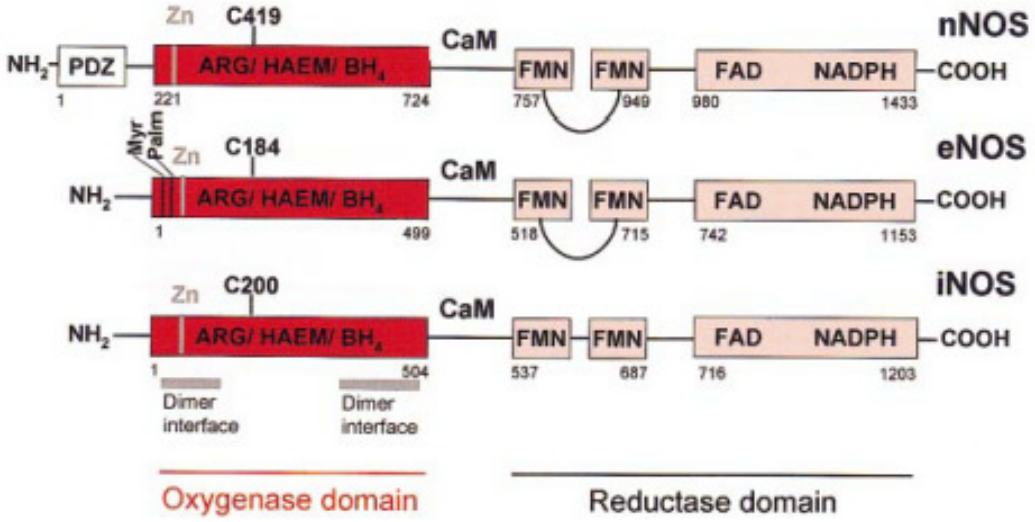


This figure shows an overview about the different groups of PDZ domain containing proteins and their various domains.

1.4. Nitric Oxide Synthase

NO is a signaling molecule generated by three different kinds of NO synthases (NOSs), which catalyze the transformation of the amino acid L-arginine to L-citrulline. NO is participating in physiological and pathophysiological processes. The three different isoforms are neuronal NOS (also known as NOS-1), which was first identified in neuronal tissue, inducible NOS (also known as NOS-2) being the isoform, which is inducible in numerous cells and tissues, and endothelial NOS (NOS-3), which is the isoform first found in vascular endothelial cells. Sometimes these isoforms also distinguished on the basis of their constitutive (eNOS and nNOS) versus inducible (iNOS) expression, and their calcium-dependence (eNOS and nNOS) or-independence (iNOS), see Fig.1.6.

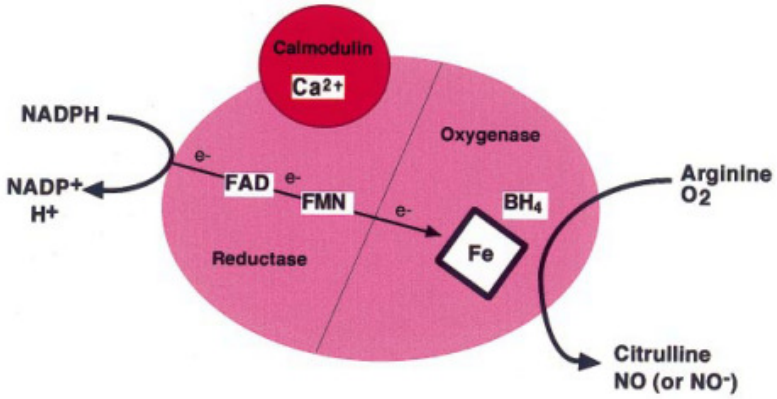
Figure1.6.: Domain structure of human nNOS, eNOS and iNOS, adapted from (Alderton et al., 2001)



In the boxes you see the oxygenase, reductase and PDZ domains. The start/end and amino acids of the different isoforms are shown. Myr = myristoylation; Palm = palmitolylation; Zn = zinc-ligating cysteines.

The NOS genes contain a similar genomic composition. NOSs demonstrate a bidomain structure in which an N-terminal oxygenase domain containing binding sites for haem, BH_4 and L-arginine is linked by a CaM-recognition site to a C-terminal reductase domain that includes binding sites for FAD, FMN and NADPH (Fig. 1.7.) (McMillan and Masters, 1995; Richards and Marletta, 1994).

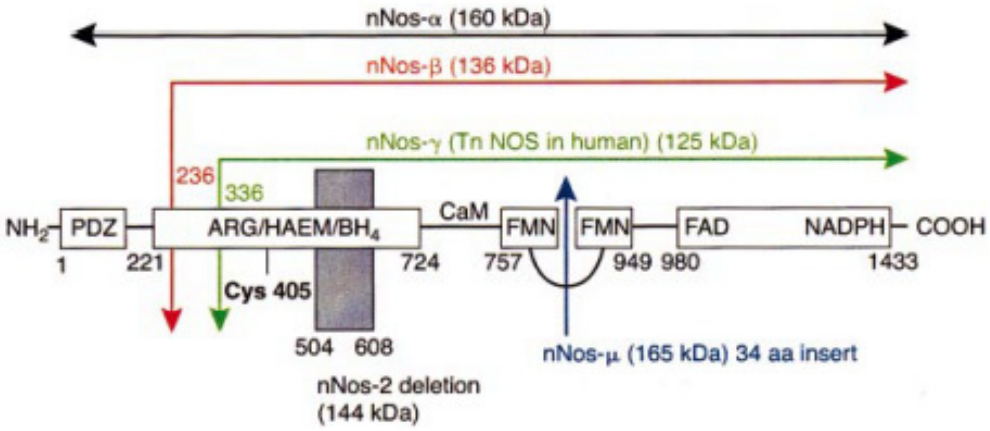
Figure 1.7.: reactions and cofactors of NOS (Alderton et al., 2001)



Electrons (e-) are donated by NADPH to the reductase domain of the enzyme and carry on via FAD and FMN redox carriers to the oxygenase domain. They interact with the haem and BH_4 at the active site to catalyze the reaction of oxygen with L-arginine, generating citrulline and NO as products. Electrons flow through the reductase domain requires the presence of bound Ca2+/CaM.

The N-terminal 220 amino-acids of nNOS are especially, because that isoform contains a PDZ domain that targets nNOS to synaptic sites in brain and skeletal muscle. The PDZ domain of nNOS interacts with the second of several similar PDZ motifs in neuron-specific PSD-95 and PSD-93. nNOS interacts with α1-syntrophin in skeletal muscles, which forms a complex with the sarcolemmal dystrophin complex. The nNOS-PDZ consensus sequence is G (D, E)-X-V (Schepens et al., 1997). Different splice variants of nNOS were descriebed. nNOSα contains the PDZ domain and is localized in various tissues. The protein has a size of 160kDa. Both nNOSβ and nNOSγ lack the PDZ domain of nNOS, which is encoded by exon 2 (Brenman et al., 1996). If translated in vivo, nNOSβ would be a 136kDa protein and nNOSγ a 125kDa protein. nNOSμ is selectively expressed in heart and is the predominant isoform in skeletal muscle (Silvagno et al., 1996). nNOSμ has additional 34 amino acids inserted between the CaM-and flavin-binding domains. nNOS-2 has been identified in mouse brain and in human neuroblastoma cells (Fujisawa et al., 1994). nNOS-2 is possibly catalytically inactive and for that reason the function plays a dominat negative role (Brenman et al., 1997). See Figure 1.8.

Figure 1.8.: Splice variants of rat nNOS (Alderton et al., 2001)



The PDZ, oxygenase and reductase domains are marked by solid boxes. The splice variants are shown by arrowed lines: black, nNOSα (amino acids 1-1433); red, nNOSβ (amino acids 236-1433); green, nNOSγ (amino acids 336-1433); blue, nNOSμ (1-1433 with a 34 amino acid insert in the FMN-bindin gdomain). The deleted amino acid residues 504-608 in nNOS-2.

Regulation of NOS activity

CaM is nessecary for the enzymatic activity of all three isoforms. The calcium-dependence of NO synthesis differentiates the NOS isoforms, so nNOS and eNOS having a much higher calcium requirement than iNOS. CaM binding increases the rate of electron transfer from NADPH to the reductase domain flavin (Gachhui et al., 1998; Gachhui et al., 1996) and artificial electron acceptors, like Ferricyanide and Cytochrom c. CaM also activates the electron transfer from the reductase domain to the haem centre (Abu-Soud et al., 1994a; Abu-Soud et al., 1994b) (Fig.1.7.). The phosphorylation of nNOS and eNOS have an effect on NOS activity. The phosphorylation of eNOS triggers an increase in electron flux through the reductase domain and an increase in NO production (McCabe et al., 2000). In contrast, the phosphorylation of nNOS processes a decrease in NOS activity (Hayashi et al., 1999).

1.5. Aim of the thesis

The Ca^{2+} channel $Ca_v1.2$ and the plasma membrane calcium ATPase PMCA4b are transmembrane proteins and operate with their C-terminal end as PDZ ligands. Both proteins play a key role in Ca^{2+} signalling and in Ca^{2+} fluctuation, for example gene expression, regulation of blood pressure, and they are involved in cardiac excitation-contraction coupling. Previous studies have shown that PMCA interacts with a few cytoskeletal proteins, as mentioned before. Also it is known that PMCA interacts with nNOS (Schuh et al., 2001). However, the molecular mechanisms responsible for spatial and temporal specificity of NO-mediated regulation of intracellular Ca^{2+} are still unclear as well as the physiological role of $Ca_v1.2$ and PMCA4b.
Based on these observations, this thesis has the following aims:

1. To screen for new interaction partners of $Ca_v1.2$ and PMCA4b via three independent assays (PDZ domain array, immunoprecipitation and pull down). Often new interaction partners could give information about signalling pathways or physiological relevance. Co-localizations of some protein interactions could support the results.

2. The nNOSα PDZ domain interacts with the C-terminus of PMCA4. About the interaction and physiological key role of nNOS and $Ca_v1.2$ via PDZ domain is little-known. To generate a heterologous system that express $Ca_v1.2$ and nNOS for electrophysiological measurements, we and co-workers from Regensburg designed patch clamp experiments to achieve information on the consequences of their interactions.

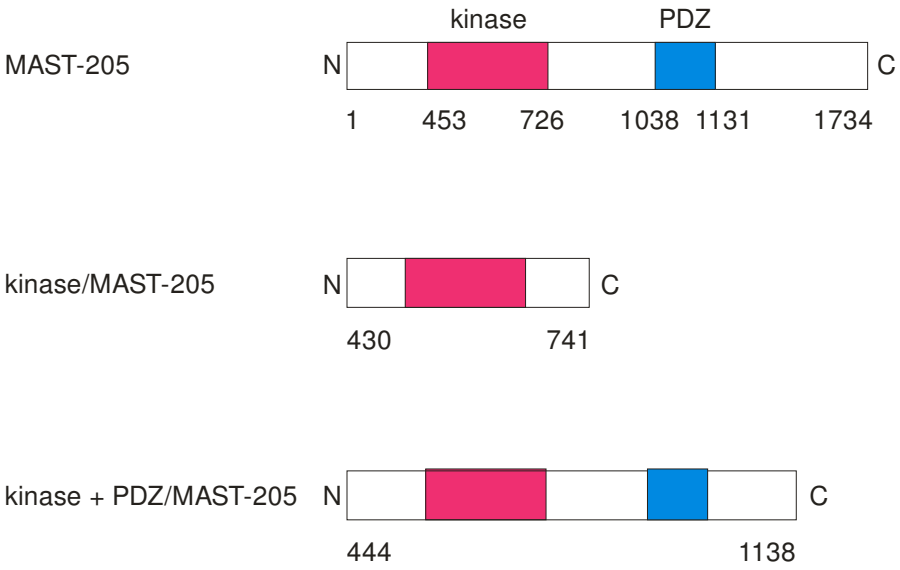
3. Nitric oxide, which is generated by nNOS, is a crucial signalling molecule in mammals. In addition, NO can interact directly with reactive thiols in many proteins, leading to post-translational modifications that induce functional changes. Such S-nitrosylations could influence $Ca_v1.2$ activity, regulated by nNOS. To verify that we established the biotin switch assay.

2 MATERIALS AND METHODS

2.1. Plasmids

Plasmid constructs were generated by standard PCR-based cloning strategies and confirmed by DNA-sequencing. PCR products were gel purified, digested with appropriate restriction enzymes (Table 2.1.), again purified from an agarose gel according to manufacturer's instructions (NucleoSpin Extract II, Macherey-Nagel), and ligated into a vector that was opened with the same restriction enzymes. Codons for the final 10 amino acids of $Ca_v1.2\alpha$ (accession no. AAI45106) and the final 15 amino acids of PMCA4b (accession no. NP_001675) were cloned into the pEXP vector (Figs. 2.4, 2.5) containing a 6 x Histidine tag (Panomics, Fremont, CA, USA) to produce 6xHis-tagged fusion proteins for PDZ Domain Arrays (Panomics) (pEXP-LTCC; pEXP-PMCA4b, see cloning constructions in appendix). The same codons of $Ca_v1.2\alpha$, the complete C-terminal cytoplasmatic tail of $Ca_v1.2\alpha$ (accession no. P15381), and approximately half of the complete C-terminal cytoplasmic tail were inserted into the pGEX-4T-3 vector (GE Healthcare Biosciences AB, Uppsala, Figs. 2.7, 2.9, 2.10) to produce Glutathion-S-Transferase (GST) fusion proteins for pull-downs (pGex-4T-3-LTCC; pGex-4T-3-Ct-$Ca_v1.2$ lang; pGex-4T-3-Ct-$Ca_v1.2$ kurz, see cloning constructions in appendix). The expression constructs pGex-4T-1-nNOS-PDZ, pcDNA3-ΔnNOS (ΔnNOS denotes the absence of the PDZ domain and was created by K. Schuh) and pcDNA3-nNOS were kind gifts from D. Bredt (University of California, San Francisco, CA), the plasmid pRK5-kinase-MAST-205, pRK5-kinase-PDZ-MAST-205 from Rafael Pulido (Centro de Investigacion Principe Felipe, Valencia, Spain, see Fig. 2.0.) and the plasmid pcDNA3-$Ca_v1.2\alpha$ a gift from Sebastian Meier (University of Wuerzburg, Germany), which was a template for plasmid constructs. Furthermore, we utilized the plasmids pBK-CMV-NHERF1 and pCMV-hPMCA4b for transfections.

Figure 2.0.: MAST-205 constructs (by Rafael Pulido)



In that figure the different recombinant proteins of HA-MAST-205 are shown. They were used for Co-immunoprecipitations and pull downs. The pink bar represents the kinase domain of MAST-205 (residues 453-726) and the blue bar shows the PDZ domain (residues 1038-1131) of that protein.

Table 2.1.: Oligonucleotide primers for plasmid constructs used for PDZ Domain Arrays and GST Fusion Proteins

Primer	Restriction site	Primer Sequence[1]
for_Ca_v1.2 LTCC expression vector pEXP	PstI	…5'- CTG CAG GAC AGC AGG TCC TAT GTC AGC AAC CTG TAG T -3'
rev_Ca_v1.2 LTCC expression vector pEXP	XbaI	…5'- TCT AGA CTA CAG GTT GCT GAC ATA GGA CCT GCT GTC -3'
for_Ca_v1.2 LTCC expression vector pGEX-4T-3	EcoRI	…5'- G AAT TCC GAC AGC AGG TCC TAT GTC AGC AAC CTG TAG -3'
rev_Ca_v1.2 LTCC expression vector pGEX-4T-3	SalI	…5'- GTC GAC CTAC AGG TTG CTG ACA TAG GAC CTG CTG ACG G -3'
GST_C_Ca_v_lang_for expression vector pGEX-4T-3	EcoRI	…5'- C GGA ATT CCC GAC AAC TTT GAC TAC CTG ACA AG -3'

GST_C_Ca_v_rev expression vector pGEX-4T-3	XhoI	…5'- CCG CTC GAG CTA CAG GCT GCT GAC GCC GGC -3'
GST_C_Ca_v_kurz_for expression vector pGEX-4T-3	EcoRI	…5'- C GGA ATT CCC AGG CAG CAT GGA AGC TCA GC -3'

[1] The restriction sites are indicated in blue. Stop codons are shown in red.

TA-Cloning

For the constructs pEXP-LTCC; pGex-4T-3-LTCC; pGex-4T-3-Ct-Cav1.2_lang and pGex-4T-3-Ct-Cav1-2_kurz we used the Topo-TA-cloning kit (Invitrogen).

TA-cloning is a cloning technique without restriction enzymes. That method is based on the ability of adenine (A) and thymine (T). A and T are complementary basepairs which excist on different DNA fragments. In the presence of ligase they were ligated together. The insert is formed by PCR using Taq DNA polymerase. This polymerase lacks 3' to 5' proofreading activity and adds a single 3'– adenine overhang to each end of the PCR product. The target vector is linearized and cut with a blunt-end restriction enzyme. It is important to use dideoxythymidine triphosphate (ddTTP) to guarantee the addition of only one T residue (tailing the vector with 3'- overhang on each blunt end). If no possible restriction sites are existing or the traditional cloning is difficult, TA-cloning is often used as an alternative. A disadvantage is that directional TA-cloning is not feasible, so the gene has 50% chance of getting cloned in the reverse direction. In our case we sequenced the constructs after TA-cloning and afterwards we cut via restriction enzymes (Table 2.1.) and ligated them in the suitable vector (Table 2.1.).

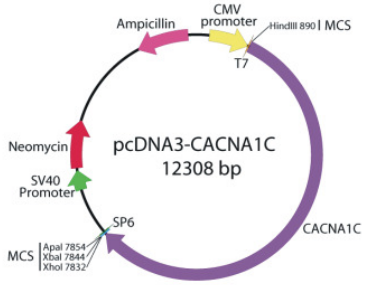


Figure 2.1: vector map pcDNA3-Ca$_v$1.2, gift from S. Meier, University of Wuerzburg, Germany

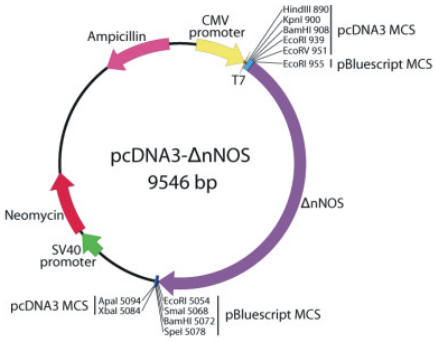


Figure 2.2: vector map pcDNA3-ΔnNOS, gift from D. Bredt, University of California, San Francisco

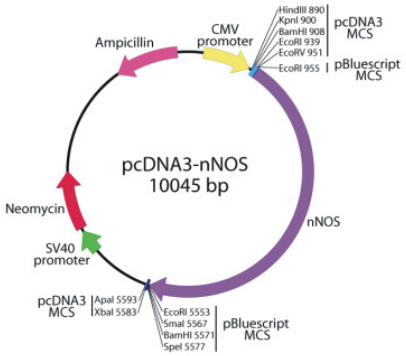


Figure 2.3: vector map pcDNA3-nNOS, gift from D. Bredt, University of California, San Francisco

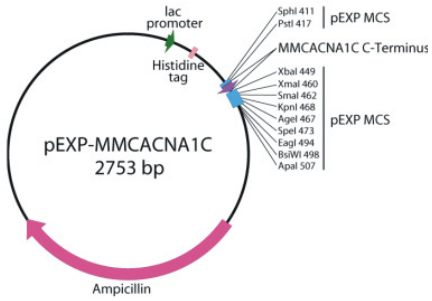


Figure 2.4: vector map pEXP-LTCC, D. Fetting, University of Wuerzburg, Germany

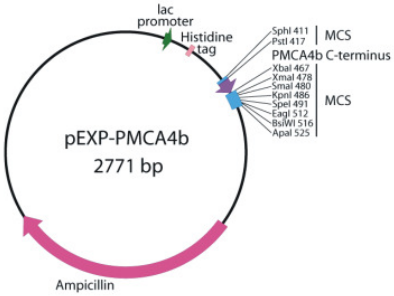


Figure 2.5: vector map pEXP-PMCA4b, K. Schuh, University of Wuerzburg, Germany

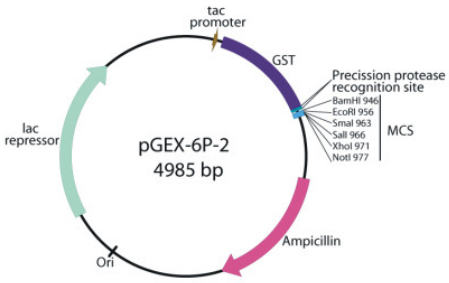


Figure 2.6: vector map pGex-6P-2 (pGex-4T-3 is the same like pGex-6P-2 just another frame), GE Healthcare. Munich

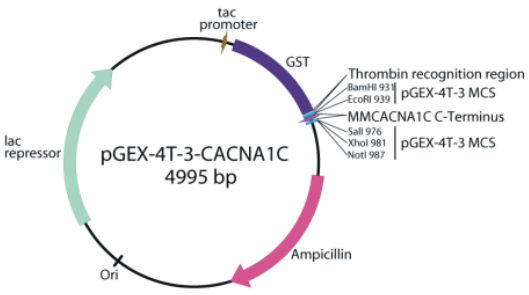


Figure 2.7: vector map pGex-4T-3-LTCC
D. Fetting, University of Wuerzburg, Germany

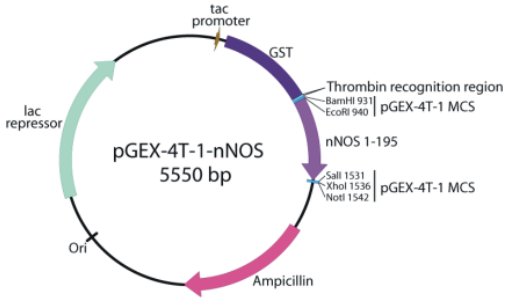


Figure 2.8: vector map pGex-4T-1-nNOS,
gift from D. Bredt, University of California,
San Francisco

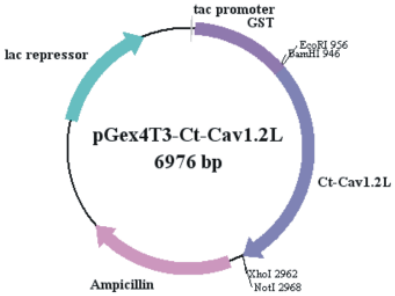


Figure 2.9: vector map pGex-4T-3-Ct-Ca_v1.2 Lang,
D. Fetting, University of Wuerzburg, Germany

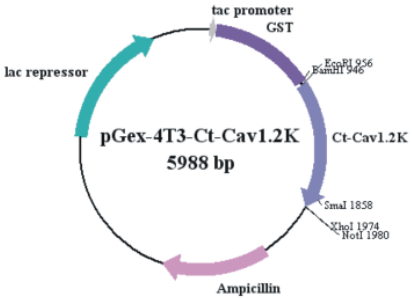


Figure 2.10: vector map pGex-4T-3-Ct-Ca_v1.2 Kurz,
D. Fetting, University of Wuerzburg, Germany

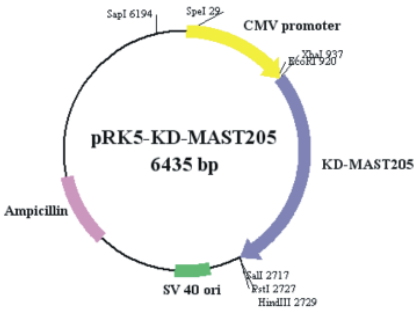


Figure 2.11: vector map pRK5-KD-MAST205,
gift from R. Pulido, Centro de Investigacion
Principe Felipe, Valencia, Spain

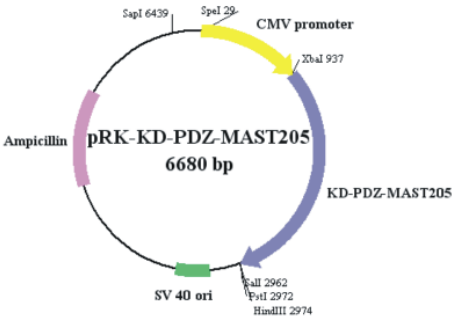
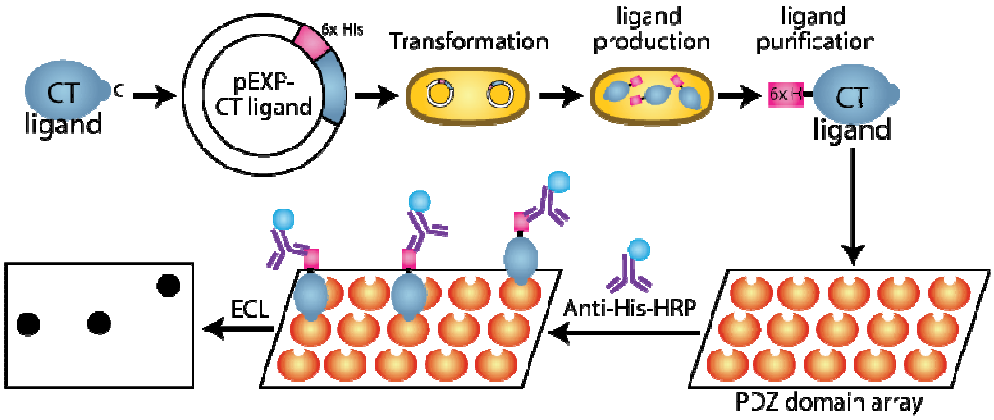


Figure 2.12: vector map pRK5-KD-PDZ-MAST205,
gift from R. Pulido, Centro de Investigacion
Principe Felipe, Valencia, Spain

2.2. TranSignal™ PDZ Domain Array

pEXP-Ca_v1.2α, pEXP-PMCA4b were transformed into *E. coli* BL21 (DE3) bacteria. The bacteria were inoculated in 3 ml of LB/Amp (100 µg/ml). Bacteria were shaken for one hour at 37 °C at 300 rpm. When OD600 of the bacterial culture 0.5-0.8 was attained, 1 mM isopropyl-1-thio-β-D-galactopyranoside (IPTG) was added to the bacteria (grow 3-4 h at 37 °C). Cells were collected by centrifugation (4000xg for 10 min at 4 °C). The pellet was resuspended in 2 ml Resuspension Buffer (Panomics) and lysed with a sonicator. Afterwards, cells were centrifugated (14.000 rpm for 5 min at 4 °C) and the supernatant was analyzed via bicinchoninic acid (BCA) protein assay. Each membrane (I-VI for pEXP-Ca_v1.2 and I-IV for pEXP-PMCA4b) was rinsed for 30 min with Wash Buffer (Panomics) and then blocked with Blocking Buffer (Panomics) for 1-2 h at room temperature before further washing. The membranes were incubated with diluted bacterial extract (5 µg/ml in Blocking Buffer) for 1-2 h at room temperature and washed afterwards three times with Wash Buffer for 5 min each. The PDZ membrane was incubated with 1x Anti-Histidine horse radish peroxidise (HRP) Conjugate (Panomics) diluted in Wash Buffer for 1-2 h at room temperature. Antibody complexes were detected by enhanced chemiluminescence using ECL Western blotting substrate (ECL™ Plus kit, Amersham). X-rays were scanned and analysed with ImageJ. The higher the signal intensity, the stronger is the protein-protein interaction between the PDZ protein and the C-terminal ligand. The values were standardized against the GST negative control.

Figure 2.13.: PDZ Domain Array



This figure shows the flow chart of the TranSignal PDZ Domain Array assay (drafted by P.Tng).

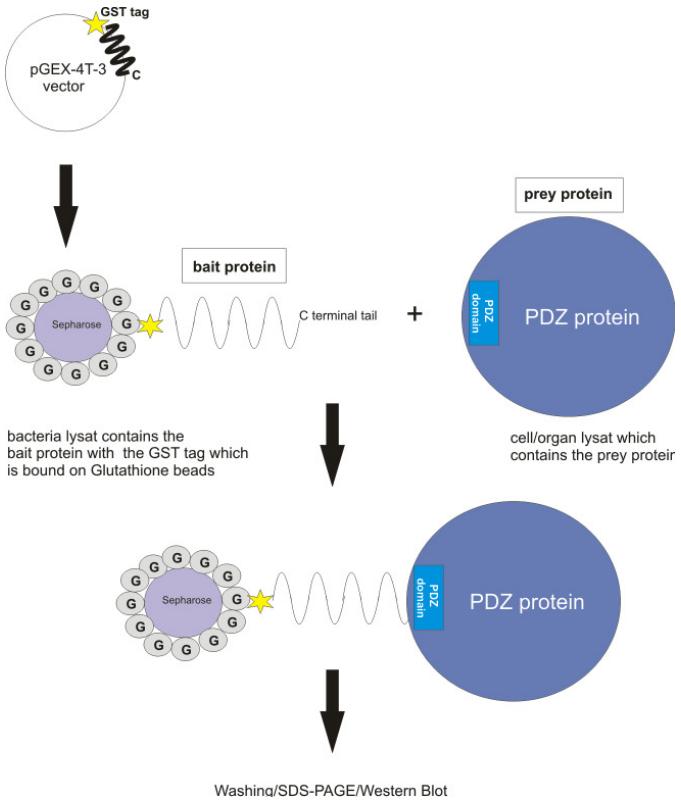
2.3. GST Fusion Proteins

GST and GST fusion proteins were expressed in *E. coli* BL21 (DE3) by induction with 1 mM IPTG for 6 h. Bacteria cells were pelleted, resuspended in PBS (137 mM NaCl, 2.7 mM KCl, 100 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4) containing protease inhibitor (complete EDTA-free Protease Inhibitor Cocktail Tablets, Roche) and lysed by addition of lysozym (1 mg/ml) and sonication. The lysate was cleared by centrifugation at 30.000xg for 20 min at 4 °C. The pellet was resupended in PBS and the resulting lysate was bound to glutathione-Sepharose (GE Healthcare) and rotated overhead for 2 h at 4 °C (Fig. 2.14.). The quantity of washed and bound fusion proteins was estimated by Coomassie Blue staining of SDS-polyacrylamide gels.

2.4. GST pull-down

To prepare tissue lysates, organs were removed from mice and immediately homogenized by a glass homogenizer in cold RIPA buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1 % Nonidet P-40, 0.5 % Na-Deoxycholate, protease inhibitor and optional 0.1 % SDS). The homogenate was centrifuged at 4000xg for 3 min. For cell lysates, the same RIPA buffer was used. The supernatant of all lysates (500 µg) and ~3 µg of bound GST or GST fusion proteins on agarose beads were rotated overnight at 4 °C (Fig. 2.14.). The beads were pelleted and washed three times in PBS with protease inhibitors. Bound proteins were eluated in 2xLaemmli buffer (4 % SDS, 20 % glycerol, 10 % 2-mercaptoethanol, 0,004 % bromphenol blue, 0,125 M Tris HCl, pH 6.8) (Laemmli, 1970) and separated on polyacrylamide-gels followed by transfer onto nitrocellulose following standard Western blotting procedures. Nitrocellulose membranes were blocked in TBST (TBS+0.1 % Tween) with 5 % milk before immunoblotting with appropriate primary and secondary antibodies. All secondary antibodies on immunoblots were detected using chemiluminescence (ECL™ Plus kit, Amersham).

Figure 2.14.: GST pull-down



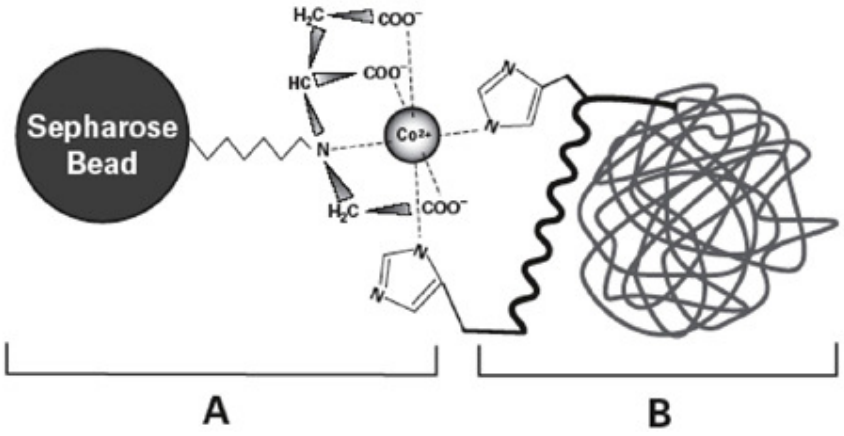
This figure demonstrates the technique of the GST pull-down. The yellow star shows the GST tag. The black wave line (with the C-terminal tail) demonstrates the ligand protein, which binds in the first step to the sepharose-glutathion beads (G). The blue circle illustrates the PDZ protein with the PDZ domain, which binds to the C-terminal tail of our ligand protein.

2.5. Talon His-Tag Purification Resins

Talon His-Tag Purification Resin is used for preparing pure his-tagged proteins from bacterial, mammalian, and yeast cells, under native or denaturating conditions. Talon is an immobilized metal affinity chromatography (IMAC) resin charged with cobalt, which binds to his-tagged proteins with higher specificity than nickel-charged resins (Fig. 2.15.). 6xHis-Tag fusion proteins (pEXP and pEXP-PMCA4b) were expressed in *E. coli* BL21 (DE3) by induction with 1 mM IPTG for 6 h. Bacteria cells were pelleted, resuspended in PBS (137 mM NaCl, 2.7 mM KCl, 100 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4) containing protease inhibitor (complete EDTA-free Protease Inhibitor Cocktail Tablets, Roche) and lysed by addition of lysozym (1 mg/ml) and sonication. The lysate was cleared by centrifugation at 30.000xg for 20 min at 4 °C. The pellet was resupended in PBS and the resulting suspension

was bound to talon resin (Clontech Laboratories) and rotated overhead for 20 min at room temperature to allow the polyhistidine-tagged proteins to bind the resin. After centrifugation, the resin was washed by adding 1x Equilibration/Wash Buffer (50 mM Na_2HPO_4, 150 mM NaCl, pH 7.0) and rotated overhead for 10 min at room temperature. The quantity of washed and bound fusion proteins was estimated by Coomassie Blue staining of SDS-polyacrylamide gels. For the His-tag pull-down of pEXP and pEXP-PMCA4b via Talon Metal resin, we followed the description in 2.4.

Figure 2.15.: Talon His-Tag Purification Resins



This figure shows the schematic diagram of the Talon metal beads. Part A: Talon Metal Affinity Resin; Sepharose bead bearing the tetradentate chelator of the Co^{2+} metal ion. Part B: Thepolyhistidine-tagged recombinant protein binds to the resin (adapted from Clontech).

2.6. Co-immunoprecipitations

HEK 293 cells (DMEM supplemented with 10 % FCS), ECV cells (DMEM supplemented with 10 % FCS, 4.5 g/l glucose) and HEK 293 cells stably expressing α_{1b} ($Ca_v1.2b$) and the $Ca_v\beta2a$ subunit of the smooth muscle L-type calcium channel (DMEM supplemented with 10% FCS, 200 µg/ml G418 plus 100 µg/ml hygromycine B) were grown to ~80 % confluence on 10-cm plates (BD Falcon). Cells were transfected with 20 µg of total DNA using Lipofectamine™ 2000 (Invitrogen) according to the manufacturer's instruction. After ~48 h, cells were rinsed with cold PBS and lysed in RIPA buffer without 0.1 % SDS. After 10 min incubation on ice, cells were scraped from the plates and were centrifugated at 13.000xg for 10 min at 4 °C. 300 µg of the lysate was used for each immunoprecipitation. 1-5 µg of antibodies: anti-$Ca_v1.2$ (Alomone Labs); anti-MAGI-3 (Abcam); anti-HA (Covance); anti-PMCA4-JA9 (Sigma) were added, respectively. After 2 h of agitation at 4 °C, 50 µl of protein A/G agarose was added to each mixture and rotated overnight at 4 °C. Protein A/G agarose was pelleted at 4.000xg for 30 s and washed twice with RIPA buffer containing protease inhibitors. Bound proteins were eluated in 2xLaemmli buffer. The bound proteins were separated on polyacrylamide gels followed by transfer onto nitrocellulose for Western blotting as described above.

2.7. Antibodies for immunoblotting

The following antibodies were used for immunoblotting: anti-ZO-1 (BD Transduction Laboratories™) used at 1:1000 dilution, anti-nNOS (Zymed Laboratories) used at 1:2000 dilution, anti-$Ca_v1.2$ (Alomone Labs) diluted 1:200, anti-CASK (BD Transduction) diluted 1:1000, anti-NHERF1 (Cell Signaling) diluted 1:1000, anti-MAGI-3 (Abcam) diluted 1:1000, anti-HA (Covance) diluted 1:1000. Secondary goat anti-mouse antibodies were purchased from Jackson Immuno Research and used at 1:5000 dilution, goat anti-rabbit (Jackson Immuno Research) used at 1:10000 dilution. From eBioscience we used rabbit IgG TrueBlot (1:1000) and mouse IgG TrueBlot (1:1000).

2.8. Immunohistochemistry

Rat heart was frozen in liquid nitrogen. Tissue was fixed with Tissue Tek (Sakura) on section blocks, and cryosections (20 µm) were cut with microtome blades. Cryosections were placed on glass slides, fixed in 4 % paraformaldehyde/PBS for 5 min, permeabilized with 0.2 % TritonX-100/PBS for 10 min, and blocked with 5 % goat serum in PBS for 1 h to reduce non-specific binding. Sections were incubated with primary antibodies overnight at 4 °C, washed thrice in PBS followed by incubation with the appropriate secondary antibodies. Stained sections were washed three times in PBS and mounted in Mowiol. The following antibodies were used: polyclonal rabbit anti-$Ca_v1.2$-ATTO 488 (Alomone Labs), polyclonal rabbit anti-NHERF1 (Cell Signaling), polyclonal rabbit anti-MAGI-3 (Abcam), monoclonal mouse anti-PMCA 5F10 (Sigma) and Alexa Fluor 488 goat anti-mouse, Alexa Fluor 594 goat anti-rabbit (Invitrogen). To test for unspecific binding, the secondary Alexa Fluor labeled antibodies were used alone. The confocal micrographs were taken with an Eclipse E600 Nikon microscope using a C1 confocal scanning head and a 60-fold oil immersion objective.

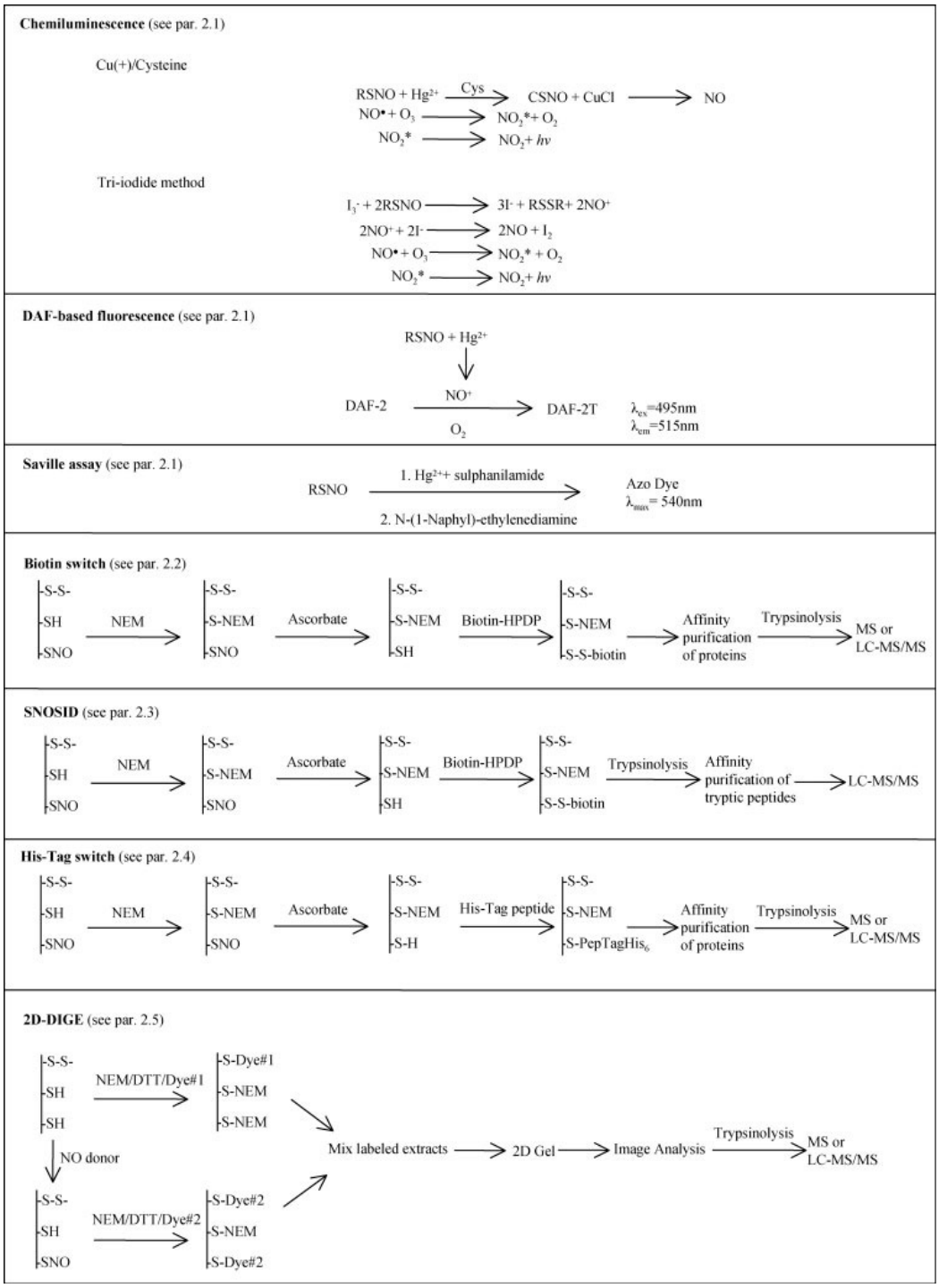
2.9. Tricine-SDS-PAGE (Schagger and von Jagow, 1987)

Proteins in the mass range between 1-100 kDa were separated with this procedure. The assembly procedure is rather similar to a standard SDS-PAGE. The follow reagents were used: anode buffer (0.2 M Tris, pH 8.0), cathode buffer (0.1 M Tris, 0.1 M Tricine (Sigma Aldrich), 0.1 % SDS, pH 8.25), gel buffer (3.0 M Tris, 0.3 % SDS, pH 8.45), separating gel monomer 16,5:1 (49.5 % T 6 % C) and stacking gel monomer 33:1 (49.5 % T 3 % C). T denotes the total percentage concentration of acrylamide and bisacrylamide (Roth) and C is the percentage concentration of the crosslinker relative to the total concentration T (Hjerten, 1962). At first, we prepared the separating gel solution (16.5 % T 6 % C), mixing 10 ml separating gel monomer, 10 ml gel buffer and 3.2 ml glycerol (Merck). We then added 100 µl of 10 % APS (Sigma Aldrich) and 10 µl TEMED (Sigma Aldrich). For the stacking gel (4 % T 3 % C) we used 1 ml stacking gel monomer, 3.1 ml gel buffer, 8.4 ml dH2O, and added 100 µl APS and 10 µl TEMED. The upper (cathode) and lower (anode) buffer chambers were filled with the appropriate buffer. Electrophoresis was performed at 4 °C at 30 V and 200 mA. After 1 h, when the sample had completely entered the stacking gel, the running conditions were set at 90 V and 300 mA for ~5 h. Afterwards, gels were stained with Coomassie Brilliant Blue G250 (Merck).

2.10. Biotin Switch Assay

Within the last few years, the research on cysteine residues represents a very dynamic and regulated event that can control a multitude of protein functions. Between the diverse oxidative modifications occurring on cysteine residues, S-nitros(yl)ation is rising as an essential nitric oxide (NO) dependent posttranslational modification that regulates a large variety of cellular functions and signalling events. We prefer to stay with the classical chemical nomenclature whereby "nitrosation" is defined as addition of an NO^+ equivalent and "nitrosylation" as addition of an NO radical to another reactant to form a nitroso or nitrosyl group. Under conditions where the mechanism is either unknown or includes both pathways, the chimera "nitros(yl)ation is used here to indicate the involvement of nitrosation and/or nitrosylation. Measuring free NO levels after cleavage of S-NO bonds or replacing the original nitrosothiols with another detectable tag (see Fig. 2.16.) for a schematic view of methods for analysis S-nitrosylation. We decided us for the Biotin Switch Assay.

Figure 2.16. Schematic illustration of the reactions occurring in the different investigational techniques employed to detect S-nitrosylation, adapted from (Torta et al., 2008).



Background of the Biotin Switch Assay

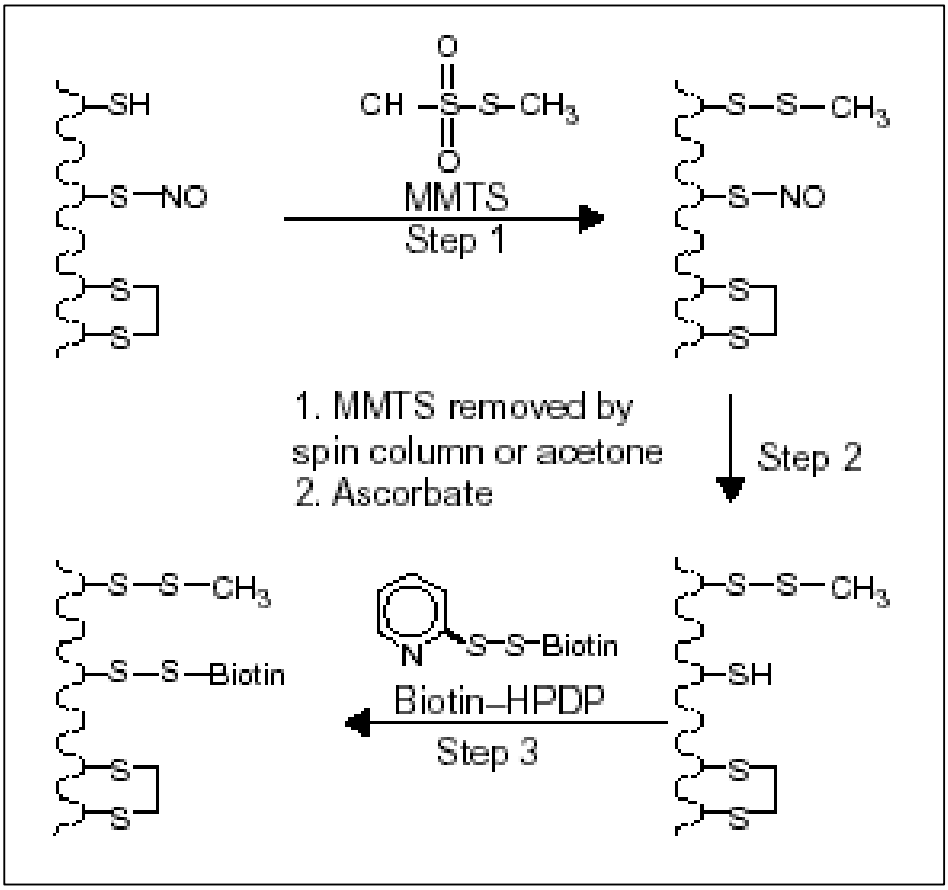
In the first experimental step proteins are treated with a thiol blocking agent, such as monomethyl thiosulphonate (MMTS) or others, to chemically block all free thiols, leaving S-nitrosylated thiols and disulphide bonds untouched. As the S-NO bond is light sensitive, all the experimental procedures should be conducted in the dark. Following the blocking step, the S-NO bond is specifically reduced to a free thiol, usually with milimolar concentrations of ascorbat (for 1 h) in the presence of the metal ion chelators ethylenediaminetetraacetic acid (EDTA) and neocuproine. Free thiols react with a thiol-specific biotinylating agent, such as biotin-HPDP (N-(6-(biotinamido)hexyl)-3-(2-pyridyldithio)propionamide)), which results in a disulphide-linked label that can be used for Western blotting. This label can be easily removed by using mercaptoethanol or dichlorodiphenyltrichloroethane (DTT).

Procedure

S-Nitrosylated proteins were detected by a modification of the biotin switch method (Jaffrey and Snyder, 2001). Cells were lysed in HEN buffer (250 mM Hepes NaOH, pH 7.1, 1 mM EDTA, 0.1 mM neocuproine from Sigma) and centrifugated at 1000xg for 10 min at 4 °C. Cell lysates (240 µg) were added to four volumes of blocking buffer (nine volumes of HEN buffer plus 1 volume 25 % SDS, adjusted to 20 mM methyl methanethiosulfonate (MMTS from Fluka) with a 2 M stock prepared in dimethylformamide DMF from Sigma) at 50 °C for 20 min with frequent vortexing. The MMTS was then removed by adding four volumes acetone and the proteins precipitated at -20 °C for 20 min. The proteins were recovered by centrifugation 5.000xg for 5 min, followed by rinsing of the pellet with 4 x 1 ml 70 % acetone/H_2O. After removal of acetone, the proteins were resuspended in 240 µl of HENS buffer (HEN buffer containing 1 % SDS). To the suspension we added biotin-HPDP (Thermo Fisher Scientific) prepared fresh as a 4 mM solution in dimethylsulfoxid (DMSO from Sigma) from a 50 mM stock suspension in DMF. Sodium ascorbate was added to a final concentration of 1 mM. Labeling reaction was performed in the dark unless otherwise indicated. After incubation for 1 h at 25 °C, biotinylated proteins were precipitated by streptavidin-agarose beads (Fluka). To detect an individual SNO protein from lysates, the labeling reaction was acetone-precipitated as previously described. The washed pellet was resuspended in 250 µl of HEN/10 buffer (HEN diluted 10-fold into H_2O containing 1 % SDS) followed by addition of 750 µl of neutralization buffer (25 mM Hepes, 100 mM NaCl, 1 mM

EDTA, 1 % Triton X-100, pH 7.5) This material was incubated overnight at 4 °C with 50 µl of a streptavidin-agarose slurry. The beads were washed with 4 x 1 ml of wash buffer (neutralization buffer plus 500 mM NaCl), followed by 2 x 1 ml of neutralization buffer. The beads were eluated with 50 µl of HEN/10 + 1% β-mercaptoethanol at room temperature for 20 min. The eluated mixture was then analyzed by SDS-PAGE, followed by immunoblotting with anti-$Ca_v1.2$.

Figure 2.17.: Biotin Switch Assay



The schematic diagram shows the important chemical steps of the Biotin Switch Assay (Proteomics).

2.11. Current Recordings (that part was done by Olaf Strauss and his group)

Transfection

For patch clamp experiments HEK 293 cells were transiently transfected with either GFP alone or nNOS and GFP or ΔNOS and GFP. All transfections were carried out using Lipofectamine transfection reagent (Invitrogen) following the manufacturer's instructions. Cells were analyzed at 36 h after transfection.

Patch-Clamp recordings

Membrane currents were measured in the whole-cell configuration of the patch-clamp technique. During the recordings, transfected cells were superfused by a bath solution containing (mM): NaCl 82, TEA-Cl 20, $BaCl_2$ 30, CsCl 5.4, $MgCl_2$ 1, EGTA 0.1, Glucose 10, HEPES 5, pH 7.4 adjusted with NaOH; 302.9 mOsm. The perfusion chamber was mounted onto a stage of an inverted fluorescence microscope. Transfected cells were selected by their GFP fluorescence. For whole-cell recordings, patch-pipettes of 3-5 MΩ were made from borosilicate tubes using a DMZ-Universal Puller (Zeitz). Pipettes were filled with a pipette-solution containing (mM): CsCl 102, TEA-Cl 10, EGTA 10, $MgCl_2$ 1, Na_2ATP 3, HEPES 5, pH 7.4 adjusted with CsOH; 248 mOsm. Membrane currents were recorded using an EPC-10 computer-controlled patch-clamp amplifier in conjunction with the software TIDA for data acquisition and analysis. The access resistance was compensated for to values lower than 10 MΩ. For analysis of voltage-dependent activation steady-state currents were plotted against the membrane potentials of the electrical stimulation. Plots of each individual cell were fitted using the Boltzmann equation.

Statistical analysis

Statistical significance was tested using one-way analysis of variance (ANOVA). All data were given as mean ± SEM. n = number of independent experiments, * = statistical significance with $p < 0.05$. Mean values of data obtained from Boltzmann fits calculated for each individual cell.

3 RESULTS

3.1. PDZ domain arrays

3.1.1. Expression of the PDZ array ligands

To discover novel PDZ domain containing protein interaction partners of $Ca_v1.2\alpha$ and PMCA4b, the nucleotide sequences coding for the C-termini of these proteins were cloned into pEXP bacterial expression vectors (Fig. 3.1.B). Verification of expression and size of His-tagged recombinant proteins in bacteria via tricine gel analysis confirmed high expression levels and expected sizes of proteins, i.e. for the pEXP read-through, pEXP-$Ca_v1.2\alpha$ and pEXP-PMCA4b, 9 kDa as calculated, 8.47 kDa, and 8.97 kDa, respectively (Fig. 3.1.B). Probing the PDZ domain arrays with these bacterial lysates and successive detection of interactions with anti-6xHis antibodies revealed a series of positive spots on all PDZ arrays tested.

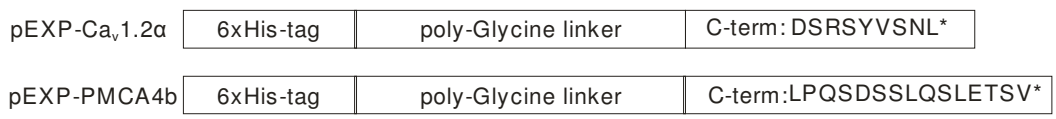
Figure 3.1.: Expression of the PDZ array ligands $Ca_v1.2\alpha$ and PMCA4b

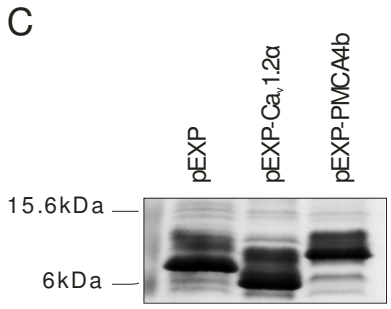
A

C-terminal end $Ca_v1.2$	
human	VSSL*
mouse	VSNL*
rat	VSNL*
consensus	VSXL*

C-terminal end PMCA4b	
human	ETSV*
mouse	ETPV*
rat	ETPV*
consensus	ETXV*

B

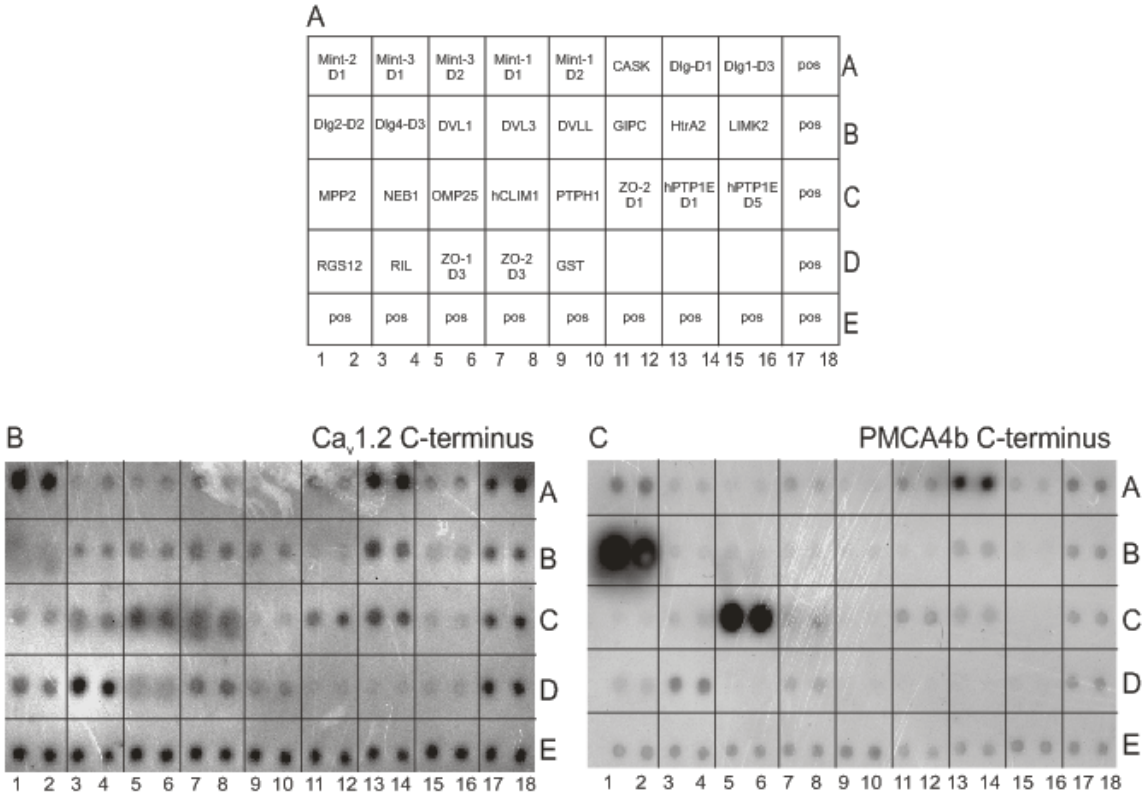




A: C-terminal binding motif of $Ca_v1.2$ and PMCA4b. The X represents any amino acid and the * = stop, possess different C-terminal ends and resulting in interactions with different PDZ domain containing proteins **B**: the last 10 amino acid residues of $Ca_v1.2\alpha$ and the final 15 amino acid residues of PMCA4b were expressed with a 6x Histidine tag linked by a poly-Glycine linker through insertion into the expression vector pEXP. $Ca_v1.2\alpha$ (VSNL*) and PMCA4b (ETSV*) **C**: Tricine-SDS-PAGE to validate the expression of recombinant protein ligands in BL21 bacteria. The shifts between the lanes pEXP (~9 kDa), pEXP-$Ca_v1.2\alpha$ (8.47 kDa), pEXP-PMCA4b (8.97 kDa), where the read through product, pEXP, is larger than the PDZ ligands, confirmed their successful expression. Each lane contained 4 µl of bacterial lysate.

3.1.2. PDZ domain array I

Figure 3.2.: PDZ domain array I



A: schematic chart of the TranSignal PDZ domain array I. 100 ng of the PDZ domain containing proteins are spotted in duplicate on the array. pos – positive control (Histidine-tagged ligand), negative control – Glutathione-S-Transferase (GST). **B**: PDZ domain array I was treated with bacterial extract containing the Histidine-tagged recombinant protein, pEXP-Ca$_v$1.2α. **C**: PDZ domain array I was treated with bacterial lysate containing the Histidine-tagged recombinant protein, pEXP-PMCA4b. Both bacterial extracts had a concentration of 5 mg/ml. PDZ domain and ligand interactions were visualized with anti-Histidine antibodies.

Incubation of the PDZ Domain Array I, on which mainly PDZ domains of synaptic proteins were spotted (overview in Fig. 3.2.A), with the C-terminal PDZ ligands of Ca$_v$1.2α and PMCA4b revealed a panel of additional positive PDZ spots, representing possible interaction partners of the Ca$_v$1.2α and the PMCA4b (Figs. 3.2.B and C). The ImageJ analysis of these signal intensities is listed in Table 3.1 and Fig. 3.3. In this case, the Ca$_v$1.2α and PMCA4b C-termini interacted strongly with the PDZ domains of Mint-2-D1, OMP25 and Dlg-D1 (also called SAP97-D1). In addition to these bindings, a promiscuous binding of the Ca$_v$1.2α C-terminus to HtrA2, hCLIM1, hPTP1E-D1, RIL, and ZO-2-D3 was identified. Moreover, binding of the PMCA4b C-terminus to PDZ domains of CASK and an interaction with the

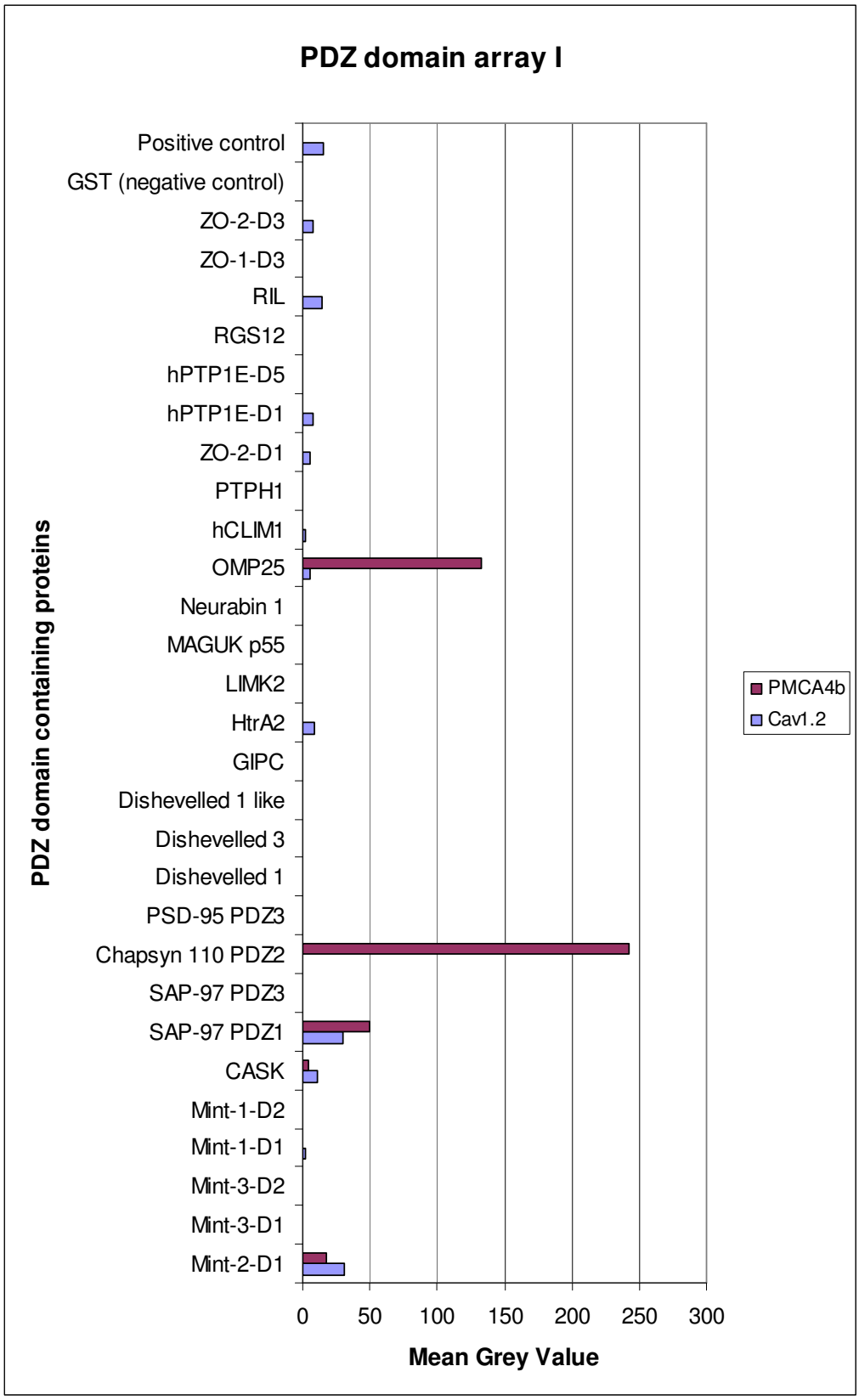
Chapsyn-110 (Dlg PDZ domain 2) was very prominent and the signal strength was much stronger than the positive controls of the array.

Table 3.1.: PDZ domain array I

PDZ domain	full name of protein	accession	mean grey values $Ca_v1.2$	mean grey values PMCA4b
Mint-2-D1	X11L protein PDZ Domain 1	Q99767	31.5	17.7
Mint-3-D1	X11L2 protein, PDZ Domain 1	O96018	0	0.5
Mint-3-D2	X11L2 protein, PDZ Domain 2	O96018	0	0
Mint-1-D1	X11 protein, PDZ Domain 1	Q02410	2.2	0.3
Mint-1-D2	X11 protein, PDZ Domain 2	Q02410	0.1	0.9
CASK	Calcium/Calmodulin-dependent serine protein kinase	O14936	11.0	4.4
Dlg-D1	Synapse-associated protein 97 PDZ-Domain 1	Q12959	30.4	49.8
Dlg1-D3	Synapse-associated protein 97 PDZ-Domain 3	Q12959	0	0.3
Dlg2-D2	Channel associated protein of synapse-110, PDZ Domain 2, PSD-93	Q15700	0.1	242.8
Dlg4-D3	Human postsynaptic density-95, PDZ Domain 3	P78352	0.2	0.2
DVL1	Dishevelled 1	O14640	0.2	0.9
DVL3	Dishevelled 3	Q92997	0.3	0
DVLL	Dishevelled-1-like	P54792	0.3	0
GIPC	GAIP C-terminus interacting protein GIPC	O14908	0.2	0
HtrA2	High temperature requirement protein A2	O43464	0	0
LIMK2	LIM motif-containing protein kinase-2	P53671	8.9	0
MPP2	MAGUK p55 subfamily member 2	Q14168	0	0
OMP25	Mitochondrial outer membrane protein 25	P57105	0.3	0.1
NEB1	Neurabin-I, neural tissue-specific F-actin-binding protein I	Q9ULJ8	0.2	0.1
hCLIM1	Human 36kDa carboxyl terminal LIM domain protein	O00151	5.4	132.5
PTPH1	Protein-tyrosine phosphatase H1	P26045	2.3	0
ZO-2-D1	Zonula occludens protein 2, PDZ Domain 1	Q9UDY2	0	0
hPTP1E-D1	Protein-tyrosine phosphatase 1E, PDZ Domain 1	Q12923	5.2	0
hPTP1E-D5	Protein-tyrosein phosphatase 1E, PDZ Domain 5	Q12923	7.9	0
RGS12	Regulator of G-protein signaling 12	O14924	0	0.1
RIL	Reversion-induced LIM protein	P50479	14.8	0.9
ZO-1-D3	Zonula occludens protein 1, PDZ Domain 3	Q07157	0.3	0
ZO-2-D3	Zonula occludens protein 2, PDZ Domain 3	Q9UDY2	8.2	0.1
pos	PDZ Domain positive control for Kv1.4 ligand		102.2	153.3
GST	Glutathione-S-Transferase (negative control)		0.0	0.0

PDZ domain containing proteins from membrane I. This table lists signal intensities of the visualised spots. We incubated the membrane I with bacterial lysate containing pEXP-$Ca_v1.2$ and pEXP-PMCA4b.

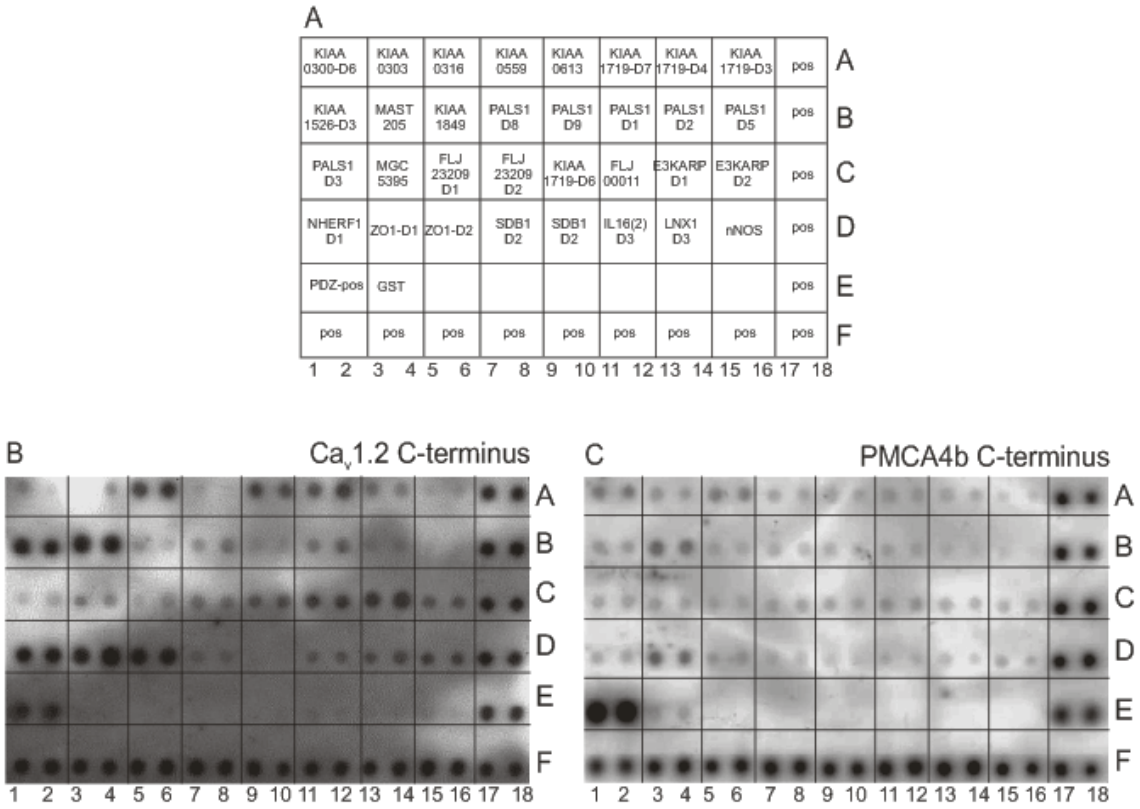
Figure 3.3.: Bar chart of spot intensities of PDZ domain array I



Blue = incubated with $Ca_v1.2\alpha$ C-terminus. Red = incubated with PMCA4b C-terminus.

3.1.3. PDZ domain array II

Figure 3.4.: PDZ domain array II



A: The arrangement of the TranSignal PDZ domain array II. The controls were the same as described in Fig. 3.2. **B**: PDZ domain array II was incubated with the bacterial extract containing pEXP-Ca$_v$1.2α. **C**: PDZ domain array II was incubated with bacterial lysate containing pEXP-PMCA4b.

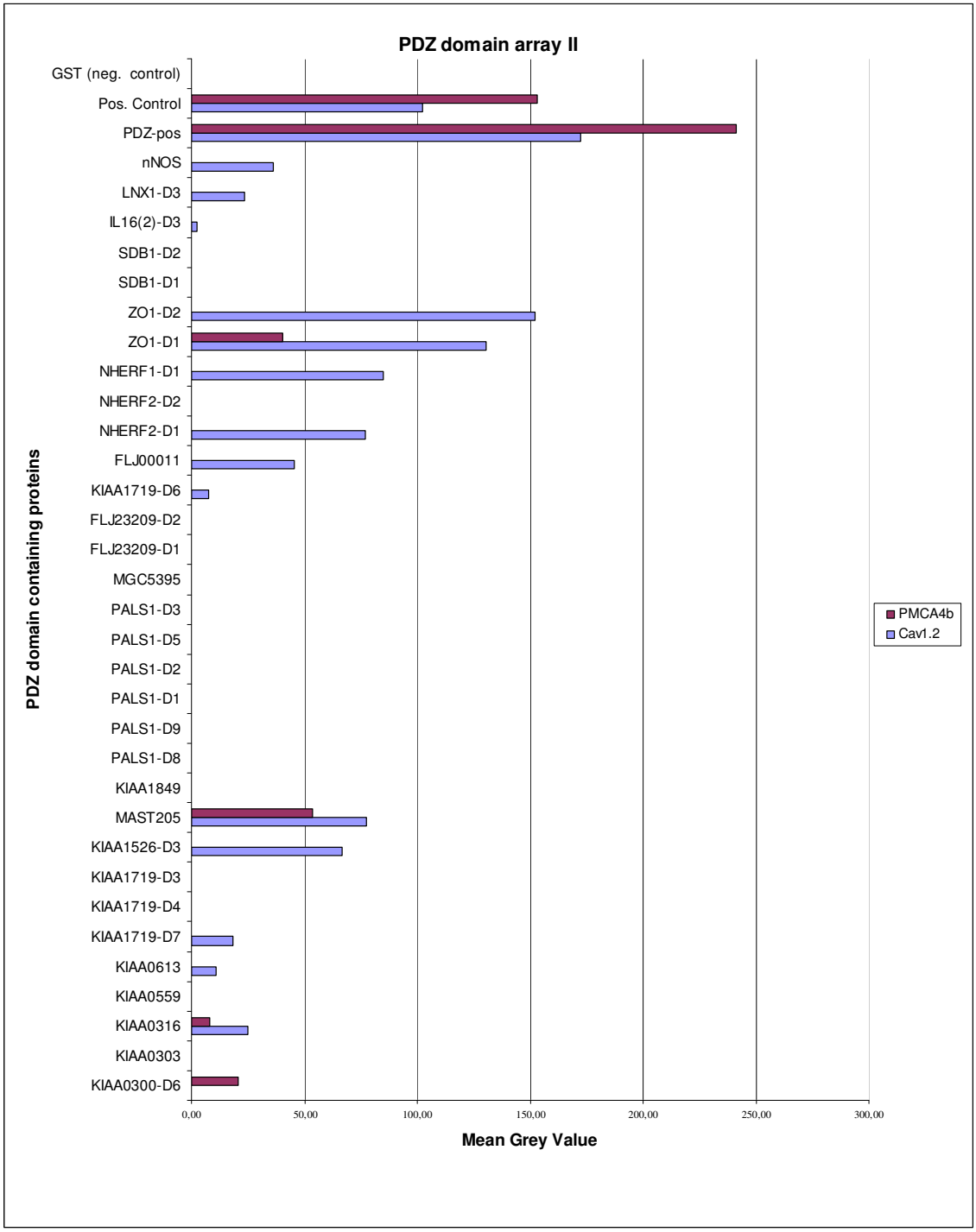
The PDZ Domain Array II is organized as shown in Fig. 3.4.A, which includes some tight junction proteins, sodium/hydrogen exchanger proteins and further PDZ domains. Strong interactions for both PDZ ligands were observed for ZO-1-D1, MAST-205 and, of course, for the PDZ positive controls (SAP-102). Ca$_v$1.2α C-terminus had a high affinity for 4 additional PDZ domains as well: ZO-1-D2, NHERF1-D1, NHERF2-D1/D2, several KIAA proteins and nNOS (Figs. 3.4.B and C and analyses of signal intensities summarized in Table 3.2. and Fig. 3.5.).

Table 3.2.: PDZ domain array II

PDZ domain	full name of protein	accession	mean grey values $Ca_v1.2$	mean grey values PMCA4b
KIAA0300-D6	KIAA0300 protein, Domain 6	O15018	0	20.8
KIAA0303	KIAA0303 protein	O15021	0	0
KIAA0316	KIAA0316 protein	Q14CM0	24.8	8.2
KIAA0559	KIAA0559 protein	Q9Y6V0	0	0
KIAA0613	KIAA0613 protein, Domain 7	O75112	10.8	0
KIAA1719-D7	KIAA1719 protein, Domain 7	Q9C0E4	18.2	0
KIAA1719-D4	KIAA1719 protein, Domain 4	Q9C0E4	0	0
KIAA1719-D3	KIAA1719 protein, Domain 3	Q9C0E4	0	0
KIAA1526-D3	KIAA1526 protein, Domain 3	Q9P202	66.5	0
MAST205	microtubule associated testis specific serine/threonine protein kinase	Q6P0Q8	77.7	53.5
KIAA1849	hypothetical protein KIAA1849	Q96JH8	0	0
PALS1-D8	Pals1-associated tight junction protein, Domain 8	Q8NI35	0	0
PALS1-D9	Pals1-associated tight junction protein, Domain 9	Q8NI35	0	0
PALS1-D1	Pals1-associated tight junction protein, Domain 1	Q8NI35	0	0
PALS1-D2	Pals1-associated tight junction protein, Domain 2	Q8NI35	0	0
PALS1-D5	Pals1-associated tight junction protein, Domain 5	Q8NI35	0	0
PALS1-D3	Pals1-associated tight junction protein, Domain 3	Q8NI35	0	0
MGC5395	similar to hypothetical protein MGC5395	AAH12477	0	0
FLJ23209-D1	hypothetical protein FLJ23209, Domain 1	NP_079171	0	0
FLJ23209-D2	hypothetical protein FLJ23209, Domain 2	NP_079171	0	0
KIAA1719-D6	KIAA1719 protein, Domain 6	Q9C0E4	7.6	0
FLJ00011	FLJ00011 protein (Fragment)	Q9H7Q6	45.5	0
NHERF2-D1	solute carrier family 9 (sodium/hydrogen exchanger), 3 regulatory factor 2, Domain 1	Q15599	77.0	0
NHERF2-D2	solute carrier family 9 (sodium/hydrogen exchnager), 3 regulatory factor 2, Domain 2	Q15599	0	0
NHERF1-D1	solute carrier family 9 (sodium/hydrogen exchanger) 3 regulatory factor 1, Domain 1	O14745	84.8	0
Z01-D1	tight junction protein 1 (zona occludens), Domain 1	Q07157	130.7	40.6
Z01-D2	tight junction protein 1 (zona occludens), Domain 2	Q07157	152.0	0
SDB1-D1	syndecan binding protein (syntenin), melanoma differentiation associated protein-9, Pro-TGF-alphacytoplasmic domain interacting protein 18 (TACIP18), Domain 1	NP_005616S	0	0
SDB1-D2	syndecan binding protein (syntenin), melanoma differentiation associated protein-9, Pro-TGF-alpha cytoplasmic domain interacting protein 18 (TACIP18), Domain 2	NP_005616S	0	0
IL16(2)-D3	interleukin 16 isoform 2; lymphocyte chemoattractant factor, Domain 3	Q14005	2.5	0
LNX1-D3	numb-binding protein 1; ligand of numb-protein, Domain 3	Q8TBB1	23.5	0
nNOS	nitric oxide synthase 1 (neuronal), Domain 5	P29475	36.2	0
PDZ-pos	PDZ Domain positive control for Kv1.4 ligand synapse associated protein 102		172.4	241.4
pos	PDZ Domain positive control for Kv1.4 ligand		102.2	153.3
GST	Glutathione-S-Transferase (negative control)		0.0	0.0

PDZ domain containing proteins from membrane II. This table gives signal intensities of protein spots. We incubated the membrane II with bacterial lysates containing pEXP-$Ca_v1.2$ and pEXP-PMCA4b.

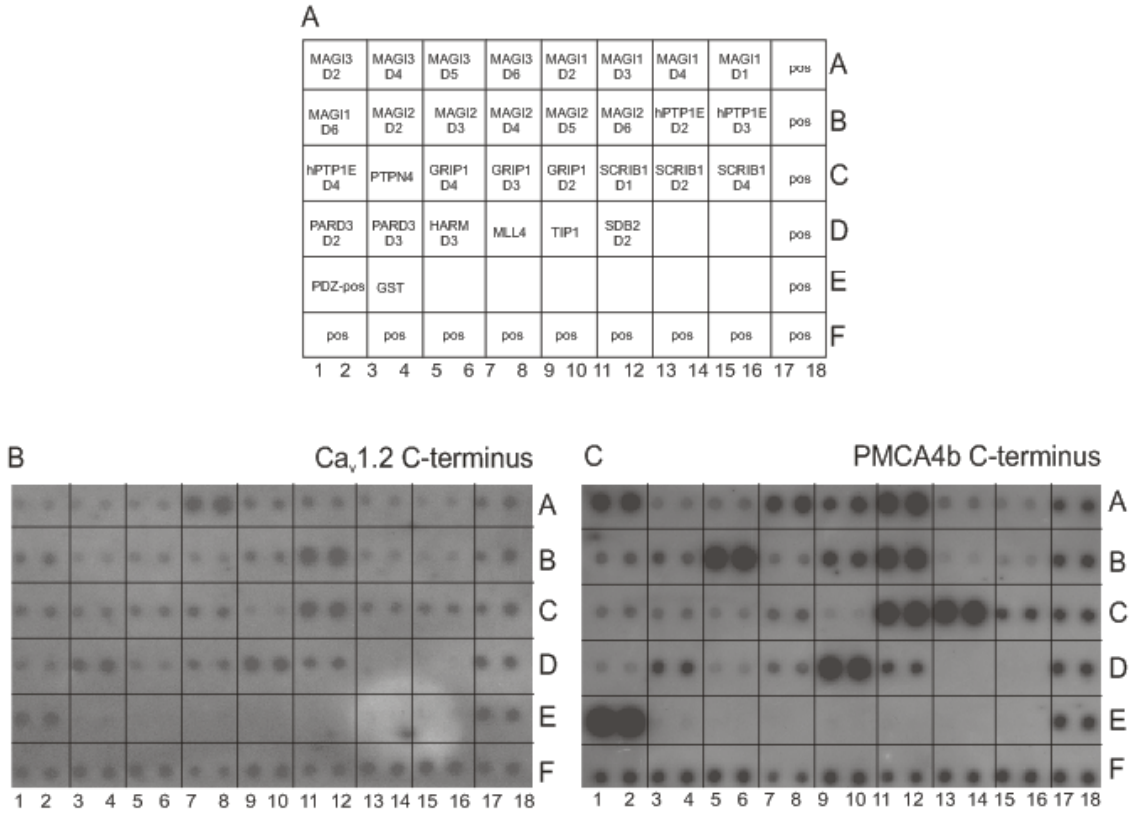
Figure 3.5.: Bar chart of spot intensities of PDZ domain array II



Blue = incubated with $Ca_v1.2\alpha$ C-terminus. Red = incubated with PMCA4b C-terminus.

3.1.4. PDZ domain array III

Figure 3.6.: PDZ domain array III



A: Schematic representation of the TranSignal PDZ domain array III. 100 ng of the PDZ domain containing proteins are spotted in duplicate on the array. pos - positive control (Histidine-tagged ligand), negative control - Glutathione-S-Transferase (GST). **B**: PDZ domain array III was incubated with the bacterial lysate containing the Histidine-tagged recombinant protein, pEXP-Ca$_v$1.2α. **C**: PDZ domain array III was incubated with the bacterial lysate containing the Histidine-tagged recombinant protein, pEXP-PMCA4b. Both bacterial extracts had a concentration of 5 µg/ml. PDZ domain and ligand interactions were visualized with anti-Histidine antibodies.

Figure 3.6.A gives an overview of the arrangement of the PDZ Domain Array III and the corresponding results for Ca$_v$1.2 and PMCA4b are shown in Figs. 3.6.B and C, respectively. The PDZ Domain Array III was mainly spotted with PDZ domains of scaffolding proteins, especially MAGUKs. ImageJ quantification (Table 3.3. and Fig. 3.7.) revealed that both PDZ ligands interacted strongly with different domains of MAGI-1 (also called BAI-1, brain-specific angiogenesis inhibitor-associated protein 1), MAGI-2 (also called AIP-1, atrophin 1 interacting protein), and MAGI-3, domains of SCRIB1, and TIP1.

Table 3.3.: PDZ domain array III

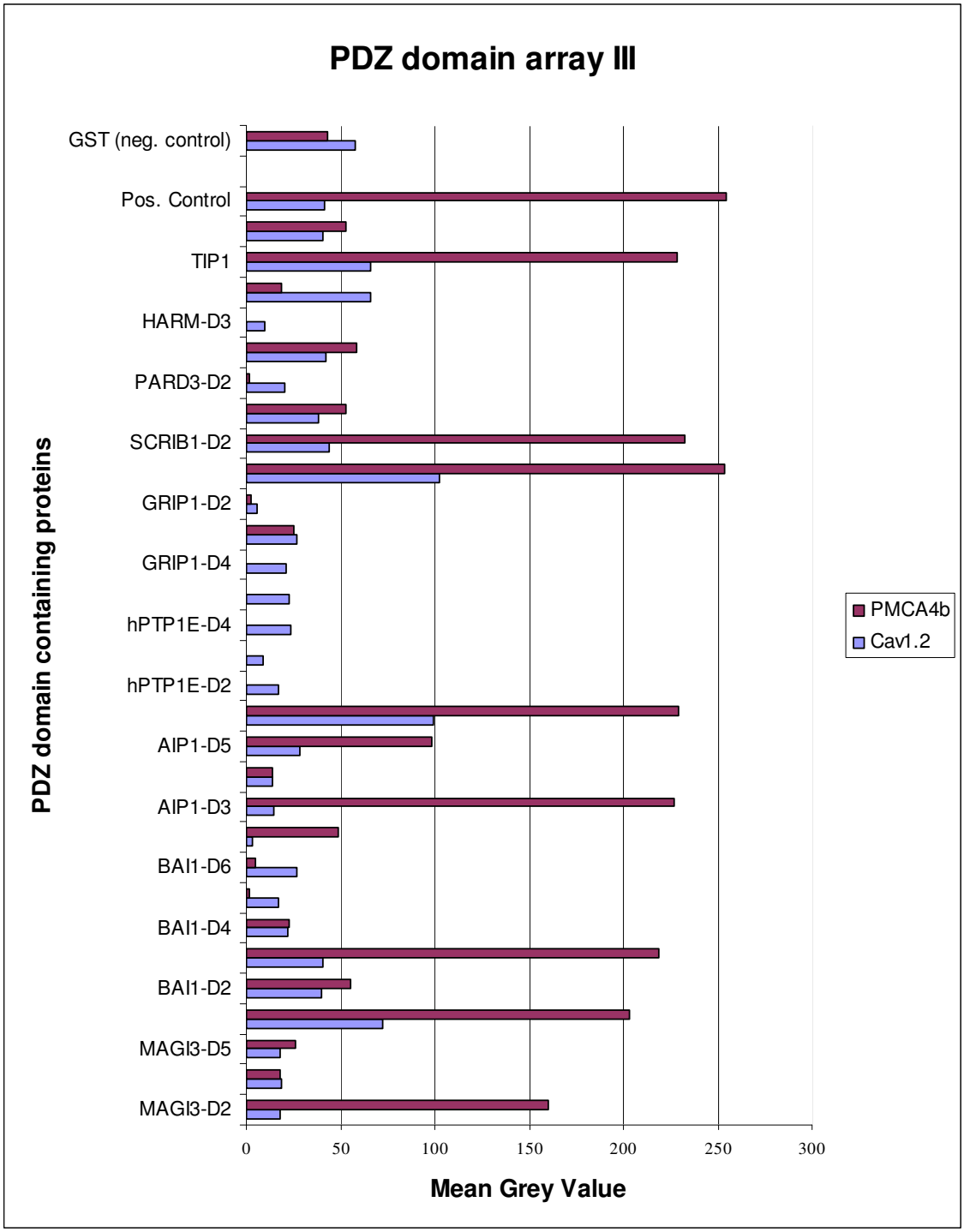
PDZ domain	full name of protein	accession	mean grey values $Ca_v1.2$	mean grey values PMCA4b
MAGI3-D2	membrane-associated guanylate kinase-related 3, Domain 2	Q5TCQ9	17.9	160.3
MAGI3-D4	membrane-associated guanylate kinase-related 3, Domain 4	Q5TCQ9	18.3	17.9
MAGI3-D5	membrane-associated guanylate kinase-related 3, Domain 5	Q5TCQ9	18.1	25.7
MAGI3-D6	membrane-associated guanylate kinase-related 3, Domain 6	Q5TCQ9	72.7	203.3
MAGI1-D2	membrane-associated guanylate kinase inverted 1; brain-specific angiogenesis inhibitor-associated protein 1; WW domain-containing protein 3; atrophin-1 interacting protein 3, Domain 2	Q96QZ7	39.5	55.5
MAGI1-D3	membrane-associated guanylate kinase inverted 1; brain-specific angiogenesis inhibitor-associated protein 1; WW domain-containing protein 3; atrophin-1 interacting protein 3, Domain 3	Q96QZ7	41.0	218.7
MAGI1-D4	membrane-associated guanylate kinase inverted 1; brain-specific angiogenesis inhibitor-associated protein 1; WW domain-containing protein 3; atrophin-1 interacting protein 3, Domain 4	Q96QZ7	22.3	22.5
MAGI1-D1	membrane-associated guanylate kinase inverted 1; brain-specific angiogenesis inhibitor-associated protein 1; WW domain-containing protein 3; atrophin-1 interacting protein 3, Domain 1	Q96QZ7	17.0	1.8
MAGI1-D6	membrane-associated guanylate kinase inverted 1; brain-specific angiogenesis inhibitor-associated protein 1; WW domain-containing protein 3; atrophin-1 interacting protein 3, Domain 6	Q96QZ7	26.7	4.7
MAGI2-D2	membrane-associated guanylate kinase inverted 1; atrophin-1 interacting protein 1, Domain 2	Q86UL8	2.9	48.5
MAGI2-D3	membrane-associated guanylate kinase inverted 1; atrophin-1 interacting protein 1, Domain 3	Q86UL8	14.9	226.5
MAGI2-D4	membrane-associated guanylate kinase inverted 1; atrophin-1 interacting protein 1, Domain 4	Q86UL8	13.5	13.7
MAGI2-D5	membrane-associated guanylate kinase inverted 1; atrophin-1 interacting protein 1, Domain 5	Q86UL8	28.3	98.6
MAGI2-D6	membrane-associated guanylate kinase inverted 1; atrophin-1 interacting protein 1, Domain 6	Q86UL8	98.9	229.2
hPTP1E-D2	protein tyrosine phosphatase, non-receptor type 13 isoform 4; protein-tyrosine phosphatase PTPL1;protein tyrosine phosphatase 1E; Fas-associated phosphatase-1; protein-tyrosine phosphatase 1, Fas-associated APO-1/CD95 (Fas)-associated phosphatase, Domain 2	Q12923	17.4	0.1
hPTP1E-D3	protein tyrosine phosphatase, non-receptor type 13 isoform 4; protein-tyrosine phosphatase PTPL1; protein tyrosine phosphatase 1E; Fas-associated phosphatase-1; protein-tyrosine phosphatase 1, Fas-associated APO-1/CD95 (Fas)-associated phosphatase, Domain 3	Q12923	8.8	0.1
hPTP1E-D4	protein tyrosine phosphatase, non-receptor type 13 isoform 4; protein-tyrosine phosphatase PTPL1; protein tyrosine phosphatase 1E; Fas-associated phosphatase-1; protein-tyrosine phosphatase 1, Fas-associated APO-1/CD95 (Fas)-associated phosphatase, Domain 4	Q12923	23.7	0.5
PTPN4	protein tyrosine phosphatase, non-receptor type 4; megakaryocyte phosphatase; PTPase-MEG1	P29074	22.8	0.1

Table 3.3.: continued from previous page

PDZ domain	full name of protein	accession	mean grey values $Ca_v1.2$	PMCA4b
GRIP1-D4	glutamate receptor-interacting protein1; GRIP1 protein, Domain 4	Q9Y3R0	20.9	0.3
GRIP1-D3	glutamate receptor-interacting protein1; GRIP1 protein, Domain 3	Q9Y3R0	27.1	25.4
GRIP1-D2	glutamate receptor-interacting protein1; GRIP1 protein, Domain 2	Q9Y3R0	5.9	2.1
SCRIB1-D1	Scribble, Domain 1	Q14160	102.6	253.3
SCRIB1-D2	Scribble, Domain 2	Q14160	43.6	232.8
SCRIB1-D4	Scribble, Domain 4	Q14160	38.3	52.9
PARD3-D2	partitioning-defective protein 3 homolog; atypical PKC isotype-specific interacting protein, Domain 2	Q8TEW0	20.1	1.4
PARD3-D3	partitioning-defective protein 3 homolog; atypical PKC isotype-specific interacting protein, Domain 3	Q8TEW0	41.9	58.3
HARM-D3	harmonin; PDZ-73 protein; antigen NY-CO-38, Domain 3	Q9Y6N9	9.9	0.0
MLL4	myeloid/lymphoid or mixed-lineage leukemia, translocated to, 4	Q9UMN6	64.9	18.8
TIP1	Tax interaction protein 1	O14907	65.6	228.2
SDB2D2	syntenin-2beta; syntenin-2; similar to syndecan binding protein, Domain 2	Q9H190	40.6	52.6
PDZ-pos	PDZ Domain positive control for Kv1.4 ligand synapse associated protein 102		41.4	254.5
pos	PDZ Domain positive control for Kv1.4 ligand		57.8	42.9
GST	Glutathione-S-Transferase (negative control)		0.1	0.0

PDZ domain containing proteins from membrane III. This table shows signal intensities of visualised spots. We incubated the membrane III with bacterial lysate containing pEXP-$Ca_v1.2$ and pEXP-PMCA4b.

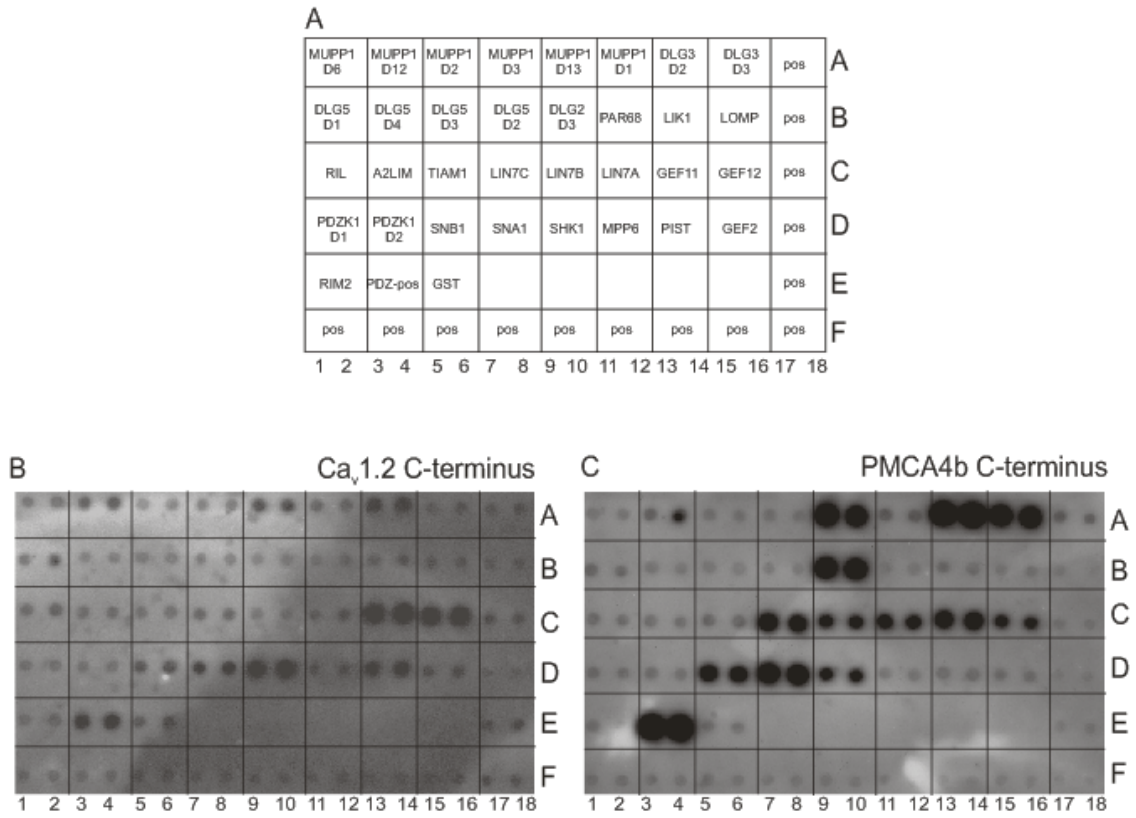
Figure 3.7.: Bar chart of spot intensities of PDZ domain array III



Blue = incubated with $Ca_v1.2\alpha$ C-terminus. Red = incubated with PMCA4b C-terminus.

3.1.5. PDZ domain array IV

Figure 3.8.: PDZ domain array IV



A: arrangement of the TranSignal PDZ domain array IV. It received the same treatment as described in figure 3.2. **B**: PDZ domain array IV was incubated with bacterial lysates containing pEXP-$Ca_v1.2\alpha$. **C**: PDZ domain IV was incubated with bacterial extract containing pEXP-PMCA4b.

Probing the PDZ Domain Array IV, which consists, for the most part, of a variety of scaffolding proteins, MAGUKs, Lin-7 proteins, nucleotide exchange factors, and synthrophins (Fig. 3.8.A), revealed strong interaction of the PMCA4b C-terminus with PDZ domains of MUPP1, Dlg2 (Chapsyn-110), Dlg3 (SAP-102), LIN7A, LIN7B, LIN7C, SNA1, and SNB1. Both PDZ ligands interact with the PDZ domains of GEF11, GEF12 and SHK1, and the $Ca_v1.2\alpha$ C-terminus binds specifically to that of PIST (Figs. 3.8.B and C and initial quantification of dot intensities summarized in Table 3.4. and Fig. 3.9.).

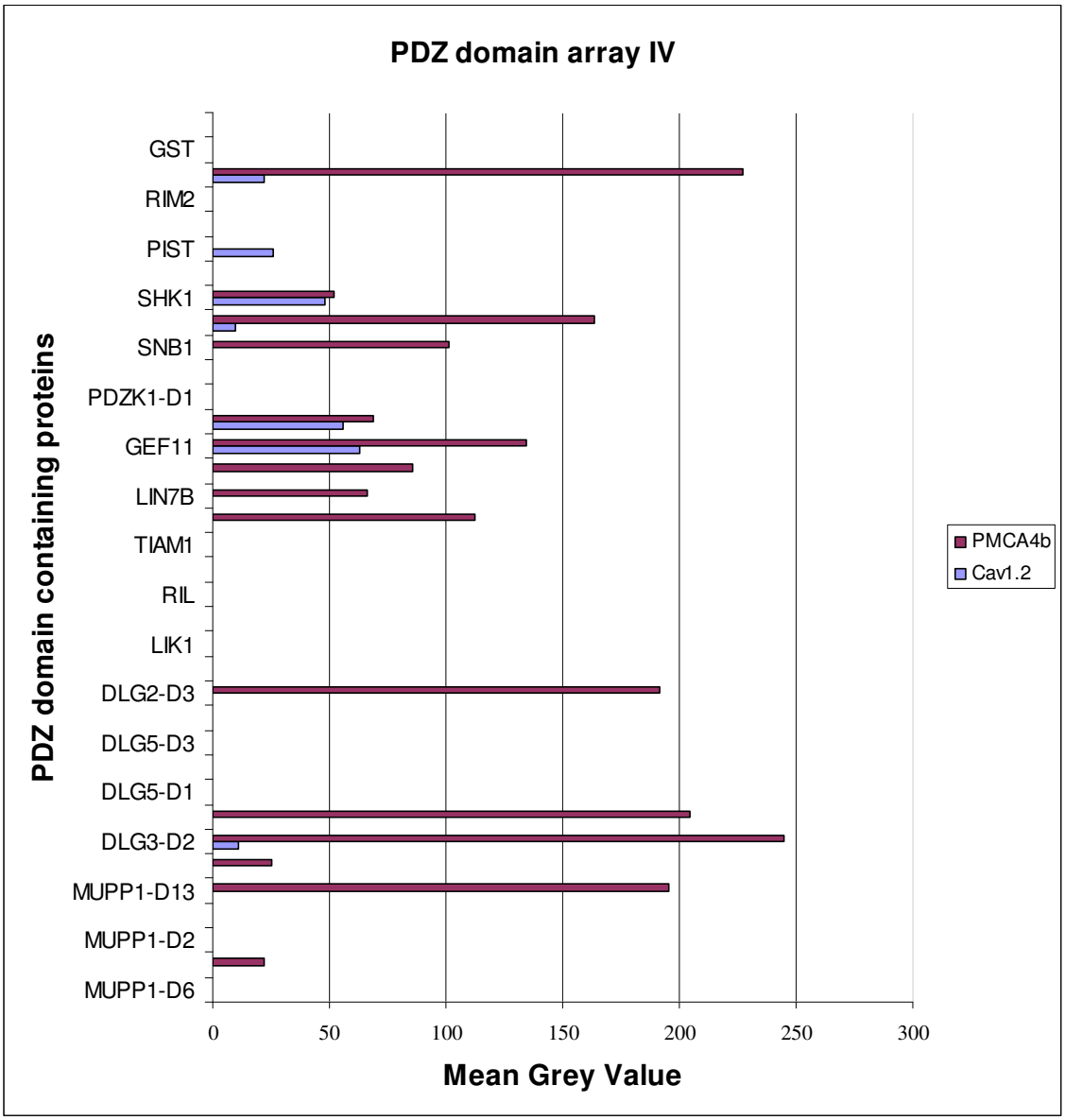
For additional analyses, several of the already established- and some of the observed-interaction partners were selected (listed in Table 3.5.) and the interactions were verified in co-immunoprecipitation and co-localization experiments.

Table 3.4.: PDZ domain array IV

PDZ domain	full name of protein	accession	mean grey values $Ca_v1.2$	PMCA4b
MUPP1-D6	multiple PDZ domain protein, Domain 6	O75970	0	0
MUPP1-D12	multiple PDZ domain protein, Domain 12	O75970	0	22.3
MUPP1-D2	multiple PDZ domain protein, Domain 2	O75970	0	0
MUPP1-D3	multiple PDZ domain protein, Domain 3	O75970	0	0
MUPP1-D13	multiple PDZ domain protein, Domain 13	O75970	0	195.2
MUPP1-D1	multiple PDZ domain protein, Domain 1	O75970	0	25.3
Dlg3-D2	synapse-associated protein 102; neuroendocrine dlg, discs large homolog 3, Domain 2	Q92796	10.9	244.5
Dlg3-D3	synapse-associated protein 102; neuroendocrine dlg, discs large homolog 3, Domain 3	Q92796	0	204.5
Dlg5-D1	discs, large homolog 5, Domain 1	Q8TDM6	0	0
Dlg5-D4	discs, large homolog 5, Domain 4	Q8TDM6	0	0
Dlg5-D3	discs, large homolog 5, Domain 3	Q8TDM6	0	0
Dlg5-D2	discs, large homolog 5, Domain 2	Q8TDM6	0	0
Dlg2-D3	Channel associated protein of synapse-110 (Chapsyn-110), Domain 3	Q15700	0	191.7
PAR6B	Partitioning defective-6 homolog beta, Domain 1	Q9BYG5	0	0
LIK1	LIM domain kinase 1 isoform; LIM motif containing protein kinase	P53667	0	0
LOMP	LIM domain only 7 isoform a; KIAA0858 protein	Q8WWI1	0	0
RIL	LIM protein RIL (Reversion-induced LIM protein)	P50479	0	0
A2LIM	alpha-actinin-2-associated LIM protein; enigma homolog	Q53GG5	0	0
TIAM1	T-cell lymphoma invasion and metastasis 1	Q13009	0	0
LIN7C	Lin-7 homolog C	Q9NUP9	0	112.6
LIN7B	Lin-7 homolog B	Q9HAP6	0	66.9
LIN7A	Lin-7 homolog A	O14910	0	85.9
GEF11	Rho guanine exchange factor (GEF) 11; glutamate transporter EAAT4-asociated protein 48; KIAA0380 protein	O15085	62.9	134.6
GEF12	Rho guanine exchange factor (GEF) 12; leukemia-associated GEF; similar to mouse Lsc oncogene	Q9NZN5	55.9	68.9
PDZK1-D1	PDZ domain containing 1, Domain 1	Q5T2W1	0	0
PDZK1-D2	PDZ domain containing 1, Domain 2	Q5T2W1	0	0
SNB1	Beta-1-syntrophin; tax interaction protein 43; dystrophin-associated protein A1,59kD, basic component 1	Q13884	0	101.1
SNA1	acidic alpha 1 syntrophin; dystrophin-associated protein A1, 59kD, acidic component; pro-TGF-alpha cytoplasmic domain-interacting protein 1	Q13424	10.0	163.6
SHK1	somatostatin receptor-interacting protein; Sh3 and multiple ankyrin repeat domains 1	Q9Y566	47.8	52.1
MPP6	membrane protein, palmitoylated 6; protein associated with Lin7 2; VELI-associated MAGUK 1; MAGUK protein p55T	Q9NZW5	0.5	0
PIST	Golgi associated and coiled-coil motif containing protein; CFTR-associated PDZ/coiled-coil domain binding partner for the rho-family GTPase Tc10; fused in glioblastoma; Golgi associated PDZ an coiled-coil motif containing protein	Q9HD26	26.0	0
GEF2	Rap guanine nucleotide exchange factor; PDZ domain-containing guanine nucleotide exchange factor 1	Q8TEU7	0.3	0
RIM2	regulating synaptic membrane exocytosis 2; RAB3 interacting protein 3; KIAA0751 protein	Q9UQ26	0	0
PDZ-pos	PDZ Domain positive control for Kv1.4 ligand synapse associated protein 102		172.4	241.4
pos	PDZ Domain positive control for Kv1.4 ligand		102.2	153.3
GST	Glutathione-S-Transferase (negative control)		0.0	0.0

PDZ domain containing protein from membrane IV. This table shows signal intensities of protein spots. We incubated the membrane IV with bacteria lysate containing pEXP-$Ca_v1.2$ and pEXP-PMCA4b.

Figure 3.9.: Bar chart of spot intensities of PDZ domain array IV



Blue = incubated with $Ca_v1.2\alpha$ C-terminus. Red = incubated with PMCA4b C-terminus.

Table 3.5.: Summary of certain potential interaction partners of $Ca_v1.2\alpha$ and PMCA4b.

PDZ domain	full name of protein	accession	mean grey values $Ca_v1.2$	mean grey values PMCA4b
Dlg2-D2	Channel associated protein of synapse-110, PDZ Domain 2, PSD-93	Q15700	0.1	242.8
CASK	Calcium/Calmodulin-dependent serine protein kinase	O14936	11.0	4.4
hCLIM1	Human 36kDa carboxyl terminal LIM domain protein	O00151	5.4	132.5
MAST205	microtubule associated testis specific serine/threonine protein kinase	Q6P0Q8	77.7	53.5
NHERF1-D1	solute carrier family 9 (sodium/hydrogen exchanger) 3 regulatory factor 1, Domain 1	O14745	84.8	0
Z01-D1	tight junction protein 1 (zona occludens), Domain 1	Q07157	130.7	40.6
nNOS	nitric oxide synthase 1 (neuronal), Domain 5	P29475	36.2	0
MAGI3-D6	membrane-associated guanylate kinase-related 3, Domain 6	Q5TCQ9	72.7	203.3
MAGI1-D3	membrane-associated guanylate kinase inverted 1; brain-specific angiogenesis inhibitor-associated protein 1; WW domain-containing protein 3; atrophin-1 interacting protein 3, Domain 3	Q96QZ7	41.0	218.7
MAGI2-D6	membrane-associated guanylate kinase inverted 1; atrophin-1 interacting protein 1, Domain 6	Q86UL8	98.9	229.2
SCRIB1-D1	Scribble, Domain 1	Q14160	102.6	253.3
GEF11	Rho guanine exchange factor (GEF) 11; glutamate transporter EAAT4-asociated protein 48; KIAA0380 protein	O15085	62.9	134.6
GEF12	Rho guanine exchange factor (GEF) 12; leukemia-associated GEF; similar to mouse Lsc oncogene	Q9NZN5	55.9	68.9

Signal intensities of positive protein spots on the PDZ domain membranes incubated with lysates of bacteria expressing pEXP-$Ca_v1.2$ and pEXP-PMCA4b. Comment: Please note that not all high score partners were listed here. For more details, please refer to the tables before.

3.2. Co-immunoprecipitation of $Ca_v1.2\alpha$

3.2.1. Co-immunoprecipitations of $Ca_v1.2\alpha$ with putative interaction partners

The interaction of $Ca_v1.2\alpha$ with diverse members of MAGUKs (CASK, MAGI-3 and ZO-1), and the proteins NHERF1 and MAST-205, was established by co-immunoprecipitations. CASK, a 112 kDa protein, is expressed at neuronal synapses, where it interacts with neurexin, and in renal epithelial cells (Hata et al., 1996). Consequently, we examined the putative interaction between $Ca_v1.2\alpha$ and CASK in mouse brain lysates, which naturally express both proteins, and lysates of HEK 293 cells, which were transfected with $Ca_v1.2\alpha$. Interaction of CASK with $Ca_v1.2\alpha$ was observed in both lysate types, and in ordinary, untransfected HEK 293 cells (Fig. 3.10.A).

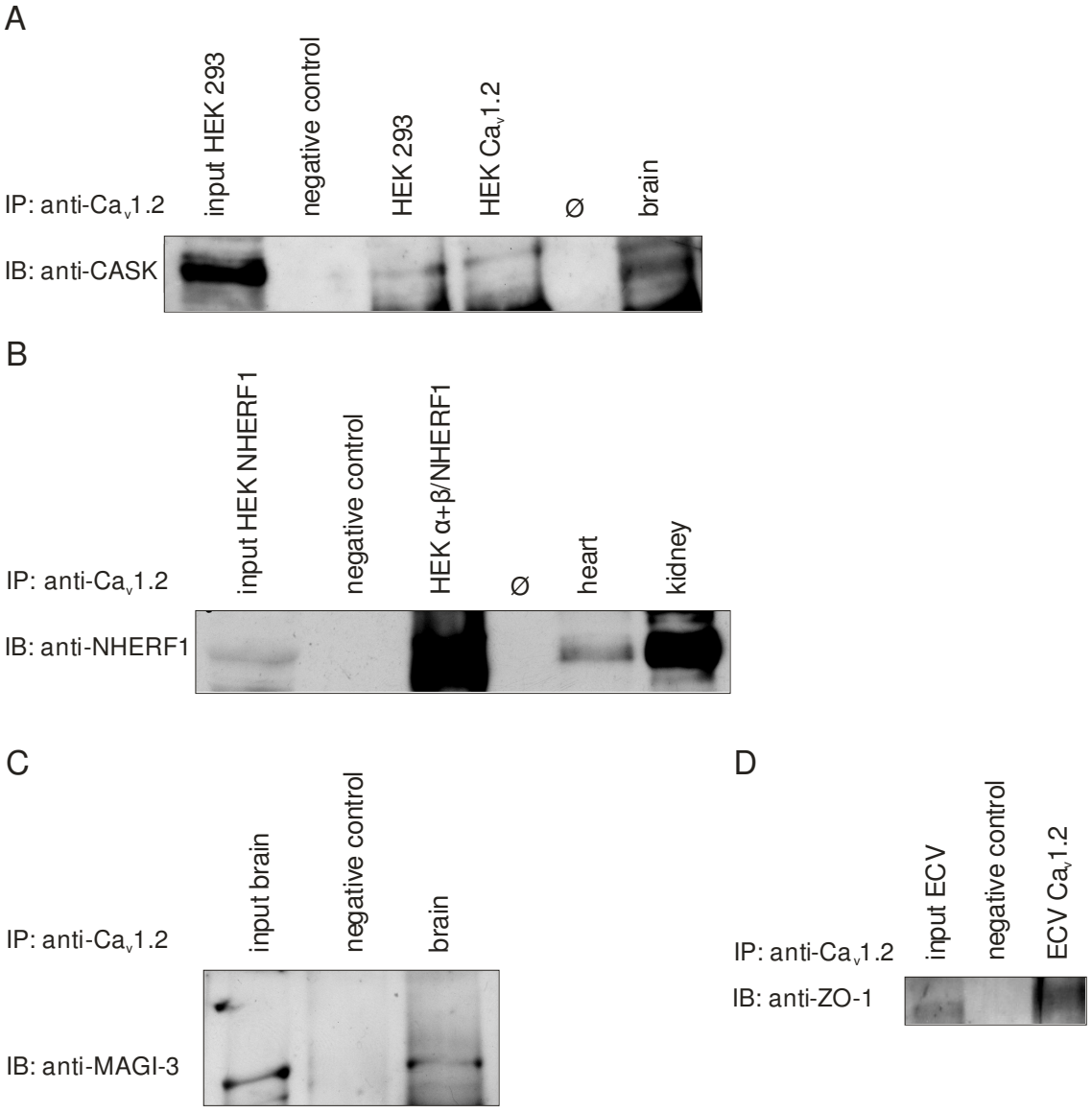
NHERF1, also well-known as ezrin binding protein 50, is a 55 kDa phosphoprotein, which contains two PDZ domains (Weinman et al., 1998). Co-immunoprecipitations were performed to test for interaction of full length proteins in heart and kidney lysates. HEK 293 cells with stable overexpression of the α and β subunits of $Ca_v1.2$ were subsequently transfected with NHERF1 and probed as well. It was possible to co-precipitate NHERF1 with the $Ca_v1.2$-specific antibody in the organ lysates and the transfected cells (Fig. 3.10.B).

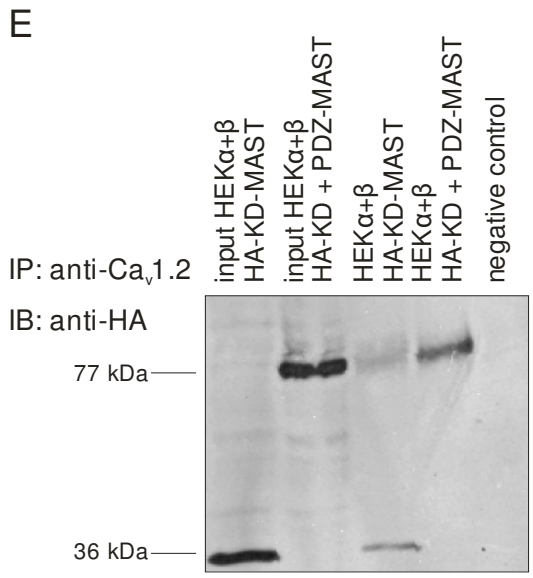
MAGI-3 (160 kDa) is predominantly expressed in a variety of tissues including the brain (Nakanishi et al., 1997; Wood et al., 1998; Wu et al., 2000b). Co-immunoprecipitations discovered potential interaction between $Ca_v1.2\alpha$ and MAGI-3 in mouse brain lysates (Fig. 3.10.C).

The tight junction protein ZO-1 is found in epithelial cells (Anderson et al., 1988b; Stevenson et al., 1986), therefore, we searched for an interaction between $Ca_v1.2\alpha$ and ZO-1 in ECV endothelial cells transfected with $Ca_v1.2$. As depicted in Fig. 3.10.D, the prominent band suggested a possible interaction of $Ca_v1.2\alpha$ and ZO-1 in ECV cells.

The serine/threonine kinase (Ser/Thr kinase) MAST-205 is expressed in testis, brain and kidney tissues (Walden and Cowan, 1993; Wang et al., 2006). To test for protein interactions between $Ca_v1.2\alpha$ and MAST-205, we used the HA-tag constructs pRK5-kinase-MAST205 (36 kDa) and pRK5-kinase-PDZ-MAST205 (77 kDa), performed co-immunoprecipitations as above and used a $Ca_v1.2$-specific antibody. We found that the $Ca_v1.2$ antibody co-precipitated MAST-205 kinase domain (KD) + PDZ domain from transfected HEK 293 cells with stable overexpression of the α and β subunits of $Ca_v1.2$ (Fig. 3.10.E). In all immunoprecipitations, the positive controls were inputs of the respective protein and the negative controls were the relevant protein samples with protein A/G-agarose beads and an irrelevant antibody (α-AT_2 (H-143)).

Figure 3.10.: Co-immunoprecipitation of $Ca_v1.2\alpha$





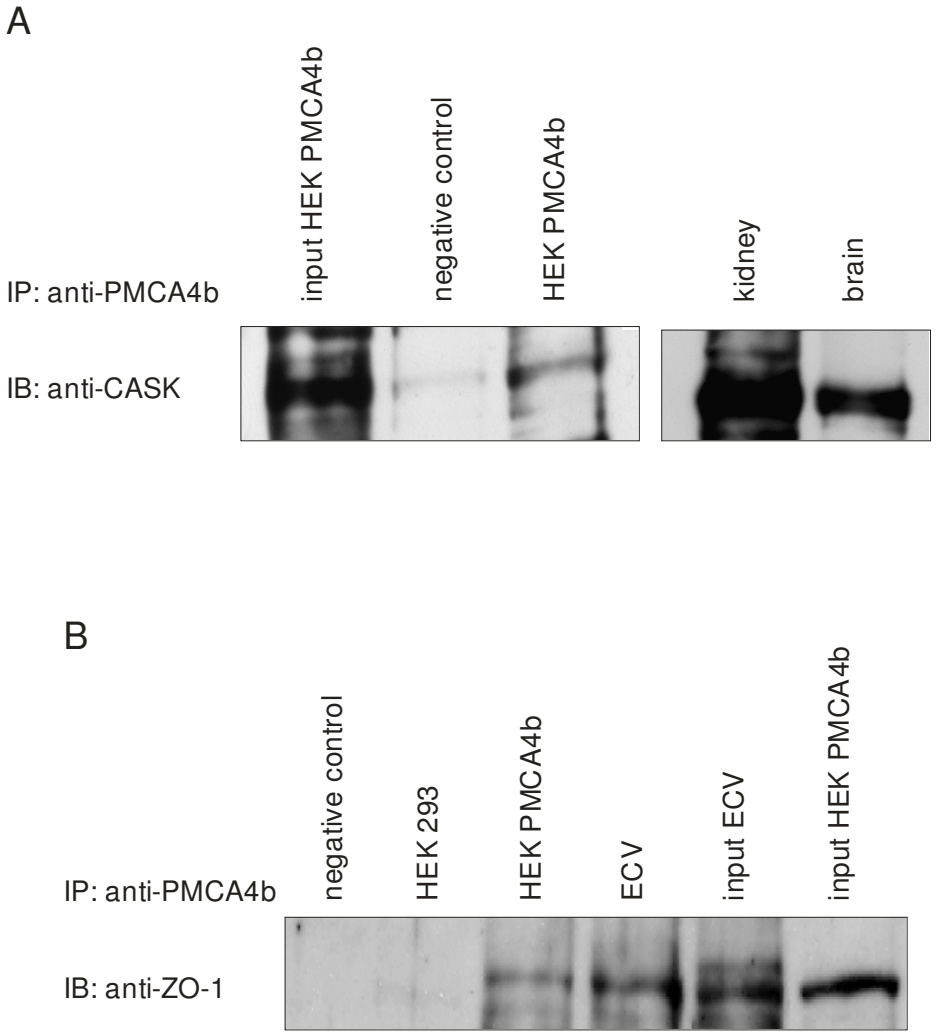
A: Co-immunoprecipitation representing an interaction of $Ca_v1.2\alpha$ with CASK. CASK was expressed in HEK 293 cells, which were additionally transfected with pcDNA3-$Ca_v1.2\alpha$. We also probed mouse brain lysates. These lysates were precipitated with polyclonal α-$Ca_v1.2$ antibody and probed with monoclonal α-CASK antibody for immunoblotting (IB). The positive control (input) consisted of 20 µg of HEK 293 lysate. The negative control was HEK 293 cells immunoprecipitated (IP) with an irrelevant antibody (α-NFATc2). **B**: Interaction between $Ca_v1.2$ and NHERF1. The positive control was HEK 293 cells transfected with pcDNA3-NHERF1, and the negative control was incubated with an irrelevant antibody (α-AT2). We precipitated HEK 293 cells stably expressing NHERF1 with polyclonal α-$Ca_v1.2$ antibody, and also tissue lysates of heart and kidney. For IB we used α-NHERF1 antibody. **C**: IP revealed an interaction of $Ca_v1.2\alpha$ with MAGI-3. Positive and negative controls are as described above. $Ca_v1.2\alpha$ antibody was used for IP and MAGI-3 antibody for IB. **D**: Interaction between $Ca_v1.2\alpha$ and ZO-1. ZO-1 protein is expressed in ECV cells, hence the positive control was non-transfected ECV cells, the negative control contained ECV cells immunoprecipitated with an irrelevant antibody (α-NFATc2), and for the IP we used $Ca_v1.2$ antibody for precipitation and α-ZO-1 for the IB. **E**: HEK 293 cells with stable overexpression of the α and β subunits of $Ca_v1.2$ transfected with HA-KD (36 kDa) or HA-KD+PDZ domain of MAST205 (77 kDa) were incubated with α-$Ca_v1.2$ and protein complexes were subsequently precipitated with protein A/G beads. Western blots were probed with HA antibodies. Irrelevant antibodies were used in negative controls and non-precipitated HEK lysates as positive controls.

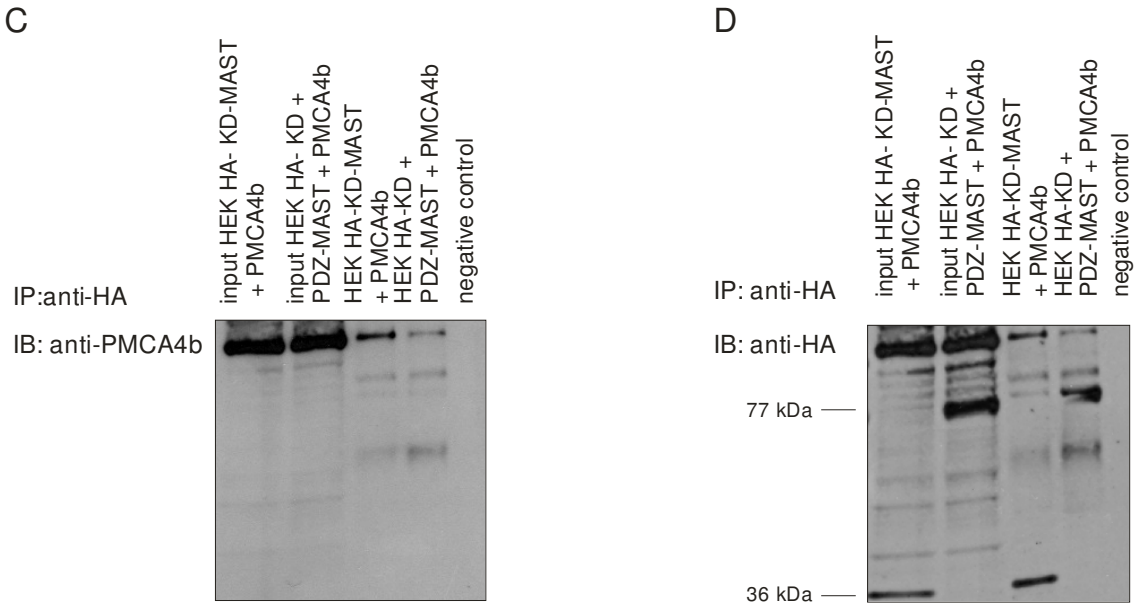
3.3. Co-immunoprecipitation of PMCA4b

3.3.1. Co-immunoprecipitations of PMCA4b with putative interaction partners

To confirm our data from the PDZ arrays, we tested PMCA4b and CASK by co-immunoprecipitations from kidney and brain lysates, and transfected HEK 293 cells (Fig. 3.11.A). The results confirmed an interaction of PMCA4b and CASK, as previously verified (Schuh et al., 2003). Binding of the proteins PMCA4b and ZO-1 was demonstrated in extracts from various sources. The PMCA-specific antibody co-precipitated the 220 kDa protein ZO-1 in all cell lysates tested (Fig. 3.11.B). We also checked the interaction between PMCA4b and MAST-205 by co-immunoprecipitation. The HEK 293 cells were two-double transfected, thereby expressing the proteins PMCA4b and HA-KD from MAST-205 or PMCA4b and HA-KD + PDZ domain from MAST-205. The HA-specific antibody co-precipitated the 136 kDa protein PMCA4b (Fig. 3.11.C). Figure 3.11.D shows a transfection control of the HA-tag constructs pRK5-kinase-MAST205 (36 kDa) and pRK5-kinase-PDZ-MAST205 (77 kDa). In immunoblots A and B, the positive controls were inputs of PMCA4b-transfected HEK 293 cells, the positive controls in the blots C and D were inputs of double transfected HEK 293 cells with PMCA4b and HA-KD MAST-205 or HA-KD + PDZ domain of MAST-205. The negative controls in all blots were the positive controls incubated with A/G-agarose beads and an irrelevant antibody (α-NF-ATc2).

Figure 3.11.: Co-immunoprecipitation of PMCA4b





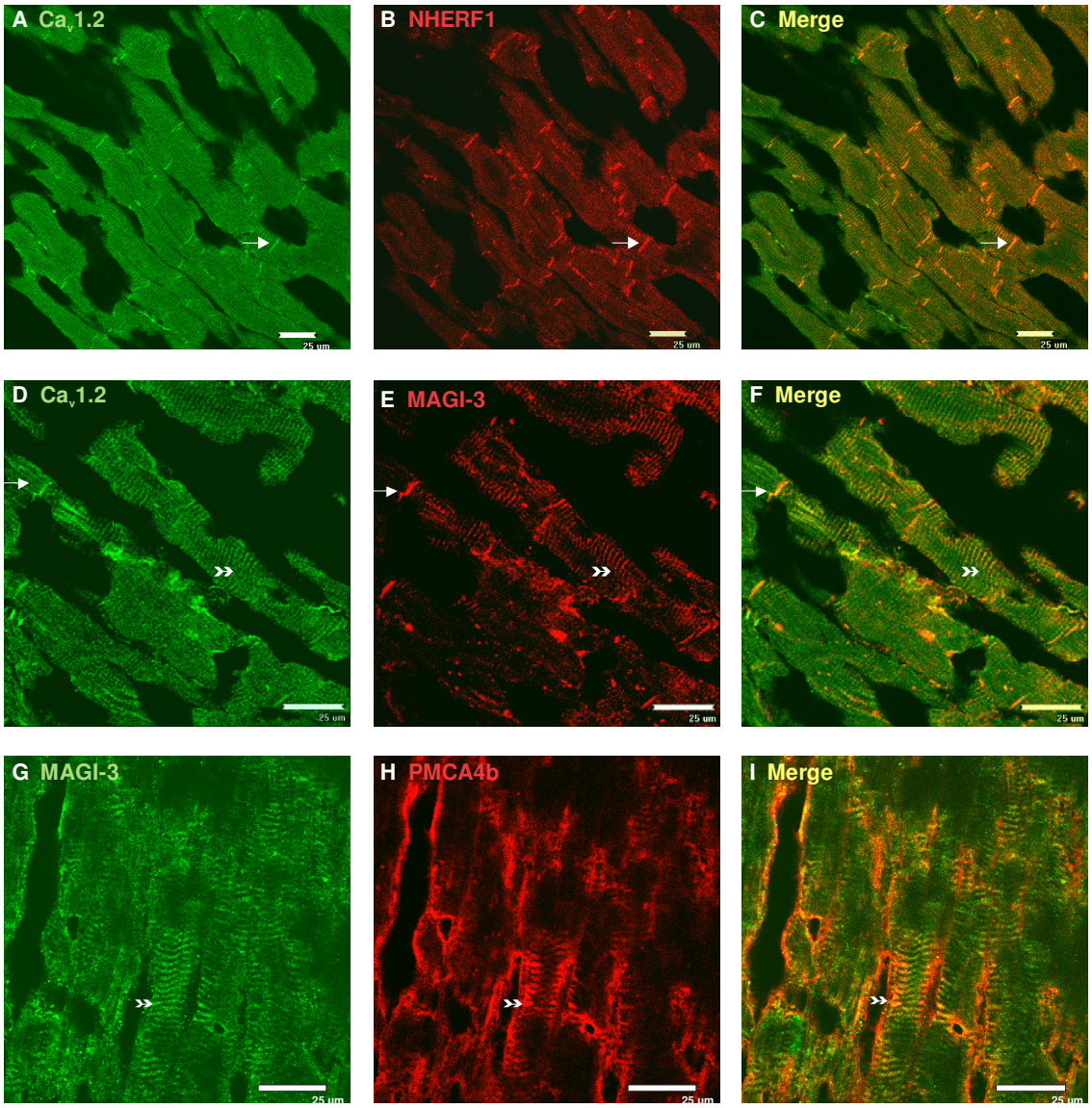
A: Lysates of transfected HEK 293-PMCA4b cells, kidney and brain were incubated with monoclonal antibody specific for PMCA4b (for details please refer to Materials and Methods section). Protein complexes were then precipitated with protein A/G beads. Western blots of precipitated proteins were probed with a CASK-specific antibody. Irrelevant antibodies were used in negative control (α-NFATc2), and transfected HEK 293-PMCA4b cells were used as positive control. **B**: Co-immunoprecipitation demonstrated an interaction of PMCA4b and ZO-1. For the IP, lysates of transfected HEK 293-PMCA4b cells, HEK 293 cells and ECV cells were precipitated with monoclonal antibody specific for PMCA4b and subsequently IB was performed with antibodies against ZO-1. The negative control was ECV cell lysate incubated with an irrelevant antibody (α-NFATc2) and the positive control an input of the same cell lysate. **C**: lysates of the double transfected HEK 293 cells (PMCA4b + HA-KD MAST, PMCA4b + HA-KD + PDZ-MAST, respectively) were incubated with monoclonal antibody specific for HA. The IB of precipitated proteins were probed with a PMCA4b-specific antibody. **D**: It is exactly the same blot like in C, only the detecting antibody for the IB was an HA-specific antibody. That picture demonstrated the positive transfection of the HEK 293 cells with the HA constructs. The negative control of C and D were HEK 293 cell lysatesincubated with an irrelevant antibody (α-NFATc2) and the positive control an input of the same cell lysate.

3.4. Co-localization

3.4.1. Co-localization of $Ca_v1.2$ and NHERF1, $Ca_v1.2$ and MAGI-3 as well as PMCA4b and MAGI-3 in rat cardiac myocytes

Confocal laser scanning microscopy studies of rat heart sections attested congruent distribution of $Ca_v1.2$, NHERF1 and MAGI-3 in cardiac tissue. All of them were localized at intercalated discs (Figs. 3.12.A-F), and $Ca_v1.2$ and MAGI-3 were additionally expressed at the transverse tubules (Figs. 3.12.D-F). As shown in Figs. 3.12.G-I, PMCA4b and MAGI-3 were partially co-localized at the plasma membrane and the transverse tubules of rat cardiac myocytes.

Figure 3.12.: Co-localization of $Ca_v1.2$ and NHERF1, $Ca_v1.2$ and MAGI-3 as well as PMCA4b and MAGI 3 in rat cardiac myocytes



Double immunofluorescent staining of $Ca_v1.2$ and NHERF1 (A-C), MAGI-3 and $Ca_v1.2$ (D-F), and of PMCA4b and MAGI-3 (G-I) in rat cardiomyocytes. For heart sections the following antibodies were used: polyclonal rabbit anti-$Ca_v1.2$-ATTO 488 (A, D), polyclonal rabbit anti-NHERF1 (B), polyclonal rabbit anti-MAGI-3 (E, G), monoclonal mouse anti-PMCA 5F10 (H) followed by Alexa Fluor 488 goat anti-mouse, or Alexa Fluor 594 goat anti-rabbit, where appropriate. C, F and I, are merged images. $Ca_v1.2$ and NHERF1 are coexpressed at the intercalated discs of cardiomyocytes (see arrow →), $Ca_v1.2$ and MAGI-3 at the intercalated discs and transverse tubules (T-tubuli) (see arrow »). PMCA4b and MAGI-3 are located at the T-tubules as well (see arrow »).

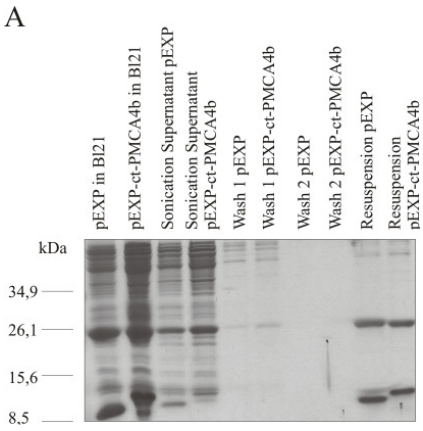
3.5. Talon His-Tag Purification Resins

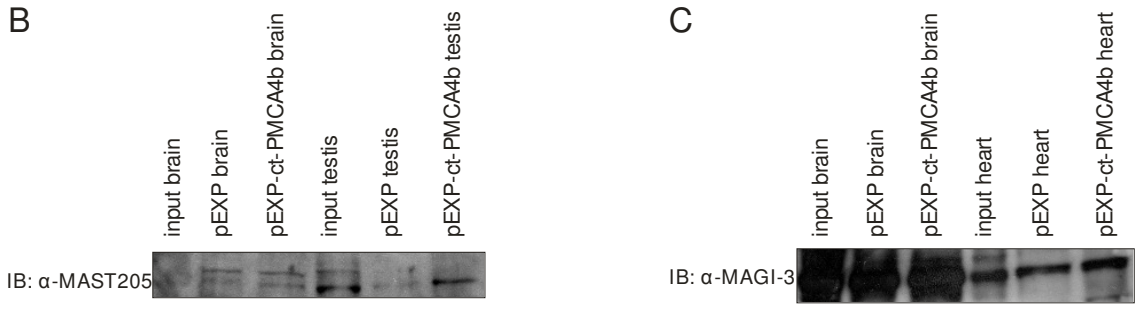
3.5.1. pull-down via Talon Metal Affinity Resins

To verify our data from the PDZ arrays II and III (Figs. 3.4. and 3.6.) and co-immunoprecipitations (Fig. 3.11.C), we checked interactions between the C-terminus of PMCA4b and MAGI-3 and/or MAST-205 by a special His-tag pull-down via Talon Metal Affinity Resins. Our cloned pEXP bacterial expression vector pEXP-PMCA4b and pEXP (see Fig. 3.1.A and B) were bound with a 6x His-Tag to the talon metal resin. Verification of expression and size of His-tagged recombinant proteins in bacteria BL21 and on the beads via tricine gel analysis confirmed expression levels. The size of pEXP expression vector is 9 kDa and pEXP-PMCA4b 8.97 kDa (Fig. 3.13.A). Therefore, the pEXP and pEXP-PMCA4b lysates showed a clear shift (Fig. 3.13.A) and were ready for the pull-down.

We performed pull-down assays from mouse brain, testis and heart extracts. As shown in Figs. 3.13.B and 3.13.C, the resulting pull-downs demonstrated unspecific binding between the C-terminus of PMCA4b, and MAST-205 and MAGI-3 protein.

Figure 3.13.: His-Tag pull-down





A: Tricine-SDS-PAGE (Coomassie dyed) to verify the expression of recombinant protein ligands in BL21 bacteria and to proof the binding of His-Tag proteins to the talon resin. The shifts between the resuspended pEXP (~9 kDa) and resuspended pEXP-PMCA4b (~8.97 kDa) confirmed their successful expression and binding to the beads. **B**: Lysates from mouse organs (brain, testis) were incubated with polyhistidine-tagged talon metal beads containing equal amounts of pEXP and pEXP-PMCA4b. For detection, we used α-MAST-205 antibody (205 kDa). Signals were observed between PMCA4b fusion proteins pEXP, and pEXP-PMCA4b. **C**: Lysates of mouse brain and heart were used for the pull-down. The negative control was pEXP without C-terminus of PMCA4b. For detection, we used α-MAGI-3 antibody (160 kDa). An interaction was observed between MAGI-3 and the fusion protein pEXP-PMCA4b, where the final C-terminal 15 amino acids were fused to the His-Tag.

3.6. GST pull-downs

3.6.1. Interaction C-terminus of $Ca_v1.2$ kurz with PDZ domain containing protein MAST-205 via GST pull-down

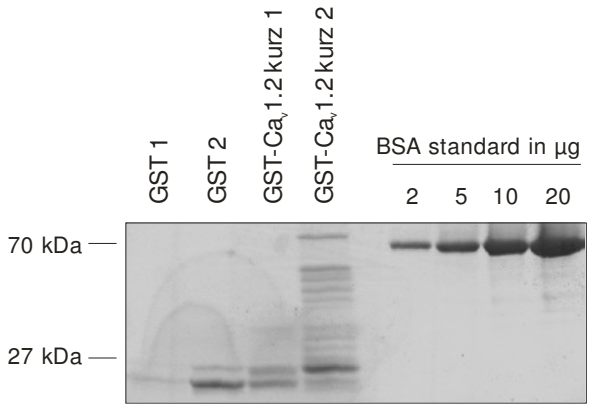
It has been described before that the PDZ domain of MAST-205 interacts with the C-terminal tail of $Ca_v1.2$ (see Figs. 3.4.B and 3.10.E). To proof this interaction by another assay, we used affinity-purified GST fusion proteins containing the C-terminus of $Ca_v1.2\alpha$ (encoded by the plasmid pGEX-4T-3-$Ca_v1.2$ kurz). We performed pull-down assays from stably transfected HEK 293 cells, which were additionally transfected with pRK5-KD-MAST-205 or pRK5-KD + PDZ-MAST-205). As shown in Fig. 3.14.B the Coomassie gel shows GST alone (27 kDa) and the expression of the GST fusion protein $Ca_v1.2$ kurz (~70 kDa). The BSA standard helps to assess the protein amount of the GST fusion proteins. The GST pull-down in Fig. 3.14.C demonstrated an interaction between kinase domain (without PDZ domain) from MAST-205 and the C-terminus of $Ca_v1.2$. But the GST + HEK KD-MAST-205 signal was slightly weaker than that of GST-$Ca_v1.2$ + HEK KD-MAST-205. Surprisingly, there was no interaction between PDZ domain of MAST-205 and $Ca_v1.2$ detectable (see Fig. 3.14.C).

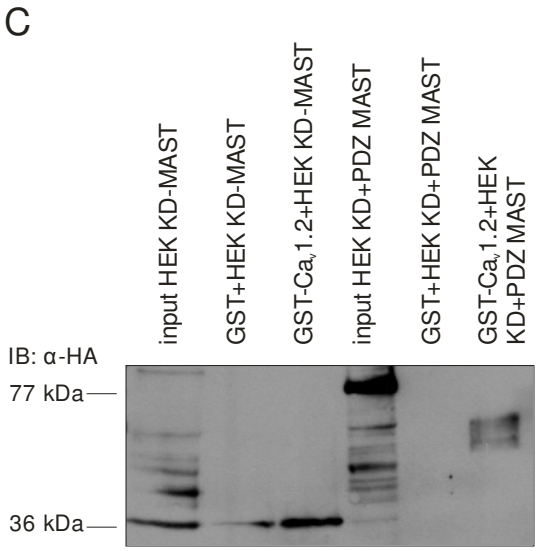
Figure 3.14.: GST pull-down MAST-205 and $Ca_v1.2$

A

```
tcctccaaaatcggatctggaagttctgttccaggggcccctgggatccccaggaattcc
 S  S  K  I  G  S  G  S  S  V  P  G  A  P  G  I  P  R  N  S
caggcagcatggaagctcagctccaagagatgccactcccaggagagccagatagccatg
 Q  A  A  W  K  L  S  S  K  R  C  H  S  Q  E  S  Q  I  A  M
gcgtgtcaggagggcgcatcccaggacgacaactacgacgtgaggatcggtgaagatgca
 A  C  Q  E  G  A  S  Q  D  D  N  Y  D  V  R  I  G  E  D  A
gagtgctgcagtgagcccagcctgctctccacagagatgctctcctaccaggatgacgaa
 E  C  C  S  E  P  S  L  L  S  T  E  M  L  S  Y  Q  D  D  E
aaccgacaactggcgccccccggaggaggagaagcgggacatcaggctgtctccaaagaag
 N  R  Q  L  A  P  P  E  E  E  K  R  D  I  R  L  S  P  K  K
ggtttcctgcgctccgcatcactgggtcgaagggcttccttccacctggagtgtctgaag
 G  F  L  R  S  A  S  L  G  R  R  A  S  F  H  L  E  C  L  K
cggcagaagaatcaaggggggagacatctctcagaagacagtcctgcccctgcatctggtc
 R  Q  K  N  Q  G  G  D  I  S  Q  K  T  V  L  P  L  H  L  V
caccaccaggcattggcagtggcgggcctgagtcccctcctgcagagaagccattccccc
 H  H  Q  A  L  A  V  A  G  L  S  P  L  L  Q  R  S  H  S  P
acctcgctccctaggccctgtgccacgccccctgccacaccgggcagccgaggctggccc
 T  S  L  P  R  P  C  A  T  P  P  A  T  P  G  S  R  G  W  P
ccacagcccatccccaccctgcggctggaggggggccgactccagtgagaaactcaacagc
 P  Q  P  I  P  T  L  R  L  E  G  A  D  S  S  E  K  L  N  S
agcttcccgtccatccactgcggctcatggtctggggagaacagcccctgcagaggggac
 S  F  P  S  I  H  C  G  S  W  S  G  E  N  S  P  C  R  G  D
agcagcgccgcccggagagcccggcccgtctccctcactgtgcccagccaggctggggcc
 S  S  A  A  R  R  A  R  P  V  S  L  T  V  P  S  Q  A  G  A
caggggagacagttccatggcagcgccagcagcctggtggaagcggtcttgatttccgaa
 Q  G  R  Q  F  H  G  S  A  S  S  L  V  E  A  V  L  I  S  E
ggactggggcagtttgctcaagatcccaagttcatcgaggtcacgacccaggagctggct
 G  L  G  Q  F  A  Q  D  P  K  F  I  E  V  T  T  Q  E  L  A
gacgcctgcgatctgaccatagaggagatggagaacgcggccgacgacattctcagcggg
 D  A  C  D  L  T  I  E  E  M  E  N  A  A  D  D  I  L  S  G
ggcgcccggcagagccccaatggcaccctgttacccttgtgaaccgcagggacccgggc
 G  A  R  Q  S  P  N  G  T  L  L  P  F  V  N  R  R  D  P  G
cgggacagagcggggcagaacgagcaggacgcgagcggcgcatgcgccccagggtgcggg
 R  D  R  A  G  Q  N  E  Q  D  A  S  G  A  C  A  P  G  C  G
cagagcgaggaggccctcgcggaccgcagggccggcgtcagcagcctgtagctcgagcgg
 Q  S  E  E  A  L  A  D  R  R  A  G  V  S  S  L  -  L  E  R
```

B



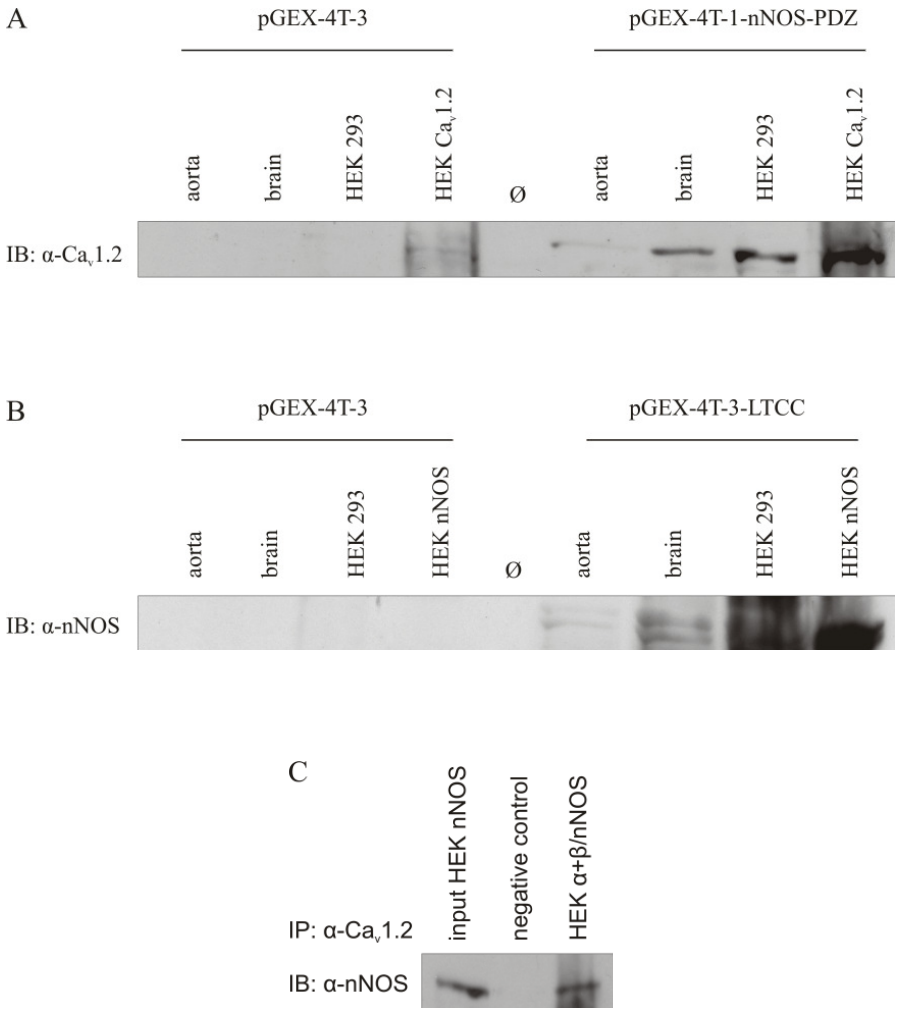


A: Amino acid-and protein sequence (in yellow) of the C-terminal tail from $Ca_v1.2\alpha$, which is encoded by the plasmid pGEX-4T-3 (pGEX-4T-3-$Ca_v1.2$ kurz). The green marked sequence presents the EcoRI cutting site, the blue bar shows the XhoI cutting sequence. The pink double CC are inserted amino acids to allow a clear open reading frame. The red tag presents the stop codon. **B**: The Coomassie Blue staining of SDS-polyacrylamide gel ascertained the expression of the GST fusion proteins pGEX-4T-3 (~27 kDa) (GST 1, GST 2) and pGEX-4T-3-$Ca_v1.2$ kurz (~70 kDa) in BL21 bacteria. GST 1 are 7 µl of the bead-slurry, GST 2 are 21 µl of the bead-slurry, GST-$Ca_v1.2$ kurz 1 are 50 µl of the slurry and GST-$Ca_v1.2$ kurz 2 are 150 µl of the slurry. The BSA standard helps to assess the protein amount of the GST fusion proteins. **C**: lysates from stably transfected HEK 293 cells (expressing the α and β subunits of $Ca_v1.2$) cotransfected with pRK5-KD-MAST-205 or pRK5-KD + PDZ-MAST-205 were incubated with glutathione-Sepharose beads containing equal amounts of GST and GST-$Ca_v1.2$. For detection we used α-HA (KD = 36 kDa; KD + PDZ = 77 kDa). An interaction was observed between KD of MAST-205 and GST-$Ca_v1.2$ and a little weaker between KD of MAST-205 and GST alone. No interaction was detected between the KD + PDZ domain of MAST-205 and GST-$Ca_v1.2$. The positive control were the stably cotransfected HEK 293 cells and the negative control was GST (pGEX-4T-3).

3.6.2. Confirmation of the interaction of $Ca_v1.2\alpha$ with the PDZ domain containing protein nNOS via GST pull-downs and co-immunoprecipitaton.

It has been previously reported that the PDZ domain of nNOS interacts with the C-terminal end of PMCA4b (Schuh et al., 2001). The Domain Array II (Fig. 3.4.B) indicated that the PDZ domain of nNOS may also interact with $Ca_v1.2\alpha$. To assess this interaction in more detail, we used affinity-purified GST fusion proteins containing the C-terminus of $Ca_v1.2\alpha$ (encoded by the plasmid pGEX-4T-3-LTCC), and the PDZ domain of nNOS (plasmid pGEX-4T-1-nNOS-PDZ). We performed pull-down assays from mouse brain and aorta extracts, and HEK 293 cell lysates, either normal and untransfected, or transfected with pcDNA3-$Ca_v1.2\alpha$ or pcDNA3-nNOS. As shown in Fig. 3.15.B, the resulting GST pull-down clearly demonstrates binding between the C-terminus of $Ca_v1.2\alpha$ and the nNOS protein. This interaction was ascertained in reverse, confirming the interaction of nNOS and $Ca_v1.2\alpha$ (Fig. 3.15.A). Additionally, using an independent assay, we could prove this protein-protein interaction by conventional co-immunoprecipitations. In this assay, we cotransfected HEK 293 cells with the α and β subunits of $Ca_v1.2$ and nNOS. Subsequent precipitation with $Ca_v1.2\alpha$-specific antibodies pulled nNOS down as well (Fig. 3.15.C).

Figure 3.15.: $Ca_v1.2\alpha$ C-terminal end interaction with PDZ domain of nNOS.



A: lysates from mouse organs (aorta, brain), and lysates from untransfected HEK 293 cells and HEK 293 cells transfected with pcDNA3-$Ca_v1.2\alpha$ were incubated with glutathione-Sepharose beads containing equal amounts of GST (pGEX-4T-3) and the nNOS PDZ domain fused to GST (pGEX-4T-1-nNOS-PDZ). For detection we used α-$Ca_v1.2$ antibody from Alomone Labs (190-210 kDa). An interaction is observed between $Ca_v1.2\alpha$ and the GST fusion protein pGEX-4T-1-nNOS-PDZ, but not with GST. **B**: the lysates were the same as described above. Here the HEK 293 cells were transfected with pcDNA3-nNOS. The negative control was GST (pGEX-4T-3). For detection, we used α-nNOS antibody from Zymed (160 kDa). An interaction between nNOS to the fusion protein pGEX-4T-1-LTCC, where the final C-terminal 10 amino acids were fused to GST, was shown. **C**: Co-immunoprecipitation demonstrated an interaction of $Ca_v1.2\alpha$ with nNOS. HEK 293 cells were transfected with pcDNA3-nNOS. 20µg of the protein lysate was used directly as input for SDS-polyacrylamide gel electrophoresis. The negative controls were HEK 293 cells transfected with nNOS expression constructs immunoprecipitated with an irrelevant α-rabbit antibody (α-AT2), and the last lane contained HEK 293 cell lysate (stably expressing the α and β subunits of $Ca_v1.2$) cotransfected with pcDNA3-nNOS, and immunoprecipitated with α-$Ca_v1.2$ antibody. For the immunoblot we used the antibody α-nNOS.

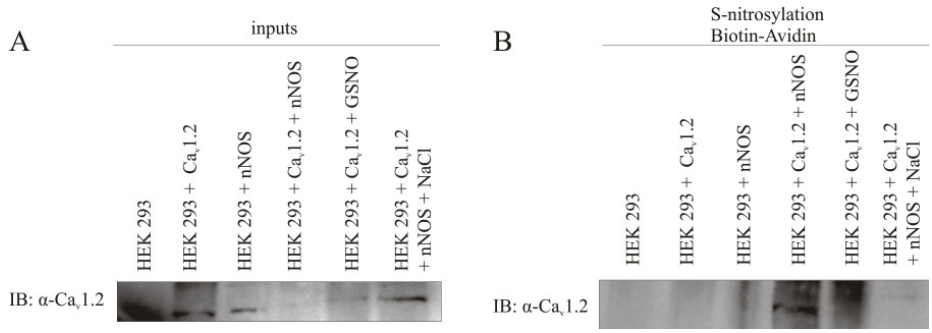
3.7. S-nitrosylation

It is well established that the redox state of cysteins represents a very dynamic and regulated balance, which can strongly influence not only the functional activity of a protein, but also its interactions with other protein partners as well as its subcellular distribution. Because of that we were looking via Biotin Switch Assay for S-nitrosylation in HEK cell lysates, which were transfected with nNOS and $Ca_v1.2\alpha$.

$Ca_v1.2\alpha$ is S-nitrosylated in transfected HEK 293 cells treated with 20mM ascorbat. To investigate S-nitrosylation of $Ca_v1.2\alpha$, we used HEK 293 cells co-transfected with nNOS and $Ca_v1.2\alpha$ because the function and importance of the NO-cGMP pathway is well defined: NO made by NO-synthases is a transmitter molecule and activates intracellular receptors, first of all the soluble guanylate cyclase (sGC) (Snyder, 1992). The signal transmission for relaxation of smooth muscle cells and hence the vasodilatation are effected by the second messenger cyclic 3', 5'-guanosin monophosphat (cGMP) (Garbers, 1979; Hardman and Sutherland, 1969). Thus sGC is stimulated by exogenic NO thereby the cGMP level increase and cause a relaxation of smooth muscles (Arnold et al., 1977).

The HEK 293 lysate (transfected with $Ca_v1.2\alpha$) was pretreated with 40 µM GSNO (S-nitrosoglutathione = NO Donor) in the dark for 1h (the S-NO bonds are light sensitive) as a positive control. For the negative control we used HEK 293 co-transfected with $Ca_v1.2\alpha$ and nNOS, and treated that with 200 mM NaCl instead ascorbat. S-nitrosylation was assessed by the biotin switch followed by avidin purification and detection by anti-$Ca_v1.2$ antibody. Before we treated all the various lysates with avidin beads, we used them as protein inputs and so we did WB with anti-$Ca_v1.2$ (Fig. 3.17.A). In Figure 3.17.A the HEK 293 lysates expressed the relevant protein $Ca_v1.2$. Figure 3.17.B showed the S-nitrosylation of $Ca_v1.2$ (see lane 4 in that figure). The lysates HEK 293; HEK 293 transfected with $Ca_v1.2$ alone and HEK 293 transfected only with nNOS showed no signal. So we conclude that an S-nitrosylation of the calcium channel $Ca_v1.2$ is possible.

Figure 3.17.: S-nitrosylation of the $Ca_v1.2$



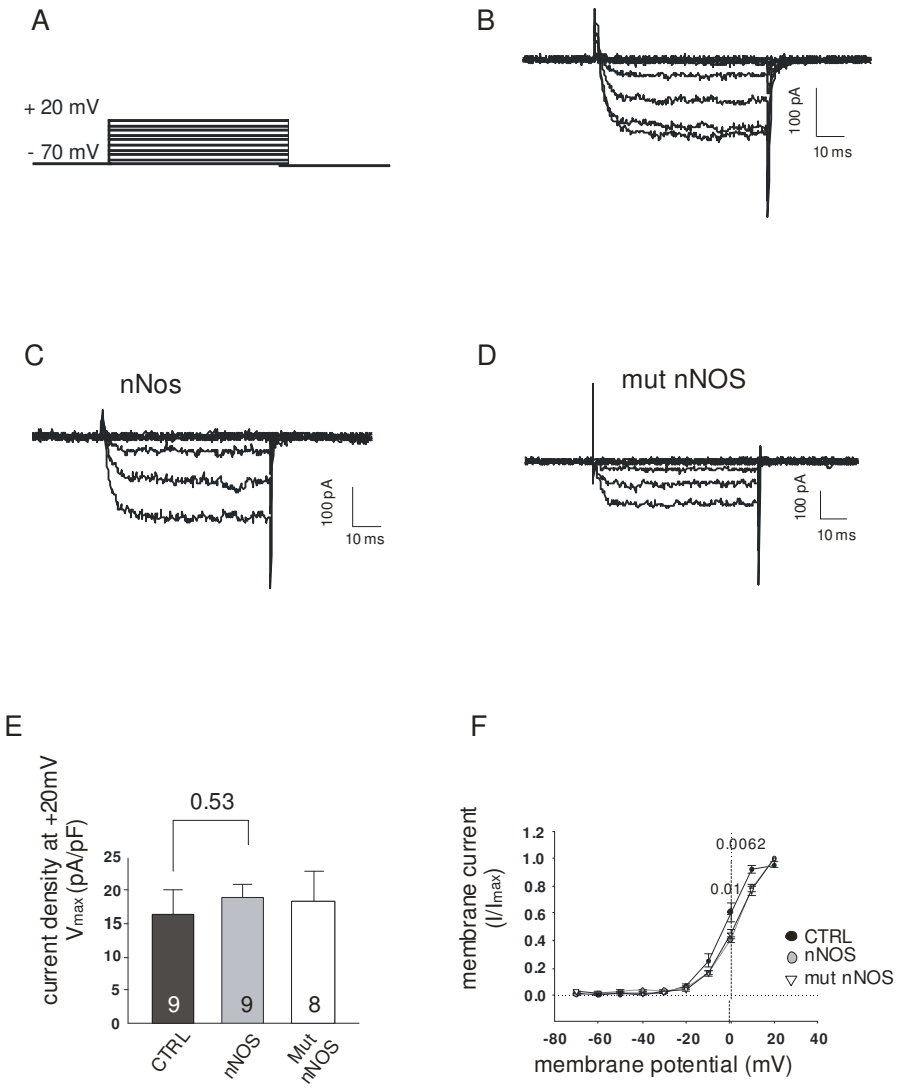
The calcium channel $Ca_v1.2$ is S-nitrosylated in the presence of nNOS. **A**: Wetsern blot with anti-$Ca_v1.2$. The inputs indicated similar $Ca_v1.2$ levels in each sample. **B**: Western blot analysis with anti-$Ca_v1.2$ of a biotin switch assay followed by Avidin purification confirming that $Ca_v1.2$ is S-nitrosylated by nNOS. The positive control was performed with 40 µM GSNO and the negative control with 200 mM NaCl instead of ascorbat.

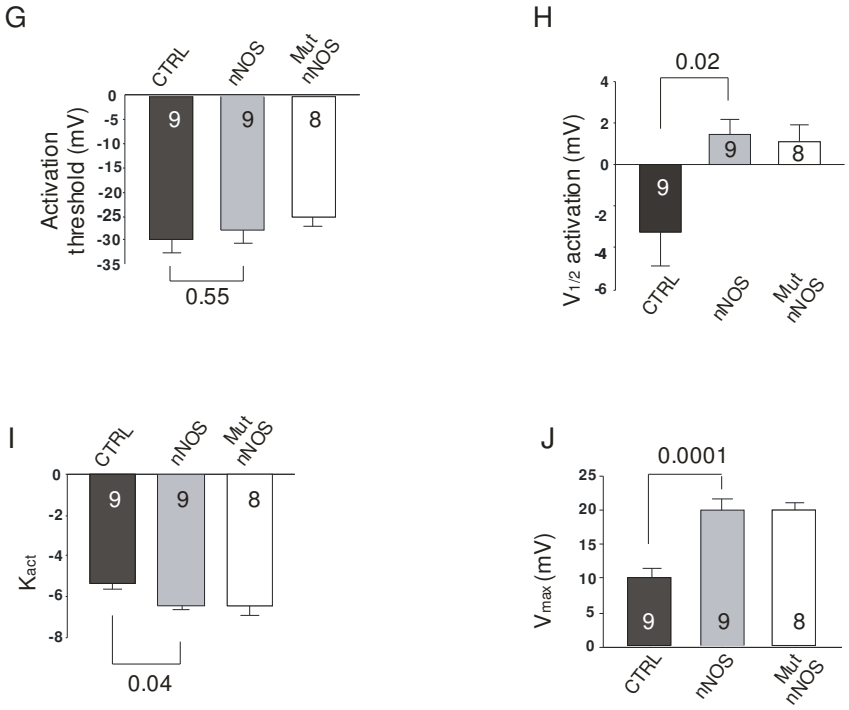
3.8. Current Recordings

Electrophysiological properties

To examine the electrophysiological properties of $Ca_V1.2$ subunits, Ba^{2+} currents were measured in the whole-cell configuration of the patch-clamp technique. To activate voltage-dependent Ca^{2+} channel membrane potential of cells was clamped to a holding potential of -70 mV. From this holding potential, the cells were depolarized stepwise by 9 voltage-steps of +10 mV increment and 50 ms duration (Fig. 3.16.A). HEK cells, which stably express the $Ca_V1.2$ subunit, showed voltage-dependent inwardly directed Ba^{2+} currents with a fast time-dependent activation with no inactivation (Fig. 3.16.B). The kinetic behavior of the $Ca_V1.2$ currents was not changed when the cells were additionally transfected with wild-type nNOS (Fig. 3.16.C). Also, in cells, which have been transfected with mutant nNOS, the kinetic behavior of Ba^{2+}currents was not different to currents in cells transfected with wild-type nNOS (Fig. 3.16.D). No differences in the overall activity of the currents were detected: $Ca_V1.2$ currents under control conditions, in the presence of nNOS or in the presence of the mutant nNOS showed no statistical different current densities (Fig. 3.16.E). However, the presence of nNOS led to a change in the voltage-dependence of $Ca_V1.2$ Ba^{2+} currents (Fig. 3.16.F). To analyze the voltage-dependence, normalized currents were plotted against their corresponding voltages of electrical stimulation and the curve was fitted by the Boltzmann function to calculate basic parameters of voltage-dependence such as potential of half maximal activation ($V_{1/2}$) and the steepness of the curve (k_{act}) (Fig. 3.16.G-J; Table 3.6.). nNOS presence led to shift of the voltage-dependent activation and potential of maximal current amplitude towards more positive voltages. Statistical analysis of parameters of voltage-dependence of wild-type $Ca_V1.2$ currents showed that activation threshold of the currents was not changed (Fig. 3.16.G) but the potential of half-maximal activation $V_{1/2}$ was shifted from -3 to + 1.5 mV (Fig. 3.16.H), which was due to a shift of the slope of the Boltzmann fitted curve (Fig. 3.16.I) and not due to a shift of the activation threshold. The same characteristics in nNOS-dependent modulation of the voltage-dependence was observed in currents of the $Ca_V1.2$ mutant. This shift in voltage-dependence resulted in a different potential of maximal current amplitude (Fig. 3.16.J).

Figure 3.16.: Current Recordings





Electrophysiological properties of Ba^{2+} currents from $Ca_V1.2$ subunits: **A**: Pattern of electrical stimulation. The membrane potential was clamped to a holding potential of -70 mV. From the holding potential the cells were depolarized by nine voltage-steps with +10 mV incrementing amplitude and 50 ms duration. **B**: Ba^{2+} currents induced by the electrical stimulation shown in A in a cell expressing wild-type $Ca_V1.2$ channels. **C**: $Ca_V1.2$ channel Ba^{2+} currents induced by the electrical stimulation shown in A in a cell expressing wild-type nNOS. **D**: $Ca_V1.2$ channel Ba^{2+} currents induced by the electrical stimulation shown in A in a cell expressing mutant nNOS. **E**: Maximal current density of control $Ca_V1.2$ currents, $Ca_V1.2$ subunits in the presence of wild-tpye nNOS and $Ca_V1.2$ subunits in the presence of mutant nNOS. **F**: Voltage-dependence of Ba^{2+} currents: currents were normalized to the maximal current amplitude and plotted against the potentials of the electrical stimulation; the curve was fitted using the Boltzmann equation. **G**: Activation threshold of Ba^{2+} currents from $Ca_V1.2$ subunits, $Ca_V1.2$ subunits in the presence of wild-type nNOS and $Ca_V1.2$ subunits in the presence of mutant nNOS; the number indicate the level of significance. **H**: Voltage of half maximal activation Ba^{2+} currents from $Ca_V1.2$ subunits, $Ca_V1.2$ subunits in the presence of wild-type nNOS and $Ca_V1.2$ subunits in the presence of mutant nNOS; the potentials of half maximal activation were significantly shifted towards positive potentials in the presence of nNOS or mutant nNOS. **I**: Slope of Boltzmann curve (k_{act}) of Ba^{2+} currents from $Ca_V1.2$ subunits, $Ca_V1.2$ subunits in the presence of wild-type nNOS and $Ca_V1.2$ subunits in the presence of mutant nNOS; the k_{act} values were significantly larger in the presence of nNOS or mutant nNOS. **J**: Comparison of the voltages of maximal current amplitudes (V_{max}); in the presence of nNOS or mutant nNOS V_{max} was shifted towards more positive voltages of currents from $Ca_V1.2$ subunits.

4 DISCUSSION

The voltage-gated L-type calcium channel, $Ca_v1.2$, and the plasma membrane calcium ATPase, PMCA4b, play major roles in excitable and non-excitable cells. $Ca_v1.2$ regulates the calcium entry into cells upon depolarization, while PMCA4b controls cellular calcium homeostasis by calcium extrusion.

Both are important functional proteins in the heart and brain, but the specific tasks and the precise mechanisms of action are still investigated. The present studies were initiated to understand the regulatory consequence and the physiological background of the interactions from the C-terminal ligands $Ca_v1.2$ and PMCA4b with PDZ domain containing proteins. Three independent assays (PDZ Domain Array, GST pull-down, and immunoprecipitation) and co-localizations showed the interaction of a multiplicity of PDZ domain containing proteins and their ligands, $Ca_v1.2$ and PMCA4b. These interactions connect both proteins to signaling networks implicated in synaptic transmission, cell adhesion and apoptosis, which may help present new indications about the physiological functions of $Ca_v1.2$ and PMCA4b in intra- and intercellular signaling. PDZ domains are protein-interaction domains that are specialized for binding to short peptide motifs at the carboxy C-termini of other proteins. You can find them in many proteins (more that 400 in humans or mice) (Kim and Sheng, 2004). PDZ domains are often arranged in tandem arrays and/or associated with other interaction domains (for example SH3 domains, WW domains) to form multidomain scaffold proteins.

Furthermore, PDZ motifs are protein binding adapters that play key roles in targeting proteins to the cytoskeleton or in regulating the intrinsic activities of enzymes (Brenman et al., 1996; Kim et al., 1995; Kornau et al., 1995; Niethammer et al., 1996; Sato et al., 1995; Shieh and Zhu, 1996). PDZ domain proteins regulate traffic and targeting of proteins, assembly of signaling complexes and networks designed for efficient and specific signal transduction (Nourry et al., 2003). For instance, the effectiveness of certain channels seems to increase by their association with PDZ containing proteins. The K^+ channel Kir 4.1 express much higher current density when cotransfected with the multivalent PDZ domain protein CIIP (Kurschner

et al., 1998). Presently, some of the described interaction partners of PMCA4b belong to the family of MAGUKs (DeMarco and Strehler, 2001; Kim et al., 1998; Schuh et al., 2003) but, in addition, nNOS and NHERF2 have previously been identified as interacting partners of PMCA C-termini (DeMarco et al., 2002; Schuh et al., 2001). The C-terminal end of the PMCA splice variant 4b (ETSV*, the asterisk indicates the COOH-terminal residue) differs from other b variants (DeMarco et al., 2002; Penniston and Enyedi, 1998; Strehler and Zacharias, 2001), suggesting that the C-termini determine the specificity of interactions with other proteins (DeMarco et al., 2002). We identified new PDZ protein interaction partners of PMCA4b, whereby ZO-1, MAGI-1-3, Mint-2, and MAST-205 are of primary importance. For $Ca_v1.2$, we detected the same combination of interacting proteins, with the addition of CASK, NHERF1, NHERF2 and nNOS.

4.1. PDZ Domain Arrays and IPs

Zonula occludens proteins are regulators of tight junction (TJ) assembly, and new investigations have shown that these proteins also promote adherens junction (AJ) assembly (Fanning and Anderson, 2009). AJs are crucial for certain signaling pathways like growth, cell morphology, and cell differentiation, and these junctions mediate cell-cell adhesion (Halbleib and Nelson, 2006). Important proteins of the AJs are Cadherines which are calcium-dependent transmembrane proteins. Cadherines are connected with different anchor proteins, like Vinculin and α-Actinin, and the actin cytoskeleton (Geiger and Ayalon, 1992; Geiger et al., 1990; Geiger et al., 1987; Yap et al., 1997). TJs control the fluctuation of ions, macromolecules, and immune cells through the paracellular space (Anderson et al., 2004; Lee et al., 2006). ZO-1 has an N-terminus with a structure similar to other MAGUKs, with three PDZ domains, an SH3 domain, and a region of homology to guanylate kinase (GUK) (Fanning, 2006; Schneeberger and Lynch, 2004). ZO-1 is enriched at the TJ of epithelial and endothelial cells but also in nonepithelial cells, such as astrocytes, Schwann cells, fibroblasts, glioma and myeloma cell lines (Anderson et al., 1988a; Itoh et al., 1991). The relative mass of that protein is species-dependent and between 210 and 225 kDa (Anderson et al., 1988a). The disruption of ZO-1 in mice resulted in embryonic lethality and was associated with disruption of the paracellular barrier and the structure of cell junctions (Damsky and Ilic, 2002; Daniel, 2007). The protein binding between the PDZ domain 1 of ZO-1 and the C-terminus of PMCA4b is insofar expectable because both proteins are located at the membrane (Fig. 3.4., 3.5., 3.11.B, Table 3.2.). A similar interaction was observed between the C-terminus of $Ca_v1.2$ and the PDZ domains 1 and 2 of ZO-1 (Fig. 3.4., 3.5., 3.10.D, Table 3.2.). Recently, it

was demonstrated that calcium calmodulin binds to the GUK domain of ZO-1 (CaM binds also on calcium channels and plasma calcium ATPase), suggesting the novel assumption that intracellular calcium levels control various ZO-1 functions (Fanning and Anderson, 2009; Paarmann et al., 2008). We suggest that this protein interaction is probably important for the regulation of calcium ions and cytoskeletal dynamics at cell junctions and the plasma membrane. In our experiments we detected that the three MAGI proteins (MAGI-1 domain 3; MAGI-2 and MAGI-3 domain 6) bind to the C-terminus of PMCA4b and $Ca_v1.2$. MAGI-1 and MAGI-3 are widely expressed in tissues like brain, heart, lung, and colon, but tend to be localized to tight junctions between adjacent epithelial cells (Franklin et al., 2005; Laura et al., 2002) (see Fig. 3.6., 3.7., 3.10.C, Table 3.3.). MAGI-2 is exclusively widespread in neuronal tissue (Iida et al., 2004). In the early stages of PDZ domain protein research, people thought scaffold proteins like MAGI-1, MAGI-2, and MAGI-3 were static scaffolds at the cell surface. Now we know that these proteins cooperate dynamically with PDZ targets at the cell surface for a dynamic and mobile cell structure (van Ham and Hendriks, 2003). The group of Hall (He et al., 2006; Xu et al., 2001) reported that the β1 adrenergic receptor (β1AR) binds MAGI-2 and MAGI-3. βARs are a subfamily of G protein coupled receptors (GPCRs). That subfamily includes the subtypes β1AR, β2AR, and β3AR and mediates physiological responses to epinephrine (also known as adrenaline) and norepinephrine (noradrenaline) (Hall, 2004). MAGI-2 enhances the receptor's association with β-Catenin and its internalization, while MAGI-3 inhibits G_i-mediated ERK activation by β1AR. G_i alpha subunit (or G_i/G_0) is a heterotrimeric G protein subunit that inhibits the production of cAMP from ATP. An interesting point is the coexpression with β1AR and MAGI-3 at the plasma membrane, whereas MAGI-3 expressed alone in the nucleus of certain cell types (He et al., 2006). The group of Hall and coworkers hypothesize that MAGI-3 play a physiological role in the nucleus, and the MAGI-3 localization via association with transmembrane proteins like β1AR could represent a novel and specific mechanism by which such PDZ interacting transmembrane proteins can control nuclear function (Adamsky et al., 2003; He et al., 2006). MAGI-2 and MAGI-3 also bind to the tumor suppressor gene product of PTEN (phoshatase and tensin homolog). PTEN works as tumor suppressor gene through the action of its phosphatase protein product. That phosphatase is involved in the regulation of the cell cycle, preventing cells from growing and dividing too rapidly (Chu and Tarnawski, 2004). These MAGI proteins support PTEN suppression of Akt/PKB (a pleckstrin homology domain-containing serine/threonine kinase), which is involved in apoptosis suppression and growth induction (Wu et al., 2000a; Wu et al., 2000b). These findings and the interaction of PMCA4b

and $Ca_v1.2$ with MAGI suggested the involvement of both proteins in cell death and growth regulation. While PDZ proteins function as specific membrane subdomains, they also operate in cell compartments and support trafficking of PDZ target proteins to the cell surface. Such an example would be the interaction of MAGI-3 with transforming growth factor α (TGFα) (Franklin et al., 2005). TGFα is upregulated in several human cancers. This growth factor occurrs in macrophages, brain cells, and keratinocytes, and is responsible for epithelial development. TGFα is closely related to epidermal grow factor (EGF), and bind to the EGF receptor with similar effects (Franklin et al., 2005). However, MAGI-3 is localized to diverse cellular compartments including the nucleus, cytoplasm, and junctional complexes at the cell surface (Adamsky et al., 2003), making it into a central modulator of its function as scaffold protein. It is interesting that both investigated ligands, PMCA4b and $Ca_v1.2$, interacted with all three MAGI proteins since the scaffolds are components of signaling complexes implicated in processes that require calcium. PMCA4b and $Ca_v1.2$ may play key roles in the arrangement of calcium dependent AJs, and may thus be responsible for cell growth, cell morphology and cell differentiation. The Mint protein family (munc18-interacting protein) has three members, Mint-1, Mint-2, and Mint-3 (Okamoto and Sudhof, 1997, 1998). All three proteins contain a phosphotyrosine binding (PTB) domain and two PDZ domains (Borg et al., 1996; Okamoto and Sudhof, 1997, 1998). Mint-1 and Mint-2 are expressed in neuronal tissues (Okamoto and Sudhof, 1997), and new investigations show that Mint-1 is also expressed in insulin-secreting β-cells (Zhang et al., 2004). Mint-3 is ubiquitously expressed (Okamoto and Sudhof, 1998). The C-terminal PDZ domains of Mint mediate an interaction with the neuronal surface protein neurexin and the N-type Ca^{2+} channel (Borg et al., 1999; Maximov et al., 1999). The Mint family plays a role in the arrangement of multiprotein complexes, and its ability to control the signaling and trafficking of membrane proteins (Rogelj et al., 2006). Mints bind to munc-18, a protein necessary for synaptic vesicle exocytosis, and to CASK, which is involved in targeting and localization of synaptic membrane proteins (Butz et al., 1998; Ferro-Novick and Jahn, 1994; Hill et al., 2003; Martin, 1997; Okamoto and Sudhof, 1997; Sudhof, 1995; Zucker, 1996). They also regulate β-amyloid precursor protein (β-APP) metabolism, trafficking and Aβ (39-43 amino acid β-amylopeptid) production. Mint-2 presence in neurons is associated with Alzheimer's disease amyloid plaques (see for reviews: (Hardy, 1997; Mattson, 1997; McLoughlin et al., 1999). The mechanism by which Mints inhibit β-APP processing is not well understood but the Mints and their binding partners have appeared as potential therapeutic targets for the treatment of Alzheimer disease. The presence of PDZ domains in Mints indicates a potential involvement of these proteins in connecting

synaptic vesicles to the sites of synaptic intercellular junctions (Gomperts, 1996). The multiprotein complex between our investigated ligands and Mint proteins could play a role in the exocytosis of synaptic vesicles, as the process requires a Ca^{2+} trigger and the resultant release of neurotransmitters is a Ca^{2+}-dependent reaction. Synaptic vesicle exocytosis initiates with the docking of the vesicles, subsequently they are primed for Ca^{2+} in a complex reaction that may involve partial fusion of the vesicles. In conclusion, Ca^{2+} rapidly triggers the release of neurotransmitters. Additionally, we and other groups have seen that the association of Mint proteins with the plasma membrane could be mediated by the binding of its PDZ domains (Gomperts, 1996; Okamoto and Sudhof, 1997; van der Geer and Pawson, 1995) (see Fig. 3.2., 3.3., Table 3.1.). MAST-205 (microtubule associated Serine/Threonine kinase) is highly expressed in testis (Walden and Cowan, 1993) and in kidney, adrenal glands, hindbrain, small intestine and colon tissues (Wang et al., 2006). This protein possesses a Ser/Thr kinase and one PDZ domain. Few protein interactions with MAST-205 have been identified. One such interaction is that of β2-synthrophin at the neuromuscular junction via its PDZ domain to the PDZ domain of MAST-205 (Lumeng et al., 1999). That observation suggests that the synthrophins may operate as a link between the dystrophin/utrophin network and a family of microtubule-associated protein kinases in the membrane cytoskeleton. The PDZ domain of MAST-205 additionally binds to PTEN, a tumor suppressor phosphatase, which regulates the cell growth and apoptosis. The phosphorylation of PTEN by the kinase domain of MAST-205, suggests that PTEN could be a physiological substrate, PTEN also interacts with MAGI-1-3 (Valiente et al., 2005), with MAGI-2 and 3 promoting its suppression of Akt, another major player in apoptosis and growth (Wu et al., 2000a; Wu et al., 2000b). Recent studies showed that this Ser/Thr kinase plays a role in interleukin-12 synthesis and NF-κB activation via interaction with TRAF6 (a member of the tumor necrosis factor receptor (TNFR)-associated factor (TRAF) family that mediates cytokine signaling pathways) (Takeda and Akira, 2004; Xiong et al., 2004; Zhou et al., 2004). So, these authors conclude that MAST-205 might be a regulator between the adaptive and innate immune response. The group of Yun demonstrated that MAST205 modulates the transport activity of Na^+/H^+ exchanger (NHE3) in the renal proximal tubule, and this regulation was dependent on the presence of the kinase motif in MAST-205 (Wang et al., 2006). Our studies suggest that the C-terminal tail of PMCA4b and $Ca_v1.2$ may act as component for specific and efficient PDZ domain recognition, which could be important in the control of PMCA4b and $Ca_v1.2$ protein phosphorylation, stability, and function (Fig. 3.4., Fig. 3.10., Fig. 3.11). We have identified the PDZ domain containing protein CASK as a functional interaction partner of $Ca_v1.2$.

Additionally, the interaction between CASK and PMCA4b was confirmed (Schuh et al., 2003). The MAGUK protein CASK consists of a Ca^{2+} -calmodulin kinase, a PDZ domain, a SH3 domain, and an inactive guanylate kinase domain. It is mainly expressed at the neuronal presynaptic membrane, interacting with neuroligin associated neurexin (Atasoy et al., 2007; Irie et al., 1997; Suckow et al., 2008), and additionally expressed in epithelial cells (Hata et al., 1996). CASK is the mammalian homolog of *Caenorhabditis elegans* LIN-2. In vertebrates, CASK is found at the lateral face of epithelial cells and binds syndecan-2 at the C terminal tail (EFYA) (Cohen et al., 1998). Syndecans are heparin sulphate proteoglycans, which are able to bind to the extracellular matrix and growth factors such as FGF (fibroblast growth factor). CASK is a regulator of epidermal progenitor cells and participates in the maintenance of epidermal homeostasis (Ojeh et al., 2008). Mutation or deletion of CASK results in unusual synaptic function and perinatal death in mice (Atasoy et al., 2007), verifying its importance for brain development and function. CASK controls synapse formation and synaptic strength, and mutation or deletion in the gene leads to mental retardation (Hsueh, 2009). Maximov and Bezprozvanny (Bezprozvanny and Maximov, 2001) showed that the C-terminus splice region of N-type calcium channels is capable of interacting with the adaptor proteins Mint-1 (by PDZ domain) and CASK (by SH3 domain). And so they might be play a role in the synaptic vesicle release machinery. Note that CASK is not a neuron-specific protein; it also presents key functions in non-neural tissue like kidney. All these studies indicate that $Ca_v1.2$ and PMCA4b in conjunction with CASK may play vital roles in the targeting of protein complexes in brain and epithelial cells, and in the modulation of synaptic transmission (see Figs. 3.2., 3.3., 3.10.A, 3.11.A, Table 3.1.). Another interesting interaction partner is the sodium-hydrogen exchanger regulatory factor, NHERF1 (also named ezrin binding protein 50, EBP50) and NHERF2 (called E3KARP), both containing two tandem PDZ domains (PDZ1 and PDZ2), besides to an ERM domain that links the proteins to the cytoskeleton. NHERF1 is 52% identical to NHERF2. The PDZ domains bind to a varity of membrane proteins together with ion transport proteins, tyrosine kinase receptors (e.g. platelet derived growth factor receptor, PDGFR), and the G-protein-coupled receptors (e.g. β2 adrenergic receptor, β2AR). For more details see reviews (Shenolikar et al., 2004; Weinman et al., 2006). NHERF1/2 are structurally related protein adapters that are highly expressed in epithelial tissues. In coexistence, they possess overlapping function as regulators of transmembrane receptors, transporters, and other proteins localized at or near the plasma membrane. The ERM (ezrin, radixin, moesin and merlin) family of membrane cytoskeletal adapters is a crucial cellular target of NHERF (Murthy et al., 1998; Reczek et al., 1997b). The

other isoforms of NHERF family (NHERF3 and NHERF4) contain four PDZ domains without any additional regulatory or interaction domain like the ERM binding region (Seidler et al., 2009). To regulate NHE3 signaling with cAMP, NHERF1 (or NHERF2), ezrin, and protein kinase A form a multiprotein signal complex connecting NHE3 to the actin cytoskeleton. This complex is proposed to facilitate the phosphorylation and downregulation of NHE3 (Reczek et al., 1997b; Weinman, 2001; Weinman et al., 2000). There it plays a crucial role in the proximal tubule, because H^+ is secreted into the lumen by NHE3, essentially maintaining the acid base balance of the kidney. One more central aspect is the relationship between NHERF and CFTR (cystic fibrosis transmembrane regulator). CFTR is the intestinal Cl^- transporter and is located in the apical membrane of different tissues. CFTR is a channel that allows the flow of chloride (and bicarbonate) from the epithelial cells into the lumen. CFTR is expressed in epithelia of airways, secretory glands, epididymis, bile ducts and intestine. The genetic disease cystic fibrosis (CF) is caused by mutations in the CFTR gene (Sheppard et al., 1999). CFTR chloride channels in lung epithelium guarantee the secretion of chloride ions and, as secondary effect, of water in the airway fluid. In CF airway epithelia, chloride secretion is reduced and sodium absorption is enhanced, resulting in the formation of dry and thick mucus (Knowles et al., 1983). The interaction between CFTR and NHERF may explain CFTRs ability to regulate other transport proteins, including the epithelial sodium channel, the renal outer medullary potassium channel, and NHE3 (Moyer et al., 1999; Raghuram et al., 2001). NHERF bear also a function in growth factor signaling. The activation of the PDGF (platelet derived growth factor) receptor tyrosine kinase is stabilized by the binding to the NHERF PDZ1-domain. The acquirement of NHERF to built homodimers could support PDGFR activation and initiate mitogenic signals through the PI 3-kinase (phosphatidylinositol 3-kinase) and MAP kinase (mitogen-activated protein kinase) pathways. MAP kinases are protein serine and threonine kinases that play central roles in cell development, differentiation, survival, and in calcium stability (Ishizuya et al., 1997; Schindeler and Little, 2006; Sneddon et al., 2000). One knockout mouse model for NHERF1 inactivation demonstrated that NHERF1 is essential for stabilizing active phosphorylated ERM proteins at the apical membrane of the polarized epithelia of the kidney and small intestine (Morales et al., 2004). NHERF1 knockout mice introduce structural deficiencies of the intestinal brush border membrane that is similar to the failures found in ezrin knockout mice (Morales et al., 2004). A further feature of NHERF1 is its function as tumour suppressor in human breast cancer (Dai et al., 2004; Reczek et al., 1997a; Weinman et al., 2000). Mangia et al. (Mangia et al., 2009) detected that NHERF1 is overexpressed in aggressive human

breast tumours and that it has the ability to enhance cell invasion and generate an invasive phenotype in breast cancer cells in vitro, either alone or in synergy with modifications of the tumour metabolic microenvironment. That study indicates that NHERF1 seems to operate as tumour suppressor when localized at the apical level of the membrane, and as an oncogenic protein when localized in the cytoplasm or nucleus. Loss of heterozygosity (LOH) at the NHERF1 locus is established in more than 50% of breast tumours. Additionally, NHERF1 is mutated in selective primary breast tumours and breast cancer cell lines. LOH at the NHERF1 locus is associated with aggressive characteristics of breast tumours, thereby defining NHERF1 as a haploinsufficieny tumour suppressor gene (Pan et al., 2006). DeMarco et al. 2000 suggested that the PDZ domains of NHERF1/2 recognize the D-(S/T)-X-L motif (X represents any amino acid) at the C terminus. Therefore, PMCA1b-3b (motif ETSL) interacts with NHERF1/2, with the exception of PMCA4b, which has the ETSV motif. We confirmed these results with the PDZ domain array (Fig. 3.4., 3.5., Table 3.2.). Additionally, we identified a new interaction between NHERF1/2 with $Ca_v1.2$ (motif VSNL) in Fig. 3.4., 3.5., 3.10.B. Our findings and previous studies from other groups, emphasize the importance of a C-terminal leucine residue for high affinity peptide interaction with NHERF (DeMarco et al., 2002; Hall et al., 1998; Moyer et al., 2000). The complex of $Ca_v1.2$ and NHERF1/2 may provide an indirect link between the Ca^{2+} channel and the actin cytoskeletal network, especially to stabilize the channel along the membrane and allowing its regulation by co-assembled cAMP-dependent protein kinases. The PDZ domain 1 of NHERF1 is associated with SOCs (store operated calcium channel), Trp4, Trp5, as well as the phospholipases Cβ1 and Cβ2 (Tang et al., 2000), suggesting that NHERF can link the functions of SOCs to PLCβ to organize calcium and phosphoinositide metabolism, and control cell metabolism and growth. Our new results suggest an involvement of NHERF1/2 in the regulation of Ca^{2+} transport as well.

Concluding remarks for the PDZ array

All novel identified interactions between our ligands ($Ca_v1.2$ and PMCA4b) and the PDZ domain containing proteins demonstrate the potential of this proteomics approach to identify physiologically important interactions between signaling molecules. Nevertheless, the PDZ array tool is not without limitations. The binding of C-terminal peptides to isolated PDZ domains does not explain secondary interactions that could contribute to binding affinity and specificity. Maybe, not all identified interactions will be physiologically relevant. The interacting partners have to be expressed in parallel in the same cell for obvious evidence.

But, that uncomplicated array has the potential to test objectively all possible interactions with both membrane bound and cytosolic signaling proteins. That array is potent enough to allow identification of interactions with specific PDZ domains in a protein that includes numerous such domains. So, it is possible to identify potential relations between multiple signaling molecules.

4.2. Co-localizations in rat cardiomyocytes

The co-localization of $Ca_v1.2$ and NHERF1 at the intercalated disc in rat cardiomyocytes, and of $Ca_v1.2$ and MAGI-3 at the intercalated discs (Fig. 3.12.) and the transversale tubules is worthy of note. Intercalated discs are complex structures, which connect single cardiac myocytes to an electrochemical syncytium. They are primarily responsible for pulse transmission for the duration of muscle contraction and for stabilization. These discs support quick swell of action potentials and the coordinated contraction of the myocardium. Intercalated discs exhibit three special types of cell-cell junctions: the actin filament anchoring adherens junctions (fascia adherens), the intermediate filament anchoring desmosomes (macula adherens) and gap junctions. The transversale tubules (T-tubules) are invaginations of the sarcolemma. These invaginations facilitate rapid transfer of a depolarization from the plasmamembrane to the core of the cell. The same applies for PMCA4b and MAGI-3 at the transverse tubules (Fig. 3.12.). T-tubules are important for the coupling of excitation and contraction. The L-type calcium channels occurred at these invaginations in a large number. So they trigger in response to electrical stimulation: their opening allows calcium flow driven by electrochemical gradient and into the cell. An interesting study is from P. Day and B. Kobilka (Day and Kobilka, 2006). They have shown an expression of MAGI-3 in cardiac myocytes and have detected a co-expression in HEK 293 cells between MAGI-3 and β1-adrenocepter. MAGI-3 is localized in the nucleus in the absence of β1-adrenoceptors, but targeted to the plasmamembrane when both were expressed in HEK 293 cells. The interaction between MAGI-3 and β1-and β2-adrenoceptors are important physiological tasks in neonatal cardiac myocytes (Xiang et al., 2002; Xiang and Kobilka, 2003). The β1-adrenoceptor does not undertake internalization and links only to the α- subunit of the G-protein (activates cAMP dependent pathway) in these myocytes (Devic et al., 2001). MAGI-3 also binds to frizzled transmembrane receptors 4 and 7, both of these are expressed in the heart and may play a role in cardiac remodeling in response to injury (Yao et al., 2004). PMCA4b and $Ca_v1.2$ are both expressed at the caveolae and at the plasmamembrane, as mentioned above. Little is known about the function of MAGI-3 and

NHERF1 in the heart. However, we would conclude that our findings of co-localizations play a role in signal transmission of cell contraction and stabilization at the membrane.

We also did immunhistochemistry (data not shown) in $Ca_v1.2$ transfected ECV cells and checked the co-localization with ZO-1 and also in double-transfected HEK 293 cells (transfected with $Ca_v1.2$ and nNOS). Both co-localizations have not confirmed clear results. ZO-1 was well seen at the membrane but $Ca_v1.2$ was diffuse and more expressed in the cytoplasm. An explanation could be that $Ca_v1.2$ expression vector contains only the α-subunit of the channel. It is known from various studies that the β-subunit is essential to express that protein on the membrane (Catterall, 2000). The same outcome was obtained for the localization of $Ca_v1.2$ and nNOS in HEK 293 cells. Daniel et al. (Daniel et al., 2001) observed in canine lower esophageal sphincter (LES) that colocalization of nNOS and L-type Ca^{2+} channel takes place in the caveolae. However, until now, no well-defined localization of nNOS in cells was detected.

4.3. Interaction of $Ca_v1.2$ with PDZ domain containing protein nNOS

NO is an important effector in the cardiovascular system and it exerts many myocardial functions, like modulation of contractile function, energetics, substrate metabolism, cell growth and survival (Massion et al., 2003; Sears et al., 2004). NO causes vascular relaxation through funtional interaction with soluble guanylyl cyclase (sGC) in the blood vessel wall, but also disperses into the lumen of the vessel where it interacts with a number of other cell types. In smooth muscles, the sGC is the primary target for NO, also in platelets and inflammatory cells. In mammalian myocardium, eNOS and nNOS are expressed. eNOS is found in coronary and endocardial endothelial cells and cardiomyocytes (Feron et al., 1996). nNOS has been localized in cardiac autonomic nerves, ganglia and cardiomyocytes (Danson et al., 2005; Xu et al., 1999a). Both NOS enzymes are expressed in divergent subcellular compartments in cardiomyocytes (Feron et al., 1996; Williams et al., 2006a; Xu et al., 1999a) where they are expected to couple to different effector molecules and exert diverse effects following enzyme activation. nNOS regulates excitation-contraction coupling (Barouch et al., 2002; Sears et al., 2003), β-adrenergic inotropic response (Barouch et al., 2002), and the development of heart failure (Bendall et al., 2004; Damy et al., 2004). nNOS-derived NO may play a role in the physiological regulation of myocardial contraction and Ca^{2+} fluxes. nNOS was identified in the sarcoplasmic reticulum (SR) in the myocardium, where it was initially found to inhibit Ca^{2+} uptake through the SR Ca^{2+} pump (SERCA2a) in SR microvesicles (Schuh et al., 2001; Xu et al., 1999b; Xu et al., 2003a). nNOS also controls the opening of L-

type Ca^{2+} channel (Sears et al., 2003). A controvers discussion has aroused about the interaction of nNOS with the SR Ca^{2+} release channel (Ryanodine receptor, RyR2) in the heart. Burkard et al. (Burkard et al., 2007) have not seen an interaction between these two proteins. Other groups co-immunoprecipitated nNOS and RyR2 (Barouch et al., 2002; Bendall et al., 2004; Damy et al., 2003; Damy et al., 2004). Myocardial nNOS has also been localized to the sarcolemma (Williams et al., 2006a), particularly in the left ventricular (LV) myocardium of remodelled and failing hearts (Bendall et al., 2004; Damy et al., 2003; Damy et al., 2004). The subcellular localization of nNOS is dependent on interactions between its PDZ domain and scaffold adaptor proteins, for example Dystrophin, α-Syntrophin (Williams et al., 2006a) and Caveolin-3 (Venema et al., 1997). Overexpression of nNOS has been found to trigger a decrease in Ca^{2+} currents in sinoatrial node cells (Heaton et al., 2006). In the same way, myocardial-specific nNOS overexpression has been correlated with a reduction in Ca^{2+} current density, [Ca2+]i transient amplitude and cell shortening in isolated myocytes and in vivo (Burkard et al., 2007). nNOS deletion or inhibition causes an increase in Ca^{2+} currents through the L-type Ca^{2+} channels and a reduction in SERCA activity, leading to enhancement of contraction and impairment of relaxation (Seddon et al., 2007). We confirmed these studies by measurement of Ca^{2+} currents in HEK 293 cells stably expressing $Ca_v1.2$ and co-transfected with nNOS (Fig. 3.16.). And we observed that the nNOS PDZ domain is possibly important for protein interaction (Fig. 3.15.). It has been shown that NO modulates ion channel activity by nitrosylation of sulfhydryl groups on the channel protein (Bolotina et al., 1994; Campbell et al., 1996a; Hu et al., 1997b). Summers et al. detected that NO inhibits L-type voltage-gated Ca^{2+} channels (Summers et al., 1999). So we postulate that the interaction between the C-terminus of $Ca_v1.2$ and the PDZ domain of nNOS inhibits the currents by S-nitrosylation of the channel protein (Fig. 3.17.). All these data propose that under basal conditions nNOS-derived NO may exert an inhibitory effect on Ca^{2+} influx and myocardial contraction thereby promoting relaxation. NO binds and stimulates sGC, which leads to the production of cGMP, which than activates a cascade of signalling events through activation of Protein kinase G. NO is also thought to control heart function through a number of cGMP-independent pathways including nitrosylation of various proteins involved in excitation-contraction coupling and the generation of peroxynitrite.

4.4. His-Tag pull-down

The interactions between the C-terminus of PMCA4b and the proteins MAST-205 and MAGI-3 were verified by His-tag pull-down via Talon Metal Affinity Resins (see 2.5.). The pull-down assays were performed from brain, testis and heart extracts (Figs. 3.13.B and 3.13.C). From the unspecific binding between the control vector pEXP and our expression vector pEXP-PMCA4b. The binding pattern was seen in every lane, whether or not pEXP, pEXP-PMCA4b, respectively. Particularly, the Coomassie Blue staining of SDS-polyacrylamide gel showed an explicit shift from the bound fusion proteins. The explanation for vague binding results could be on one hand the lysates of the organs which are often highly concentrated and contain so many different proteins, peptides, fats and fatty acids. So, an unspecific binding is not unusual. On the other hand both proteins (MAST-205, MAGI-3) are not very common proteins. As a consequence of this, there exists only one company which sell these antibodies. So maybe the antibodies were not good working. A solution for the His-tag pull down might be a construct enabling of MAST-205 protein and MAGI-3 protein for cell transfection.

4.5. Interaction $Ca_v1.2$ kurz with MAST-205 via GST pull-down

The interacting of $Ca_v1.2$ with the PDZ domain of MAST-205 posed the question of wether MAST kinase may phosphorylate $Ca_v1.2$. To explore this possibility, we tested the ability of the kinase domain (KD/MAST-205) and the kinase plus the PDZ domain of MAST-205 (KD + PDZ/MAST-205) to interact physically with $Ca_v1.2$ (Fig. 3.14. B). Stable HEK cells were transfected with plasmids encoding HA KD/MAST-205 or HA KD + PDZ/MAST-205 protein, and pull-down assays were performed using GST-$Ca_v1.2$ kurz purified from bacteria (Fig. 3.14. A). We expected that $Ca_v1.2$ co-precipitate the kinase + PDZ/MAST-205 protein. Unfortunately, our results did not show clearly that interaction. Probably it is a technical problem: the GST protein expression could be stronger and more clearly. However, the GST pull-down present tendencies in binding of $Ca_v1.2$ to the PDZ domain of MAST-205 (Fig. 3.14. B, last lane). The signal in the last lane showed a disaggregation of the protein lysate. After optimizing the GST protein expression in bacteria and the pull-down protocol, a positive result might be expected. $Ca_v1.2$ as well interacted with kinase/MAST-205 (Fig. 3.14.B, lane 3), suggesting that $Ca_v1.2$ could be a substrate for MAST-205. The future step could be MAST-kinase assay. The procedure includes incubation of GST-$Ca_v1.2$ with purified HA kinase/MAST-205 in the presence of [γ-^{32}P]ATP, and the phosphorylated proteins might be detected by SDS-polyacrylamide gels. Together, our results demonstrate for

the first time a binding of the C-terminal end of $Ca_v1.2$ to the PDZ domain of MAST-205. A new approach testing if the calcium channel could be phosphorylated by the kinase domain of MAST-205 would be of interest.

4.6. S-nitrosylation of $Ca_v1.2$ via Biotin Switch Assay

We discussed in 4.3. the intracellular signaling pathways together with the effects of nNOS-derived NO. Nitric oxide does not act as a freely diffusible messenger. Either NO stimulates the activity of guanylate cyclase, that produces cGMP from GTP or NO nitrosylates tyrosine and thiol groups of cysteine in proteins. During nitrosylation, proteins could modify their properties, equivalent to the changes induced by phosphorylation and palmitoylation (Hess et al., 2005; Mannick and Schonhoff, 2002; Martinez-Ruiz and Lamas, 2004). Cellular proteins that may undergo S-nitrosylation are L-type Ca^{2+} channel (Campbell et al., 1996b; Hu et al., 1997a; Sun et al., 2006), potassium channel $K_v1.5$, SERCA and RyR2 (Eu et al., 1999; Lokuta et al., 2005; Nunez et al., 2006; Xu et al., 1998). It is also known that NO plays a significant role in modulating myocardial function in both health and disease (Hare and Stamler, 2005) and so, the nitrosative and oxidative stress play essential roles in the regulation of cardiac myocyte function and survival (Hare and Stamler, 2005). Under physiological oxidative stress, NO might provide protection to cells by S-nitrosylation of some critical protein thiols, preventing them from additional oxidative damage. This NO-induced post-translational modification of proteins serves as a key effector of NO bioactivity and is an imperative regulator of cellular signal transduction. In our study, we would conclude that S-nitrosylation of the $Ca_v1.2$ channel is possible (Fig. 3.17.). The result (Fig. 3.17.B) confirmed a clear banding pattern in the lane 4 with the $Ca_v1.2$ and nNOS lysate. Even if the controls were not clearly (Fig. 3.17.B lane 5/6). NO is a highly diffusible and short-living physiological messenger, obviously the influential factor that supports S-nitrosylation. The detection of protein S-nitrosylation is not simply performed with traditional methods such as IP or IB, where the S-NO bond is broken during the SDS-PAGE. The largest part of the works in this field have been prepared using indirect methods, measuring free NO levels after cleavage of S-NO bonds or changing the unique nitrosothiols with a different detectable tag (see scheme 2.16.). These methods are sensitive but lack specificity. The first and most commonly method for the specific tagging of S-nitrosylated proteins was developed by Jaffrey et al. (Jaffrey et al., 2001) and named Biotin Switch. Their suggestion was to convert nitrosylated cysteins into biotinylated cysteins that could then be identified afterwards via streptavidin or a specific antibody without difficulty (see 2.10.). However, there are some critical points to discuss,

which were potentially the reason for our imprecise controls in that assay. On the one hand, the Biotin Switch assay is very light sensitive, meaning the assay has to be entirely conducted in darkness. On the other hand, ascorbate, which is used in the labeling step, is a poor reducer of protein S-NO and long incubation times are necessary to realize a quantitative reaction (Zhang et al., 2005). Furthermore, it has been reported that use of ascorbate can initiate false positive signals (Landino et al., 2006). Despite these controversial discussions, the Biotin Switch is the most commonly used method of detection of S-nitrosylated proteins in biological samples, which have been effectively used to identify the S-nitrosylated sites.

We conclude that $Ca_v1.2$ interacts physically with nNOS-, MAST-205-, MAGI-3-, NHERF1-, and ZO-1-PDZ domains; and PMCA4b with MAST-205- and MAGI-3-PDZ domains, all demonstrated via different assays (PDZ array, GST pull-down, IP). The partial co-localization of $Ca_v1.2$ and MAGI-3, $Ca_v1.2$ and NHERF1, and PMCA4b and MAGI-3 in rat cardiomyocytes indicates that an interaction of these proteins is highly possible. From our results, we conclude that $Ca_v1.2$ and PMCA4b bind promiscuously to a variety of PDZ domains. The physiological consequences of some of these interactions remain to be investigated.

5 OUTLOOK

$Ca_v1.2$ and PMCA4b are important in heart, brain, smooth and skeletal muscles. Despite numerous studies, their physiological functions have not yet been completely clearified. The novel discovered interaction partners of both protein ligands have possible influences on to their intercellular signaling, cell adhesion, and angiogenesis. Further investigations, like co-localizations and co-immunoprecipitations should be conducted for these interactions, for example an IP of $Ca_v1.2$ with ΔnNOS (mutant nNOS without PDZ domain). The physiological roles must be more recessed. We measured calcium currents in stably $Ca_v1.2$ transfected HEK 293 cells (co-transfected with nNOS, ΔnNOS respectively). In future, we should extend these experiments, e.g. with NO donors. In order to prove that the MAST kinase affects the $Ca_v1.2$ channel a physiological experiment could be a MAST-205 kinase assay. More experiments should also be done with the S-nitrosylation assays. Overexpression and knock-down research with the interacting partners could also be a part of future projects. These steps would expose further physiological functions of $Ca_v1.2$ and PMCA4b in the cardiovascular system and in the nervous system.

BIBLIOGRAPHY

Abu-Soud, H.M., Feldman, P.L., Clark, P., and Stuehr, D.J. (1994a). Electron transfer in the nitric-oxide synthases. Characterization of L-arginine analogs that block heme iron reduction. J Biol Chem *269*, 32318-32326.

Abu-Soud, H.M., Yoho, L.L., and Stuehr, D.J. (1994b). Calmodulin controls neuronal nitric-oxide synthase by a dual mechanism. Activation of intra- and interdomain electron transfer. J Biol Chem *269*, 32047-32050.

Adamsky, K., Arnold, K., Sabanay, H., and Peles, E. (2003). Junctional protein MAGI-3 interacts with receptor tyrosine phosphatase beta (RPTP beta) and tyrosine-phosphorylated proteins. J Cell Sci *116*, 1279-1289.

Alderton, W.K., Cooper, C.E., and Knowles, R.G. (2001). Nitric oxide synthases: structure, function and inhibition. Biochem J *357*, 593-615.

Anderson, C.R. (1998). Identification of cardiovascular pathways in the sympathetic nervous system. Clin Exp Pharmacol Physiol *25*, 449-452.

Anderson, J.M., Stevenson, B.R., Jesaitis, L.A., Goodenough, D.A., and Mooseker, M.S. (1988a). Characterization of ZO-1, a protein component of the tight junction from mouse liver and Madin-Darby canine kidney cells. J Cell Biol *106*, 1141-1149.

Anderson, J.M., Stevenson, B.R., Jesaitis, L.A., Goodenough, D.A., and Mooseker, M.S. (1988b). Characterization of ZO-1, a protein component of the tight junction from mouse liver and Madin-Darby canine kidney cells. J Cell Biol *106*, 1141-1149.

Anderson, J.M., Van Itallie, C.M., and Fanning, A.S. (2004). Setting up a selective barrier at the apical junction complex. Curr Opin Cell Biol *16*, 140-145.

Armesilla, A.L., Williams, J.C., Buch, M.H., Pickard, A., Emerson, M., Cartwright, E.J., Oceandy, D., Vos, M.D., Gillies, S., Clark, G.J.*, et al.* (2004). Novel functional interaction between the plasma membrane Ca2+ pump 4b and the proapoptotic tumor suppressor Ras-associated factor 1 (RASSF1). J Biol Chem *279*, 31318-31328.

Arnold, W.P., Mittal, C.K., Katsuki, S., and Murad, F. (1977). Nitric oxide activates guanylate cyclase and increases guanosine 3':5'-cyclic monophosphate levels in various tissue preparations. Proc Natl Acad Sci U S A *74*, 3203-3207.

Atasoy, D., Schoch, S., Ho, A., Nadasy, K.A., Liu, X., Zhang, W., Mukherjee, K., Nosyreva, E.D., Fernandez-Chacon, R., Missler, M.*, et al.* (2007). Deletion of CASK in mice is lethal and impairs synaptic function. Proc Natl Acad Sci U S A *104*, 2525-2530.

Barouch, L.A., Harrison, R.W., Skaf, M.W., Rosas, G.O., Cappola, T.P., Kobeissi, Z.A., Hobai, I.A., Lemmon, C.A., Burnett, A.L., O'Rourke, B.*, et al.* (2002). Nitric oxide regulates the heart by spatial confinement of nitric oxide synthase isoforms. Nature *416*, 337-339.

Bean, B.P. (1989). Classes of calcium channels in vertebrate cells. Annu Rev Physiol *51*, 367-384.

Bendall, J.K., Damy, T., Ratajczak, P., Loyer, X., Monceau, V., Marty, I., Milliez, P., Robidel, E., Marotte, F., Samuel, J.L.*, et al.* (2004). Role of myocardial neuronal nitric oxide synthase-derived nitric oxide in beta-adrenergic hyporesponsiveness after myocardial infarction-induced heart failure in rat. Circulation *110*, 2368-2375.

Berridge, M.J. (2002). The endoplasmic reticulum: a multifunctional signaling organelle. Cell Calcium *32*, 235-249.

Bers, D.M. (2002). Cardiac excitation-contraction coupling. Nature *415*, 198-205.

Bezprozvanny, I., and Maximov, A. (2001). PDZ domains: More than just a glue. Proc Natl Acad Sci U S A *98*, 787-789.

Biel, M., Ruth, P., Bosse, E., Hullin, R., Stuhmer, W., Flockerzi, V., and Hofmann, F. (1990). Primary structure and functional expression of a high voltage activated calcium channel from rabbit lung. FEBS Lett *269*, 409-412.

Bodi, I., Mikala, G., Koch, S.E., Akhter, S.A., and Schwartz, A. (2005). The L-type calcium channel in the heart: the beat goes on. J Clin Invest *115*, 3306-3317.

Bolotina, V.M., Najibi, S., Palacino, J.J., Pagano, P.J., and Cohen, R.A. (1994). Nitric oxide directly activates calcium-dependent potassium channels in vascular smooth muscle. Nature *368*, 850-853.

Bootman, M.D., and Berridge, M.J. (1995). The elemental principles of calcium signaling. Cell *83*, 675-678.

Bootman, M.D., Collins, T.J., Peppiatt, C.M., Prothero, L.S., MacKenzie, L., De Smet, P., Travers, M., Tovey, S.C., Seo, J.T., Berridge, M.J.*, et al.* (2001a). Calcium signalling--an overview. Semin Cell Dev Biol *12*, 3-10.

Bootman, M.D., Lipp, P., and Berridge, M.J. (2001b). The organisation and functions of local Ca(2+) signals. J Cell Sci *114*, 2213-2222.

Borg, J.P., Lopez-Figueroa, M.O., de Taddeo-Borg, M., Kroon, D.E., Turner, R.S., Watson, S.J., and Margolis, B. (1999). Molecular analysis of the X11-mLin-2/CASK complex in brain. J Neurosci *19*, 1307-1316.

Borg, J.P., Ooi, J., Levy, E., and Margolis, B. (1996). The phosphotyrosine interaction domains of X11 and FE65 bind to distinct sites on the YENPTY motif of amyloid precursor protein. Mol Cell Biol *16*, 6229-6241.

Brenman, J.E., Chao, D.S., Gee, S.H., McGee, A.W., Craven, S.E., Santillano, D.R., Wu, Z., Huang, F., Xia, H., Peters, M.F.*, et al.* (1996). Interaction of nitric oxide synthase with the postsynaptic density protein PSD-95 and alpha1-syntrophin mediated by PDZ domains. Cell *84*, 757-767.

Brenman, J.E., Xia, H., Chao, D.S., Black, S.M., and Bredt, D.S. (1997). Regulation of neuronal nitric oxide synthase through alternative transcripts. Dev Neurosci *19*, 224-231.

Brini, M. (2009). Plasma membrane Ca(2+)-ATPase: from a housekeeping function to a versatile signaling role. Pflugers Arch *457*, 657-664.

Brodin, P., Falchetto, R., Vorherr, T., and Carafoli, E. (1992). Identification of two domains which mediate the binding of activating phospholipids to the plasma-membrane Ca2+ pump. Eur J Biochem *204*, 939-946.

Brone, B., and Eggermont, J. (2005). PDZ proteins retain and regulate membrane transporters in polarized epithelial cell membranes. Am J Physiol Cell Physiol *288*, C20-29.

Buch, M.H., Pickard, A., Rodriguez, A., Gillies, S., Maass, A.H., Emerson, M., Cartwright, E.J., Williams, J.C., Oceandy, D., Redondo, J.M.*, et al.* (2005). The sarcolemmal calcium pump inhibits the calcineurin/nuclear factor of activated T-cell pathway via interaction with the calcineurin A catalytic subunit. J Biol Chem *280*, 29479-29487.

Budde, T., Meuth, S., and Pape, H.C. (2002). Calcium-dependent inactivation of neuronal calcium channels. Nat Rev Neurosci *3*, 873-883.

Burkard, N., Rokita, A.G., Kaufmann, S.G., Hallhuber, M., Wu, R., Hu, K., Hofmann, U., Bonz, A., Frantz, S., Cartwright, E.J.*, et al.* (2007). Conditional neuronal nitric oxide synthase overexpression impairs myocardial contractility. Circ Res *100*, e32-44.

Butz, S., Okamoto, M., and Sudhof, T.C. (1998). A tripartite protein complex with the potential to couple synaptic vesicle exocytosis to cell adhesion in brain. Cell *94*, 773-782.

Campbell, D.L., Stamler, J.S., and Strauss, H.C. (1996a). Redox modulation of L-type calcium channels in ferret ventricular myocytes. Dual mechanism regulation by nitric oxide and S-nitrosothiols. J Gen Physiol *108*, 277-293.

Campbell, D.L., Stamler, J.S., and Strauss, H.C. (1996b). Redox modulation of L-type calcium channels in ferret ventricular myocytes. Dual mechanism regulation by nitric oxide and S-nitrosothiols. J Gen Physiol *108*, 277-293.

Carafoli, E., Kessler, F., Falchetto, R., Heim, R., Quadroni, M., Krebs, J., Strehler, E.E., and Vorherr, T. (1992). The molecular basis of the modulation of the plasma membrane calcium pump by calmodulin. Ann N Y Acad Sci *671*, 58-68; discussion 68-59.

Carafoli, E., Santella, L., Branca, D., and Brini, M. (2001). Generation, control, and processing of cellular calcium signals. Crit Rev Biochem Mol Biol *36*, 107-260.

Carafoli, E., and Stauffer, T. (1994). The plasma membrane calcium pump: functional domains, regulation of the activity, and tissue specificity of isoform expression. J Neurobiol *25*, 312-324.

Cartwright, E.J., Oceandy, D., and Neyses, L. (2007). Plasma membrane calcium ATPase and its relationship to nitric oxide signaling in the heart. Ann N Y Acad Sci *1099*, 247-253.

Cartwright, E.J., Oceandy, D., and Neyses, L. (2009). Physiological implications of the interaction between the plasma membrane calcium pump and nNOS. Pflugers Arch *457*, 665-671.

Catterall, W.A. (2000). Structure and regulation of voltage-gated Ca2+ channels. Annu Rev Cell Dev Biol *16*, 521-555.

Chicka, M.C., and Strehler, E.E. (2003). Alternative splicing of the first intracellular loop of plasma membrane Ca2+-ATPase isoform 2 alters its membrane targeting. J Biol Chem *278*, 18464-18470.

Cho, K.O., Hunt, C.A., and Kennedy, M.B. (1992). The rat brain postsynaptic density fraction contains a homolog of the Drosophila discs-large tumor suppressor protein. Neuron *9*, 929-942.

Christopherson, K.S., Hillier, B.J., Lim, W.A., and Bredt, D.S. (1999). PSD-95 assembles a ternary complex with the N-methyl-D-aspartic acid receptor and a bivalent neuronal NO synthase PDZ domain. J Biol Chem *274*, 27467-27473.

Chu, E.C., and Tarnawski, A.S. (2004). PTEN regulatory functions in tumor suppression and cell biology. Med Sci Monit *10*, RA235-241.

Clapham, D.E. (2007). Calcium signaling. Cell *131*, 1047-1058.

Cohen, A.R., Woods, D.F., Marfatia, S.M., Walther, Z., Chishti, A.H., and Anderson, J.M. (1998). Human CASK/LIN-2 binds syndecan-2 and protein 4.1 and localizes to the basolateral membrane of epithelial cells. J Cell Biol *142*, 129-138.

Cowburn, D. (1997). Peptide recognition by PTB and PDZ domains. Curr Opin Struct Biol *7*, 835-838.

Cribbs, L.L., Lee, J.H., Yang, J., Satin, J., Zhang, Y., Daud, A., Barclay, J., Williamson, M.P., Fox, M., Rees, M.*, et al.* (1998). Cloning and characterization of alpha1H from human heart, a member of the T-type Ca2+ channel gene family. Circ Res *83*, 103-109.

Dai, J.L., Wang, L., Sahin, A.A., Broemeling, L.D., Schutte, M., and Pan, Y. (2004). NHERF (Na+/H+ exchanger regulatory factor) gene mutations in human breast cancer. Oncogene *23*, 8681-8687.

Damsky, C.H., and Ilic, D. (2002). Integrin signaling: it's where the action is. Curr Opin Cell Biol *14*, 594-602.

Damy, T., Ratajczak, P., Robidel, E., Bendall, J.K., Oliviero, P., Boczkowski, J., Ebrahimian, T., Marotte, F., Samuel, J.L., and Heymes, C. (2003). Up-regulation of cardiac nitric oxide synthase 1-derived nitric oxide after myocardial infarction in senescent rats. Faseb J *17*, 1934-1936.

Damy, T., Ratajczak, P., Shah, A.M., Camors, E., Marty, I., Hasenfuss, G., Marotte, F., Samuel, J.L., and Heymes, C. (2004). Increased neuronal nitric oxide synthase-derived NO production in the failing human heart. Lancet *363*, 1365-1367.

Daniel, E.E., Jury, J., and Wang, Y.F. (2001). nNOS in canine lower esophageal sphincter: colocalized with Cav-1 and Ca2+-handling proteins? Am J Physiol Gastrointest Liver Physiol *281*, G1101-1114.

Daniel, J.M. (2007). Dancing in and out of the nucleus: p120(ctn) and the transcription factor Kaiso. Biochim Biophys Acta *1773*, 59-68.

Danson, E.J., Choate, J.K., and Paterson, D.J. (2005). Cardiac nitric oxide: emerging role for nNOS in regulating physiological function. Pharmacol Ther *106*, 57-74.

Day, P., and Kobilka, B. (2006). PDZ-domain arrays for identifying components of GPCR signaling complexes. Trends Pharmacol Sci *27*, 509-511.

DeMarco, S.J., Chicka, M.C., and Strehler, E.E. (2002). Plasma membrane Ca2+ ATPase isoform 2b interacts preferentially with Na+/H+ exchanger regulatory factor 2 in apical plasma membranes. J Biol Chem *277*, 10506-10511.

DeMarco, S.J., and Strehler, E.E. (2001). Plasma membrane Ca2+-atpase isoforms 2b and 4b interact promiscuously and selectively with members of the membrane-associated guanylate kinase family of PDZ (PSD95/Dlg/ZO-1) domain-containing proteins. J Biol Chem *276*, 21594-21600.

Devic, E., Xiang, Y., Gould, D., and Kobilka, B. (2001). Beta-adrenergic receptor subtype-specific signaling in cardiac myocytes from beta(1) and beta(2) adrenoceptor knockout mice. Mol Pharmacol *60*, 577-583.

Di Leva, F., Domi, T., Fedrizzi, L., Lim, D., and Carafoli, E. (2008). The plasma membrane Ca2+ ATPase of animal cells: structure, function and regulation. Arch Biochem Biophys *476*, 65-74.

Doyle, D.A., Lee, A., Lewis, J., Kim, E., Sheng, M., and MacKinnon, R. (1996). Crystal structures of a complexed and peptide-free membrane protein-binding domain: molecular basis of peptide recognition by PDZ. Cell *85*, 1067-1076.

Ertel, E.A., Campbell, K.P., Harpold, M.M., Hofmann, F., Mori, Y., Perez-Reyes, E., Schwartz, A., Snutch, T.P., Tanabe, T., Birnbaumer, L.*, et al.* (2000). Nomenclature of voltage-gated calcium channels. Neuron *25*, 533-535.

Eu, J.P., Xu, L., Stamler, J.S., and Meissner, G. (1999). Regulation of ryanodine receptors by reactive nitrogen species. Biochem Pharmacol *57*, 1079-1084.

Fabiato, A. (1983). Calcium-induced release of calcium from the cardiac sarcoplasmic reticulum. Am J Physiol *245*, C1-14.

Falchetto, R., Vorherr, T., Brunner, J., and Carafoli, E. (1991). The plasma membrane Ca2+ pump contains a site that interacts with its calmodulin-binding domain. J Biol Chem *266*, 2930-2936.

Fan, J.S., and Zhang, M. (2002). Signaling complex organization by PDZ domain proteins. Neurosignals *11*, 315-321.

Fanning, A., ed. (2006). ZO proteins and tight junction assembly., 64-65 edn (New York, Landes Bioscience).

Fanning, A.S., and Anderson, J.M. (1996). Protein-protein interactions: PDZ domain networks. Curr Biol *6*, 1385-1388.

Fanning, A.S., and Anderson, J.M. (2009). Zonula occludens-1 and -2 are cytosolic scaffolds that regulate the assembly of cellular junctions. Ann N Y Acad Sci *1165*, 113-120.

Feron, O., Belhassen, L., Kobzik, L., Smith, T.W., Kelly, R.A., and Michel, T. (1996). Endothelial nitric oxide synthase targeting to caveolae. Specific interactions with caveolin isoforms in cardiac myocytes and endothelial cells. J Biol Chem *271*, 22810-22814.

Ferro-Novick, S., and Jahn, R. (1994). Vesicle fusion from yeast to man. Nature *370*, 191-193.

Franklin, J.L., Yoshiura, K., Dempsey, P.J., Bogatcheva, G., Jeyakumar, L., Meise, K.S., Pearsall, R.S., Threadgill, D., and Coffey, R.J. (2005). Identification of MAGI-3 as a transforming growth factor-alpha tail binding protein. Exp Cell Res *303*, 457-470.

Freise, D., Held, B., Wissenbach, U., Pfeifer, A., Trost, C., Himmerkus, N., Schweig, U., Freichel, M., Biel, M., Hofmann, F*., et al.* (2000). Absence of the gamma subunit of the skeletal muscle dihydropyridine receptor increases L-type Ca2+ currents and alters channel inactivation properties. J Biol Chem *275*, 14476-14481.

Fujimoto, T. (1993). Calcium pump of the plasma membrane is localized in caveolae. J Cell Biol *120*, 1147-1157.

Fujisawa, H., Ogura, T., Kurashima, Y., Yokoyama, T., Yamashita, J., and Esumi, H. (1994). Expression of two types of nitric oxide synthase mRNA in human neuroblastoma cell lines. J Neurochem *63*, 140-145.

Furuta, H., Luo, L., Hepler, K., and Ryan, A.F. (1998). Evidence for differential regulation of calcium by outer versus inner hair cells: plasma membrane Ca-ATPase gene expression. Hear Res *123*, 10-26.

Gachhui, R., Abu-Soud, H.M., Ghosha, D.K., Presta, A., Blazing, M.A., Mayer, B., George, S.E., and Stuehr, D.J. (1998). Neuronal nitric-oxide synthase interaction with calmodulin-troponin C chimeras. J Biol Chem *273*, 5451-5454.

Gachhui, R., Presta, A., Bentley, D.F., Abu-Soud, H.M., McArthur, R., Brudvig, G., Ghosh, D.K., and Stuehr, D.J. (1996). Characterization of the reductase domain of rat neuronal nitric oxide synthase generated in the methylotrophic yeast Pichia pastoris. Calmodulin response is complete within the reductase domain itself. J Biol Chem *271*, 20594-20602.

Garbers, D.L. (1979). Purification of soluble guanylate cyclase from rat lung. J Biol Chem *254*, 240-243.

Garcia, M.L., and Strehler, E.E. (1999). Plasma membrane calcium ATPases as critical regulators of calcium homeostasis during neuronal cell function. Front Biosci *4*, D869-882.

Garner, C.C., Nash, J., and Huganir, R.L. (2000). PDZ domains in synapse assembly and signalling. Trends Cell Biol *10*, 274-280.

Gee, S.H., Sekely, S.A., Lombardo, C., Kurakin, A., Froehner, S.C., and Kay, B.K. (1998). Cyclic peptides as non-carboxyl-terminal ligands of syntrophin PDZ domains. J Biol Chem *273*, 21980-21987.

Geiger, B., and Ayalon, O. (1992). Cadherins. Annu Rev Cell Biol *8*, 307-332.

Geiger, B., Ginsberg, D., Salomon, D., and Volberg, T. (1990). The molecular basis for the assembly and modulation of adherens-type junctions. Cell Differ Dev *32*, 343-353.

Geiger, B., Volk, T., Volberg, T., and Bendori, R. (1987). Molecular interactions in adherens-type contacts. J Cell Sci Suppl *8*, 251-272.

Giallourakis, C., Cao, Z., Green, T., Wachtel, H., Xie, X., Lopez-Illasaca, M., Daly, M., Rioux, J., and Xavier, R. (2006). A molecular-properties-based approach to understanding PDZ domain proteins and PDZ ligands. Genome Res *16*, 1056-1072.

Goellner, G.M., DeMarco, S.J., and Strehler, E.E. (2003). Characterization of PISP, a novel single-PDZ protein that binds to all plasma membrane Ca2+-ATPase b-splice variants. Ann N Y Acad Sci *986*, 461-471.

Gomperts, S.N. (1996). Clustering membrane proteins: It's all coming together with the PSD-95/SAP90 protein family. Cell *84*, 659-662.

Gregg, R.G., Messing, A., Strube, C., Beurg, M., Moss, R., Behan, M., Sukhareva, M., Haynes, S., Powell, J.A., Coronado, R.*, et al.* (1996). Absence of the beta subunit (cchb1) of the skeletal muscle dihydropyridine receptor alters expression of the alpha 1 subunit and eliminates excitation-contraction coupling. Proc Natl Acad Sci U S A *93*, 13961-13966.

Gurnett, C.A., De Waard, M., and Campbell, K.P. (1996). Dual function of the voltage-dependent Ca2+ channel alpha 2 delta subunit in current stimulation and subunit interaction. Neuron *16*, 431-440.

Halbleib, J.M., and Nelson, W.J. (2006). Cadherins in development: cell adhesion, sorting, and tissue morphogenesis. Genes Dev *20*, 3199-3214.

Hall, R.A. (2004). Beta-adrenergic receptors and their interacting proteins. Semin Cell Dev Biol *15*, 281-288.

Hall, R.A., Ostedgaard, L.S., Premont, R.T., Blitzer, J.T., Rahman, N., Welsh, M.J., and Lefkowitz, R.J. (1998). A C-terminal motif found in the beta2-adrenergic receptor, P2Y1 receptor and cystic fibrosis transmembrane conductance regulator determines binding to the Na+/H+ exchanger regulatory factor family of PDZ proteins. Proc Natl Acad Sci U S A *95*, 8496-8501.

Hammes, A., Oberdorf, S., Strehler, E.E., Stauffer, T., Carafoli, E., Vetter, H., and Neyses, L. (1994). Differentiation-specific isoform mRNA expression of the calmodulin-dependent plasma membrane Ca(2+)-ATPase. Faseb J *8*, 428-435.

Hardman, J.G., and Sutherland, E.W. (1969). Guanyl cyclase, an enzyme catalyzing the formation of guanosine 3',5'-monophosphate from guanosine trihosphate. J Biol Chem *244*, 6363-6370.

Hardy, J. (1997). The Alzheimer family of diseases: many etiologies, one pathogenesis? Proc Natl Acad Sci U S A *94*, 2095-2097.

Hare, J.M., and Stamler, J.S. (2005). NO/redox disequilibrium in the failing heart and cardiovascular system. J Clin Invest *115*, 509-517.

Harris, B.Z., and Lim, W.A. (2001). Mechanism and role of PDZ domains in signaling complex assembly. J Cell Sci *114*, 3219-3231.

Harrison, S.C. (1996). Peptide-surface association: the case of PDZ and PTB domains. Cell *86*, 341-343.

Hata, Y., Butz, S., and Sudhof, T.C. (1996). CASK: a novel dlg/PSD95 homolog with an N-terminal calmodulin-dependent protein kinase domain identified by interaction with neurexins. J Neurosci *16*, 2488-2494.

Hayashi, Y., Nishio, M., Naito, Y., Yokokura, H., Nimura, Y., Hidaka, H., and Watanabe, Y. (1999). Regulation of neuronal nitric-oxide synthase by calmodulin kinases. J Biol Chem *274*, 20597-20602.

He, J., Bellini, M., Inuzuka, H., Xu, J., Xiong, Y., Yang, X., Castleberry, A.M., and Hall, R.A. (2006). Proteomic analysis of beta1-adrenergic receptor interactions with PDZ scaffold proteins. J Biol Chem *281*, 2820-2827.

Heaton, D.A., Lei, M., Li, D., Golding, S., Dawson, T.A., Mohan, R.M., and Paterson, D.J. (2006). Remodeling of the cardiac pacemaker L-type calcium current and its beta-adrenergic responsiveness in hypertension after neuronal NO synthase gene transfer. Hypertension *48*, 443-452.

Hess, D.T., Matsumoto, A., Kim, S.O., Marshall, H.E., and Stamler, J.S. (2005). Protein S-nitrosylation: purview and parameters. Nat Rev Mol Cell Biol *6*, 150-166.

Hill, J.K., Williams, D.E., LeMasurier, M., Dumont, R.A., Strehler, E.E., and Gillespie, P.G. (2006). Splice-site A choice targets plasma-membrane Ca2+-ATPase isoform 2 to hair bundles. J Neurosci *26*, 6172-6180.

Hill, K., Li, Y., Bennett, M., McKay, M., Zhu, X., Shern, J., Torre, E., Lah, J.J., Levey, A.I., and Kahn, R.A. (2003). Munc18 interacting proteins: ADP-ribosylation factor-dependent coat proteins that regulate the traffic of beta-Alzheimer's precursor protein. J Biol Chem *278*, 36032-36040.

Hillier, B.J., Christopherson, K.S., Prehoda, K.E., Bredt, D.S., and Lim, W.A. (1999). Unexpected modes of PDZ domain scaffolding revealed by structure of nNOS-syntrophin complex. Science *284*, 812-815.

Hjerten, S. (1962). A new method for preparation of agaraose for gel electrophoresis. Biochim Biophys Acta *62*, 445-449.

Hofmann, F., Biel, M., and Flockerzi, V. (1994). Molecular basis for Ca2+ channel diversity. Annu Rev Neurosci *17*, 399-418.

Hofmann, F., Lacinova, L., and Klugbauer, N. (1999). Voltage-dependent calcium channels: from structure to function. Rev Physiol Biochem Pharmacol *139*, 33-87.

Hsueh, Y.P. (2009). Calcium/calmodulin-dependent serine protein kinase and mental retardation. Ann Neurol *66*, 438-443.

Hu, H., Chiamvimonvat, N., Yamagishi, T., and Marban, E. (1997a). Direct inhibition of expressed cardiac L-type Ca2+ channels by S-nitrosothiol nitric oxide donors. Circ Res *81*, 742-752.

Hu, H., Chiamvimonvat, N., Yamagishi, T., and Marban, E. (1997b). Direct inhibition of expressed cardiac L-type Ca2+ channels by S-nitrosothiol nitric oxide donors. Circ Res *81*, 742-752.

Hung, A.Y., and Sheng, M. (2002). PDZ domains: structural modules for protein complex assembly. J Biol Chem *277*, 5699-5702.

Iida, J., Hirabayashi, S., Sato, Y., and Hata, Y. (2004). Synaptic scaffolding molecule is involved in the synaptic clustering of neuroligin. Mol Cell Neurosci *27*, 497-508.

Irie, M., Hata, Y., Takeuchi, M., Ichtchenko, K., Toyoda, A., Hirao, K., Takai, Y., Rosahl, T.W., and Sudhof, T.C. (1997). Binding of neuroligins to PSD-95. Science *277*, 1511-1515.

Ishizuya, T., Yokose, S., Hori, M., Noda, T., Suda, T., Yoshiki, S., and Yamaguchi, A. (1997). Parathyroid hormone exerts disparate effects on osteoblast differentiation depending on exposure time in rat osteoblastic cells. J Clin Invest *99*, 2961-2970.

Itoh, M., Yonemura, S., Nagafuchi, A., and Tsukita, S. (1991). A 220-kD undercoat-constitutive protein: its specific localization at cadherin-based cell-cell adhesion sites. J Cell Biol *115*, 1449-1462.

Jaffrey, S.R., Erdjument-Bromage, H., Ferris, C.D., Tempst, P., and Snyder, S.H. (2001). Protein S-nitrosylation: a physiological signal for neuronal nitric oxide. Nat Cell Biol *3*, 193-197.

Jaffrey, S.R., and Snyder, S.H. (2001). The biotin switch method for the detection of S-nitrosylated proteins. Sci STKE *2001*, pl1.

James, P., Maeda, M., Fischer, R., Verma, A.K., Krebs, J., Penniston, J.T., and Carafoli, E. (1988). Identification and primary structure of a calmodulin binding domain of the Ca2+ pump of human erythrocytes. J Biol Chem *263*, 2905-2910.

Kamp, T.J., and Hell, J.W. (2000). Regulation of cardiac L-type calcium channels by protein kinase A and protein kinase C. Circ Res *87*, 1095-1102.

Kennedy, M.B. (1995). Origin of PDZ (DHR, GLGF) domains. Trends Biochem Sci *20*, 350.

Kim, D.Y., and Kim, K.K. (2005). Structure and function of HtrA family proteins, the key players in protein quality control. J Biochem Mol Biol *38*, 266-274.

Kim, E., DeMarco, S.J., Marfatia, S.M., Chishti, A.H., Sheng, M., and Strehler, E.E. (1998). Plasma membrane Ca2+ ATPase isoform 4b binds to membrane-associated guanylate kinase (MAGUK) proteins via their PDZ (PSD-95/Dlg/ZO-1) domains. J Biol Chem *273*, 1591-1595.

Kim, E., Niethammer, M., Rothschild, A., Jan, Y.N., and Sheng, M. (1995). Clustering of Shaker-type K+ channels by interaction with a family of membrane-associated guanylate kinases. Nature *378*, 85-88.

Kim, E., and Sheng, M. (2004). PDZ domain proteins of synapses. Nat Rev Neurosci *5*, 771-781.

Klugbauer, N., Marais, E., Lacinova, L., and Hofmann, F. (1999). A T-type calcium channel from mouse brain. Pflugers Arch *437*, 710-715.

Knowles, M.R., Stutts, M.J., Spock, A., Fischer, N., Gatzy, J.T., and Boucher, R.C. (1983). Abnormal ion permeation through cystic fibrosis respiratory epithelium. Science *221*, 1067-1070.

Kornau, H.C., Schenker, L.T., Kennedy, M.B., and Seeburg, P.H. (1995). Domain interaction between NMDA receptor subunits and the postsynaptic density protein PSD-95. Science *269*, 1737-1740.

Kozel, P.J., Friedman, R.A., Erway, L.C., Yamoah, E.N., Liu, L.H., Riddle, T., Duffy, J.J., Doetschman, T., Miller, M.L., Cardell, E.L.*, et al.* (1998). Balance and hearing deficits in mice with a null mutation in the gene encoding plasma membrane Ca2+-ATPase isoform 2. J Biol Chem *273*, 18693-18696.

Krebs, G., ed. (1996). Biomemranes.

Krebs, J., Vasak, M., Scarpa, A., and Carafoli, E. (1987). Conformational differences between the E1 and E2 states of the calcium adenosinetriphosphatase of the erythrocyte plasma membrane as revealed by circular dichroism and fluorescence spectroscopy. Biochemistry *26*, 3921-3926.

Kurschner, C., Mermelstein, P.G., Holden, W.T., and Surmeier, D.J. (1998). CIPP, a novel multivalent PDZ domain protein, selectively interacts with Kir4.0 family members, NMDA receptor subunits, neurexins, and neuroligins. Mol Cell Neurosci *11*, 161-172.

Kurzchalia, T.V., and Parton, R.G. (1999). Membrane microdomains and caveolae. Curr Opin Cell Biol *11*, 424-431.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature *227*, 680-685.

Landino, L.M., Koumas, M.T., Mason, C.E., and Alston, J.A. (2006). Ascorbic acid reduction of microtubule protein disulfides and its relevance to protein S-nitrosylation assays. Biochem Biophys Res Commun *340*, 347-352.

Laskowski, R.A., Watson, J.D., and Thornton, J.M. (2005). Protein function prediction using local 3D templates. J Mol Biol *351*, 614-626.

Lasky, L.A., Skelton, N. J., Siduh S. S. (2005). PDZ Domains: Intracellular Mediators of Carboxy-terminal Protein Recognition and Scaffolding. In Modular Protein Domains, G. Cesareni, Gimona M., Sudol, M., Yaffe, M. , ed. (Weinheim, WILEY-VCH Verlag GmbH & Co), pp. 257-273.

Laura, R.P., Ross, S., Koeppen, H., and Lasky, L.A. (2002). MAGI-1: a widely expressed, alternatively spliced tight junction protein. Exp Cell Res *275*, 155-170.

Lee, D.B., Huang, E., and Ward, H.J. (2006). Tight junction biology and kidney dysfunction. Am J Physiol Renal Physiol *290*, F20-34.

Leung, A.T., Imagawa, T., Block, B., Franzini-Armstrong, C., and Campbell, K.P. (1988). Biochemical and ultrastructural characterization of the 1,4-dihydropyridine receptor from rabbit skeletal muscle. Evidence for a 52,000 Da subunit. J Biol Chem *263*, 994-1001.

Llinas, R.R., Sugimori, M., and Cherksey, B. (1989). Voltage-dependent calcium conductances in mammalian neurons. The P channel. Ann N Y Acad Sci *560*, 103-111.

Lokuta, A.J., Maertz, N.A., Meethal, S.V., Potter, K.T., Kamp, T.J., Valdivia, H.H., and Haworth, R.A. (2005). Increased nitration of sarcoplasmic reticulum Ca2+-ATPase in human heart failure. Circulation *111*, 988-995.

Long, J.F., Tochio, H., Wang, P., Fan, J.S., Sala, C., Niethammer, M., Sheng, M., and Zhang, M. (2003). Supramodular structure and synergistic target binding of the N-terminal tandem PDZ domains of PSD-95. J Mol Biol *327*, 203-214.

Lumeng, C., Phelps, S., Crawford, G.E., Walden, P.D., Barald, K., and Chamberlain, J.S. (1999). Interactions between beta 2-syntrophin and a family of microtubule-associated serine/threonine kinases. Nat Neurosci *2*, 611-617.

Mangia, A., Chiriatti, A., Bellizzi, A., Malfettone, A., Stea, B., Zito, F.A., Reshkin, S.J., Simone, G., and Paradiso, A. (2009). Biological role of NHERF1 protein expression in breast cancer. Histopathology *55*, 600-608.

Mannick, J.B., and Schonhoff, C.M. (2002). Nitrosylation: the next phosphorylation? Arch Biochem Biophys *408*, 1-6.

Martin, T.F. (1997). Stages of regulated exocytosis. Trends Cell Biol *7*, 271-276.

Martinez-Dunst, C., Michaels, R.L., and Fuchs, P.A. (1997). Release sites and calcium channels in hair cells of the chick's cochlea. J Neurosci *17*, 9133-9144.

Martinez-Ruiz, A., and Lamas, S. (2004). S-nitrosylation: a potential new paradigm in signal transduction. Cardiovasc Res *62*, 43-52.

Massion, P.B., Feron, O., Dessy, C., and Balligand, J.L. (2003). Nitric oxide and cardiac function: ten years after, and continuing. Circ Res *93*, 388-398.

Mattson, M.P. (1997). Cellular actions of beta-amyloid precursor protein and its soluble and fibrillogenic derivatives. Physiol Rev *77*, 1081-1132.

Maxfield, F.R. (2002). Plasma membrane microdomains. Curr Opin Cell Biol *14*, 483-487.

Maximov, A., Sudhof, T.C., and Bezprozvanny, I. (1999). Association of neuronal calcium channels with modular adaptor proteins. J Biol Chem *274*, 24453-24456.

McCabe, T.J., Fulton, D., Roman, L.J., and Sessa, W.C. (2000). Enhanced electron flux and reduced calmodulin dissociation may explain "calcium-independent" eNOS activation by phosphorylation. J Biol Chem *275*, 6123-6128.

McLoughlin, D.M., Irving, N.G., Brownlees, J., Brion, J.P., Leroy, K., and Miller, C.C. (1999). Mint2/X11-like colocalizes with the Alzheimer's disease amyloid precursor protein and is associated with neuritic plaques in Alzheimer's disease. Eur J Neurosci *11*, 1988-1994.

McMillan, K., and Masters, B.S. (1995). Prokaryotic expression of the heme- and flavin-binding domains of rat neuronal nitric oxide synthase as distinct polypeptides: identification of the heme-binding proximal thiolate ligand as cysteine-415. Biochemistry *34*, 3686-3693.

Mikami, A., Imoto, K., Tanabe, T., Niidome, T., Mori, Y., Takeshima, H., Narumiya, S., and Numa, S. (1989). Primary structure and functional expression of the cardiac dihydropyridine-sensitive calcium channel. Nature *340*, 230-233.

Milani, D., Malgaroli, A., Guidolin, D., Fasolato, C., Skaper, S.D., Meldolesi, J., and Pozzan, T. (1990). Ca2+ channels and intracellular Ca2+ stores in neuronal and neuroendocrine cells. Cell Calcium *11*, 191-199.

Monteith, G.R., and Roufogalis, B.D. (1995). The plasma membrane calcium pump--a physiological perspective on its regulation. Cell Calcium *18*, 459-470.

Moosmang, S., Lenhardt, P., Haider, N., Hofmann, F., and Wegener, J.W. (2005). Mouse models to study L-type calcium channel function. Pharmacol Ther *106*, 347-355.

Moosmang, S., Schulla, V., Welling, A., Feil, R., Feil, S., Wegener, J.W., Hofmann, F., and Klugbauer, N. (2003). Dominant role of smooth muscle L-type calcium channel Cav1.2 for blood pressure regulation. Embo J *22*, 6027-6034.

Morais Cabral, J.H., Petosa, C., Sutcliffe, M.J., Raza, S., Byron, O., Poy, F., Marfatia, S.M., Chishti, A.H., and Liddington, R.C. (1996). Crystal structure of a PDZ domain. Nature *382*, 649-652.

Morales, F.C., Takahashi, Y., Kreimann, E.L., and Georgescu, M.M. (2004). Ezrin-radixin-moesin (ERM)-binding phosphoprotein 50 organizes ERM proteins at the apical membrane of polarized epithelia. Proc Natl Acad Sci U S A *101*, 17705-17710.

Moyer, B.D., Denton, J., Karlson, K.H., Reynolds, D., Wang, S., Mickle, J.E., Milewski, M., Cutting, G.R., Guggino, W.B., Li, M.*, et al.* (1999). A PDZ-interacting domain in CFTR is an apical membrane polarization signal. J Clin Invest *104*, 1353-1361.

Moyer, B.D., Duhaime, M., Shaw, C., Denton, J., Reynolds, D., Karlson, K.H., Pfeiffer, J., Wang, S., Mickle, J.E., Milewski, M.*, et al.* (2000). The PDZ-interacting domain of cystic fibrosis transmembrane conductance regulator is required for functional expression in the apical plasma membrane. J Biol Chem *275*, 27069-27074.

Murthy, A., Gonzalez-Agosti, C., Cordero, E., Pinney, D., Candia, C., Solomon, F., Gusella, J., and Ramesh, V. (1998). NHE-RF, a regulatory cofactor for Na(+)-H+ exchange, is a common interactor for merlin and ERM (MERM) proteins. J Biol Chem *273*, 1273-1276.

Nakanishi, H., Obaishi, H., Satoh, A., Wada, M., Mandai, K., Satoh, K., Nishioka, H., Matsuura, Y., Mizoguchi, A., and Takai, Y. (1997). Neurabin: a novel neural tissue-specific actin filament-binding protein involved in neurite formation. J Cell Biol *139*, 951-961.

Namkung, Y., Smith, S.M., Lee, S.B., Skrypnyk, N.V., Kim, H.L., Chin, H., Scheller, R.H., Tsien, R.W., and Shin, H.S. (1998). Targeted disruption of the Ca2+ channel beta3 subunit reduces N- and L-type Ca2+ channel activity and alters the voltage-dependent activation of P/Q-type Ca2+ channels in neurons. Proc Natl Acad Sci U S A *95*, 12010-12015.

Neely, A., Wei, X., Olcese, R., Birnbaumer, L., and Stefani, E. (1993). Potentiation by the beta subunit of the ratio of the ionic current to the charge movement in the cardiac calcium channel. Science *262*, 575-578.

Niethammer, M., Kim, E., and Sheng, M. (1996). Interaction between the C terminus of NMDA receptor subunits and multiple members of the PSD-95 family of membrane-associated guanylate kinases. J Neurosci *16*, 2157-2163.

Nourry, C., Grant, S.G., and Borg, J.P. (2003). PDZ domain proteins: plug and play! Sci STKE *2003*, RE7.

Nunez, L., Vaquero, M., Gomez, R., Caballero, R., Mateos-Caceres, P., Macaya, C., Iriepa, I., Galvez, E., Lopez-Farre, A., Tamargo, J.*, et al.* (2006). Nitric oxide blocks hKv1.5 channels by S-nitrosylation and by a cyclic GMP-dependent mechanism. Cardiovasc Res *72*, 80-89.

Ojeh, N., Pekovic, V., Jahoda, C., and Maatta, A. (2008). The MAGUK-family protein CASK is targeted to nuclei of the basal epidermis and controls keratinocyte proliferation. J Cell Sci *121*, 2705-2717.

Okamoto, M., and Sudhof, T.C. (1997). Mints, Munc18-interacting proteins in synaptic vesicle exocytosis. J Biol Chem *272*, 31459-31464.

Okamoto, M., and Sudhof, T.C. (1998). Mint 3: a ubiquitous mint isoform that does not bind to munc18-1 or -2. Eur J Cell Biol *77*, 161-165.

Okunade, G.W., Miller, M.L., Pyne, G.J., Sutliff, R.L., O'Connor, K.T., Neumann, J.C., Andringa, A., Miller, D.A., Prasad, V., Doetschman, T.*, et al.* (2004). Targeted ablation of plasma membrane Ca2+-ATPase (PMCA) 1 and 4 indicates a major housekeeping function for PMCA1 and a critical role in hyperactivated sperm motility and male fertility for PMCA4. J Biol Chem *279*, 33742-33750.

Paarmann, I., Lye, M.F., Lavie, A., and Konrad, M. (2008). Structural requirements for calmodulin binding to membrane-associated guanylate kinase homologs. Protein Sci *17*, 1946-1954.

Pallen, M.J., and Ponting, C.P. (1997). PDZ domains in bacterial proteins. Mol Microbiol *26*, 411-413.

Pan, Y., Wang, L., and Dai, J.L. (2006). Suppression of breast cancer cell growth by Na+/H+ exchanger regulatory factor 1 (NHERF1). Breast Cancer Res *8*, R63.

Pawson, T. (2007). Dynamic control of signaling by modular adaptor proteins. Curr Opin Cell Biol *19*, 112-116.

Pawson, T., and Nash, P. (2003). Assembly of cell regulatory systems through protein interaction domains. Science *300*, 445-452.

Penniston, J.T., and Enyedi, A. (1998). Modulation of the plasma membrane Ca2+ pump. J Membr Biol *165*, 101-109.

Perez-Reyes, E., Cribbs, L.L., Daud, A., Lacerda, A.E., Barclay, J., Williamson, M.P., Fox, M., Rees, M., and Lee, J.H. (1998). Molecular characterization of a neuronal low-voltage-activated T-type calcium channel. Nature *391*, 896-900.

Peterson, B.Z., DeMaria, C.D., Adelman, J.P., and Yue, D.T. (1999). Calmodulin is the Ca2+ sensor for Ca2+ -dependent inactivation of L-type calcium channels. Neuron *22*, 549-558.

Petit, C.M., Zhang, J., Sapienza, P.J., Fuentes, E.J., and Lee, A.L. (2009). Hidden dynamic allostery in a PDZ domain. Proc Natl Acad Sci U S A *106*, 18249-18254.

Platzer, J., Engel, J., Schrott-Fischer, A., Stephan, K., Bova, S., Chen, H., Zheng, H., and Striessnig, J. (2000). Congenital deafness and sinoatrial node dysfunction in mice lacking class D L-type Ca2+ channels. Cell *102*, 89-97.

Raghuram, V., Mak, D.O., and Foskett, J.K. (2001). Regulation of cystic fibrosis transmembrane conductance regulator single-channel gating by bivalent PDZ-domain-mediated interaction. Proc Natl Acad Sci U S A *98*, 1300-1305.

Randall, A., and Tsien, R.W. (1995). Pharmacological dissection of multiple types of Ca2+ channel currents in rat cerebellar granule neurons. J Neurosci *15*, 2995-3012.

Reczek, D., Berryman, M., and Bretscher, A. (1997a). Identification of EBP50: A PDZ-containing phosphoprotein that associates with members of the ezrin-radixin-moesin family. J Cell Biol *139*, 169-179.

Reczek, D., Berryman, M., and Bretscher, A. (1997b). Identification of EBP50: A PDZ-containing phosphoprotein that associates with members of the ezrin-radixin-moesin family. J Cell Biol *139*, 169-179.

Reuter, H. (1979). Properties of two inward membrane currents in the heart. Annu Rev Physiol *41*, 413-424.

Richards, M.K., and Marletta, M.A. (1994). Characterization of neuronal nitric oxide synthase and a C415H mutant, purified from a baculovirus overexpression system. Biochemistry *33*, 14723-14732.

Rimessi, A., Coletto, L., Pinton, P., Rizzuto, R., Brini, M., and Carafoli, E. (2005). Inhibitory interaction of the 14-3-3{epsilon} protein with isoform 4 of the plasma membrane Ca(2+)-ATPase pump. J Biol Chem *280*, 37195-37203.

Rogelj, B., Mitchell, J.C., Miller, C.C., and McLoughlin, D.M. (2006). The X11/Mint family of adaptor proteins. Brain Res Rev *52*, 305-315.

Rothberg, K.G., Heuser, J.E., Donzell, W.C., Ying, Y.S., Glenney, J.R., and Anderson, R.G. (1992). Caveolin, a protein component of caveolae membrane coats. Cell *68*, 673-682.

Saras, J., and Heldin, C.H. (1996). PDZ domains bind carboxy-terminal sequences of target proteins. Trends Biochem Sci *21*, 455-458.

Sato, T., Irie, S., Kitada, S., and Reed, J.C. (1995). FAP-1: a protein tyrosine phosphatase that associates with Fas. Science *268*, 411-415.

Schagger, H., and von Jagow, G. (1987). Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa. Anal Biochem *166*, 368-379.

Schatzmann, H.J. (1966). ATP-dependent Ca++-extrusion from human red cells. Experientia *22*, 364-365.

Schepens, J., Cuppen, E., Wieringa, B., and Hendriks, W. (1997). The neuronal nitric oxide synthase PDZ motif binds to -G(D,E)XV* carboxyterminal sequences. FEBS Lett *409*, 53-56.

Schindeler, A., and Little, D.G. (2006). Ras-MAPK signaling in osteogenic differentiation: friend or foe? J Bone Miner Res *21*, 1331-1338.

Schneeberger, E.E., and Lynch, R.D. (2004). The tight junction: a multifunctional complex. Am J Physiol Cell Physiol *286*, C1213-1228.

Schuh, K., Cartwright, E.J., Jankevics, E., Bundschu, K., Liebermann, J., Williams, J.C., Armesilla, A.L., Emerson, M., Oceandy, D., Knobeloch, K.P., *et al.* (2004). Plasma membrane Ca2+ ATPase 4 is required for sperm motility and male fertility. J Biol Chem *279*, 28220-28226.

Schuh, K., Uldrijan, S., Gambaryan, S., Roethlein, N., and Neyses, L. (2003). Interaction of the plasma membrane Ca2+ pump 4b/CI with the Ca2+/calmodulin-dependent membrane-associated kinase CASK. J Biol Chem *278*, 9778-9783.

Schuh, K., Uldrijan, S., Telkamp, M., Rothlein, N., and Neyses, L. (2001). The plasmamembrane calmodulin-dependent calcium pump: a major regulator of nitric oxide synthase I. J Cell Biol *155*, 201-205.

Schultz, J., Hoffmuller, U., Krause, G., Ashurst, J., Macias, M.J., Schmieder, P., Schneider-Mergener, J., and Oschkinat, H. (1998). Specific interactions between the syntrophin PDZ domain and voltage-gated sodium channels. Nat Struct Biol *5*, 19-24.

Sears, C.E., Ashley, E.A., and Casadei, B. (2004). Nitric oxide control of cardiac function: is neuronal nitric oxide synthase a key component? Philos Trans R Soc Lond B Biol Sci *359*, 1021-1044.

Sears, C.E., Bryant, S.M., Ashley, E.A., Lygate, C.A., Rakovic, S., Wallis, H.L., Neubauer, S., Terrar, D.A., and Casadei, B. (2003). Cardiac neuronal nitric oxide synthase isoform regulates myocardial contraction and calcium handling. Circ Res *92*, e52-59.

Seddon, M., Shah, A.M., and Casadei, B. (2007). Cardiomyocytes as effectors of nitric oxide signalling. Cardiovasc Res *75*, 315-326.

Segal, S.S., Brett, S.E., and Sessa, W.C. (1999). Codistribution of NOS and caveolin throughout peripheral vasculature and skeletal muscle of hamsters. Am J Physiol *277*, H1167-1177.

Seidler, U., Singh, A.K., Cinar, A., Chen, M., Hillesheim, J., Hogema, B., and Riederer, B. (2009). The role of the NHERF family of PDZ scaffolding proteins in the regulation of salt and water transport. Ann N Y Acad Sci *1165*, 249-260.

Seino, S., Chen, L., Seino, M., Blondel, O., Takeda, J., Johnson, J.H., and Bell, G.I. (1992). Cloning of the alpha 1 subunit of a voltage-dependent calcium channel expressed in pancreatic beta cells. Proc Natl Acad Sci U S A *89*, 584-588.

Seisenberger, C., Specht, V., Welling, A., Platzer, J., Pfeifer, A., Kuhbandner, S., Striessnig, J., Klugbauer, N., Feil, R., and Hofmann, F. (2000). Functional embryonic cardiomyocytes after disruption of the L-type alpha1C (Cav1.2) calcium channel gene in the mouse. J Biol Chem *275*, 39193-39199.

Sheng, M., and Sala, C. (2001). PDZ domains and the organization of supramolecular complexes. Annu Rev Neurosci *24*, 1-29.

Shenolikar, S., Voltz, J.W., Cunningham, R., and Weinman, E.J. (2004). Regulation of ion transport by the NHERF family of PDZ proteins. Physiology (Bethesda) *19*, 362-369.

Sheppard, L., Levy, D., Norris, G., Larson, T.V., and Koenig, J.Q. (1999). Effects of ambient air pollution on nonelderly asthma hospital admissions in Seattle, Washington, 1987-1994. Epidemiology *10*, 23-30.

Shieh, B.H., and Zhu, M.Y. (1996). Regulation of the TRP Ca2+ channel by INAD in Drosophila photoreceptors. Neuron *16*, 991-998.

Silvagno, F., Xia, H., and Bredt, D.S. (1996). Neuronal nitric-oxide synthase-mu, an alternatively spliced isoform expressed in differentiated skeletal muscle. J Biol Chem *271*, 11204-11208.

Sneddon, W.B., Liu, F., Gesek, F.A., and Friedman, P.A. (2000). Obligate mitogen-activated protein kinase activation in parathyroid hormone stimulation of calcium transport but not calcium signaling. Endocrinology *141*, 4185-4193.

Snyder, S.H. (1992). Nitric oxide: first in a new class of neurotransmitters. Science *257*, 494-496.

Song, E., Gao, S., Tian, R., Ma, S., Huang, H., Guo, J., Li, Y., Zhang, L., and Gao, Y. (2006). A high efficiency strategy for binding property characterization of peptide-binding domains. Mol Cell Proteomics *5*, 1368-1381.

Songyang, Z., Fanning, A.S., Fu, C., Xu, J., Marfatia, S.M., Chishti, A.H., Crompton, A., Chan, A.C., Anderson, J.M., and Cantley, L.C. (1997). Recognition of unique carboxyl-terminal motifs by distinct PDZ domains. Science *275*, 73-77.

Spaller, M.R. (2006). Act globally, think locally: systems biology addresses the PDZ domain. ACS Chem Biol *1*, 207-210.

Splawski, I., Timothy, K.W., Decher, N., Kumar, P., Sachse, F.B., Beggs, A.H., Sanguinetti, M.C., and Keating, M.T. (2005). Severe arrhythmia disorder caused by cardiac L-type calcium channel mutations. Proc Natl Acad Sci U S A *102*, 8089-8096; discussion 8086-8088.

Splawski, I., Timothy, K.W., Sharpe, L.M., Decher, N., Kumar, P., Bloise, R., Napolitano, C., Schwartz, P.J., Joseph, R.M., Condouris, K.*, et al.* (2004). Ca(V)1.2 calcium channel dysfunction causes a multisystem disorder including arrhythmia and autism. Cell *119*, 19-31.

Stevenson, B.R., Siliciano, J.D., Mooseker, M.S., and Goodenough, D.A. (1986). Identification of ZO-1: a high molecular weight polypeptide associated with the tight junction (zonula occludens) in a variety of epithelia. J Cell Biol *103*, 755-766.

Stieber, J., Herrmann, S., Feil, S., Loster, J., Feil, R., Biel, M., Hofmann, F., and Ludwig, A. (2003). The hyperpolarization-activated channel HCN4 is required for the generation of pacemaker action potentials in the embryonic heart. Proc Natl Acad Sci U S A *100*, 15235-15240.

Strehler, B.L. (1991). Where is the self? A neuroanatomical theory of consciousness. Synapse *7*, 44-91.

Strehler, E.E., Caride, A.J., Filoteo, A.G., Xiong, Y., Penniston, J.T., and Enyedi, A. (2007a). Plasma membrane Ca2+ ATPases as dynamic regulators of cellular calcium handling. Ann N Y Acad Sci *1099*, 226-236.

Strehler, E.E., Filoteo, A.G., Penniston, J.T., and Caride, A.J. (2007b). Plasma-membrane Ca(2+) pumps: structural diversity as the basis for functional versatility. Biochem Soc Trans *35*, 919-922.

Strehler, E.E., Heim, R., and Carafoli, E. (1991). Molecular characterization of plasma membrane calcium pump isoforms. Adv Exp Med Biol *307*, 251-261.

Strehler, E.E., and Zacharias, D.A. (2001). Role of alternative splicing in generating isoform diversity among plasma membrane calcium pumps. Physiol Rev *81*, 21-50.

Striessnig, J. (1999). Pharmacology, structure and function of cardiac L-type Ca(2+) channels. Cell Physiol Biochem *9*, 242-269.

Striessnig, J., Bolz, H.J., and Koschak, A. (2010). Channelopathies in Cav1.1, Cav1.3, and Cav1.4 voltage-gated L-type Ca2+ channels. Pflugers Arch *460*, 361-374.

Striessnig, J., Goll, A., Moosburger, K., and Glossmann, H. (1986). Purified calcium channels have three allosterically coupled drug receptors. FEBS Lett *197*, 204-210.

Suckow, A.T., Comoletti, D., Waldrop, M.A., Mosedale, M., Egodage, S., Taylor, P., and Chessler, S.D. (2008). Expression of neurexin, neuroligin, and their cytoplasmic binding partners in the pancreatic beta-cells and the involvement of neuroligin in insulin secretion. Endocrinology *149*, 6006-6017.

Sudhof, T.C. (1995). The synaptic vesicle cycle: a cascade of protein-protein interactions. Nature *375*, 645-653.

Summers, B.A., Overholt, J.L., and Prabhakar, N.R. (1999). Nitric oxide inhibits L-type Ca2+ current in glomus cells of the rabbit carotid body via a cGMP-independent mechanism. J Neurophysiol *81*, 1449-1457.

Sun, J., Picht, E., Ginsburg, K.S., Bers, D.M., Steenbergen, C., and Murphy, E. (2006). Hypercontractile female hearts exhibit increased S-nitrosylation of the L-type Ca2+ channel alpha1 subunit and reduced ischemia/reperfusion injury. Circ Res *98*, 403-411.

Takeda, K., and Akira, S. (2004). TLR signaling pathways. Semin Immunol *16*, 3-9.

Tanabe, T., Beam, K.G., Powell, J.A., and Numa, S. (1988). Restoration of excitation-contraction coupling and slow calcium current in dysgenic muscle by dihydropyridine receptor complementary DNA. Nature *336*, 134-139.

Tang, Y., Tang, J., Chen, Z., Trost, C., Flockerzi, V., Li, M., Ramesh, V., and Zhu, M.X. (2000). Association of mammalian trp4 and phospholipase C isozymes with a PDZ domain-containing protein, NHERF. J Biol Chem *275*, 37559-37564.

Torta, F., Usuelli, V., Malgaroli, A., and Bachi, A. (2008). Proteomic analysis of protein S-nitrosylation. Proteomics *8*, 4484-4494.

Ullmer, C., Schmuck, K., Figge, A., and Lubbert, H. (1998). Cloning and characterization of MUPP1, a novel PDZ domain protein. FEBS Lett *424*, 63-68.

Valiente, M., Andres-Pons, A., Gomar, B., Torres, J., Gil, A., Tapparel, C., Antonarakis, S.E., and Pulido, R. (2005). Binding of PTEN to specific PDZ domains contributes to PTEN protein stability and phosphorylation by microtubule-associated serine/threonine kinases. J Biol Chem *280*, 28936-28943.

van der Geer, P., and Pawson, T. (1995). The PTB domain: a new protein module implicated in signal transduction. Trends Biochem Sci *20*, 277-280.

van Ham, M., and Hendriks, W. (2003). PDZ domains-glue and guide. Mol Biol Rep *30*, 69-82.

Venema, V.J., Ju, H., Zou, R., and Venema, R.C. (1997). Interaction of neuronal nitric-oxide synthase with caveolin-3 in skeletal muscle. Identification of a novel caveolin scaffolding/inhibitory domain. J Biol Chem *272*, 28187-28190.

Vorherr, T., Chiesi, M., Schwaller, R., and Carafoli, E. (1992). Regulation of the calcium ion pump of sarcoplasmic reticulum: reversible inhibition by phospholamban and by the calmodulin binding domain of the plasma membrane calcium ion pump. Biochemistry *31*, 371-376.

Vorherr, T., James, P., Krebs, J., Enyedi, A., McCormick, D.J., Penniston, J.T., and Carafoli, E. (1990). Interaction of calmodulin with the calmodulin binding domain of the plasma membrane Ca2+ pump. Biochemistry *29*, 355-365.

Walden, P.D., and Cowan, N.J. (1993). A novel 205-kilodalton testis-specific serine/threonine protein kinase associated with microtubules of the spermatid manchette. Mol Cell Biol *13*, 7625-7635.

Wang, D., Lee, H.J., Cooper, D.S., Cebotaro, L., Walden, P.D., Choi, I., and Yun, C.C. (2006). Coexpression of MAST205 inhibits the activity of Na+/H+ exchanger NHE3. Am J Physiol Renal Physiol *290*, F428-437.

Wang, N.X., Lee, H.J., and Zheng, J.J. (2008). Therapeutic use of PDZ protein-protein interaction antagonism. Drug News Perspect *21*, 137-141.

Weinman, E.J. (2001). New functions for the NHERF family of proteins. J Clin Invest *108*, 185-186.

Weinman, E.J., Hall, R.A., Friedman, P.A., Liu-Chen, L.Y., and Shenolikar, S. (2006). The association of NHERF adaptor proteins with g protein-coupled receptors and receptor tyrosine kinases. Annu Rev Physiol *68*, 491-505.

Weinman, E.J., Minkoff, C., and Shenolikar, S. (2000). Signal complex regulation of renal transport proteins: NHERF and regulation of NHE3 by PKA. Am J Physiol Renal Physiol *279*, F393-399.

Weinman, E.J., Steplock, D., Tate, K., Hall, R.A., Spurney, R.F., and Shenolikar, S. (1998). Structure-function of recombinant Na/H exchanger regulatory factor (NHE-RF). J Clin Invest *101*, 2199-2206.

Williams, J.C., Armesilla, A.L., Mohamed, T.M., Hagarty, C.L., McIntyre, F.H., Schomburg, S., Zaki, A.O., Oceandy, D., Cartwright, E.J., Buch, M.H.*, et al.* (2006a). The sarcolemmal calcium pump, alpha-1 syntrophin, and neuronal nitric-oxide synthase are parts of a macromolecular protein complex. J Biol Chem *281*, 23341-23348.

Williams, J.P., Stewart, T., Li, B., Mulloy, R., Dimova, D., and Classon, M. (2006b). The retinoblastoma protein is required for Ras-induced oncogenic transformation. Mol Cell Biol *26*, 1170-1182.

Williams, M.E., Feldman, D.H., McCue, A.F., Brenner, R., Velicelebi, G., Ellis, S.B., and Harpold, M.M. (1992). Structure and functional expression of alpha 1, alpha 2, and beta subunits of a novel human neuronal calcium channel subtype. Neuron *8*, 71-84.

Witcher, D.R., De Waard, M., Sakamoto, J., Franzini-Armstrong, C., Pragnell, M., Kahl, S.D., and Campbell, K.P. (1993). Subunit identification and reconstitution of the N-type Ca2+ channel complex purified from brain. Science *261*, 486-489.

Wood, J.D., Yuan, J., Margolis, R.L., Colomer, V., Duan, K., Kushi, J., Kaminsky, Z., Kleiderlein, J.J., Sharp, A.H., and Ross, C.A. (1998). Atrophin-1, the DRPLA gene product, interacts with two families of WW domain-containing proteins. Mol Cell Neurosci *11*, 149-160.

Woods, D.F., and Bryant, P.J. (1993). ZO-1, DlgA and PSD-95/SAP90: homologous proteins in tight, septate and synaptic cell junctions. Mech Dev *44*, 85-89.

Wu, X., Hepner, K., Castelino-Prabhu, S., Do, D., Kaye, M.B., Yuan, X.J., Wood, J., Ross, C., Sawyers, C.L., and Whang, Y.E. (2000a). Evidence for regulation of the PTEN tumor suppressor by a membrane-localized multi-PDZ domain containing scaffold protein MAGI-2. Proc Natl Acad Sci U S A *97*, 4233-4238.

Wu, Y., Dowbenko, D., Spencer, S., Laura, R., Lee, J., Gu, Q., and Lasky, L.A. (2000b). Interaction of the tumor suppressor PTEN/MMAC with a PDZ domain of MAGI3, a novel membrane-associated guanylate kinase. J Biol Chem *275*, 21477-21485.

Xiang, Y., Devic, E., and Kobilka, B. (2002). The PDZ binding motif of the beta 1 adrenergic receptor modulates receptor trafficking and signaling in cardiac myocytes. J Biol Chem *277*, 33783-33790.

Xiang, Y., and Kobilka, B. (2003). The PDZ-binding motif of the beta2-adrenoceptor is essential for physiologic signaling and trafficking in cardiac myocytes. Proc Natl Acad Sci U S A *100*, 10776-10781.

Xiong, H., Li, H., Chen, Y., Zhao, J., and Unkeless, J.C. (2004). Interaction of TRAF6 with MAST205 regulates NF-kappaB activation and MAST205 stability. J Biol Chem *279*, 43675-43683.

Xu, J., Paquet, M., Lau, A.G., Wood, J.D., Ross, C.A., and Hall, R.A. (2001). beta 1-adrenergic receptor association with the synaptic scaffolding protein membrane-associated

guanylate kinase inverted-2 (MAGI-2). Differential regulation of receptor internalization by MAGI-2 and PSD-95. J Biol Chem *276*, 41310-41317.

Xu, K.Y., Huso, D.L., Dawson, T.M., Bredt, D.S., and Becker, L.C. (1999a). Nitric oxide synthase in cardiac sarcoplasmic reticulum. Proc Natl Acad Sci U S A *96*, 657-662.

Xu, K.Y., Huso, D.L., Dawson, T.M., Bredt, D.S., and Becker, L.C. (1999b). Nitric oxide synthase in cardiac sarcoplasmic reticulum. Proc Natl Acad Sci U S A *96*, 657-662.

Xu, K.Y., Kuppusamy, S.P., Wang, J.Q., Li, H., Cui, H., Dawson, T.M., Huang, P.L., Burnett, A.L., Kuppusamy, P., and Becker, L.C. (2003a). Nitric oxide protects cardiac sarcolemmal membrane enzyme function and ion active transport against ischemia-induced inactivation. J Biol Chem *278*, 41798-41803.

Xu, L., Eu, J.P., Meissner, G., and Stamler, J.S. (1998). Activation of the cardiac calcium release channel (ryanodine receptor) by poly-S-nitrosylation. Science *279*, 234-237.

Xu, M., Welling, A., Paparisto, S., Hofmann, F., and Klugbauer, N. (2003b). Enhanced expression of L-type Cav1.3 calcium channels in murine embryonic hearts from Cav1.2-deficient mice. J Biol Chem *278*, 40837-40841.

Yaari, Y., Hamon, B., and Lux, H.D. (1987). Development of two types of calcium channels in cultured mammalian hippocampal neurons. Science *235*, 680-682.

Yao, R., Natsume, Y., and Noda, T. (2004). MAGI-3 is involved in the regulation of the JNK signaling pathway as a scaffold protein for frizzled and Ltap. Oncogene *23*, 6023-6030.

Yap, A.S., Brieher, W.M., and Gumbiner, B.M. (1997). Molecular and functional analysis of cadherin-based adherens junctions. Annu Rev Cell Dev Biol *13*, 119-146.

Zhang, M., and Wang, W. (2003). Organization of signaling complexes by PDZ-domain scaffold proteins. Acc Chem Res *36*, 530-538.

Zhang, W., Lilja, L., Bark, C., Berggren, P.O., and Meister, B. (2004). Mint1, a Munc-18-interacting protein, is expressed in insulin-secreting beta-cells. Biochem Biophys Res Commun *320*, 717-721.

Zhang, Y., Keszler, A., Broniowska, K.A., and Hogg, N. (2005). Characterization and application of the biotin-switch assay for the identification of S-nitrosated proteins. Free Radic Biol Med *38*, 874-881.

Zhou, H., Xiong, H., Li, H., Plevy, S.E., Walden, P.D., Sassaroli, M., Prestwich, G.D., and Unkeless, J.C. (2004). Microtubule-associated serine/threonine kinase-205 kDa and Fc gamma receptor control IL-12 p40 synthesis and NF-kappa B activation. J Immunol *172*, 2559-2568.

Zucker, R.S. (1996). Exocytosis: a molecular and physiological perspective. Neuron *17*, 1049-1055.

Zuhlke, R.D., Pitt, G.S., Tsien, R.W., and Reuter, H. (2000). Ca2+-sensitive inactivation and facilitation of L-type Ca2+ channels both depend on specific amino acid residues in a consensus calmodulin-binding motif in the(alpha)1C subunit. J Biol Chem *275*, 21121-21129.

Zvaritch, E., James, P., Vorherr, T., Falchetto, R., Modyanov, N., and Carafoli, E. (1990). Mapping of functional domains in the plasma membrane Ca2+ pump using trypsin proteolysis. Biochemistry *29*, 8070-8076.

APPENDIX

A.1. Sequence alignments of vector constructs

A.1.1. pEXP-LTCC

```
1     AGC GCC CAA TAC GCA AAC CGC CTC TCC CCG CGC GTT GGC CGA TTC   45
1     Ser Ala Gln Tyr Ala Asn Arg Leu Ser Pro Arg Val Gly Arg Phe   15

46    ATT AAT GCA GCT GGC ACG ACA GGT TTC CCG ACT GGA AAG CGG GCA   90
16    Ile Asn Ala Ala Gly Thr Thr Gly Phe Pro Thr Gly Lys Arg Ala   30

91    GTG AGC GCA ACG CAA TTA ATG TGA GTT AGC TCA CTC ATT AGG CAC   135
31    Val Ser Ala Thr Gln Leu Met End Val Ser Ser Leu Ile Arg His   45

136   CCC AGG CTT TAC ACT TTA TGC TTC CGG CTC GTA TGT TGT GTG GAA   180
46    Pro Arg Leu Tyr Thr Leu Cys Phe Arg Leu Val Cys Cys Val Glu   60

181   TTG TGA GCG GAT AAC AAT TTC ACA CAG GAA ACA GCT ATG ACC ATG   225
61    Leu End Ala Asp Asn Asn Phe Thr Gln Glu Thr Ala Met Thr Met   75
```

Start Histidin-tag (241-258)

```
226   ATT ACG CCA AGC TTG CAT CAC CAT CAC CAT CAC AAG AAG AAA CCA   270
76    Ile Thr Pro Ser Leu His His His His His His Lys Lys Lys Pro   90

271   CTG GAT GGA GAA TAT TTC ACC CTT CAG ATC CGT GGG CGT GAG CGC   315
91    Leu Asp Gly Glu Tyr Phe Thr Leu Gln Ile Arg Gly Arg Glu Arg   105

316   TTC GAG ATG TTC CGA GAG CTG AAT GAG GCC TTG GAA CTC AAG GAT   360
106   Phe Glu Met Phe Arg Glu Leu Asn Glu Ala Leu Glu Leu Lys Asp   120

361   GCC CAG GCT GGG AAG GAG CCA GGG GGT GGT GGT GGT GGT GGT GGT   405
121   Ala Gln Ala Gly Lys Glu Pro Gly Gly Gly Gly Gly Gly Gly Gly   135
```

Multiple Cloning Side, MCS (406-483)

GAC AGC AGG TCC TAT GTC AGC AAC CTG TAG (LTCC) 418-447, Sequence of C-terminal tail of $Ca_v1.2$

(restriction enzymes) **PstI** ↓ **XbaI**, the red arrow indicate the position of the insert

```
406   GCA TGC CTG CAG TCT AGA GGA TCC CCG GGT ACC GGT ACT           450
136   Ala Cys Leu Gln Val Asp Ser Arg Gly Ser Pro Gly Thr Gly Thr   150

451   AGT AGA AAA AAT GAG TAA CGG CCG TAC GGG CCC TTT CGT CTC GCG   495
151   Ser Arg Lys Asn Glu End Arg Pro Tyr Gly Pro Phe Arg Leu Ala   165

496   CGT TTC GGT GAT GAC GGT GAA AAC CTC TGA CAC ATG CAG CTC CCG   540
```

```
166   Arg Phe Gly Asp Asp Gly Glu Asn Leu End His Met Gln Leu Pro   180

541   GAG ACG GTC ACA GCT TGT CTG TAA GCG GAT GCC GGG AGC AGA CAA   585
181   Glu Thr Val Thr Ala Cys Leu End Ala Asp Ala Gly Ser Arg Gln   195

586   GCC CGT CAG GGC GCG TCA GCG GGT GTT GGC GGG TGT CGG GGC TGG   630
196   Ala Arg Gln Gly Ala Ser Ala Gly Val Gly Gly Cys Arg Gly Trp   210

631   CTT AAC TAT GCG GCA TCA GAG CAG ATT GTA CTG AGA GTG CAC CAT   675
211   Leu Asn Tyr Ala Ala Ser Glu Gln Ile Val Leu Arg Val His His   225

676   ATG CGG TGT GAA ATA CCG CAC AGA TGC GTA AGG AGA AAA TAC CGC   720
226   Met Arg Cys Glu Ile Pro His Arg Cys Val Arg Arg Lys Tyr Arg   240

721   ATC AGG CGG CCT TAA GGG CCT CGT GAT ACG CCT ATT TTT ATA GGT   765
241   Ile Arg Arg Pro End Gly Pro Arg Asp Thr Pro Ile Phe Ile Gly   255

766   TAA TGT CAT GAT AAT AAT GGT TTC TTA GAC GTC AGG TGG CAC TTT   810
256   End Cys His Asp Asn Asn Gly Phe Leu Asp Val Arg Trp His Phe   270

811   TCG GGG AAA TGT GCG CGG AAC CCC TAT TTG TTT ATT TTT CTA AAT   855
271   Ser Gly Lys Cys Ala Arg Asn Pro Tyr Leu Phe Ile Phe Leu Asn   285

856   ACA TTC AAA TAT GTA TCC GCT CAT GAG ACA ATA ACC CTG ATA AAT   900
286   Thr Phe Lys Tyr Val Ser Ala His Glu Thr Ile Thr Leu Ile Asn   300

901   GCT TCA ATA ATA TTG AAA AAG GAA GAG TAT GAG TAT TCA ACA TTT   945
301   Ala Ser Ile Ile Leu Lys Lys Glu Glu Tyr Glu Tyr Ser Thr Phe   315

946   CCG TGT CGC CCT TAT TCC CTT TTT TGC GGC ATT TTG CCT TCC TGT   990
316   Pro Cys Arg Pro Tyr Ser Leu Phe Cys Gly Ile Leu Pro Ser Cys   330

991   TTT TGC TCA CCC AGA AAC GCT GGT GAA AGT AAA AGA TGC TGA AGA   1035
331   Phe Cys Ser Pro Arg Asn Ala Gly Glu Ser Lys Arg Cys End Arg   345

1036  TCA GTT GGG TGC ACG AGT GGG TTA CAT CGA ACT GGA TCT CAA CAG   1080
346   Ser Val Gly Cys Thr Ser Gly Leu His Arg Thr Gly Ser Gln Gln   360

1081  CGG TAA GAT CCT TGA GAG TTT TCG CCC CGA AGA ACG TTT TCC AAT   1125
361   Arg End Asp Pro End Glu Phe Ser Pro Arg Arg Thr Phe Ser Asn   375

1126  GAT GAG CAC TTT TAA AGT TCT GCT ATG TGG CGC GGT ATT ATC CCG   1170
376   Asp Glu His Phe End Ser Ser Ala Met Trp Arg Gly Ile Ile Pro   390

1171  TAT TGA CGC CGG GCA AGA GCA ACT CGG TCG CCG CAT ACA CTA TTC   1215
391   Tyr End Arg Arg Ala Arg Ala Thr Arg Ser Pro His Thr Leu Phe   405

1216  TCA GAA TGA CTT GGT TGA GTA CTC ACC AGT CAC AGA AAA GCA TCT   1260
406   Ser Glu End Leu Gly End Val Leu Thr Ser His Arg Lys Ala Ser   420

1261  TAC GGA TGG CAT GAC AGT AAG AGA ATT ATG CAG TGC TGC CAT AAC   1305
421   Tyr Gly Trp His Asp Ser Lys Arg Ile Met Gln Cys Cys His Asn   435

1306  CAT GAG TGA TAA CAC TGC GGC CAA CTT ACT TCT GAC AAC GAT CGG   1350
436   His Glu End End His Cys Gly Gln Leu Thr Ser Asp Asn Asp Arg   450

1351  AGG ACC GAA GGA GCT AAC CGC TTT TTT GCA CAA CAT GGG GGA TCA   1395
451   Arg Thr Glu Gly Ala Asn Arg Phe Phe Ala Gln His Gly Gly Ser   465

1396  TGT AAC TCG CCT TGA TCG TTG GGA ACC GGA GCT GAA TGA AGC CAT   1440
466   Cys Asn Ser Pro End Ser Leu Gly Thr Gly Ala Glu End Ser His   480

1441  ACC AAA CGA CGA GCG TGA CAC CAC GAT GCC TGT AGC AAT GGC AAC   1485
```

```
481   Thr Lys Arg Arg Ala End His His Asp Ala Cys Ser Asn Gly Asn   495

1486  AAC GTT GCG CAA ACT ATT AAC TGG CGA ACT ACT TAC TCT AGC TTC   1530
496   Asn Val Ala Gln Thr Ile Asn Trp Arg Thr Thr Tyr Ser Ser Phe   510

1531  CCG GCA ACA ATT AAT AGA CTG GAT GGA GGC GGA TAA AGT TGC AGG   1575
511   Pro Ala Thr Ile Asn Arg Leu Asp Gly Gly Gly End Ser Cys Arg   525

1576  ACC ACT TCT GCG CTC GGC CCT TCC GGC TGG CTG GTT TAT TGC TGA   1620
526   Thr Thr Ser Ala Leu Gly Pro Ser Gly Trp Leu Val Tyr Cys End   540

1621  TAA ATC TGG AGC CGG TGA GCG TGG GTC TCG CGG TAT CAT TGC AGC   1665
541   End Ile Trp Ser Arg End Ala Trp Val Ser Arg Tyr His Cys Ser   555

1666  ACT GGG GCC AGA TGG TAA GCC CTC CCG TAT CGT AGT TAT CTA CAC   1710
556   Thr Gly Ala Arg Trp End Ala Leu Pro Tyr Arg Ser Tyr Leu His   570

1711  GAC GGG GAG TCA GGC AAC TAT GGA TGA ACG AAA TAG ACA GAT CGC   1755
571   Asp Gly Glu Ser Gly Asn Tyr Gly End Thr Lys End Thr Asp Arg   585

1756  TGA GAT AGG TGC CTC ACT GAT TAA GCA TTG GTA ACT GTC AGA CCA   1800
586   End Asp Arg Cys Leu Thr Asp End Ala Leu Val Thr Val Arg Pro   600

1801  AGT TTA CTC ATA TAT ACT TTA GAT TGA TTT AAA ACT TCA TTT TTA   1845
601   Ser Leu Leu Ile Tyr Thr Leu Asp End Phe Lys Thr Ser Phe Leu   615

1846  ATT TAA AAG GAT CTA GGT GAA GAT CCT TTT TGA TAA TCT CAT GAC   1890
616   Ile End Lys Asp Leu Gly Glu Asp Pro Phe End End Ser His Asp   630

1891  CAA AAT CCC TTA ACG TGA GTT TTC GTT CCA CTG AGC GTC AGA CCC   1935
631   Gln Asn Pro Leu Thr End Val Phe Val Pro Leu Ser Val Arg Pro   645

1936  CGT AGA AAA GAT CAA AGG ATC TTC TTG AGA TCC TTT TTT TCT GCG   1980
646   Arg Arg Lys Asp Gln Arg Ile Phe Leu Arg Ser Phe Phe Ser Ala   660

1981  CGT AAT CTG CTG CTT GCA AAC AAA AAA ACC ACC GCT ACC AGC GGT   2025
661   Arg Asn Leu Leu Leu Ala Asn Lys Lys Thr Thr Ala Thr Ser Gly   675

2026  GGT TTG TTT GCC GGA TCA AGA GCT ACC AAC TCT TTT TCC GAA GGT   2070
676   Gly Leu Phe Ala Gly Ser Arg Ala Thr Asn Ser Phe Ser Glu Gly   690

2071  AAC TGG CTT CAG CAG AGC GCA GAT ACC AAA TAC TGT CCT TCT AGT   2115
691   Asn Trp Leu Gln Gln Ser Ala Asp Thr Lys Tyr Cys Pro Ser Ser   705

2116  GTA GCC GTA GTT AGG CCA CCA CTT CAA GAA CTC TGT AGC ACC GCC   2160
706   Val Ala Val Val Arg Pro Pro Leu Gln Glu Leu Cys Ser Thr Ala   720

2161  TAC ATA CCT CGC TCT GCT AAT CCT GTT ACC AGT GGC TGC TGC CAG   2205
721   Tyr Ile Pro Arg Ser Ala Asn Pro Val Thr Ser Gly Cys Cys Gln   735

2206  TGG CGA TAA GTC GTG TCT TAC CGG GTT GGA CTC AAG ACG ATA GTT   2250
736   Trp Arg End Val Val Ser Tyr Arg Val Gly Leu Lys Thr Ile Val   750

2251  ACC GGA TAA GGC GCA GCG GTC GGG CTG AAC GGG GGG TTC GTG CAC   2295
751   Thr Gly End Gly Ala Ala Val Gly Leu Asn Gly Gly Phe Val His   765

2296  ACA GCC CAG CTT GGA GCG AAC GAC CTA CAC CGA ACT GAG ATA CCT   2340
766   Thr Ala Gln Leu Gly Ala Asn Asp Leu His Arg Thr Glu Ile Pro   780

2341  ACA GCG TGA GCT ATG AGA AAG CGC CAC GCT TCC CGA AGG GAG AAA   2385
781   Thr Ala End Ala Met Arg Lys Arg His Ala Ser Arg Arg Glu Lys   795

2386  GGC GGA CAG GTA TCC GGT AAG CGG CAG GGT CGG AAC AGG AGA GCG   2430
```

```
796   Gly Gly Gln Val Ser Gly Lys Arg Gln Gly Arg Asn Arg Arg Ala   810

2431  CAC GAG GGA GCT TCC AGG GGG AAA CGC CTG GTA TCT TTA TAG TCC   2475
811   His Glu Gly Ala Ser Arg Gly Lys Arg Leu Val Ser Leu End Ser   825

2476  TGT CGG GTT TCG CCA CCT CTG ACT TGA GCG TCG ATT TTT GTG ATG   2520
826   Cys Arg Val Ser Pro Pro Leu Thr End Ala Ser Ile Phe Val Met   840

2521  CTC GTC AGG GGG GCG GAG CCT ATG GAA AAA CGC CAG CAA CGC GGC   2565
841   Leu Val Arg Gly Ala Glu Pro Met Glu Lys Arg Gln Gln Arg Gly   855

2566  CTT TTT ACG GTT CCT GGC CTT TTG CTG GCC TTT TGC TCA CAT GTT   2610
856   Leu Phe Thr Val Pro Gly Leu Leu Leu Ala Phe Cys Ser His Val   870

2611  CTT TCC TGC GTT ATC CCC TGA TTC TGT GGA TAA CCG TAT TAC CGC   2655
871   Leu Ser Cys Val Ile Pro End Phe Cys Gly End Pro Tyr Tyr Arg   885

2656  CTT TGA GTG AGC TGA TAC CGC TCG CCG CAG CCG AAC GAC CGA GCG   2700
886   Leu End Val Ser End Tyr Arg Ser Pro Gln Pro Asn Asp Arg Ala   900

2701  CAG CGA GTC AGT GAG CGA GGA AGC GGA   2727
901   Gln Arg Val Ser Glu Arg Gly Ser Gly
```

A.1.2. pGEX-4T-3-LTCC

pGEX-4T-3

```
3     GTT ATC GAC TGC ACG GTG CAC CAA TGC TTC TGG CGT CAG GCA GCC   47
0     Val Ile Asp Cys Thr Val His Gln Cys Phe Trp Arg Gln Ala Ala   14

48    ATC GGA AGC TGT GGT ATG GCT GTG CAG GTC GTA AAT CAC TGC ATA   92
15    Ile Gly Ser Cys Gly Met Ala Val Gln Val Val Asn His Cys Ile   29

93    ATT CGT GTC GCT CAA GGC GCA CTC CCG TTC TGG ATA ATG TTT TTT   137
30    Ile Arg Val Ala Gln Gly Ala Leu Pro Phe Trp Ile Met Phe Phe   44

138   GCG CCG ACA TCA TAA CGG TTC TGG CAA ATA TTC TGA AAT GAG CTG   182
45    Ala Pro Thr Ser End Arg Phe Trp Gln Ile Phe End Asn Glu Leu   59

183   TTG ACA ATT AAT CAT CGG CTC GTA TAA TGT GTG GAA TTG TGA GCG   227
60    Leu Thr Ile Asn His Arg Leu Val End Cys Val Glu Leu End Ala   74
```

Startcodon GST

```
228   GAT AAC AAT TTC ACA CAG GAA ACA GTA TTC ATG TCC CCT ATA CTA   272
75    Asp Asn Asn Phe Thr Gln Glu Thr Val Phe Met Ser Pro Ile Leu   89

273   GGT TAT TGG AAA ATT AAG GGC CTT GTG CAA CCC ACT CGA CTT CTT   317
90    Gly Tyr Trp Lys Ile Lys Gly Leu Val Gln Pro Thr Arg Leu Leu   104

318   TTG GAA TAT CTT GAA GAA AAA TAT GAA GAG CAT TTG TAT GAG CGC   362
105   Leu Glu Tyr Leu Glu Glu Lys Tyr Glu Glu His Leu Tyr Glu Arg   119

363   GAT GAA GGT GAT AAA TGG CGA AAC AAA AAG TTT GAA TTG GGT TTG   407
120   Asp Glu Gly Asp Lys Trp Arg Asn Lys Lys Phe Glu Leu Gly Leu   134

408   GAG TTT CCC AAT CTT CCT TAT TAT ATT GAT GGT GAT GTT AAA TTA   452
135   Glu Phe Pro Asn Leu Pro Tyr Tyr Ile Asp Gly Asp Val Lys Leu   149

453   ACA CAG TCT ATG GCC ATC ATA CGT TAT ATA GCT GAC AAG CAC AAC   497
150   Thr Gln Ser Met Ala Ile Ile Arg Tyr Ile Ala Asp Lys His Asn   164
```

```
498   ATG TTG GGT GGT TGT CCA AAA GAG CGT GCA GAG ATT TCA ATG CTT   542
165   Met Leu Gly Gly Cys Pro Lys Glu Arg Ala Glu Ile Ser Met Leu   179

543   GAA GGA GCG GTT TTG GAT ATT AGA TAC GGT GTT TCG AGA ATT GCA   587
180   Glu Gly Ala Val Leu Asp Ile Arg Tyr Gly Val Ser Arg Ile Ala   194

588   TAT AGT AAA GAC TTT GAA ACT CTC AAA GTT GAT TTT CTT AGC AAG   632
195   Tyr Ser Lys Asp Phe Glu Thr Leu Lys Val Asp Phe Leu Ser Lys   209

633   CTA CCT GAA ATG CTG AAA ATG TTC GAA GAT CGT TTA TGT CAT AAA   677
210   Leu Pro Glu Met Leu Lys Met Phe Glu Asp Arg Leu Cys His Lys   224

678   ACA TAT TTA AAT GGT GAT CAT GTA ACC CAT CCT GAC TTC ATG TTG   722
225   Thr Tyr Leu Asn Gly Asp His Val Thr His Pro Asp Phe Met Leu   239

723   TAT GAC GCT CTT GAT GTT GTT TTA TAC ATG GAC CCA ATG TGC CTG   767
240   Tyr Asp Ala Leu Asp Val Val Leu Tyr Met Asp Pro Met Cys Leu   254

768   GAT GCG TTC CCA AAA TTA GTT TGT TTT AAA AAA CGT ATT GAA GCT   812
255   Asp Ala Phe Pro Lys Leu Val Cys Phe Lys Lys Arg Ile Glu Ala   269

813   ATC CCA CAA ATT GAT AAG TAC TTG AAA TCC AGC AAG TAT ATA GCA   857
270   Ile Pro Gln Ile Asp Lys Tyr Leu Lys Ser Ser Lys Tyr Ile Ala   284

858   TGG CCT TTG CAG GGC TGG CAA GCC ACG TTT GGT GGT GGC GAC CAT   902
285   Trp Pro Leu Gln Gly Trp Gln Ala Thr Phe Gly Gly Gly Asp His   299
```

Start Multiple Cloning Side MCS

LTCC:GACAGCAGGTCCTATGTCAGCAACCTGTAG (sequence of C-terminal tail og $Ca_v1.2$)

(EcoRI and SalI)

the green arrow indicate the position of the insert

IN FRAME

```
903   CCT CCA AAA TCG GAT CTG GTT CCG CGT GGA TCC CCG AAT TCC CGG   947
300   Pro Pro Lys Ser Asp Leu Val Pro Arg Gly Ser Pro Asn Ser Arg   314
```

End MCS

```
948   GTC GAC TCG AGC GGC CGC ATC GTG ACT GAC TGA CGA TCT GCC TCG   992
315   Val Asp Ser Ser Gly Arg Ile Val Thr Asp End Arg Ser Ala Ser   329

993   CGC GTT TCG GTG ATG ACG GTG AAA ACC TCT GAC ACA TGC AGC TCC   1037
330   Arg Val Ser Val Met Thr Val Lys Thr Ser Asp Thr Cys Ser Ser   344

1038  CGG AGA CGG TCA CAG CTT GTC TGT AAG CGG ATG CCG GGA GCA GAC   1082
345   Arg Arg Arg Ser Gln Leu Val Cys Lys Arg Met Pro Gly Ala Asp   359

1083  AAG CCC GTC AGG GCG CGT CAG CGG GTG TTG GCG GGT GTC GGG GCG   1127
360   Lys Pro Val Arg Ala Arg Gln Arg Val Leu Ala Gly Val Gly Ala   374

1128  CAG CCA TGA CCC AGT CAC GTA GCG ATA GCG GAG TGT ATA ATT CTT   1172
375   Gln Pro End Pro Ser His Val Ala Ile Ala Glu Cys Ile Ile Leu   389

1173  GAA GAC GAA AGG GCC TCG TGA TAC GCC TAT TTT TAT AGG TTA ATG   1217
390   Glu Asp Glu Arg Ala Ser End Tyr Ala Tyr Phe Tyr Arg Leu Met   404

1218  TCA TGA TAA TAA TGG TTT CTT AGA CGT CAG GTG GCA CTT TTC GGG   1262
405   Ser End End End Trp Phe Leu Arg Arg Gln Val Ala Leu Phe Gly   419
```

```
1263  GAA ATG TGC GCG GAA CCC CTA TTT GTT TAT TTT TCT AAA TAC ATT   1307
420   Glu Met Cys Ala Glu Pro Leu Phe Val Tyr Phe Ser Lys Tyr Ile   434

1308  CAA ATA TGT ATC CGC TCA TGA GAC AAT AAC CCT GAT AAA TGC TTC   1352
435   Gln Ile Cys Ile Arg Ser End Asp Asn Asn Pro Asp Lys Cys Phe   449

1353  AAT AAT ATT GAA AAA GGA AGA GTA TGA GTA TTC AAC ATT TCC GTG   1397
450   Asn Asn Ile Glu Lys Gly Arg Val End Val Phe Asn Ile Ser Val   464

1398  TCG CCC TTA TTC CCT TTT TTG CGG CAT TTT GCC TTC CTG TTT TTG   1442
465   Ser Pro Leu Phe Pro Phe Leu Arg His Phe Ala Phe Leu Phe Leu   479

1443  CTC ACC CAG AAA CGC TGG TGA AAG TAA AAG ATG CTG AAG ATC AGT   1487
480   Leu Thr Gln Lys Arg Trp End Lys End Lys Met Leu Lys Ile Ser   494

1488  TGG GTG CAC GAG TGG GTT ACA TCG AAC TGG ATC TCA ACA GCG GTA   1532
495   Trp Val His Glu Trp Val Thr Ser Asn Trp Ile Ser Thr Ala Val   509

1533  AGA TCC TTG AGA GTT TTC GCC CCG AAG AAC GTT TTC CAA TGA TGA   1577
510   Arg Ser Leu Arg Val Phe Ala Pro Lys Asn Val Phe Gln End End   524

1578  GCA CTT TTA AAG TTC TGC TAT GTG GCG CGG TAT TAT CCC GTG TTG   1622
525   Ala Leu Leu Lys Phe Cys Tyr Val Ala Arg Tyr Tyr Pro Val Leu   539

1623  ACG CCG GGC AAG AGC AAC TCG GTC GCC GCA TAC ACT ATT CTC AGA   1667
540   Thr Pro Gly Lys Ser Asn Ser Val Ala Ala Tyr Thr Ile Leu Arg   554

1668  ATG ACT TGG TTG AGT ACT CAC CAG TCA CAG AAA AGC ATC TTA CGG   1712
555   Met Thr Trp Leu Ser Thr His Gln Ser Gln Lys Ser Ile Leu Arg   569

1713  ATG GCA TGA CAG TAA GAG AAT TAT GCA GTG CTG CCA TAA CCA TGA   1757
570   Met Ala End Gln End Glu Asn Tyr Ala Val Leu Pro End Pro End   584

1758  GTG ATA ACA CTG CGG CCA ACT TAC TTC TGA CAA CGA TCG GAG GAC   1802
585   Val Ile Thr Leu Arg Pro Thr Tyr Phe End Gln Arg Ser Glu Asp   599

1803  CGA AGG AGC TAA CCG CTT TTT TGC ACA ACA TGG GGG ATC ATG TAA   1847
600   Arg Arg Ser End Pro Leu Phe Cys Thr Thr Trp Gly Ile Met End   614

1848  CTC GCC TTG ATC GTT GGG AAC CGG AGC TGA ATG AAG CCA TAC CAA   1892
615   Leu Ala Leu Ile Val Gly Asn Arg Ser End Met Lys Pro Tyr Gln   629

1893  ACG ACG AGC GTG ACA CCA CGA TGC CTG CAG CAA TGG CAA CAA CGT   1937
630   Thr Thr Ser Val Thr Pro Arg Cys Leu Gln Gln Trp Gln Gln Arg   644

1938  TGC GCA AAC TAT TAA CTG GCG AAC TAC TTA CTC TAG CTT CCC GGC   1982
645   Cys Ala Asn Tyr End Leu Ala Asn Tyr Leu Leu End Leu Pro Gly   659

1983  AAC AAT TAA TAG ACT GGA TGG AGG CGG ATA AAG TTG CAG GAC CAC   2027
660   Asn Asn End End Thr Gly Trp Arg Arg Ile Lys Leu Gln Asp His   674

2028  TTC TGC GCT CGG CCC TTC CGG CTG GCT GGT TTA TTG CTG ATA AAT   2072
675   Phe Cys Ala Arg Pro Phe Arg Leu Ala Gly Leu Leu Leu Ile Asn   689

2073  CTG GAG CCG GTG AGC GTG GGT CTC GCG GTA TCA TTG CAG CAC TGG   2117
690   Leu Glu Pro Val Ser Val Gly Leu Ala Val Ser Leu Gln His Trp   704

2118  GGC CAG ATG GTA AGC CCT CCC GTA TCG TAG TTA TCT ACA CGA CGG   2162
705   Gly Gln Met Val Ser Pro Pro Val Ser End Leu Ser Thr Arg Arg   719

2163  GGA GTC AGG CAA CTA TGG ATG AAC GAA ATA GAC AGA TCG CTG AGA   2207
720   Gly Val Arg Gln Leu Trp Met Asn Glu Ile Asp Arg Ser Leu Arg   734
```

```
2208  TAG GTG CCT CAC TGA TTA AGC ATT GGT AAC TGT CAG ACC AAG TTT   2252
735   End Val Pro His End Leu Ser Ile Gly Asn Cys Gln Thr Lys Phe   749

2253  ACT CAT ATA TAC TTT AGA TTG ATT TAA AAC TTC ATT TTT AAT TTA   2297
750   Thr His Ile Tyr Phe Arg Leu Ile End Asn Phe Ile Phe Asn Leu   764

2298  AAA GGA TCT AGG TGA AGA TCC TTT TTG ATA ATC TCA TGA CCA AAA   2342
765   Lys Gly Ser Arg End Arg Ser Phe Leu Ile Ile Ser End Pro Lys   779

2343  TCC CTT AAC GTG AGT TTT CGT TCC ACT GAG CGT CAG ACC CCG TAG   2387
780   Ser Leu Asn Val Ser Phe Arg Ser Thr Glu Arg Gln Thr Pro End   794

2388  AAA AGA TCA AAG GAT CTT CTT GAG ATC CTT TTT TTC TGC GCG TAA   2432
795   Lys Arg Ser Lys Asp Leu Leu Glu Ile Leu Phe Phe Cys Ala End   809

2433  TCT GCT GCT TGC AAA CAA AAA AAC CAC CGC TAC CAG CGG TGG TTT   2477
810   Ser Ala Ala Cys Lys Gln Lys Asn His Arg Tyr Gln Arg Trp Phe   824

2478  GTT TGC CGG ATC AAG AGC TAC CAA CTC TTT TTC CGA AGG TAA CTG   2522
825   Val Cys Arg Ile Lys Ser Tyr Gln Leu Phe Phe Arg Arg End Leu   839

2523  GCT TCA GCA GAG CGC AGA TAC CAA ATA CTG TCC TTC TAG TGT AGC   2567
840   Ala Ser Ala Glu Arg Arg Tyr Gln Ile Leu Ser Phe End Cys Ser   854

2568  CGT AGT TAG GCC ACC ACT TCA AGA ACT CTG TAG CAC CGC CTA CAT   2612

855   Arg Ser End Ala Thr Thr Ser Arg Thr Leu End His Arg Leu His   869

2613  ACC TCG CTC TGC TAA TCC TGT TAC CAG TGG CTG CTG CCA GTG GCG   2657
870   Thr Ser Leu Cys End Ser Cys Tyr Gln Trp Leu Leu Pro Val Ala   884

2658  ATA AGT CGT GTC TTA CCG GGT TGG ACT CAA GAC GAT AGT TAC CGG   2702
885   Ile Ser Arg Val Leu Pro Gly Trp Thr Gln Asp Asp Ser Tyr Arg   899

2703  ATA AGG CGC AGC GGT CGG GCT GAA CGG GGG GTT CGT GCA CAC AGC   2747
900   Ile Arg Arg Ser Gly Arg Ala Glu Arg Gly Val Arg Ala His Ser   914

2748  CCA GCT TGG AGC GAA CGA CCT ACA CCG AAC TGA GAT ACC TAC AGC   2792
915   Pro Ala Trp Ser Glu Arg Pro Thr Pro Asn End Asp Thr Tyr Ser   929

2793  GTG AGC TAT GAG AAA GCG CCA CGC TTC CCG AAG GGA GAA AGG CGG   2837
930   Val Ser Tyr Glu Lys Ala Pro Arg Phe Pro Lys Gly Glu Arg Arg   944

2838  ACA GGT ATC CGG TAA GCG GCA GGG TCG GAA CAG GAG AGC GCA CGA   2882
945   Thr Gly Ile Arg End Ala Ala Gly Ser Glu Gln Glu Ser Ala Arg   959

2883  GGG AGC TTC CAG GGG GAA ACG CCT GGT ATC TTT ATA GTC CTG TCG   2927
960   Gly Ser Phe Gln Gly Glu Thr Pro Gly Ile Phe Ile Val Leu Ser   974

2928  GGT TTC GCC ACC TCT GAC TTG AGC GTC GAT TTT TGT GAT GCT CGT   2972
975   Gly Phe Ala Thr Ser Asp Leu Ser Val Asp Phe Cys Asp Ala Arg   989

2973  CAG GGG GGC GGA GCC TAT GGA AAA ACG CCA GCA ACG CGG CCT TTT   3017
990   Gln Gly Gly Gly Ala Tyr Gly Lys Thr Pro Ala Thr Arg Pro Phe   1004

3018  TAC GGT TCC TGG CCT TTT GCT GGC CTT TTG CTC ACA TGT TCT TTC   3062
1005  Tyr Gly Ser Trp Pro Phe Ala Gly Leu Leu Leu Thr Cys Ser Phe   1019

3063  CTG CGT TAT CCC CTG ATT CTG TGG ATA ACC GTA TTA CCG CCT TTG   3107
1020  Leu Arg Tyr Pro Leu Ile Leu Trp Ile Thr Val Leu Pro Pro Leu   1034

3108  AGT GAG CTG ATA CCG CTC GCC GCA GCC GAA CGA CCG AGC GCA GCG   3152
1035  Ser Glu Leu Ile Pro Leu Ala Ala Ala Glu Arg Pro Ser Ala Ala   1049
```

```
3153  AGT CAG TGA GCG AGG AAG CGG AAG AGC GCC TGA TGC GGT ATT TTC   3197
1050  Ser Gln End Ala Arg Lys Arg Lys Ser Ala End Cys Gly Ile Phe   1064

3198  TCC TTA CGC ATC TGT GCG GTA TTT CAC ACC GCA TAA ATT CCG ACA   3242
1065  Ser Leu Arg Ile Cys Ala Val Phe His Thr Ala End Ile Pro Thr   1079

3243  CCA TCG AAT GGT GCA AAA CCT TTC GCG GTA TGG CAT GAT AGC GCC   3287
1080  Pro Ser Asn Gly Ala Lys Pro Phe Ala Val Trp His Asp Ser Ala   1094

3288  CGG AAG AGA GTC AAT TCA GGG TGG TGA ATG TGA AAC CAG TAA CGT   3332
1095  Arg Lys Arg Val Asn Ser Gly Trp End Met End Asn Gln End Arg   1109

3333  TAT ACG ATG TCG CAG AGT ATG CCG GTG TCT CTT ATC AGA CCG TTT   3377
1110  Tyr Thr Met Ser Gln Ser Met Pro Val Ser Leu Ile Arg Pro Phe   1124

3378  CCC GCG TGG TGA ACC AGG CCA GCC ACG TTT CTG CGA AAA CGC GGG   3422
1125  Pro Ala Trp End Thr Arg Pro Ala Thr Phe Leu Arg Lys Arg Gly   1139

3423  AAA AAG TGG AAG CGG CGA TGG CGG AGC TGA ATT ACA TTC CCA ACC   3467
1140  Lys Lys Trp Lys Arg Arg Trp Arg Ser End Ile Thr Phe Pro Thr   1154

3468  GCG TGG CAC AAC AAC TGG CGG GCA AAC AGT CGT TGC TGA TTG GCG   3512
1155  Ala Trp His Asn Asn Trp Arg Ala Asn Ser Arg Cys End Leu Ala   1169

3513  TTG CCA CCT CCA GTC TGG CCC TGC ACG CGC CGT CGC AAA TTG TCG   3557
1170  Leu Pro Pro Pro Val Trp Pro Cys Thr Arg Arg Arg Lys Leu Ser   1184

3558  CGG CGA TTA AAT CTC GCG CCG ATC AAC TGG GTG CCA GCG TGG TGG   3602
1185  Arg Arg Leu Asn Leu Ala Pro Ile Asn Trp Val Pro Ala Trp Trp   1199

3603  TGT CGA TGG TAG AAC GAA GCG GCG TCG AAG CCT GTA AAG CGG CGG   3647
1200  Cys Arg Trp End Asn Glu Ala Ala Ser Lys Pro Val Lys Arg Arg   1214

3648  TGC ACA ATC TTC TCG CGC AAC GCG TCA GTG GGC TGA TCA TTA ACT   3692
1215  Cys Thr Ile Phe Ser Arg Asn Ala Ser Val Gly End Ser Leu Thr   1229

3693  ATC CGC TGG ATG ACC AGG ATG CCA TTG CTG TGG AAG CTG CCT GCA   3737
1230  Ile Arg Trp Met Thr Arg Met Pro Leu Leu Trp Lys Leu Pro Ala   1244

3738  CTA ATG TTC CGG CGT TAT TTC TTG ATG TCT CTG ACC AGA CAC CCA   3782
1245  Leu Met Phe Arg Arg Tyr Phe Leu Met Ser Leu Thr Arg His Pro   1259

3783  TCA ACA GTA TTA TTT TCT CCC ATG AAG ACG GTA CGC GAC TGG GCG   3827
1260  Ser Thr Val Leu Phe Ser Pro Met Lys Thr Val Arg Asp Trp Ala   1274

3828  TGG AGC ATC TGG TCG CAT TGG GTC ACC AGC AAA TCG CGC TGT TAG   3872
1275  Trp Ser Ile Trp Ser His Trp Val Thr Ser Lys Ser Arg Cys End   1289

3873  CGG GCC CAT TAA GTT CTG TCT CGG CGC GTC TGC GTC TGG CTG GCT   3917
1290  Arg Ala His End Val Leu Ser Arg Arg Val Cys Val Trp Leu Ala   1304

3918  GGC ATA AAT ATC TCA CTC GCA ATC AAA TTC AGC CGA TAG CGG AAC   3962
1305  Gly Ile Asn Ile Ser Leu Ala Ile Lys Phe Ser Arg End Arg Asn   1319

3963  GGG AAG GCG ACT GGA GTG CCA TGT CCG GTT TTC AAC AAA CCA TGC   4007
1320  Gly Lys Ala Thr Gly Val Pro Cys Pro Val Phe Asn Lys Pro Cys   1334

4008  AAA TGC TGA ATG AGG GCA TCG TTC CCA CTG CGA TGC TGG TTG CCA   4052
1335  Lys Cys End Met Arg Ala Ser Phe Pro Leu Arg Cys Trp Leu Pro   1349

4053  ACG ATC AGA TGG CGC TGG GCG CAA TGC GCG CCA TTA CCG AGT CCG   4097
1350  Thr Ile Arg Trp Arg Trp Ala Gln Cys Ala Pro Leu Pro Ser Pro   1364
```

```
4098  GGC TGC GCG TTG GTG CGG ATA TCT CGG TAG TGG GAT ACG ACG ATA   4142
1365  Gly Cys Ala Leu Val Arg Ile Ser Arg End Trp Asp Thr Thr Ile   1379

4143  CCG AAG ACA GCT CAT GTT ATA TCC CGC CGT TAA CCA CCA TCA AAC   4187
1380  Pro Lys Thr Ala His Val Ile Ser Arg Arg End Pro Pro Ser Asn   1394

4188  AGG ATT TTC GCC TGC TGG GGC AAA CCA GCG TGG ACC GCT TGC TGC   4232
1395  Arg Ile Phe Ala Cys Trp Gly Lys Pro Ala Trp Thr Ala Cys Cys   1409

4233  AAC TCT CTC AGG GCC AGG CGG TGA AGG GCA ATC AGC TGT TGC CCG   4277
1410  Asn Ser Leu Arg Ala Arg Arg End Arg Ala Ile Ser Cys Cys Pro   1424

4278  TCT CAC TGG TGA AAA GAA AAA CCA CCC TGG CGC CCA ATA CGC AAA   4322
1425  Ser His Trp End Lys Glu Lys Pro Pro Trp Arg Pro Ile Arg Lys   1439

4323  CCG CCT CTC CCC GCG CGT TGG CCG ATT CAT TAA TGC AGC TGG CAC   4367
1440  Pro Pro Leu Pro Ala Arg Trp Pro Ile His End Cys Ser Trp His   1454

4368  GAC AGG TTT CCC GAC TGG AAA GCG GGC AGT GAG CGC AAC GCA ATT   4412
1455  Asp Arg Phe Pro Asp Trp Lys Ala Gly Ser Glu Arg Asn Ala Ile   1469

4413  AAT GTG AGT TAG CTC ACT CAT TAG GCA CCC CAG GCT TTA CAC TTT   4457
1470  Asn Val Ser End Leu Thr His End Ala Pro Gln Ala Leu His Phe   1484

4458  ATG CTT CCG GCT CGT ATG TTG TGT GGA ATT GTG AGC GGA TAA CAA   4502
1485  Met Leu Pro Ala Arg Met Leu Cys Gly Ile Val Ser Gly End Gln   1499

4503  TTT CAC ACA GGA AAC AGC TAT GAC CAT GAT TAC GGA TTC ACT GGC   4547
1500  Phe His Thr Gly Asn Ser Tyr Asp His Asp Tyr Gly Phe Thr Gly   1514

4548  CGT CGT TTT ACA ACG TCG TGA CTG GGA AAA CCC TGG CGT TAC CCA   4592
1515  Arg Arg Phe Thr Thr Ser End Leu Gly Lys Pro Trp Arg Tyr Pro   1529

4593  ACT TAA TCG CCT TGC AGC ACA TCC CCC TTT CGC CAG CTG GCG TAA   4637
1530  Thr End Ser Pro Cys Ser Thr Ser Pro Phe Arg Gln Leu Ala End   1544

4638  TAG CGA AGA GGC CCG CAC CGA TCG CCC TTC CCA ACA GTT GCG CAG   4682
1545  End Arg Arg Gly Pro His Arg Ser Pro Phe Pro Thr Val Ala Gln   1559

4683  CCT GAA TGG CGA ATG GCG CTT TGC CTG GTT TCC GGC ACC AGA AGC   4727
1560  Pro Glu Trp Arg Met Ala Leu Cys Leu Val Ser Gly Thr Arg Ser   1574

4728  GGT GCC GGA AAG CTG GCT GGA GTG CGA TCT TCC TGA GGC CGA TAC   4772
1575  Gly Ala Gly Lys Leu Ala Gly Val Arg Ser Ser End Gly Arg Tyr   1589

4773  TGT CGT CGT CCC CTC AAA CTG GCA GAT GCA CGG TTA CGA TGC GCC   4817
1590  Cys Arg Arg Pro Leu Lys Leu Ala Asp Ala Arg Leu Arg Cys Ala   1604

4818  CAT CTA CAC CAA CGT AAC CTA TCC CAT TAC GGT CAA TCC GCC GTT   4862
1605  His Leu His Gln Arg Asn Leu Ser His Tyr Gly Gln Ser Ala Val   1619

4863  TGT TCC CAC GGA GAA TCC GAC GGG TTG TTA CTC GCT CAC ATT TAA   4907
1620  Cys Ser His Gly Glu Ser Asp Gly Leu Leu Leu Ala His Ile End   1634

4908  TGT TGA TGA AAG CTG GCT ACA GGA AGG CCA GAC GCG AAT TAT TTT   4952
1635  Cys End End Lys Leu Ala Thr Gly Arg Pro Asp Ala Asn Tyr Phe   1649

4953  TGA TGG CGT TGG AAT   4967
1650  End Trp Arg Trp Asn   1654
```

A.1.3. pGEX-4T-3-Ct-Ca$_v$1.2 lang/kurz

Proteinsequence Ca$_v$1.2 alpha subunit rabbit

Swiss-Prot: P15381.1

RecName: Full=Voltage-dependent L-type calcium channel subunit alpha-1C; AltName: Full=Voltage-gated calcium channel subunit alpha Cav1.2; AltName: Full=Calcium channel, L type, alpha-1 polypeptide, isoform 1, cardiac muscle

LOCUS P15381 2171 aa linear MAM 02-MAR-2010
DEFINITION RecName: Full=Voltage-dependent L-type calcium channel subunit alpha-1C; AltName: Full=Voltage-gated calcium channel subunit alpha Cav1.2; AltName: Full=Calcium channel, L type, alpha-1 polypeptide, isoform 1, cardiac muscle; AltName: Full=Smooth muscle calcium channel blocker receptor; Short=CACB-receptor.
ACCESSION P15381

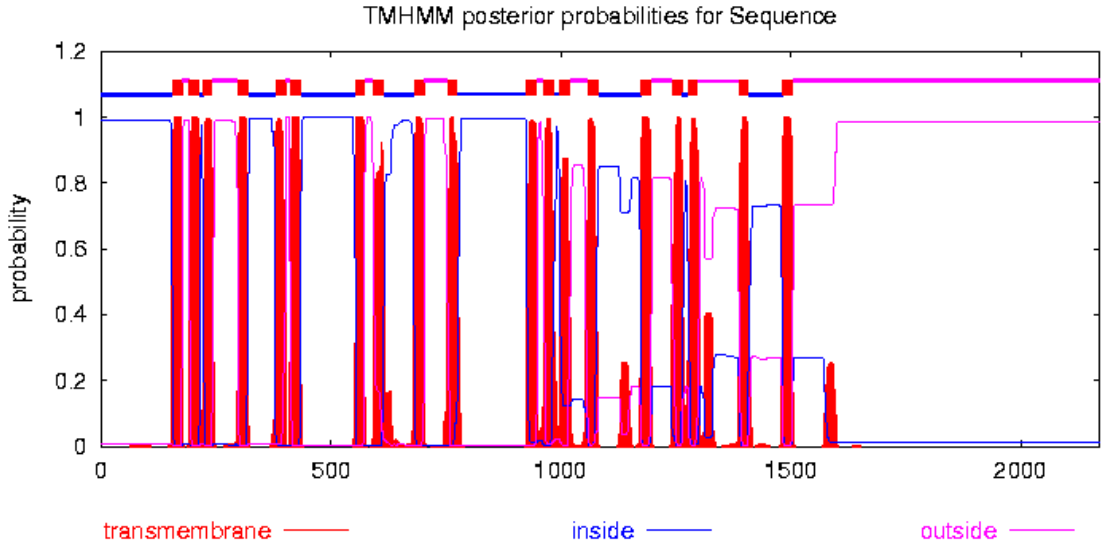
mlralvqpatpayqplpshlsaetestckgtvvheaqlnhfyispggsnygsprpahanm
nanaaaglapehiptpgaalswqaaidaarqaklmgsagnatistvsstqrkrqqygkpk
kqgsttatrpprallcltlknpirracisivewkpfeiiilltifancvalaiyipfped
dsnatnsnlerveylfliiftveaflkviaygllfhpnaylrngwnlldfiivvvglfsa
ileqatkadganalggkgagfdvkalrafrvlrplrlvsgvpslqvvlnsiikamvpllh
iallvlfviiiyaiiglelfmgkmhktcynqegvadvpaeddpspcaletghgrqcqngt
vckpgwdgpkhgitnfdnfafamltvfqcitmegwtdvlywmqdamgyelpwvyfvslvi
fgsffvlnlvlgvlsgefskerekakargdfqklrekqqleedlkgyldwitqaedidpe
nedegmdeekprnmsmptsetesvntenvaggdiegencgarlahriskskfsrywrrwn
rfcrrkcraavksnvfywlviflvflntltiasehynqphwltevqdtankallalftae
mllkmyslglqayfvslfnrfdcfivcggiletilvetkvmsplgisvlrcvrllrifki
trywnslsnlvasllnsvrsiasllllflfiiifsllgmqlfggkfnfdemqtrrstfd
nfpqslltvfqiltgedwnsvmydgimayggpsfpgmlvciyfiilficgnyillnvfla
iavdnladaesltsaqkeeeeekerkklartaspekkqevvgkpaleeakeekielksit
adgesppttkinmddlqpnesedkspypnpettgeedeeepempvgprprplselhlkek
avpmpeasaffifspnnrfrlqchrivndtiftnlilffillssislaaedpvqhtsfrn
hilfyfdivfttiftieialkmtaygaflhkgsfcrnyfnildllvvsvslisfgiqssa
invvkilrvlrvlrplrainrakglkhvvqcvfvairtignivivttllqfmfacigvql
fkgklytcsdsskqteaeckgnyitykdgevdhpiiqprswenskfdfdnvlaammalft
vstfegwpellyrsidshtedkgpiynyrveisiffiiyiiiiaffmmnifvgfvivtfq
eqgeqeyknceldknqrqcveyalkarplrryipknqhqykvwyvvnstyfeylmfvlil
lnticlamqhygqsclfkiamnilnmlftglftvemilkliafkpkgyfsdpwnvfdfli
vigsiidvilsetnpaehtqcspsmnaeensrisitffrlfrvmrlvkllsrgegirtll
wtfiksfqalpyvallivmlffiyavigmqvfgkialndtteinrnnnfqtfpqavlllf
rcatgeawqdimlacmpgkkcapesephnstegetpcgss**favfyfisfymlcafliinl**
fvavimdnfdyltrdwsilgphhldefkriwaeydpeakgrikhldvvtllrriqpplgf
gklcphrvackrlvsmnmplnsdgtvmfnatlfalvrtalriktegnleqaneelraiik
kiwkrtsmklldqvvppagddevtvgkfyatfliqeyfrkfkkrkeqglvgkpsqrnals
lqaglrtlhdigpeirraisgdltaeeeldkamkeavsaaseddifrragglfgnhvsyy
qsdsrsafpqtfttqrplhiskagnnqgdtespsheklvdstftpssysstgsnaninna
nntalgrlprpagypstvstveghgsplspavraqeaawklsskrchsqesqiamacqeg
asqddnydvrigedaeccsepsllstemlsyqddenrqlappeeekrdirlspkkgflrs
aslgrrasfhleclkrqknqggdisqktvlplhlvhhqalavaglspllqrshsptslpr
pcatppatpgsrgwppqpiptlrlegadsseklnssfpsihcgswsgenspcrgdssaar
rarpvsltvpsqagaqgrqfhgsasslveavliseglgqfaqdpkfievttqeladacdl
tieemenaaddilsggarqspngtllpfvnrrdpgrdragqneqdasgacapgcgqseea
ladrragvssl

part of protein sequence of C-terminal end Ca$_v$1.2 (transmembranhelix)

TMHMM result (bioinformatic tool server), prediction of transmembrane helices in proteins

```
# Sequence Length: 2171
# Sequence Number of predicted TMHs:  19
# Sequence Exp number of AAs in TMHs: 437.523389999999999999999999999
# Sequence Exp number, first 60 AAs:  0.00042
# Sequence Total prob of N-in:        0.99076
Sequence        TMHMM2.0        inside      1   154
Sequence        TMHMM2.0        TMhelix   155   177
Sequence        TMHMM2.0        outside   178   191
Sequence        TMHMM2.0        TMhelix   192   214
Sequence        TMHMM2.0        inside    215   222
Sequence        TMHMM2.0        TMhelix   223   242
Sequence        TMHMM2.0        outside   243   297
Sequence        TMHMM2.0        TMhelix   298   320
Sequence        TMHMM2.0        inside    321   379
Sequence        TMHMM2.0        TMhelix   380   402
Sequence        TMHMM2.0        outside   403   411
Sequence        TMHMM2.0        TMhelix   412   434
Sequence        TMHMM2.0        inside    435   553
Sequence        TMHMM2.0        TMhelix   554   573
Sequence        TMHMM2.0        outside   574   592
Sequence        TMHMM2.0        TMhelix   593   615
Sequence        TMHMM2.0        inside    616   680
Sequence        TMHMM2.0        TMhelix   681   703
Sequence        TMHMM2.0        outside   704   752
Sequence        TMHMM2.0        TMhelix   753   775
Sequence        TMHMM2.0        inside    776   925
Sequence        TMHMM2.0        TMhelix   926   948
Sequence        TMHMM2.0        outside   949   962
Sequence        TMHMM2.0        TMhelix   963   985
Sequence        TMHMM2.0        inside    986   997
Sequence        TMHMM2.0        TMhelix   998  1020
Sequence        TMHMM2.0        outside  1021  1058
Sequence        TMHMM2.0        TMhelix  1059  1081
Sequence        TMHMM2.0        inside   1082  1174
Sequence        TMHMM2.0        TMhelix  1175  1197
Sequence        TMHMM2.0        outside  1198  1241
Sequence        TMHMM2.0        TMhelix  1242  1264
Sequence        TMHMM2.0        inside   1265  1275
Sequence        TMHMM2.0        TMhelix  1276  1298
Sequence        TMHMM2.0        outside  1299  1388
Sequence        TMHMM2.0        TMhelix  1389  1408
Sequence        TMHMM2.0        inside   1409  1482
Sequence        TMHMM2.0        TMhelix  1483  1505
Sequence        TMHMM2.0        outside  1506  2171
```

protein sequence transmembranhelix of $Ca_v1.2$



Hmmtop (program for transmembrane topology prediction)

```
Length:  2171
N-terminus: OUT
Number of transmembrane helices: 17
Transmembrane helices: 158-177 196-215 224-242 298-317 413-436 549-572 625-
649 680-699 758-782 926-949 998-1021 1052-1076 1172-1196 1244-1268 1279-
1303 1390-1409 1481-1505
Total entropy of the model:  17.0257
Entropy of the best path:  17.0356

The best path:

     seq  MLRALVQPAT PAYQPLPSHL SAETESTCKG TVVHEAQLNH FYISPGGSNY    50
     pred OOOOOOOOOO OOOOOOOOOO OOOOOOOOOO OOOOOOOOOO OOOOOOOOOO

     seq  GSPRPAHANM NANAAAGLAP EHIPTPGAAL SWQAAIDAAR QAKLMGSAGN   100
     pred OOOOOOOOOO OOOOOOOOOO OOOOOOOOOO OOOOOOOOOO OOOOOOOOOO

     seq  ATISTVSSTQ RKRQQYGKPK KQGSTTATRP PRALLCLTLK NPIRRACISI   150
     pred OOOOOOOOOO OOOOOOOOOO OOOOOOOOOO OOOOOOOOOO OOoooooooo

     seq  VEWKPFEIII LLTIFANCVA LAIYIPFPED DSNATNSNLE RVEYLFLIIF   200
     pred ooooooHHH HHHHHHHHHH HHHHHHHiii iiiiiiiiii iiiiiHHHHH

     seq  TVEAFLKVIA YGLLFHPNAY LRNGWNLLDF IIVVVGLFSA ILEQATKADG   250
     pred HHHHHHHHHH HHHHHooooo oooHHHHHHH HHHHHHHHHH HHiiiiiiii

     seq  ANALGGKGAG FDVKALRAFR VLRPLRLVSG VPSLQVVLNS IIKAMVPLLH   300
     pred iiiiiiiIII IIIIIIIIII IIIIIIIIII IIiiiiiiii iiiiiiiHHH

     seq  IALLVLFVII IYAIIGLELF MGKMHKTCYN QEGVADVPAE DDPSPCALET   350
     pred HHHHHHHHHH HHHHHHHooo oooooooooo ooOOOOOOOO OOOOOOOOOO

     seq  GHGRQCQNGT VCKPGWDGPK HGITNFDNFA FAMLTVFQCI TMEGWTDVLY   400
     pred OOOOOOOOOO OOOOOOOOOO OOOOOOOOOO OOOOOOOOOO OOOOOOOooo

     seq  WMQDAMGYEL PWVYFVSLVI FGSFFVLNLV LGVLSGEFSK EREKAKARGD   450
     pred oooooooooo ooHHHHHHHH HHHHHHHHHH HHHHHHiiii iiiiiiiiii

     seq  FQKLREKQQL EEDLKGYLDW ITQAEDIDPE NEDEGMDEEK PRNMSMPTSE   500
     pred iIIIIIIIII IIIIIIIIII IIIIIIIIII IIIIIIIIII IIIIIIIIII

     seq  TESVNTENVA GGDIEGENCG ARLAHRISKS KFSRYWRRWN RFCRRKCRAA   550
     pred IIIIIIIIII IIIIIIIIII IIIIIIIIII IIIiiiiiii iiiiiiiiHH

     seq  VKSNVFYWLV IFLVFLNTLT IASEHYNQPH WLTEVQDTAN KALLALFTAE   600
     pred HHHHHHHHHH HHHHHHHHHH HHoooooooo oooooooOOO OOOOOOOOOO

     seq  MLLKMYSLGL QAYFVSLFNR FDCFIVCGGI LETILVETKV MSPLGISVLR   650
     pred OOOOOOOOOo oooooooooo ooooHHHHHH HHHHHHHHHH HHHHHHHHHi

     seq  CVRLLRIFKI TRYWNSLSNL VASLLNSVRS IASLLLLLFL FIIIFSLLGM   700
     pred iiiiiiiiii iiiiiiiiii iiiiiiiiiH HHHHHHHHHH HHHHHHHHHo

     seq  QLFGGKFNFD EMQTRRSTFD NFPQSLLTVF QILTGEDWNS VMYDGIMAYG   750
     pred oooooooooo ooooOOOOOO OOOOOOOOOO OOOOOOOOOO OOoooooooo

     seq  GPSFPGMLVC IYFIILFICG NYILLNVFLA IAVDNLADAE SLTSAQKEEE   800
     pred ooooooHHH HHHHHHHHHH HHHHHHHHHH HHiiiiiiii iiiiiiiIII

     seq  EEKERKKLAR TASPEKKQEV VGKPALEEAK EEKIELKSIT ADGESPPTTK   850
     pred IIIIIIIIII IIIIIIIIII IIIIIIIIII IIIIIIIIII IIIIIIIIII

     seq  INMDDLQPNE SEDKSPYPNP ETTGEEDEEE PEMPVGPRPR PLSELHLKEK   900
     pred IIIIIIIIII IIIIIIIIII IIIIIIIIII IIIIIIIIII IIIIIIIIII

     seq  AVPMPEASAF FIFSPNNRFR LQCHRIVNDT IFTNLILFFI LLSSISLAAE   950
     pred IIIIIIIIII iiiiiiiiii iiiiiHHHHH HHHHHHHHHH HHHHHHHHHo

     seq  DPVQHTSFRN HILFYFDIVF TTIFTIEIAL KMTAYGAFLH KGSFCRNYFN  1000
```

```
pred oooooooooo ooooOOOOOO OOOOOOOOOO OOoooooooo oooooooHHH

seq  ILDLLVVSVS LISFGIQSSA INVVKILRVL RVLRPLRAIN RAKGLKHVVQ  1050
pred HHHHHHHHHH HHHHHHHHHH Hiiiiiiiii iiiiiiiiii iiiiiiiiii

seq  CVFVAIRTIG NIVIVTTLLQ FMFACIGVQL FKGKLYTCSD SSKQTEAECK  1100
pred iHHHHHHHHH HHHHHHHHHH HHHHHHoooo oooooooooo oOOOOOOOOO

seq  GNYITYKDGE VDHPIIQPRS WENSKFDFDN VLAAMMALFT VSTFEGWPEL  1150
pred OOOOOOOOOO OOOOOOOOOO OOOOOOOOOO OOOOOOOOOO OOOOOOOOOO

seq  LYRSIDSHTE DKGPIYNYRV EISIFFIIYI IIIAFFMMNI FVGFVIVTFQ  1200
pred OOOOOOoooo oooooooooo oHHHHHHHHH HHHHHHHHHH HHHHHHiiii

seq  EQGEQEYKNC ELDKNQRQCV EYALKARPLR RYIPKNQHQY KVWYVVNSTY  1250
pred iiiiiiiiii iIIIIIIIII IIIIIIIIii iiiiiiiiii iiiHHHHHHH

seq  FEYLMFVLIL LNTICLAMQH YGQSCLFKIA MNILNMLFTG LFTVEMILKL  1300
pred HHHHHHHHHH HHHHHHHHoo oooooooooHH HHHHHHHHHH HHHHHHHHHH

seq  IAFKPKGYFS DPWNVFDFLI VIGSIIDVIL SETNPAEHTQ CSPSMNAEEN  1350
pred HHHiiiiiii iiiiiiiiII IIIIIIIIII IIIIIIIIII IIIIIIIIII

seq  SRISITFFRL FRVMRLVKLL SRGEGIRTLL WTFIKSFQAL PYVALLIVML  1400
pred IIIIIIIIII IIIIIIIIII IIIiiiiiii iiiiiiiiiH HHHHHHHHHH

seq  FFIYAVIGMQ VFGKIALNDT TEINRNNNFQ TFPQAVLLLF RCATGEAWQD  1450
pred HHHHHHHHHo oooooooooo ooooOOOOOO OOOOOOOOOO OOOOOOOOOO

seq  IMLACMPGKK CAPESEPHNS TEGETPCGSS FAVFYFISFY MLCAFLIINL  1500
pred OOOOOOOOOO OOOOOooooo oooooooooo HHHHHHHHHH HHHHHHHHHH

seq  FVAVIMDNFD YLTRDWSILG PHHLDEFKRI WAEYDPEAKG RIKHLDVVTL  1550
pred HHHHHiiiii iiiiiiiiii IIIIIIIIII IIIIIIIIII IIIIIIIIII

seq  LRRIQPPLGF GKLCPHRVAC KRLVSMNMPL NSDGTVMFNA TLFALVRTAL  1600
pred IIIIIIIIII IIIIIIIIII IIIIIIIIII IIIIIIIIII IIIIIIIIII

seq  RIKTEGNLEQ ANEELRAIIK KIWKRTSMKL LDQVVPPAGD DEVTVGKFYA  1650
pred IIIIIIIIII IIIIIIIIII IIIIIIIIII IIIIIIIIII IIIIIIIIII

seq  TFLIQEYFRK FKKRKEQGLV GKPSQRNALS LQAGLRTLHD IGPEIRRAIS  1700
pred IIIIIIIIII IIIIIIIIII IIIIIIIIII IIIIIIIIII IIIIIIIIII

seq  GDLTAEEELD KAMKEAVSAA SEDDIFRRAG GLFGNHVSYY QSDSRSAFPQ  1750
pred IIIIIIIIII IIIIIIIIII IIIIIIIIII IIIIIIIIII IIIIIIIIII

seq  TFTTQRPLHI SKAGNNQGDT ESPSHEKLVD STFTPSSYSS TGSNANINNA  1800
pred IIIIIIIIII IIIIIIIIII IIIIIIIIII IIIIIIIIII IIIIIIIIII

seq  NNTALGRLPR PAGYPSTVST VEGHGSPLSP AVRAQEAAWK LSSKRCHSQE  1850
pred IIIIIIIIII IIIIIIIIII IIIIIIIIII IIIIIIIIII IIIIIIIIII

seq  SQIAMACQEG ASQDDNYDVR IGEDAECCSE PSLLSTEMLS YQDDENRQLA  1900
pred IIIIIIIIII IIIIIIIIII IIIIIIIIII IIIIIIIIII IIIIIIIIII

seq  PPEEEKRDIR LSPKKGFLRS ASLGRRASFH LECLKRQKNQ GGDISQKTVL  1950
pred IIIIIIIIII IIIIIIIIII IIIIIIIIII IIIIIIIIII IIIIIIIIII

seq  PLHLVHHQAL AVAGLSPLLQ RSHSPTSLPR PCATPPATPG SRGWPPQPIP  2000
pred IIIIIIIIII IIIIIIIIII IIIIIIIIII IIIIIIIIII IIIIIIIIII

seq  TLRLEGADSS EKLNSSFPSI HCGSWSGENS PCRGDSSAAR RARPVSLTVP  2050
pred IIIIIIIIII IIIIIIIIII IIIIIIIIII IIIIIIIIII IIIIIIIIII

seq  SQAGAQGRQF HGSASSLVEA VLISEGLGQF AQDPKFIEVT TQELADACDL  2100
pred IIIIIIIIII IIIIIIIIII IIIIIIIIII IIIIIIIIII IIIIIIIIII

seq  TIEEMENAAD DILSGGARQS PNGTLLPFVN RRDPGRDRAG QNEQDASGAC  2150
pred IIIIIIIIII IIIIIIIIII IIIIIIIIII IIIIIIIIII IIIIIIIIII
```

part of protein sequence transmembran helix $Ca_v1.2$

```
seq  APGCGQSEEA LADRRAGVSS L 2171
pred IIIIIIIIII IIIIIIIIII I
```

cacna 1C, PHDhtm result

PHD transmembrane helix prediction result for : UNK_69740

Abstract Rost B, Casadio R, Fariselli P, Sander C : Transmembrane helices predicted at 95% accuracy. Protein Sci. 1995 Mar;4(3):521-33.

```
        10        20        30        40        50        60        70
         |         |         |         |         |         |         |
MLRALVQPATPAYQPLPSHLSAETESTCKGTVVHEAQLNHFYISPGGSNYGSPRPAHANMNANAAAGLAP

EHIPTPGAALSWQAAIDAARQAKLMGSAGNATISTVSSTQRKRQQYGKPKKQGSTTATRPPRALLCLTLK

NPIRRACISIVEWKPFEIIILLTIFANCVALAIYIPFPEDDSNATNSNLERVEYLFLIIFTVEAFLKVIA
              HHHHHHHHHHHHHHHHHH                        HHHHHHHHHHHHHHHH
YGLLFHPNAYLRNGWNLLDFIIVVVGLFSAILEQATKADGANALGGKGAGFDVKALRAFRVLRPLRLVSG
HHHH             HHHHHHHHHHHHH
VPSLQVVLNSIIKAMVPLLHIALLVLFVIIIYAIIGLELFMGKMHKTCYNQEGVADVPAEDDPSPCALET
    HHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHHH
GHGRQCQNGTVCKPGWDGPKHGITNFDNFAFAMLTVFQCITMEGWTDVLYWMQDAMGYELPWVYFVSLVI
                                HHHHHH HHH                    HHHHHHHHHH
FGSFFVLNLVLGVLSGEFSKEREKAKARGDFQKLREKQQLEEDLKGYLDWITQAEDIDPENEDEGMDEEK
HHHHHHHHHHHHH
PRNMSMPTSETESVNTENVAGGDIEGENCGARLAHRISKSKFSRYWRRWNRFCRRKCRAAVKSNVFYWLV
                                                                HHHHHHH
IFLVFLNTLTIASEHYNQPHWLTEVQDTANKALLALFTAEMLLKMYSLGLQAYFVSLFNRFDCFIVCGGI
HHHHHHHHH                       HHHHHHHHHHHHHHH                 HHHHHHH
LETILVETKVMSPLGISVLRCVRLLRIFKITRYWNSLSNLVASLLNSVRSIASLLLLLFLFIIIFSLLGM
HHHH             HH HH                    HHHHHHHHHHHHHHHHHHHHHHHHHHHHH
QLFGGKFNFDEMQTRRSTFDNFPQSLLTVFQILTGEDWNSVMYDGIMAYGGPSFPGMLVCIYFIILFICG
H                                                   HHHHHHHHHHHHHHH
NYILLNVFLAIAVDNLADAESLTSAQKEEEEEKERKKLARTASPEKKQEVVGKPALEEAKEEKIELKSIT
HHHHHHHHHHHH
ADGESPPTTKINMDDLQPNESEDKSPYPNPETTGEEDEEEPEMPVGPRPRPLSELHLKEKAVPMPEASAF

FIFSPNNRFRLQCHRIVNDTIFTNLILFFILLSSISLAAEDPVQHTSFRNHILFYFDIVFTTIFTIEIAL
                    HHHHHHHHHHHHHHHH                    HHHHHHHHHHHH
KMTAYGAFLHKGSFCRNYFNILDLLVVSVSLISFGIQSSAINVVKILRVLRVLRPLRAINRAKGLKHVVQ
HHHH                HHHHHHHHHHHHHHHHHH HHHHH
CVFVAIRTIGNIVIVTTLLQFMFACIGVQLFKGKLYTCSDSSKQTEAECKGNYITYKDGEVDHPIIQPRS
 HHHHHHHHHHHHHHHHHHHHHHHHHHHHHH
WENSKFDFDNVLAAMMALFTVSTFEGWPELLYRSIDSHTEDKGPIYNYRVEISIFFIIYIIIIAFFMMNI
                                                    HHHHHHHHHHHHHHHHHHH
FVGFVIVTFQEQGEQEYKNCELDKNQRQCVEYALKARPLRRYIPKNQHQYKVWYVVNSTYFEYLMFVLIL
HHHHHH                                                      HHHHHHHHHHH
LNTICLAMQHYGQSCLFKIAMNILNMLFTGLFTVEMILKLIAFKPKGYFSDPWNVFDFLIVIGSIIDVIL
HHHHHH                  HHHHHHHHHHHHHHHHHH                HHHHHHHHHH
SETNPAEHTQCSPSMNAEENSRISITFFRLFRVMRLVKLLSRGEGIRTLLWTFIKSFQALPYVALLIVML
                                H                    HHHHHHHHHHHHHHHHHH
FFIYAVIGMQVFGKIALNDTTEINRNNNFQTFPQAVLLLFRCATGEAWQDIMLACMPGKKCAPESEPHNS
HHHHHHHHHHH
TEGETPCGSSFAVFYFISFYMLCAFLIINLFVAVIMDNFDYLTRDWSILGPHHLDEFKRIWAEYDPEAKG
           HHHHHHHHHHHHHHHHHHHHHHHHH
RIKHLDVVTLLRRIQPPLGFGKLCPHRVACKRLVSMNMPLNSDGTVMFNATLFALVRTALRIKTEGNLEQ

ANEELRAIIKKIWKRTSMKLLDQVVPPAGDDEVTVGKFYATFLIQEYFRKFKKRKEQGLVGKPSQRNALS

LQAGLRTLHDIGPEIRRAISGDLTAEEELDKAMKEAVSAASEDDIFRRAGGLFGNHVSYYQSDSRSAFPQ
```

TFTTQRPLHISKAGNNQGDTESPSHEKLVDSTFTPSSYSSTGSNANINNANNTALGRLPRPAGYPSTVST

VEGHGSPLSPAVRAQEAAWKLSSKRCHSQESQIAMACQEGASQDDNYDVRIGEDAECCSEPSLLSTEMLS

YQDDENRQLAPPEEEKRDIRLSPKKGFLRSASLGRRASFHLECLKRQKNQGGDISQKTVLPLHLVHHQAL

AVAGLSPLLQRSHSPTSLPRPCATPPATPGSRGWPPQPIPTLRLEGADSSEKLNSSFPSIHCGSWSGENS

PCRGDSSAARRARPVSLTVPSQAGAQGRQFHGSASSLVEAVLISEGLGQFAQDPKFIEVTTQELADACDL

TIEEMENAADDILSGGARQSPNGTLLPFVNRRDPGRDRAGQNEQDASGACAPGCGQSEEALADRRAGVSS

L

Rabbit mRNA for cardiac dihydropyridine-sensitive calcium channel

taaaacgtaaagtattactaaaacctcaatttgcagcaatgccatatggccatgtaaact
tttgtggcactgaaatgacattacaggaatagtttcttagtcttaaaaagttacaaggag
aaaagatcacctgcagggtacttgtttagctttaaaaatcaccctgttttgtatacaact
ggaaactgacaatgcttcgagcccttgttcagccagctacgcccgcataccagccgctgc
ctagccacctgtctgctgaaacggagagtacatgtaaaggtactgtggtgcatgaagctc
aactcaaccatttctacatctctcctggaggttccaactatgggagcccacgcccagctc
atgccaacatgaatgccaacgcagctgcggggctcgcccctgagcacatccccaccccag
gggcagccctgtcctggcaggcagccatcgatgcggcccggcaggccaagctgatgggca
gtgctggcaacgcgactatctccaccgtcagctccacgcagcggaagcggcagcagtatg
ggaagcccaagaagcagggcagcaccactgccactcgcccgccccgtgccctgctctgcc
tcaccctgaagaaccccatccggagggcgtgcataagcatcgtcgagtggaaaccatttg
aaataattatttttactgactatttttgccaattgtgtggccttagcaatctatattccct
ttccagaagatgactccaatgccaccaattccaacctggaacgagtggaatatctctttc
tcataatttttactgtggaagcatttttaaaagtaatagcctatggacttctgtttcacc
ccaacgcttacctccgcaatggctggaatttactagactttataattgtggttgtagggc
tttttagtgcaattttagaacaagcaaccaaagcagacggggccaatgccctaggaggga
aaggggctggattcgacgtgaaggcgctgagggctttccgcgtgctgcgccccctgcggc
tggtgtctggagtcccgagtctccaggtggtcctgaactccatcatcaaggccatggtcc
ctctgctgcacattgccctgctagtgctgtttgtcatcatcatctatgccatcatcggcc
tggagctcttcatggggaagatgcacaagacatgctacaaccaggagggtgtagcagatg
tcccagcagaagatgatccttccccttgtgctctggagacgggccacgggcggcagtgcc
agaacggcaccgtgtgcaagcctgggtgggatggacccaagcacggcatcaccaactttg
acaattttgctttcgccatgttgacggtgttccagtgtatcaccatggagggctggaccg
acgtgctgtactggatgcaggacgctatgggctatgagctaccctgggtgtattttgtca
gtctggtcatctttggatccttttcgttctaaatctggttctcggtgtgttgagcggag
agttttccaaagagagggagaaggccaaagctcggggagatttccagaagttgcgggaga
agcagcagctggaagaggacctcaaaggctacctggactggatcactcaggcagaagaca
tcgaccctgagaatgaggatgaaggcatggatgaggagaaaccccgaaacatgagcatgc
ccacaagtgagaccgaatctgtcaacactgaaaacgtggctggaggtgacatcgaaggag
aaaactgcggggccaggctggcccaccggatctccaagtcgaaattcagccgctactggc
gccggtggaataggttctgcaggagaaagtgccgcgcagcggtcaagtcgaacgtcttct
actggctggtgatcttcctggtcttcctgaacacgctcaccattgcctctgagcactaca
accagccccactggctcacggaggtccaagacacggccaataaggctctactggccctgt
tcactgccgagatgctgctgaagatgtacagcctgggcctgcaggcctatttcgtgtccc
tcttcaaccgcttcgactgcttcattgtgtgcgggggcatcctggagaccatcctggtgg
agaccaaggtcatgtccccctgggcatctctgtgctgagatgcgtgcggctcctgagaa
tattcaaaattacaaggtactggaactccttgagcaacctggtggcctccctgctgaact
cggtgcgctccatcgcctccctgctcctgctcctcttcctcttcatcatcatcttctccc
tgctggggatgcagctgtttggaggcaagttcaacttcgatgagatgcagacccggagga
gcacgttcgacaatttcccgcagtccctgctcaccgtgtttcagatcctgaccggggagg
actggaattcggtgatgtatgatgggatcatggcttatggcggcccctcttttccaggga
tgttagtctgtatttacttcatcatcctcttcatctgtggaaattatatcctactgaatg
tgttcttggccattgctgtggacaacctggctgatgctgagagccttacttctgcccaaa
aggaagaggaagaagagaaggagagaaagaagctggccaggactgccagcccggagaaga
aacaagaggtggtagggaagccggccctggaggaggccaaggaggagaaaattgagctga
aatccattacagctgatggagagtccccgcctaccaccaagatcaacatggatgacctcc
agcccaatgagagtgaggataagagtccctaccccaacccggaaaccacaggagaagagg
atgaggaggagcctgagatgcctgtcggcccccgccctcggccactctccgagctgcacc
ttaaggagaaggccgtgcctatgccagaagccagtgcgtttttcatcttcagccccaaca

acaggttccgcctccagtgtcaccgtatcgtcaacgacacgatcttcaccaacctgatcc
tcttcttcattctgctcagcagcatttccctggctgccgaggaccctgtgcagcacacct
ccttcaggaatcacattctgttttattttgacattgtttttactaccattttcaccattg
aaattgctctcaagatgactgcgtatggggccttcctgcacaagggctctttctgcagga
actacttcaacatcctggacctgctggtggtcagcgtgtccctcatctccttcggcatcc
agtccagcgcgatcaatgtcgtgaagatcttgcgagtgctgcgagtgctcaggccactgc
gggccatcaacagggccaaggggctaaagcacgtggttcagtgtgtgttcgtggccatcc
ggaccattgggaacatcgtgattgtcaccacgctgctgcagttcatgttcgcctgcatcg
gagtccagctcttcaaggggaagctgtacacctgttcagacagttccaaacagactgagg
ctgaatgcaagggtaactacatcacctacaaagatggagaggttgaccatcccatcatcc
agccgcgcagctgggagaacagcaagtttgactttgacaacgtcctggcagccatgatgg
ccctcttcactgtctccaccttcgagggctggccagagctgctgtaccgctccatcgact
cccacacggaagacaagggccctatctacaactaccgagtggagatctccatcttcttca
tcatctacatcatcatcatcgccttcttcatgatgaacatcttcgtgggtttcgtcattg
tcaccttccaggagcaggggagcaggagtacaagaactgtgagctggacaagaaccagc
ggcagtgcgtggaatatgccctcaaggcccggcccctgcggaggtacatccccaagaacc
agcaccagtacaaagtgtggtacgtggtcaactccacctactttgagtacctgatgttcg
tcctcatcctgctcaacaccatctgcttggccatgcagcactacggccagagctgcctgt
tcaaaatcgccatgaacatcctcaacatgctcttcaccggcctcttcaccgtggaaatga
tcctgaagctcattgccttcaaacccaagggttactttagtgatccctggaatgtttttg
acttcctcatcgtaattggcagcataattgacgtcattctcagtgagactaatccagctg
aacatacccaatgctctccctctatgaacgcagaggagaactcccgcatctccatcacct
tcttccgcctgttccgggtcatgcgcctggtcaagctgctgagccgcggggagggcatcc
ggacgctgctgtggaccttcatcaagtccttccaggccctgccctatgtggctcttctga
tcgtaatgctgttcttcatctatgctgtgatcgggatgcaggtgtttgggaaaatcgccc
tgaacgacaccacggagatcaaccggaacaacaacttccagaccttcccccaagctgtgc
tgctcctcttcaggtgtgccacgggggaggcttggcaggatatcatgctggcctgcatgc
caggcaagaagtgtgccccagagtctgagccccacaacagcacagaaggggagacccccct
gcggcagcagcttcgccgtcttctacttcatcagcttctacatgctttgtgccttcctga
tcatcaatctctttgtagctgtcatcatggacaactttgactacctgacaagggactggt
caatccttggtccccaccatctggatgaatttaaaagaatctgggcagagtatgaccctg
aagccaagggtcgtatcaaacacctggatgtggtgaccctcctccggcggattcagcccc
cactgggttttgggaagctgtgccctcaccgtgtggcttgcaaacgcctggtctccatga
acatgcctctgaacagtgacgggacggtcatgttcaacgccaccctgtttgccctggtca
ggacagctctgaggatcaaaacagaaggaaacctggaacaagccaatgaggagctgcggg
ccatcatcaagaagatctggaagcggaccagcatgaagctgctggaccaagtggtgcccc
ctgcaggcgatgatgaggtcacagtcggcaagttctacgctaccttcctgatccaagagt
acttccggaaattcaagaagcgcaaagagcaagggcttgtgggcaagccctcccagagga
atgccctttccctgcaggctggcctgcgcactctgcacgacatcgggcctgagatccgac
gggccatctccggagacctgacagctgaggaagagctggacaaggccatgaaggaggctg
tgtctgctgcctctgaagatgacatcttcaggagggccggtggcctgtttggcaaccatg
tcagctactaccaaagtgacagccggagcgccttcccccagaccttcactacgcagcgcc
cactgcacatcagcaaggctggcaacaaccaaggcgacaccgagtcaccctcccacgaga
agctggtggactccactttcacccccagcagctactcgtccaccggctccaacgccaaca
tcaacaatgccaacaacactgccctgggccgcctccccgccccgccggctaccccagca
cagtcagcactgtggagggccacgggtccccccttgtctcctgccgtccgggcacaggagg
cagcatggaagctcagctccaagagatgccactcccaggagagccagatagccatggcgt
gtcaggagggcgcatcccaggacgacaactacgacgtgaggatcggtgaagatgcagagt
gctgcagtgagcccagcctgctctccacagagatgctctcctaccaggatgacgaaaacc
gacaactggcgcccccggaggaggagaagcgggacatcaggctgtctccaaagaagggtt
tcctgcgctccgcatcactgggtcgaagggcttccttccacctggagtgtctgaagcggc
agaagaatcaaggggagacatctctcagaagacagtcctgcccctgcatctggtccacc
accaggcattggcagtggcgggcctgagtcccctcctgcagagaagccattcccccacct
cgctccctaggccctgtgccacgccccctgccacaccgggcagccgaggctggcccccac
agcccatccccaccctgcggctggaggggccgactccagtgagaaactcaacagcagct
tcccgtccatccactgcggctcatggtctggggagaacagcccctgcagaggggacagca
gcgccgcccggagagcccggcccgtctccctcactgtgcccagccaggctggggcccagg
ggagacagttccatggcagcgccagcagcctggtggaagcggtcttgatttccgaaggac
tggggcagtttgctcaagatcccaagttcatcgaggtcacgacccaggagctggctgacg
cctgcgatctgaccatagaggagatggagaacgcggccgacgacattctcagcgggggcg
cccggcagagccccaatggcaccctgttacccttgtgaaccgcagggacccgggccggg
acagagcggggcagaacgagcaggacgcgagcggcgcatgcgccccagggtgcgggcaga
gcgaggaggccctcgcggaccgcagggccggcgtcagcagcctgtaggcgccagggccgg
gggtgcgggttttttatttgtctcaatgttcctaatgggttcgtttcagaagtgcctcac
tgttctcgtgacctggagttaaccggaacagcgtcttcattcatttctgttgggacgaga
cgcaggctgggaggtgtggagccctctgtgtccgcagaggcgaggaggggcggcggccg
cgggaaggggagagacccgccctcgagctctgctccaggcgcgccccgggcgggaaagag
aacctcagctttctgcggtggccctcgctcgccaaaaggaccctgaaccaaacgggtgtc
tttcaactttgcttgt

TAAAACGTAAAGTATTACTAAAACCTCAATTTGCAGCAATGCCATATGGCCATGTAAACTTTTGTGGCACTGAAA
TGACATTACAGGAATAGTTTCTTAGTCTTAAAAAGTTACAAGGAGAAAAGATCACCTGCAGGGTACTTGTTTAGC
TTTAAAAATCACCCTGTTTTGTATACAACTGGAAACTGACAATGCTTCGAGCCCTTGTTCAGCCAGCTACGCCCG
CATACCAGCCGCTGCCTAGCCACCTGTCTGCTGAAACGGAGAGTACATGTAAAGGTACTGTGGTGCATGAAGCTC
AACTCAACCATTTCTACATCTCTCCTGGAGGTTCCAACTATGGGAGCCCACGCCCAGCTCATGCCAACATGAATG
CCAACGCAGCTGCGGGGCTCGCCCCTGAGCACATCCCCACCCCAGGGGCAGCCCTGTCCTGGCAGGCAGCCATCG
ATGCGGCCCGGCAGGCCAAGCTGATGGGCAGTGCTGGCAACGCGACTATCTCCACCGTCAGCTCCACGCAGCGGA
AGCGGCAGCAGTATGGGAAGCCCAAGAAGCAGGGCAGCACCACTGCCACTCGCCCGCCCCGTGCCCTGCTCTGCC
TCACCCTGAAGAACCCCATCCGGAGGGCGTGCATAAGCATCGTCGAGTGGAAACCATTTGAAATAATTATTTTAC
TGACTATTTTTGCCAATTGTGTGGCCTTAGCAATCTATATTCCCTTTCCAGAAGATGACTCCAATGCCACCAATT
CCAACCTGGAACGAGTGGAATATCTCTTTCTCATAATTTTTACTGTGGAAGCATTTTTAAAAGTAATAGCCTATG
GACTTCTGTTTCACCCCAACGCTTACCTCCGCAATGGCTGGAATTTACTAGACTTTATAATTGTGGTTGTAGGGC
TTTTTAGTGCAATTTTAGAACAAGCAACCAAAGCAGACGGGGCCAATGCCCTAGGAGGGAAAGGGGCTGGATTCG
ACGTGAAGGCGCTGAGGGCTTTCCGCGTGCTGCGCCCCCTGCGGCTGGTGTCTGGAGTCCCGAGTCTCCAGGTGG
TCCTGAACTCCATCATCAAGGCCATGGTCCCTCTGCTGCACATTGCCCTGCTAGTGCTGTTTGTCATCATCATCT
ATGCCATCATCGGCCTGGAGCTCTTCATGGGGAAGATGCACAAGACATGCTACAACCAGGAGGGTGTAGCAGATG
TCCCAGCAGAAGATGATCCTTCCCCTTGTGCTCTGGAGACGGGCCACGGGCGGCAGTGCCAGAACGGCACCGTGT
GCAAGCCTGGGTGGGATGGACCCAAGCACGGCATCACCAACTTTGACAATTTTGCTTTCGCCATGTTGACGGTGT
TCCAGTGTATCACCATGGAGGGCTGGACCGACGTGCTGTACTGGATGCAGGACGCTATGGGCTATGAGCTACCCT
GGGTGTATTTTGTCAGTCTGGTCATCTTTGGATCCTTTTTCGTTCTAAATCTGGTTCTCGGTGTGTTGAGCGGAG
AGTTTTCCAAAGAGAGGGAGAAGGCCAAAGCTCGGGGAGATTTCCAGAAGTTGCGGGAGAAGCAGCAGCTGGAAG
AGGACCTCAAAGGCTACCTGGACTGGATCACTCAGGCAGAAGACATCGACCCTGAGAATGAGGATGAAGGCATGG
ATGAGGAGAAACCCCGAAACATGAGCATGCCCACAAGTGAGACCGAATCTGTCAACACTGAAAACGTGGCTGGAG
GTGACATCGAAGGAGAAAACTGCGGGGCCAGGCTGGCCCACCGGATCTCCAAGTCGAAATTCAGCCGCTACTGGC
GCCGGTGGAATAGGTTCTGCAGGAGAAAGTGCCGCGCAGCGGTCAAGTCGAACGTCTTCTACTGGCTGGTGATCT
TCCTGGTCTTCCTGAACACGCTCACCATTGCCTCTGAGCACTACAACCAGCCCCACTGGCTCACGGAGGTCCAAG
ACACGGCCAATAAGGCTCTACTGGCCCTGTTCACTGCCGAGATGCTGCTGAAGATGTACAGCCTGGGCCTGCAGG
CCTATTTCGTGTCCCTCTTCAACCGCTTCGACTGCTTCATTGTGTGCGGGGGCATCCTGGAGACCATCCTGGTGG
AGACCAAGGTCATGTCCCCCCTGGGCATCTCTGTGCTGAGATGCGTGCGGCTCCTGAGAATATTCAAAATTACAA
GGTACTGGAACTCCTTGAGCAACCTGGTGGCCTCCCTGCTGAACTCGGTGCGCTCCATCGCCTCCCTGCTCCTGC
TCCTCTTCCTCTTCATCATCATCTTCTCCCTGCTGGGGATGCAGCTGTTTGGAGGCAAGTTCAACTTCGATGAGA
TGCAGACCCGGAGGAGCACGTTCGACAATTTCCCGCAGTCCCTGCTCACCGTGTTTCAGATCCTGACCGGGGAGG
ACTGGAATTCGGTGATGTATGATGGGATCATGGCTTATGGCGGCCCCTCTTTTCCAGGGATGTTAGTCTGTATTT
ACTTCATCATCCTCTTCATCTGTGGAAATTATATCCTACTGAATGTGTTCTTGGCCATTGCTGTGGACAACCTGG
CTGATGCTGAGAGCCTTACTTCTGCCCAAAAGGAAGAGGAAGAAGAGAAGGAGAGAAAGAAGCTGGCCAGGACTG
CCAGCCCGGAGAAGAAACAAGAGGTGGTAGGGAAGCCGGCCCTGGAGGAGGCCAAGGAGGAGAAAATTGAGCTGA
AATCCATTACAGCTGATGGAGAGTCCCCGCCTACCACCAAGATCAACATGGATGACCTCCAGCCCAATGAGAGTG
AGGATAAGAGTCCCTACCCCAACCCGGAAACCACAGGAGAAGAGGATGAGGAGGAGCCTGAGATGCCTGTCGGCC
CCCGCCCTCGGCCACTCTCCGAGCTGCACCTTAAGGAGAAGGCCGTGCCTATGCCAGAAGCCAGTGCGTTTTTCA
TCTTCAGCCCCAACAACAGGTTCCGCCTCCAGTGTCACCGTATCGTCAACGACACGATCTTCACCAACCTGATCC
TCTTCTTCATTCTGCTCAGCAGCATTTCCCTGGCTGCCGAGGACCCTGTGCAGCACACCTCCTTCAGGAATCACA
TTCTGTTTTATTTTGACATTGTTTTTACTACCATTTTCACCATTGAAATTGCTCTCAAGATGACTGCGTATGGGG
CCTTCCTGCACAAGGGCTCTTTCTGCAGGAACTACTTCAACATCCTGGACCTGCTGGTGGTCAGCGTGTCCCTCA
TCTCCTTCGGCATCCAGTCCAGCGCGATCAATGTCGTGAAGATCTTGCGAGTGCTGCGAGTGCTCAGGCCACTGC
GGGCCATCAACAGGGCCAAGGGGCTAAAGCACGTGGTTCAGTGTGTGTTCGTGGCCATCCGGACCATTGGGAACA
TCGTGATTGTCACCACGCTGCTGCAGTTCATGTTCGCCTGCATCGGAGTCCAGCTCTTCAAGGGGAAGCTGTACA
CCTGTTCAGACAGTTCCAAACAGACTGAGGCTGAATGCAAGGGTAACTACATCACCTACAAAGATGGAGAGGTTG
ACCATCCCATCATCCAGCCGCGCAGCTGGGAGAACAGCAAGTTTGACTTTGACAACGTCCTGGCAGCCATGATGG
CCCTCTTCACTGTCTCCACCTTCGAGGGCTGGCCAGAGCTGCTGTACCGCTCCATCGACTCCCACACGGAAGACA
AGGGCCCTATCTACAACTACCGAGTGGAGATCTCCATCTTCTTCATCATCTACATCATCATCATCGCCTTCTTCA
TGATGAACATCTTCGTGGGTTTCGTCATTGTCACCTTCCAGGAGCAGGGGGAGCAGGAGTACAAGAACTGTGAGC
TGGACAAGAACCAGCGGCAGTGCGTGGAATATGCCCTCAAGGCCCGGCCCCTGCGGAGGTACATCCCCAAGAACC
AGCACCAGTACAAAGTGTGGTACGTGGTCAACTCCACCTACTTTGAGTACCTGATGTTCGTCCTCATCCTGCTCA
ACACCATCTGCTTGGCCATGCAGCACTACGGCCAGAGCTGCCTGTTCAAAATCGCCATGAACATCCTCAACATGC
TCTTCACCGGCCTCTTCACCGTGGAAATGATCCTGAAGCTCATTGCCTTCAAACCCAAGGGTTACTTTAGTGATC
CCTGGAATGTTTTTGACTTCCTCATCGTAATTGGCAGCATAATTGACGTCATTCTCAGTGAGACTAATCCAGCTG
AACATACCCAATGCTCTCCCTCTATGAACGCAGAGGAGAACTCCCGCATCTCCATCACCTTCTTCCGCCTGTTCC
GGGTCATGCGCCTGGTCAAGCTGCTGAGCCGCGGGGAGGGCATCCGGACGCTGCTGTGGACCTTCATCAAGTCCT
TCCAGGCCCTGCCCTATGTGGCTCTTCTGATCGTAATGCTGTTCTTCATCTATGCTGTGATCGGGATGCAGGTGT
TTGGGAAAATCGCCCTGAACGACACCACGGAGATCAACCGGAACAACAACTTCCAGACCTTCCCCCAAGCTGTGC
TGCTCCTCTTCAGGTGTGCCACGGGGGAGGCTTGGCAGGATATCATGCTGGCCTGCATGCCAGGCAAGAAGTGTG
CCCCAGAGTCTGAGCCCCACAACAGCACAGAAGGGGAGACCCCCTGCGGCAGCAGCTTCGCCGTCTTCTACTTCA
TCAGCTTCTACATGCTTTGTGCCTTCCTGATCATCAATCTCTTTGTAGCTGTCATCATGGACAACTTTGACTACC

C-terminal cytoplasmic tail

TGACAAGGGACTGGTCAATCCTTGGTCCCCACCATCTGGATGAATTTAAAAGAATCTGGGCAGAGTATGACCCTG
AAGCCAAGGGTCGTATCAAACACCTGGATGTGGTGACCCTCCTCCGGCGGATTCAGCCCCCACTGGGTTTTGGGA
AGCTGTGCCCTCACCGTGTGGCTTGCAAACGCCTGGTCTCCATGAACATGCCTCTGAACAGTGACGGGACGGTCA
TGTTCAACGCCACCCTGTTTGCCCTGGTCAGGACAGCTCTGAGGATCAAAACAGAAGGAAACCTGGAACAAGCCA
ATGAGGAGCTGCGGGCCATCATCAAGAAGATCTGGAAGCGGACCAGCATGAAGCTGCTGGACCAAGTGGTGCCCC
CTGCAGGCGATGATGAGGTCACAGTCGGCAAGTTCTACGCTACCTTCCTGATCCAAGAGTACTTCCGGAAATTCA
AGAAGCGCAAAGAGCAAGGGCTTGTGGGCAAGCCCTCCCAGAGGAATGCCCTTTCCCTGCAGGCTGGCCTGCGCA
CTCTGCACGACATCGGGCCTGAGATCCGACGGGCCATCTCCGGAGACCTGACAGCTGAGGAAGAGCTGGACAAGG
CCATGAAGGAGGCTGTGTCTGCTGCCTCTGAAGATGACATCTTCAGGAGGGCCGGTGGCCTGTTTGGCAACCATG
TCAGCTACTACCAAAGTGACAGCCGGAGCGCCTTCCCCCAGACCTTCACTACGCAGCGCCCACTGCACATCAGCA
AGGCTGGCAACAACCAAGGCGACACCGAGTCACCCTCCCACGAGAAGCTGGTGGACTCCACTTTCACCCCCAGCA
GCTACTCGTCCACCGGCTCCAACGCCAACATCAACAATGCCAACAACACTGCCCTGGGCCGCCTCCCCCGCCCCG
CCGGCTACCCCAGCACAGTCAGCACTGTGGAGGGCCACGGGTCCCCCTTGTCTCCTGCCGTCCGGGCACAGGAGG
CAGCATGGAAGCTCAGCTCCAAGAGATGCCACTCCCAGGAGAGCCAGATAGCCATGGCGTGTCAGGAGGGCGCAT
CCCAGGACGACAACTACGACGTGAGGATCGGTGAAGATGCAGAGTGCTGCAGTGAGCCCAGCCTGCTCTCCACAG
AGATGCTCTCCTACCAGGATGACGAAAACCGACAACTGGCGCCCCCGGAGGAGGAGAAGCGGGACATCAGGCTGT
CTCCAAAGAAGGGTTTCCTGCGCTCCGCATCACTGGGTCGAAGGGCTTCCTTCCACCTGGAGTGTCTGAAGCGGC
AGAAGAATCAAGGGGGAGACATCTCTCAGAAGACAGTCCTGCCCCTGCATCTGGTCCACCACCAGGCATTGGCAG
TGGCGGGCCTGAGTCCCCTCCTGCAGAGAAGCCATTCCCCCACCTCGCTCCCTAGGCCCTGTGCCACGCCCCCTG
CCACACCGGGCAGCCGAGGCTGGCCCCCACAGCCCATCCCCACCCTGCGGCTGGAGGGGGCCGACTCCAGTGAGA
AACTCAACAGCAGCTTCCCGTCCATCCACTGCGGCTCATGGTCTGGGGAGAACAGCCCCTGCAGAGGGGACAGCA
GCGCCGCCCGGAGAGCCCGGCCCGTCTCCCTCACTGTGCCCAGCCAGGCTGGGGCCCAGGGGAGACAGTTCCATG
GCAGCGCCAGCAGCCTGGTGGAAGCGGTCTTGATTTCCGAAGGACTGGGGCAGTTTGCTCAAGATCCCAAGTTCA
TCGAGGTCACGACCCAGGAGCTGGCTGACGCCTGCGATCTGACCATAGAGGAGATGGAGAACGCGGCCGACGACA
TTCTCAGCGGGGGCGCCCGGCAGAGCCCCAATGGCACCCTGTTACCCTTTGTGAACCGCAGGGACCCGGGCCGGG
ACAGAGCGGGGCAGAACGAGCAGGACGCGAGCGGCGCATGCGCCCCAGGGTGCGGGCAGAGCGAGGAGGCCCTCG
CGGACCGCAGGGCCGGCGTCAGCAGCCTGTAGGCGCCAGGGCCGGGGGTGCGGGTTTTTTATTTGTCTCAATGTT
CCTAATGGGTTCGTTTCAGAAGTGCCTCACTGTTCTCGTGACCTGGAGTTAACCGGAACAGCGTCTTCATTCATT
TCTGTTGGGACGAGACGCAGGCTGGGAGGTGTGGAGCCCTCTGTGTCCGCAGAGGCGAGGAGGGGGCGGCGGCCG
CGGGAAGGGGAGAGACCCGCCCTCGAGCTCTGCTCCAGGCGCGCCCCGGGCGGGAAAGAGAACCTCAGCTTTCTG
CGGTGGCCCTCGCTCGCCAAAAGGACCCTGAACCAAACGGGTGTCTTTCAACTTTGCTTGT

Cytoplasmic C-terminus only

GACAACTTTGACTACCTGACAAGGGACTGGTCAATCCTTGGTCCCCACCATCTGGATGAATTTAAAAGAATCTGG
GCAGAGTATGACCCTGAAGCCAAGGGTCGTATCAAACACCTGGATGTGGTGACCCTCCTCCGGCGGATTCAGCCC
CCACTGGGTTTTGGGAAGCTGTGCCCTCACCGTGTGGCTTGCAAACGCCTGGTCTCCATGAACATGCCTCTGAAC
AGTGACGGGACGGTCATGTTCAACGCCACCCTGTTTGCCCTGGTCAGGACAGCTCTGAGGATCAAAACAGAAGGA
AACCTGGAACAAGCCAATGAGGAGCTGCGGGCCATCATCAAGAAGATCTGGAAGCGGACCAGCATGAAGCTGCTG
GACCAAGTGGTGCCCCCTGCAGGCGATGATGAGGTCACAGTCGGCAAGTTCTACGCTACCTTCCTGATCCAAGAG
TACTTCCGGAAATTCAAGAAGCGCAAAGAGCAAGGGCTTGTGGGCAAGCCCTCCCAGAGGAATGCCCTTTCCCTG
CAGGCTGGCCTGCGCACTCTGCACGACATCGGGCCTGAGATCCGACGGGCCATCTCCGGAGACCTGACAGCTGAG
GAAGAGCTGGACAAGGCCATGAAGGAGGCTGTGTCTGCTGCCTCTGAAGATGACATCTTCAGGAGGGCCGGTGGC
CTGTTTGGCAACCATGTCAGCTACTACCAAAGTGACAGCCGGAGCGCCTTCCCCCAGACCTTCACTACGCAGCGC
CCACTGCACATCAGCAAGGCTGGCAACAACCAAGGCGACACCGAGTCACCCTCCCACGAGAAGCTGGTGGACTCC
ACTTTCACCCCCAGCAGCTACTCGTCCACCGGCTCCAACGCCAACATCAACAATGCCAACAACACTGCCCTGGGC
CGCCTCCCCCGCCCCGCCGGCTACCCCAGCACAGTCAGCACTGTGGAGGGCCACGGGTCCCCCTTGTCTCCTGCC
GTCCGGGCACAGGAGGCAGCATGGAAGCTCAGCTCCAAGAGATGCCACTCCCAGGAGAGCCAGATAGCCATGGCG
TGTCAGGAGGGCGCATCCCAGGACGACAACTACGACGTGAGGATCGGTGAAGATGCAGAGTGCTGCAGTGAGCCC
AGCCTGCTCTCCACAGAGATGCTCTCCTACCAGGATGACGAAAACCGACAACTGGCGCCCCCGGAGGAGGAGAAG
CGGGACATCAGGCTGTCTCCAAAGAAGGGTTTCCTGCGCTCCGCATCACTGGGTCGAAGGGCTTCCTTCCACCTG
GAGTGTCTGAAGCGGCAGAAGAATCAAGGGGGAGACATCTCTCAGAAGACAGTCCTGCCCCTGCATCTGGTCCAC
CACCAGGCATTGGCAGTGGCGGGCCTGAGTCCCCTCCTGCAGAGAAGCCATTCCCCCACCTCGCTCCCTAGGCCC
TGTGCCACGCCCCCTGCCACACCGGGCAGCCGAGGCTGGCCCCCACAGCCCATCCCCACCCTGCGGCTGGAGGGG
GCCGACTCCAGTGAGAAACTCAACAGCAGCTTCCCGTCCATCCACTGCGGCTCATGGTCTGGGGAGAACAGCCCC
TGCAGAGGGGACAGCAGCGCCGCCCGGAGAGCCCGGCCCGTCTCCCTCACTGTGCCCAGCCAGGCTGGGGCCCAG
GGGAGACAGTTCCATGGCAGCGCCAGCAGCCTGGTGGAAGCGGTCTTGATTTCCGAAGGACTGGGGCAGTTTGCT
CAAGATCCCAAGTTCATCGAGGTCACGACCCAGGAGCTGGCTGACGCCTGCGATCTGACCATAGAGGAGATGGAG
AACGCGGCCGACGACATTCTCAGCGGGGGCGCCCGGCAGAGCCCCAATGGCACCCTGTTACCCTTTGTGAACCGC
AGGGACCCGGGCCGGGACAGAGCGGGGCAGAACGAGCAGGACGCGAGCGGCGCATGCGCCCCAGGGTGCGGGCAG
AGCGAGGAGGCCCTCGCGGACCGCAGGGCCGGCGTCAGCAGCCTGTAG

C-terminal cytoplasmic tail

Rabbit mRNA for cardiac dihydropyridine-sensitive calcium channel

Translate

5'3' Frame 3

```
taaaacgtaaagtattactaaaacctcaatttgcagcaatgccatatggccatgtaaacttt
   K  R  K  V  L  L  K  P  Q  F  A  A  M  P  Y  G  H  V  N  F
tgtggcactgaaatgacattacaggaatagtttcttagtcttaaaaagttacaaggagaa
 C  G  T  E  M  T  L  Q  E  -  F  L  S  L  K  K  L  Q  G  E
aagatcacctgcagggtacttgtttagctttaaaaatcaccctgttttgtatacaactgg
 K  I  T  C  R  V  L  V  -  L  -  K  S  P  C  F  V  Y  N  W
aaactgacaatgcttcgagcccttgttcagccagctacgcccgcataccagccgctgcct
 K  L  T  M  L  R  A  L  V  Q  P  A  T  P  A  Y  Q  P  L  P
agccacctgtctgctgaaacggagagtacatgtaaaggtactgtggtgcatgaagctcaa
 S  H  L  S  A  E  T  E  S  T  C  K  G  T  V  V  H  E  A  Q
ctcaaccatttctacatctctcctggaggttccaactatgggagcccacgcccagctcat
 L  N  H  F  Y  I  S  P  G  G  S  N  Y  G  S  P  R  P  A  H
gccaacatgaatgccaacgcagctgcggggctcgcccctgagcacatccccaccccaggg
 A  N  M  N  A  N  A  A  A  G  L  A  P  E  H  I  P  T  P  G
gcagccctgtcctggcaggcagccatcgatgcggcccggcaggccaagctgatgggcagt
 A  A  L  S  W  Q  A  A  I  D  A  A  R  Q  A  K  L  M  G  S
gctggcaacgcgactatctccaccgtcagctccacgcagcggaagcggcagcagtatggg
 A  G  N  A  T  I  S  T  V  S  S  T  Q  R  K  R  Q  Q  Y  G
aagcccaagaagcagggcagcaccactgccactcgcccgccccgtgccctgctctgcctc
 K  P  K  K  Q  G  S  T  T  A  T  R  P  P  R  A  L  L  C  L
accctgaagaaccccatccggagggcgtgcataagcatcgtcgagtggaaaccatttgaa
 T  L  K  N  P  I  R  R  A  C  I  S  I  V  E  W  K  P  F  E
ataattattttactgactatttttgccaattgtgtggccttagcaatctatattccctttt
 I  I  I  L  L  T  I  F  A  N  C  V  A  L  A  I  Y  I  P  F
ccagaagatgactccaatgccaccaattccaacctggaacgagtggaatatctctttctc
 P  E  D  D  S  N  A  T  N  S  N  L  E  R  V  E  Y  L  F  L
ataatttttactgtggaagcatttttaaaagtaatagcctatggacttctgtttcacccc
 I  I  F  T  V  E  A  F  L  K  V  I  A  Y  G  L  L  F  H  P
aacgcttacctccgcaatggctggaatttactagactttataattgtggttgtagggctt
 N  A  Y  L  R  N  G  W  N  L  L  D  F  I  I  V  V  V  G  L
tttagtgcaattttagaacaagcaaccaaagcagacggggccaatgccctaggagggaaa
 F  S  A  I  L  E  Q  A  T  K  A  D  G  A  N  A  L  G  G  K
ggggctggattcgacgtgaaggcgctgagggctttccgcgtgctgcgccccctgcggctg
 G  A  G  F  D  V  K  A  L  R  A  F  R  V  L  R  P  L  R  L
gtgtctggagtcccgagtctccaggtggtcctgaactccatcatcaaggccatggtccct
 V  S  G  V  P  S  L  Q  V  V  L  N  S  I  I  K  A  M  V  P
ctgctgcacattgccctgctagtgctgtttgtcatcatcatctatgccatcatcggcctg
 L  L  H  I  A  L  L  V  L  F  V  I  I  I  Y  A  I  I  G  L
gagctcttcatggggaagatgcacaagacatgctacaaccaggagggtgtagcagatgtc
 E  L  F  M  G  K  M  H  K  T  C  Y  N  Q  E  G  V  A  D  V
ccagcagaagatgatccttccccttgtgctctggagacgggccacgggcggcagtgccag
 P  A  E  D  D  P  S  P  C  A  L  E  T  G  H  G  R  Q  C  Q
aacggcaccgtgtgcaagcctgggtgggatggacccaagcacggcatcaccaactttgac
 N  G  T  V  C  K  P  G  W  D  G  P  K  H  G  I  T  N  F  D
aattttgctttcgccatgttgacggtgttccagtgtatcaccatggagggctggaccgac
 N  F  A  F  A  M  L  T  V  F  Q  C  I  T  M  E  G  W  T  D
gtgctgtactggatgcaggacgctatgggctatgagctaccctgggtgtattttgtcagt
 V  L  Y  W  M  Q  D  A  M  G  Y  E  L  P  W  V  Y  F  V  S
ctggtcatctttggatccttttcgttctaaatctggttctcggtgtgttgagcggagag
 L  V  I  F  G  S  F  F  V  L  N  L  V  L  G  V  L  S  G  E
ttttccaaagagagggagaaggccaaagctcggggagatttccagaagttgcgggagaag
 F  S  K  E  R  E  K  A  K  A  R  G  D  F  Q  K  L  R  E  K
cagcagctggaagaggacctcaaaggctacctggactggatcactcaggcagaagacatc
 Q  Q  L  E  E  D  L  K  G  Y  L  D  W  I  T  Q  A  E  D  I
gaccctgagaatgaggatgaaggcatggatgaggagaaaccccgaaacatgagcatgccc
 D  P  E  N  E  D  E  G  M  D  E  E  K  P  R  N  M  S  M  P
acaagtgagaccgaatctgtcaacactgaaaacgtggctggaggtgacatcgaaggagaa
 T  S  E  T  E  S  V  N  T  E  N  V  A  G  G  D  I  E  G  E
aactgcggggccaggctggcccaccggatctccaagtcgaaattcagccgctactggcgc
 N  C  G  A  R  L  A  H  R  I  S  K  S  K  F  S  R  Y  W  R
cggtggaataggttctgcaggagaaagtgccgcgcagcggtcaagtcgaacgtcttctac
 R  W  N  R  F  C  R  R  K  C  R  A  A  V  K  S  N  V  F  Y
```

M = methionin, Start codon

```
tggctggtgatcttcctggtcttcctgaacacgctcaccattgcctctgagcactacaac
 W  L  V  I  F  L  V  F  L  N  T  L  T  I  A  S  E  H  Y  N
cagccccactggctcacggaggtccaagacacggccaataaggctctactggccctgttc
 Q  P  H  W  L  T  E  V  Q  D  T  A  N  K  A  L  L  A  L  F
actgccgagatgctgctgaagatgtacagcctgggcctgcaggcctatttcgtgtccctc
 T  A  E  M  L  L  K  M  Y  S  L  G  L  Q  A  Y  F  V  S  L
ttcaaccgcttcgactgcttcattgtgtgcgggggcatcctggagaccatcctggtggag
 F  N  R  F  D  C  F  I  V  C  G  G  I  L  E  T  I  L  V  E
accaaggtcatgtccccctgggcatctctgtgctgagatgcgtgcggctcctgagaata
 T  K  V  M  S  P  L  G  I  S  V  L  R  C  V  R  L  L  R  I
ttcaaaattacaaggtactggaactccttgagcaacctggtggcctccctgctgaactcg
 F  K  I  T  R  Y  W  N  S  L  S  N  L  V  A  S  L  L  N  S
gtgcgctccatcgcctccctgctcctgctcctcttcctcttcatcatcatcttctccctg
 V  R  S  I  A  S  L  L  L  L  L  F  L  F  I  I  I  F  S  L
ctggggatgcagctgtttggaggcaagttcaacttcgatgagatgcagacccggaggagc
 L  G  M  Q  L  F  G  G  K  F  N  F  D  E  M  Q  T  R  R  S
acgttcgacaatttcccgcagtccctgctcaccgtgtttcagatcctgaccggggaggac
 T  F  D  N  F  P  Q  S  L  L  T  V  F  Q  I  L  T  G  E  D
tggaattcggtgatgtatgatgggatcatggcttatggcggcccctcttttccagggatg
 W  N  S  V  M  Y  D  G  I  M  A  Y  G  G  P  S  F  P  G  M
ttagtctgtatttacttcatcatcctcttcatctgtggaaattatatcctactgaatgtg
 L  V  C  I  Y  F  I  I  L  F  I  C  G  N  Y  I  L  L  N  V
ttcttggccattgctgtggacaacctggctgatgctgagagccttacttctgcccaaaag
 F  L  A  I  A  V  D  N  L  A  D  A  E  S  L  T  S  A  Q  K
gaagaggaagaagagaaggagagaaagaagctggccaggactgccagcccggagaagaaa
 E  E  E  E  E  K  E  R  K  K  L  A  R  T  A  S  P  E  K  K
caagaggtggtagggaagccggccctggaggaggccaaggaggagaaaattgagctgaaa
 Q  E  V  V  G  K  P  A  L  E  E  A  K  E  E  K  I  E  L  K
tccattacagctgatggagagtccccgcctaccaccaagatcaacatggatgacctccag
 S  I  T  A  D  G  E  S  P  P  T  T  K  I  N  M  D  D  L  Q
cccaatgagagtgaggataagagtccctaccccaacccggaaaccacaggagaagaggat
 P  N  E  S  E  D  K  S  P  Y  P  N  P  E  T  T  G  E  E  D
gaggaggagcctgagatgcctgtcggcccccgccctcggccactctccgagctgcaccttt
 E  E  E  P  E  M  P  V  G  P  R  P  R  P  L  S  E  L  H  L
aaggagaaggccgtgcctatgccagaagccagtgcgtttttcatcttcagccccaacaac
 K  E  K  A  V  P  M  P  E  A  S  A  F  F  I  F  S  P  N  N
aggttccgcctccagtgtcaccgtatcgtcaacgacacgatcttcaccaacctgatcctc
 R  F  R  L  Q  C  H  R  I  V  N  D  T  I  F  T  N  L  I  L
ttcttcattctgctcagcagcatttccctggctgccgaggaccctgtgcagcacacctcc
 F  F  I  L  L  S  S  I  S  L  A  A  E  D  P  V  Q  H  T  S
ttcaggaatcacattctgttttattttgacattgtttttactaccattttcaccattgaa
 F  R  N  H  I  L  F  Y  F  D  I  V  F  T  T  I  F  T  I  E
attgctctcaagatgactgcgtatggggccttcctgcacaagggctctttctgcaggaac
 I  A  L  K  M  T  A  Y  G  A  F  L  H  K  G  S  F  C  R  N
tacttcaacatcctggacctgctggtggtcagcgtgtccctcatctccttcggcatccag
 Y  F  N  I  L  D  L  L  V  V  S  V  S  L  I  S  F  G  I  Q
tccagcgcgatcaatgtcgtgaagatcttgcgagtgctgcgagtgctcaggccactgcgg
 S  S  A  I  N  V  V  K  I  L  R  V  L  R  V  L  R  P  L  R
gccatcaacagggccaaggggctaaagcacgtggttcagtgtgtgttcgtggccatccgg
 A  I  N  R  A  K  G  L  K  H  V  V  Q  C  V  F  V  A  I  R
accattgggaacatcgtgattgtcaccacgctgctgcagttcatgttcgcctgcatcgga
 T  I  G  N  I  V  I  V  T  T  L  L  Q  F  M  F  A  C  I  G
gtccagctcttcaaggggaagctgtacacctgttcagacagttccaaacagactgaggct
 V  Q  L  F  K  G  K  L  Y  T  C  S  D  S  S  K  Q  T  E  A
gaatgcaagggtaactacatcacctacaaagatggagaggttgaccatcccatcatccag
 E  C  K  G  N  Y  I  T  Y  K  D  G  E  V  D  H  P  I  I  Q
ccgcgcagctgggagaacagcaagtttgactttgacaacgtcctggcagccatgatggcc
 P  R  S  W  E  N  S  K  F  D  F  D  N  V  L  A  A  M  M  A
ctcttcactgtctccaccttcgagggctggccagagctgctgtaccgctccatcgactcc
 L  F  T  V  S  T  F  E  G  W  P  E  L  L  Y  R  S  I  D  S
cacacggaagacaagggccctatctacaactaccgagtggagatctccatcttcttcatc
 H  T  E  D  K  G  P  I  Y  N  Y  R  V  E  I  S  I  F  F  I
atctacatcatcatcatcgccttcttcatgatgaacatcttcgtgggtttcgtcattgtc
 I  Y  I  I  I  I  A  F  F  M  M  N  I  F  V  G  F  V  I  V
accttccaggagcaggggggagcaggagtacaagaactgtgagctggacaagaaccagcgg
 T  F  Q  E  Q  G  E  Q  E  Y  K  N  C  E  L  D  K  N  Q  R
cagtgcgtggaatatgccctcaaggcccggcccctgcggaggtacatccccaagaaccag
 Q  C  V  E  Y  A  L  K  A  R  P  L  R  R  Y  I  P  K  N  Q
caccagtacaaagtgtggtacgtggtcaactccacctactttgagtacctgatgttcgtc
 H  Q  Y  K  V  W  Y  V  V  N  S  T  Y  F  E  Y  L  M  F  V
```

```
ctcatcctgctcaacaccatctgcttggccatgcagcactacggccagagctgcctgttc
 L  I  L  L  N  T  I  C  L  A  M  Q  H  Y  G  Q  S  C  L  F
aaaatcgccatgaacatcctcaacatgctcttcaccggcctcttcaccgtggaaatgatc
 K  I  A  M  N  I  L  N  M  L  F  T  G  L  F  T  V  E  M  I
ctgaagctcattgccttcaaacccaagggttactttagtgatccctggaatgtttttgac
 L  K  L  I  A  F  K  P  K  G  Y  F  S  D  P  W  N  V  F  D
ttcctcatcgtaattggcagcataattgacgtcattctcagtgagactaatccagctgaa
 F  L  I  V  I  G  S  I  I  D  V  I  L  S  E  T  N  P  A  E
catacccaatgctctccctctatgaacgcagaggagaactcccgcatctccatcaccttc
 H  T  Q  C  S  P  S  M  N  A  E  E  N  S  R  I  S  I  T  F
ttccgcctgttccgggtcatgcgcctggtcaagctgctgagccgcggggagggcatccgg
 F  R  L  F  R  V  M  R  L  V  K  L  L  S  R  G  E  G  I  R
acgctgctgtggaccttcatcaagtccttccaggccctgccctatggctcttctgatc
 T  L  L  W  T  F  I  K  S  F  Q  A  L  P  Y  V  A  L  L  I
gtaatgctgttcttcatctatgctgtgatcgggatgcaggtgtttgggaaaatcgccctg
 V  M  L  F  F  I  Y  A  V  I  G  M  Q  V  F  G  K  I  A  L
aacgacaccacggagatcaaccggaacaacaacttccagaccttcccccaagctgtgctg
 N  D  T  T  E  I  N  R  N  N  N  F  Q  T  F  P  Q  A  V  L
ctcctcttcaggtgtgccacgggggaggcttggcaggatatcatgctggcctgcatgcca
 L  L  F  R  C  A  T  G  E  A  W  Q  D  I  M  L  A  C  M  P
ggcaagaagtgtgccccagagtctgagccccacaacagcacagaaggggagacccccctgc
 G  K  K  C  A  P  E  S  E  P  H  N  S  T  E  G  E  T  P  C
ggcagcagcttcgccgtcttctacttcatcagcttctacatgctttgtgccttcctgatc
 G  S  S  F  A  V  F  Y  F  I  S  F  Y  M  L  C  A  F  L  I
atcaatctctttgtagctgtcatcatggacaactttgactacctgacaagggactggtca
 I  N  L  F  V  A  V  I  M  D  N  F  D  Y  L  T  R  D  W  S
atccttggtccccaccatctggatgaatttaaaagaatctgggcagagtatgaccctgaa
 I  L  G  P  H  H  L  D  E  F  K  R  I  W  A  E  Y  D  P  E
gccaagggtcgtatcaaacacctggatgtggtgaccctcctccggcggattcagccccca
 A  K  G  R  I  K  H  L  D  V  V  T  L  L  R  R  I  Q  P  P
ctgggttttgggaagctgtgccctcaccgtgtggcttgcaaacgcctggtctccatgaac
 L  G  F  G  K  L  C  P  H  R  V  A  C  K  R  L  V  S  M  N
Atgcctctgaacagtgacgggacggtcatgttcaacgccaccctgtttgccctggtcagg
 M  P  L  N  S  D  G  T  V  M  F  N  A  T  L  F  A  L  V  R
acagctctgaggatcaaaacagaaggaaacctggaacaagccaatgaggagctgcgggcc
 T  A  L  R  I  K  T  E  G  N  L  E  Q  A  N  E  E  L  R  A
atcatcaagaagatctggaagcggaccagcatgaagctgctggaccaagtggtgccccct
 I  I  K  K  I  W  K  R  T  S  M  K  L  L  D  Q  V  V  P  P
gcaggcgatgatgaggtcacagtcggcaagttctacgctaccttcctgatccaagagtac
 A  G  D  D  E  V  T  V  G  K  F  Y  A  T  F  L  I  Q  E  Y
ttccggaaattcaagaagcgcaaagagcaagggcttgtgggcaagccctcccagaggaat
 F  R  K  F  K  K  R  K  E  Q  G  L  V  G  K  P  S  Q  R  N
gcccttccctgcaggctggcctgcgcactctgcacgacatcgggcctgagatccgacgg
 A  L  S  L  Q  A  G  L  R  T  L  H  D  I  G  P  E  I  R  R
gccatctccggagacctgacagctgaggaagagctggacaaggccatgaaggaggctgtg
 A  I  S  G  D  L  T  A  E  E  E  L  D  K  A  M  K  E  A  V
tctgctgcctctgaagatgacatcttcaggagggccggtggcctgtttggcaaccatgtc
 S  A  A  S  E  D  D  I  F  R  R  A  G  G  L  F  G  N  H  V
agctactaccaaagtgacagccggagcgccttcccccagaccttcactacgcagcgccca
 S  Y  Y  Q  S  D  S  R  S  A  F  P  Q  T  F  T  T  Q  R  P
ctgcacatcagcaaggctggcaacaaccaaggcgacaccgagtcaccctcccacgagaag
 L  H  I  S  K  A  G  N  N  Q  G  D  T  E  S  P  S  H  E  K
ctggtggactccacttcacccccagcagctactcgtccaccggctccaacgccaacatc
 L  V  D  S  T  F  T  P  S  S  Y  S  S  T  G  S  N  A  N  I
aacaatgccaacaacactgccctgggccgcctcccccgccccgccggctaccccagcaca
 N  N  A  N  N  T  A  L  G  R  L  P  R  P  A  G  Y  P  S  T
gtcagcactgtggagggccacgggtccccctttgtctcctgccgtccgggcacaggaggca
 V  S  T  V  E  G  H  G  S  P  L  S  P  A  V  R  A  Q  E  A
gcatggaagctcagctccaagagatgccactcccaggagagccagatagccatggcgtgt
 A  W  K  L  S  S  K  R  C  H  S  Q  E  S  Q  I  A  M  A  C
caggagggcgcatcccaggacgacaactacgacgtgaggatcggtgaagatgcagagtgc
 Q  E  G  A  S  Q  D  D  N  Y  D  V  R  I  G  E  D  A  E  C
tgcagtgagcccagcctgctctccacagagatgctctcctaccaggatgacgaaaaccga
 C  S  E  P  S  L  L  S  T  E  M  L  S  Y  Q  D  D  E  N  R
caactggcgccccccggaggaggagaagcgggacatcaggctgtctccaaagaagggtttc
 Q  L  A  P  P  E  E  E  K  R  D  I  R  L  S  P  K  K  G  F
ctgcgctccgcatcactgggtcgaagggcttccttccacctggagtgtctgaagcggcag
 L  R  S  A  S  L  G  R  R  A  S  F  H  L  E  C  L  K  R  Q
aagaatcaaggggagacatctctcagaagacagtcctgcccctgcatctggtccaccac
 K  N  Q  G  G  D  I  S  Q  K  T  V  L  P  L  H  L  V  H  H
```

IQ-Motiv

C-terminal cytoplasmic tail

```
caggcattggcagtggcgggcctgagtcccctcctgcagagaagccattcccccacctcg
 Q  A  L  A  V  A  G  L  S  P  L  L  Q  R  S  H  S  P  T  S
ctccctaggccctgtgccacgccccctgccacaccgggcagccgaggctggcccccacag
 L  P  R  P  C  A  T  P  P  A  T  P  G  S  R  G  W  P  P  Q
cccatccccaccctgcggctggaggggccgactccagtgagaaactcaacagcagcttc
 P  I  P  T  L  R  L  E  G  A  D  S  S  E  K  L  N  S  S  F
ccgtccatccactgcggctcatggtctggggagaacagcccctgcagaggggacagcagc
 P  S  I  H  C  G  S  W  S  G  E  N  S  P  C  R  G  D  S  S
gccgcccggagagcccggcccgtctccctcactgtgcccagccaggctggggcccagggg
 A  A  R  R  A  R  P  V  S  L  T  V  P  S  Q  A  G  A  Q  G
agacagttccatggcagcgccagcagcctggtggaagcggtcttgatttccgaaggactg
 R  Q  F  H  G  S  A  S  S  L  V  E  A  V  L  I  S  E  G  L
gggcagtttgctcaagatcccaagttcatcgaggtcacgacccaggagctggctgacgcc
 G  Q  F  A  Q  D  P  K  F  I  E  V  T  T  Q  E  L  A  D  A
tgcgatctgaccatagaggagatggagaacgcggccgacgacattctcagcgggggcgcc
 C  D  L  T  I  E  E  M  E  N  A  A  D  D  I  L  S  G  G  A
cggcagagccccaatggcaccctgttacccttttgtgaaccgcagggacccgggccgggac
 R  Q  S  P  N  G  T  L  L  P  F  V  N  R  R  D  P  G  R  D
agagcggggcagaacgagcaggacgcgagcggcgcatgcgccccagggtgcgggcagagc
 R  A  G  Q  N  E  Q  D  A  S  G  A  C  A  P  G  C  G  Q  S
gaggaggccctcgcggaccgcagggccggcgtcagcagcctgtaggcgccagggccgggg
 E  E  A  L  A  D  R  R  A  G  V  S  S  L  ■  A  P  G  P  G
gtgcgggtttttttatttgtctcaatgttcctaatgggttcgtttcagaagtgcctcactg
 V  R  V  F  Y  L  S  Q  C  S  -  W  V  R  F  R  S  A  S  L
ttctcgtgacctggagttaaccggaacagcgtcttcattcatttctgttgggacgagacg
 F  S  -  P  G  V  N  R  N  S  V  F  I  H  F  C  W  D  E  T
caggctgggaggtgtggagccctctgtgtccgcagaggcgaggaggggcggcggccgcg
 Q  A  G  R  C  G  A  L  C  V  R  R  G  E  E  G  A  A  A  A
ggaaggggagagacccgccctcgagctctgctccaggcgcgccccgggcgggaaagagaa
 G  R  G  E  T  R  P  R  A  L  L  Q  A  R  P  G  R  E  R  E
cctcagctttctgcggtggccctcgctcgccaaaaggaccctgaaccaaacgggtgtctt
 P  Q  L  S  A  V  A  L  A  R  Q  K  D  P  E  P  N  G  C  L
tcaactttgcttgt
 S  T  L  L
```

Stop codon

pGEX-6P2 + Cytoplasmic C-Terminus of CACNA 1C (EcoRI/XhoI)

pGEX-6P-2 (4985 bp)

```
ACGTTATCGACTGCACGGTGCACCAATGCTTCTGGCGTCAGGCAGCCATCGGAAGCTGTGGTATGGCTGTGCAGG
TCGTAAATCACTGCATAATTCGTGTCGCTCAAGGCGCACTCCCGTTCTGGATAATGTTTTTTGCGCCGACATCAT
AACGGTTCTGGCAAATATTCTGAAATGAGCTGTTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAG
CGGATAACAATTTCACACAGGAAACAGTATTCATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGC
AACCCACTCGACTTCTTTTGGAATATCTTGAAGAAAAATATGAAGAGCATTTGTATGAGCGCGATGAAGGTGATA
AATGGCGAAACAAAAAGTTTGAATTGGGTTTGGAGTTTCCCAATCTTCCTTATTATATTGATGGTGATGTTAAAT
TAACACAGTCTATGGCCATCATACGTTATATAGCTGACAAGCACAACATGTTGGGTGGTTGTCCAAAAGAGCGTG
CAGAGATTTCAATGCTTGAAGGAGCGGTTTTGGATATTAGATACGGTGTTTCGAGAATTGCATATAGTAAAGACT
TTGAAACTCTCAAAGTTGATTTTCTTAGCAAGCTACCTGAAATGCTGAAAATGTTCGAAGATCGTTTATGTCATA
AAACATATTTAAATGGTGATCATGTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTATACA
TGGACCCAATGTGCCTGGATGCGTTCCCAAAATTAGTTTGTTTTAAAAAACGTATTGAAGCTATCCCACAAATTG
ATAAGTACTTGAAATCCAGCAAGTATATAGCATGGCCTTTGCAGGGCTGGCAAGCCACGTTTGGTGGTGGCGACC
ATCCTCCAAAATCGGATCTGGAAGTTCTGTTCCAGGGGCCCCTGGGATCCCCAGGAATTCCCGGGTCGACTCGAG
CGGCCGCATCGTGACTGACTGACGATCTGCCTCGCGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAG
CTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGT
GTTGGCGGGTGTCGGGGCGCAGCCATGACCCAGTCACGTAGCGATAGCGGAGTGTATAATTCTTGAAGACGAAAG
GGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTT
CGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAA
TAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATT
CCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGAT
CAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAA
GAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTGTTGACGCCGGGCAA
GAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTT
ACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTT
CTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGAT
CGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGCAGCAATGGCAACA
```

ACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCG
GATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGT
GAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACG
ACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGG
TAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCTAG
GTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCC
GTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCA
CCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGA
GCGCAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCT
ACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGAC
TCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAG
CGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAG
GCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGG
TATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGG
AGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTC
TTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGC
CGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCTGATGCGGTATTTTCTCCTTACGCAT
CTGTGCGGTATTTCACACCGCATAAATTCCGACACCATCGAATGGTGCAAAACCTTTCGCGGTATGGCATGATAG
CGCCCGGAAGAGAGTCAATTCAGGGTGGTGAATGTGAAACCAGTAACGTTATACGATGTCGCAGAGTATGCCGGT
GTCTCTTATCAGACCGTTTCCCGCGTGGTGAACCAGGCCAGCCACGTTTCTGCGAAAACGCGGGAAAAAGTGGAA
GCGGCGATGGCGGAGCTGAATTACATTCCCAACCGCGTGGCACAACAACTGGCGGGCAAACAGTCGTTGCTGATT
GGCGTTGCCACCTCCAGTCTGGCCCTGCACGCGCCGTCGCAAATTGTCGCGGCGATTAAATCTCGCGCCGATCAA
CTGGGTGCCAGCGTGGTGGTGTCGATGGTAGAACGAAGCGGCGTCGAAGCCTGTAAAGCGGCGGTGCACAATCTT
CTCGCGCAACGCGTCAGTGGGCTGATCATTAACTATCCGCTGGATGACCAGGATGCCATTGCTGTGGAAGCTGCC
TGCACTAATGTTCCGGCGTTATTTCTTGATGTCTCTGACCAGACACCCATCAACAGTATTATTTTCTCCCATGAA
GACGGTACGCGACTGGGCGTGGAGCATCTGGTCGCATTGGGTCACCAGCAAATCGCGCTGTTAGCGGGCCCATTA
AGTTCTGTCTCGGCGCGTCTGCGTCTGGCTGGCTGGCATAAATATCTCACTCGCAATCAAATTCAGCCGATAGCG
GAACGGGAAGGCGACTGGAGTGCCATGTCCGGTTTTCAACAAACCATGCAAATGCTGAATGAGGGCATCGTTCCC
ACTGCGATGCTGGTTGCCAACGATCAGATGGCGCTGGGCGCAATGCGCGCCATTACCGAGTCCGGGCTGCGCGTT
GGTGCGGATATCTCGGTAGTGGGATACGACGATACCGAAGACAGCTCATGTTATATCCCGCCGTCAACCACCATC
AAACAGGATTTTCGCCTGCTGGGGCAAACCAGCGTGGACCGCTTGCTGCAACTCTCTCAGGGCCAGGCGGTGAAG
GGCAATCAGCTGTTGCCCGTCTCACTGGTGAAAAGAAAAACCACCCTGGCGCCCAATACGCAAACCGCCTCTCCC
CGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGC
AATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGG
AATTGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGACCATGATTACGGATTCACTGGCCGTCGTTTTAC
AACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGC
GTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCTTTGCCT
GGTTTCCGGCACCAGAAGCGGTGCCGGAAAGCTGGCTGGAGTGCGATCTTCCTGAGGCCGATACTGTCGTCGTCC
CCTCAAACTGGCAGATGCACGGTTACGATGCGCCCATCTACACCAACGTAACCTATCCCATTACGGTCAATCCGC
CGTTTGTTCCCACGGAGAATCCGACGGGTTGTTACTCGCTCACATTTAATGTTGATGAAAGCTGGCTACAGGAAG
GCCAGACGCGAATTATTTTTGATGGCGTTGGAATT

pGEX-6P2 + Cytoplasmic C-Terminus of CACNA 1C

ACGTTATCGACTGCACGGTGCACCAATGCTTCTGGCGTCAGGCAGCCATCGGAAGCTGTGGTATGGCTGTGCAGG
TCGTAAATCACTGCATAATTCGTGTCGCTCAAGGCGCACTCCCGTTCTGGATAATGTTTTTTGCGCCGACATCAT
AACGGTTCTGGCAAATATTCTGAAATGAGCTGTTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAG
CGGATAACAATTTCACACAGGAAACAGTATTCATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGC
AACCCACTCGACTTCTTTTGGAATATCTTGAAGAAAAATATGAAGAGCATTTGTATGAGCGCGATGAAGGTGATA
AATGGCGAAACAAAAAGTTTGAATTGGGTTTGGAGTTTCCCAATCTTCCTTATTATATTGATGGTGATGTTAAAT
TAACACAGTCTATGGCCATCATACGTTATATAGCTGACAAGCACAACATGTTGGGTGGTTGTCCAAAAGAGCGTG
CAGAGATTTCAATGCTTGAAGGAGCGGTTTTGGATATTAGATACGGTGTTTCGAGAATTGCATATAGTAAAGACT
TTGAAACTCTCAAAGTTGATTTTCTTAGCAAGCTACCTGAAATGCTGAAAATGTTCGAAGATCGTTTATGTCATA
AAACATATTTAAATGGTGATCATGTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTATACA
TGGACCCAATGTGCCTGGATGCGTTCCCAAAATTAGTTTGTTTTAAAAAACGTATTGAAGCTATCCCACAAATTG
ATAAGTACTTGAAATCCAGCAAGTATATAGCATGGCCTTTGCAGGGCTGGCAAGCCACGTTTGGTGGTGGCGACC
ATCCTCCAAAATCGGATCTGGAAGTTCTGTTCCAGGGGCCCCTGGGATCCCCAGGAATTCCCGACAACTTTGACT
ACCTGACAAGGGACTGGTCAATCCTTGGTCCCCACCATCTGGATGAATTTAAAAGAATCTGGGCAGAGTATGACC
CTGAAGCCAAGGGTCGTATCAAACACCTGGATGTGGTGACCCTCCTCCGGCGGATTCAGCCCCCACTGGGTTTTG
GGAAGCTGTGCCCTCACCGTGTGGCTTGCAAACGCCTGGTCTCCATGAACATGCCTCTGAACAGTGACGGGACGG
TCATGTTCAACGCCACCCTGTTTGCCCTGGTCAGGACAGCTCTGAGGATCAAAACAGAAGGAAACCTGGAACAAG
CCAATGAGGAGCTGCGGGCCATCATCAAGAAGATCTGGAAGCGGACCAGCATGAAGCTGCTGGACCAAGTGGTGC

C-terminal cytoplasmic tail

IQ-Motiv

CCCCTGCAGGCGATGATGAGGTCACAGTCGGCAAGTTCTACGCTACCTTCCTGATCCAAGAGTACTTCCGGAAAT
TCAAGAAGCGCAAAGAGCAAGGGCTTGTGGGCAAGCCCTCCCAGAGGAATGCCCTTTCCCTGCAGGCTGGCCTGC
GCACTCTGCACGACATCGGGCCTGAGATCCGACGGGCCATCTCCGGAGACCTGACAGCTGAGGAAGAGCTGGACA
AGGCCATGAAGGAGGCTGTGTCTGCTGCCTCTGAAGATGACATCTTCAGGAGGGCCGGTGGCCTGTTTGGCAACC
ATGTCAGCTACTACCAAAGTGACAGCCGGAGCGCCTTCCCCCAGACCTTCACTACGCAGCGCCCACTGCACATCA
GCAAGGCTGGCAACAACCAAGGCGACACCGAGTCACCCTCCCACGAGAAGCTGGTGGACTCCACTTTCACCCCCA
GCAGCTACTCGTCCACCGGCTCCAACGCCAACATCAACAATGCCAACAACACTGCCCTGGGCCGCCTCCCCCGCC
CCGCCGGCTACCCCAGCACAGTCAGCACTGTGGAGGGCCACGGGTCCCCCTTGTCTCCTGCCGTCCGGGCACAGG
AGGCAGCATGGAAGCTCAGCTCCAAGAGATGCCACTCCCAGGAGAGCCAGATAGCCATGGCGTGTCAGGAGGGCG
CATCCCAGGACGACAACTACGACGTGAGGATCGGTGAAGATGCAGAGTGCTGCAGTGAGCCCAGCCTGCTCTCCA **TAG = STOP**
CAGAGATGCTCTCCTACCAGGATGACGAAAACCGACAACTGGCGCCCCCGGAGGAGGAGAAGCGGGACATCAGGC
TGTCTCCAAAGAAGGGTTTCCTGCGCTCCGCATCACTGGGTCGAAGGGCTTCCTTCCACCTGGAGTGTCTGAAGC
GGCAGAAGAATCAAGGGGGAGACATCTCTCAGAAGACAGTCCTGCCCCTGCATCTGGTCCACCACCAGGCATTGG
CAGTGGCGGGCCTGAGTCCCCTCCTGCAGAGAAGCCATTCCCCCACCTCGCTCCCTAGGCCCTGTGCCACGCCCC
CTGCCACACCGGGCAGCCGAGGCTGGCCCCCACAGCCCATCCCCACCCTGCGGCTGGAGGGGGCCGACTCCAGTG
AGAAACTCAACAGCAGCTTCCCGTCCATCCACTGCGGCTCATGGTCTGGGGAGAACAGCCCCTGCAGAGGGGACA
GCAGCGCCGCCCGGAGAGCCCGGCCCGTCTCCCTCACTGTGCCCAGCCAGGCTGGGGCCCAGGGGAGACAGTTCC
ATGGCAGCGCCAGCAGCCTGGTGGAAGCGGTCTTGATTTCCGAAGGACTGGGGCAGTTTGCTCAAGATCCCAAGT
TCATCGAGGTCACGACCCAGGAGCTGGCTGACGCCTGCGATCTGACCATAGAGGAGATGGAGAACGCGGCCGACG
ACATTCTCAGCGGGGGCGCCCGGCAGAGCCCCAATGGCACCCTGTTACCCTTTGTGAACCGCAGGGACCCGGGCC
GGGACAGAGCGGGGCAGAACGAGCAGGACGCGAGCGGCGCATGCGCCCCAGGGTGCGGGCAGAGCGAGGAGGCCC
TCGCGGACCGCAGGGCCGGCGTCAGCAGCCTGTAGCTCGAGCGGCCGCATCGTGACTGACTGACGATCTGCCTCG
CGCGTTTCGGTGATGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGG
ATGCCGGGAGCAGACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCGCAGCCATGACCCAGT
CACGTAGCGATAGCGGAGTGTATAATTCTTGAAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATG
TCATGATAATAATGGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTAT
TTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAA
GGAAGAGTATGAGTATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTG
CTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGG
ATCTCAACAGCGGTAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTC
TGCTATGTGGCGCGGTATTATCCCGTGTTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGA
ATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTG
CTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCG
CTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAA
ACGACGAGCGTGACACCACGATGCCTGCAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTA
CTCTAGCTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCC
TTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGG
GGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATA
GACAGATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTT
AGATTGATTTAAAACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAA
TCCCTTAACGTGAGTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTT
TTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAG
AGCTACCAACTCTTTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAGC
CGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGG
CTGCTGCCAGTGGCGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGT
CGGGCTGAACGGGGGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGC
GTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAA
CAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCT
GACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTT
TACGGTTCCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACC
GTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGG
AAGCGGAAGAGCGCCTGATGCGGTATTTTCTCCTTACGCATCTGTGCGGTATTTCACACCGCATAAATTCCGACA
CCATCGAATGGTGCAAAACCTTTCGCGGTATGGCATGATAGCGCCCGGAAGAGAGTCAATTCAGGGTGGTGAATG
TGAAACCAGTAACGTTATACGATGTCGCAGAGTATGCCGGTGTCTCTTATCAGACCGTTTCCCGCGTGGTGAACC
AGGCCAGCCACGTTTCTGCGAAAACGCGGGAAAAAGTGGAAGCGGCGATGGCGGAGCTGAATTACATTCCCAACC
GCGTGGCACAACAACTGGCGGGCAAACAGTCGTTGCTGATTGGCGTTGCCACCTCCAGTCTGGCCCTGCACGCGC
CGTCGCAAATTGTCGCGGCGATTAAATCTCGCGCCGATCAACTGGGTGCCAGCGTGGTGGTGTCGATGGTAGAAC
GAAGCGGCGTCGAAGCCTGTAAAGCGGCGGTGCACAATCTTCTCGCGCAACGCGTCAGTGGGCTGATCATTAACT
ATCCGCTGGATGACCAGGATGCCATTGCTGTGGAAGCTGCCTGCACTAATGTTCCGGCGTTATTTCTTGATGTCT
CTGACCAGACACCCATCAACAGTATTATTTTCTCCCATGAAGACGGTACGCGACTGGGCGTGGAGCATCTGGTCG
CATTGGGTCACCAGCAAATCGCGCTGTTAGCGGGCCCATTAAGTTCTGTCTCGGCGCGTCTGCGTCTGGCTGGCT
GGCATAAATATCTCACTCGCAATCAAATTCAGCCGATAGCGGAACGGGAAGGCGACTGGAGTGCCATGTCCGGTT
TTCAACAAACCATGCAAATGCTGAATGAGGGCATCGTTCCCACTGCGATGCTGGTTGCCAACGATCAGATGGCGC

TGGGCGCAATGCGCGCCATTACCGAGTCCGGGCTGCGCGTTGGTGCGGATATCTCGGTAGTGGGATACGACGATA
CCGAAGACAGCTCATGTTATATCCCGCCGTCAACCACCATCAAACAGGATTTTCGCCTGCTGGGGCAAACCAGCG
TGGACCGCTTGCTGCAACTCTCTCAGGGCCAGGCGGTGAAGGGCAATCAGCTGTTGCCCGTCTCACTGGTGAAAA
GAAAAACCACCCTGGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCAC
GACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCC
CAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAAC
AGCTATGACCATGATTACGGATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCA
ACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTC
CCAACAGTTGCGCAGCCTGAATGGCGAATGGCGCTTTGCCTGGTTTCCGGCACCAGAAGCGGTGCCGGAAAGCTG
GCTGGAGTGCGATCTTCCTGAGGCCGATACTGTCGTCGTCCCCTCAAACTGGCAGATGCACGGTTACGATGCGCC
CATCTACACCAACGTAACCTATCCCATTACGGTCAATCCGCCGTTTGTTCCCACGGAGAATCCGACGGGTTGTTA
CTCGCTCACATTTAATGTTGATGAAAGCTGGCTACAGGAAGGCCAGACGCGAATTATTTTTGATGGCGTTGGAAT
T

PCR-Primer for the GST fusion proteins:

GST_C_Cav_F_lang:

atcaatctctttgtagctgtcatcatggacaactttgactacctgacaagggactggtca
I N L F V A V I M D N F D Y L T R D W S

EcoRI

5'- c gGA ATT CCC gac aac ttt gac tac ctg aca ag - 3'

GST_C_CAV_F_kurz:

AGGCAGCATGGAAGCTCAGCTCCAAG

5'- c gGA ATT CCC agg cag cat gga agc tca gc

GST_C_CAV_R:

gaggaggccctcgcggaccgcagggccggcgtcagcagcctgtagcgccagggccgggg
E E A L A D R R A G V S S L - A P G P G

XhoI STOP

5' - ccgCTCGAGCTA CAG GCT GCT GAC GCC GGC - 3'

```
acgttatcgactgcacggtgcaccaatgcttctggcgtcaggcagccatcggaagctgtggt
   V  I  D  C  T  V  H  Q  C  F  W  R  Q  A  A  I  G  S  C  G
atggctgtgcaggtcgtaaatcactgcataattcgtgtcgctcaaggcgcactcccgttc
 M  A  V  Q  V  V  N  H  C  I  I  R  V  A  Q  G  A  L  P  F
tggataatgtttttgcgccgacatcataacggttctggcaaatattctgaaatgagctg
 W  I  M  F  F  A  P  T  S  -  R  F  W  Q  I  F  -  N  E  L
ttgacaattaatcatcggctcgtataatgtgtggaattgtgagcggataacaatttcaca
 L  T  I  N  H  R  L  V  -  C  V  E  L  *  A  D  N  N  F  T
caggaaacagtattcatgtcccctatactaggttattggaaaattaagggccttgtgcaa
 Q  E  T  V  F  M  S  P  I  L  G  Y  W  K  I  K  G  L  V  Q
cccactcgacttcttttggaatatcttgaagaaaaatatgaagagcatttgtatgagcgc
 P  T  R  L  L  L  E  Y  L  E  E  K  Y  E  E  H  L  Y  E  R
gatgaaggtgataaatggcgaaacaaaaagtttgaattgggtttggagtttcccaatctt
 D  E  G  D  K  W  R  N  K  K  F  E  L  G  L  E  F  P  N  L
ccttattatattgatggtgatgttaaattaacacagtctatggccatcatacgttatata
 P  Y  Y  I  D  G  D  V  K  L  T  Q  S  M  A  I  I  R  Y  I
gctgacaagcacaacatgttgggtggttgtccaaaagagcgtgcagagatttcaatgctt
 A  D  K  H  N  M  L  G  G  C  P  K  E  R  A  E  I  S  M  L
gaaggagcggttttggatattagatacggtgtttcgagaattgcatatagtaaagacttt
 E  G  A  V  L  D  I  R  Y  G  V  S  R  I  A  Y  S  K  D  F
gaaactctcaaagttgattttcttagcaagctacctgaaatgctgaaaatgttcgaagat
 E  T  L  K  V  D  F  L  S  K  L  P  E  M  L  K  M  F  E  D
cgtttatgtcataaaacatatttaaatggtgatcatgtaacccatcctgacttcatgttg
 R  L  C  H  K  T  Y  L  N  G  D  H  V  T  H  P  D  F  M  L
tatgacgctcttgatgttgttttatacatggacccaatgtgcctggatgcgttcccaaaa
 Y  D  A  L  D  V  V  L  Y  M  D  P  M  C  L  D  A  F  P  K
ttagtttgttttaaaaaacgtattgaagctatcccacaaattgataagtacttgaaatcc
 L  V  C  F  K  K  R  I  E  A  I  P  Q  I  D  K  Y  L  K  S
agcaagtatatagcatggccttgcagggctggcaagccacgtttggtggtggcgaccat
 S  K  Y  I  A  W  P  L  Q  G  W  Q  A  T  F  G  G  G  D  H
cctccaaaatcggatctggaagttctgttccaggggcccctgggatccccaggaattccc
 P  P  K  S  D  L  E  V  L  F  Q  G  P  L  G  S  P  G  I  P
gacaactttgactacctgacaagggactggtcaatccttggtccccaccatctggatgaa
 D  N  F  D  Y  L  T  R  D  W  S  I  L  G  P  H  H  L  D  E
tttaaaagaatctgggcagagtatgaccctgaagccaagggtcgtatcaaacacctggat
 F  K  R  I  W  A  E  Y  D  P  E  A  K  G  R  I  K  H  L  D
gtggtgaccctcctccggcggattcagccccactgggttttgggaagctgtgccctcac
 V  V  T  L  L  R  R  I  Q  P  P  L  G  F  G  K  L  C  P  H
cgtgtggcttgcaaacgcctggtctccatgaacatgcctctgaacagtgacgggacggtc
 R  V  A  C  K  R  L  V  S  M  N  M  P  L  N  S  D  G  T  V
atgttcaacgccaccctgtttgccctggtcaggacagctctgaggatcaaaacagaagga
 M  F  N  A  T  L  F  A  L  V  R  T  A  L  R  I  K  T  E  G
aacctggaacaagccaatgaggagctgcgggccatcatcaagaagatctggaagcggacc
 N  L  E  Q  A  N  E  E  L  R  A  I  I  K  K  I  W  K  R  T
agcatgaagctgctggaccaagtggtgccccctgcaggcgatgatgaggtcacagtcggc
 S  M  K  L  L  D  Q  V  V  P  P  A  G  D  D  E  V  T  V  G
aagttctacgctaccttcctgatccaagagtacttccggaaattcaagaagcgcaaagag
 K  F  Y  A  T  F  L  I  Q  E  Y  F  R  K  F  K  K  R  K  E
caagggcttgtgggcaagccctcccagaggaatgccctttccctgcaggctggcctgcgc
 Q  G  L  V  G  K  P  S  Q  R  N  A  L  S  L  Q  A  G  L  R
actctgcacgacatcgggcctgagatccgacgggccatctccggagacctgacagctgag
 T  L  H  D  I  G  P  E  I  R  R  A  I  S  G  D  L  T  A  E
gaagagctggacaaggccatgaaggaggctgtgtctgctgcctctgaagatgacatcttc
 E  E  L  D  K  A  M  K  E  A  V  S  A  A  S  E  D  D  I  F
aggagggccggtggcctgtttggcaaccatgtcagctactaccaaagtgacagccggagc
 R  R  A  G  G  L  F  G  N  H  V  S  Y  Y  Q  S  D  S  R  S
gccttcccccagaccttcactacgcagcgcccactgcacatcagcaaggctggcaacaac
 A  F  P  Q  T  F  T  T  Q  R  P  L  H  I  S  K  A  G  N  N
caaggcgacaccgagtcaccctcccacgagaagctggtggactccactttcacccccagc
 Q  G  D  T  E  S  P  S  H  E  K  L  V  D  S  T  F  T  P  S
agctactcgtccaccggctccaacgccaacatcaacaatgccaacaacactgccctgggc
 S  Y  S  S  T  G  S  N  A  N  I  N  N  A  N  N  T  A  L  G
cgcctcccccgcccccgccggctaccccagcacagtcagcactgtggagggccacgggtcc
 R  L  P  R  P  A  G  Y  P  S  T  V  S  T  V  E  G  H  G  S
cccttgtctcctgccgtccgggcacaggaggcagcatggaagctcagctccaagagatgc
 P  L  S  P  A  V  R  A  Q  E  A  A  W  K  L  S  S  K  R  C
cactcccaggagagccagatagccatggcgtgtcaggagggcgcatcccaggacgacaac
 H  S  Q  E  S  Q  I  A  M  A  C  Q  E  G  A  S  Q  D  D  N
tacgacgtgaggatcggtgaagatgcagagtgctgcagtgagcccagcctgctctccaca
 Y  D  V  R  I  G  E  D  A  E  C  C  S  E  P  S  L  L  S  T
```

M = methionin
Start codon

```
gagatgctctcctaccaggatgacgaaaaccgacaactggcgcccccggaggaggagaag
 E  M  L  S  Y  Q  D  D  E  N  R  Q  L  A  P  P  E  E  E  K
cgggacatcaggctgtctccaaagaagggtttcctgcgctccgcatcactgggtcgaagg
 R  D  I  R  L  S  P  K  K  G  F  L  R  S  A  S  L  G  R  R
gcttccttccacctggagtgtctgaagcggcagaagaatcaaggggggagacatctctcag
 A  S  F  H  L  E  C  L  K  R  Q  K  N  Q  G  G  D  I  S  Q
aagacagtcctgcccctgcatctggtccaccaccaggcattggcagtggcgggcctgagt
 K  T  V  L  P  L  H  L  V  H  H  Q  A  L  A  V  A  G  L  S
cccctcctgcagagaagccattccccacctcgctccctaggccctgtgccacgccccct
 P  L  L  Q  R  S  H  S  P  T  S  L  P  R  P  C  A  T  P  P
gccacaccgggcagccgaggctggccccacagcccatccccaccctgcggctggagggg
 A  T  P  G  S  R  G  W  P  P  Q  P  I  P  T  L  R  L  E  G
gccgactccagtgagaaactcaacagcagcttcccgtccatccactgcggctcatggtct
 A  D  S  S  E  K  L  N  S  S  F  P  S  I  H  C  G  S  W  S
ggggagaacagcccctgcagaggggacagcagcgccgcccggagagcccggcccgtctcc
 G  E  N  S  P  C  R  G  D  S  S  A  A  R  R  A  R  P  V  S
ctcactgtgcccagccaggctggggcccaggggagacagttccatggcagcgccagcagc
 L  T  V  P  S  Q  A  G  A  Q  G  R  Q  F  H  G  S  A  S  S
ctggtggaagcggtcttgatttccgaaggactggggcagtttgctcaagatcccaagttc
 L  V  E  A  V  L  I  S  E  G  L  G  Q  F  A  Q  D  P  K  F
atcgaggtcacgacccaggagctggctgacgcctgcgatctgaccatagaggagatggag
 I  E  V  T  T  Q  E  L  A  D  A  C  D  L  T  I  E  E  M  E
aacgcggccgacgacattctcagcgggggcgcccggcagagccccaatggcaccctgtta
 N  A  A  D  D  I  L  S  G  G  A  R  Q  S  P  N  G  T  L  L
ccctttgtgaaccgcagggacccgggccgggacagagcggggcagaacgagcaggacgcg
 P  F  V  N  R  R  D  P  G  R  D  R  A  G  Q  N  E  Q  D  A
agcggcgcatgcgccccagggtgcgggcagagcgaggaggccctcgcggaccgcagggcc
 S  G  A  C  A  P  G  C  G  Q  S  E  E  A  L  A  D  R  R  A
ggcgtcagcagcctgtagctcgagcggccgcatcgtgactgactgacgatctgcctcgcg
 G  V  S  S  L  *  L  E  R  P  H  R  D  -  L  T  I  C  L  A
cgtttcggtgatgacggtgaaaacctctgacacatgcagctcccggagacggtcacagct
 R  F  G  D  D  G  E  N  L  -  H  M  Q  L  P  E  T  V  T  A
tgtctgtaagcggatgccgggagcagacaagcccgtcagggcgcgtcagcgggtgttggc
 C  L  -  A  D  A  G  S  R  Q  A  R  Q  G  A  S  A  G  V  G
gggtgtcggggcgcagccatgacccagtcacgtagcgatagcggagtgtataattcttga
 G  C  R  G  A  A  M  T  Q  S  R  S  D  S  G  V  Y  N  S  -
agacgaaagggcctcgtgatacgcctatttttataggttaatgtcatgataataatggtt
 R  R  K  G  L  V  I  R  L  F  L  -  V  N  V  M  I  I  M  V
tcttagacgtcaggtggcacttttcggggaaatgtgcgcggaacccctatttgtttattt
 S  -  T  S  G  G  T  F  R  G  N  V  R  G  T  P  I  C  L  F
ttctaaatacattcaaatatgtatccgctcatgagacaataaccctgataaatgcttcaa
 F  -  I  H  S  N  M  Y  P  L  M  R  Q  -  P  -  -  M  L  Q
taatattgaaaaaggaagagtatgagtattcaacatttccgtgtcgcccttattccctttt
 -  Y  -  K  R  K  S  M  S  I  Q  H  F  R  V  A  L  I  P  F
tttgcggcattttgccttcctgtttttgctcacccagaaacgctggtgaaagtaaaagat
 F  A  A  F  C  L  P  V  F  A  H  P  E  T  L  V  K  V  K  D
gctgaagatcagttgggtgcacgagtgggttacatcgaactggatctcaacagcggtaag
 A  E  D  Q  L  G  A  R  V  G  Y  I  E  L  D  L  N  S  G  K
atccttgagagttttcgccccgaagaacgttttccaatgatgagcacttttaaagttctg
 I  L  E  S  F  R  P  E  E  R  F  P  M  M  S  T  F  K  V  L
ctatgtggcgcggtattatcccgtgttgacgccgggcaagagcaactcggtcgccgcata
 L  C  G  A  V  L  S  R  V  D  A  G  Q  E  Q  L  G  R  R  I
cactattctcagaatgacttggttgagtactcaccagtcacagaaaagcatcttacggat
 H  Y  S  Q  N  D  L  V  E  Y  S  P  V  T  E  K  H  L  T  D
ggcatgacagtaagagaattatgcagtgctgccataaccatgagtgataacactgcggcc
 G  M  T  V  R  E  L  C  S  A  A  I  T  M  S  D  N  T  A  A
aacttacttctgacaacgatcggaggaccgaaggagctaaccgcttttttgcacaacatg
 N  L  L  L  T  T  I  G  G  P  K  E  L  T  A  F  L  H  N  M
ggggatcatgtaactcgccttgatcgttgggaaccggagctgaatgaagccataccaaac
 G  D  H  V  T  R  L  D  R  W  E  P  E  L  N  E  A  I  P  N
gacgagcgtgacaccacgatgcctgcagcaatggcaacaacgttgcgcaaactattaact
 D  E  R  D  T  T  M  P  A  A  M  A  T  T  L  R  K  L  L  T
ggcgaactacttactctagcttcccggcaacaattaatagactggatggaggcggataaa
 G  E  L  L  T  L  A  S  R  Q  Q  L  I  D  W  M  E  A  D  K
gttgcaggaccacttctgcgctcggcccttccggctggctggtttattgctgataaatct
 V  A  G  P  L  L  R  S  A  L  P  A  G  W  F  I  A  D  K  S
ggagccggtgagcgtgggtctcgcggtatcattgcagcactggggccagatggtaagccc
 G  A  G  E  R  G  S  R  G  I  I  A  A  L  G  P  D  G  K  P
tcccgtatcgtagttatctacacgacggggagtcaggcaactatggatgaacgaaataga
 S  R  I  V  V  I  Y  T  T  G  S  Q  A  T  M  D  E  R  N  R
```

Stop codon

```
cagatcgctgagataggtgcctcactgattaagcattggtaactgtcagaccaagtttac
 Q  I  A  E  I  G  A  S  L  I  K  H  W  -  L  S  D  Q  V  Y
tcatatatactttagattgatttaaaacttcatttttaatttaaaaggatctaggtgaag
 S  Y  I  L  -  I  D  L  K  L  H  F  -  F  K  R  I  -  V  K
atccttttttgataatctcatgaccaaaatcccttaacgtgagttttcgttccactgagcg
 I  L  F  D  N  L  M  T  K  I  P  -  R  E  F  S  F  H  -  A
tcagaccccgtagaaaagatcaaaggatcttcttgagatccttttttttctgcgcgtaatc
 S  D  P  V  E  K  I  K  G  S  S  -  D  P  F  F  L  R  V  I
tgctgcttgcaaacaaaaaaaccaccgctaccagcggtggtttgtttgccggatcaagag
 C  C  L  Q  T  K  K  P  P  L  P  A  V  V  C  L  P  D  Q  E
ctaccaactctttttccgaaggtaactggcttcagcagagcgcagataccaaatactgtc
 L  P  T  L  F  P  K  V  T  G  F  S  R  A  Q  I  P  N  T  V
cttctagtgtagccgtagttaggccaccacttcaagaactctgtagcaccgcctacatac
 L  L  V  -  P  -  L  G  H  H  F  K  N  S  V  A  P  P  T  Y
ctcgctctgctaatcctgttaccagtggctgctgccagtggcgataagtcgtgtcttacc
 L  A  L  L  I  L  L  P  V  A  A  A  S  G  D  K  S  C  L  T
gggttggactcaagacgatagttaccggataaggcgcagcggtcgggctgaacgggggggt
 G  L  D  S  R  R  -  L  P  D  K  A  Q  R  S  G  -  T  G  G
tcgtgcacacagcccagcttggagcgaacgacctacaccgaactgagatacctacagcgt
 S  C  T  Q  P  S  L  E  R  T  T  Y  T  E  L  R  Y  L  Q  R
gagctatgagaaagcgccacgcttcccgaagggagaaaggcggacaggtatccggtaagc
 E  L  -  E  S  A  T  L  P  E  G  R  K  A  D  R  Y  P  V  S
ggcagggtcggaacaggagagcgcacgagggagcttccagggggaaacgcctggtatctt
 G  R  V  G  T  G  E  R  T  R  E  L  P  G  G  N  A  W  Y  L
tatagtcctgtcgggtttcgccacctctgacttgagcgtcgatttttgtgatgctcgtca
 Y  S  P  V  G  F  R  H  L  -  L  E  R  R  F  L  -  C  S  S
gggggggcggagcctatggaaaaacgccagcaacgcggccttttacggttcctggccttt
 G  G  R  S  L  W  K  N  A  S  N  A  A  F  L  R  F  L  A  F
tgctggccttttgctcacatgttctttcctgcgttatcccctgattctgtggataaccgt
 C  W  P  F  A  H  M  F  F  P  A  L  S  P  D  S  V  D  N  R
attaccgcctttgagtgagctgataccgctcgccgcagccgaacgaccgagcgcagcgag
 I  T  A  F  E  -  A  D  T  A  R  R  S  R  T  T  E  R  S  E
tcagtgagcgaggaagcggaagagcgcctgatgcggtattttctccttacgcatctgtgc
 S  V  S  E  E  A  E  E  R  L  M  R  Y  F  L  L  T  H  L  C
ggtatttcacaccgcataaattccgacaccatcgaatggtgcaaaacctttcgcggtatg
 G  I  S  H  R  I  N  S  D  T  I  E  W  C  K  T  F  R  G  M
gcatgatagcgcccggaagagagtcaattcagggtggtgaatgtgaaaccagtaacgtta
 A  -  -  R  P  E  E  S  Q  F  R  V  V  N  V  K  P  V  T  L
tacgatgtcgcagagtatgccggtgtctcttatcagaccgtttcccgcgtggtgaaccag
 Y  D  V  A  E  Y  A  G  V  S  Y  Q  T  V  S  R  V  V  N  Q
gccagccacgtttctgcgaaaacgcgggaaaaagtggaagcggcgatggcggagctgaat
 A  S  H  V  S  A  K  T  R  E  K  V  E  A  A  M  A  E  L  N
tacattcccaaccgcgtggcacaacaactggcgggcaaacagtcgttgctgattggcgtt
 Y  I  P  N  R  V  A  Q  Q  L  A  G  K  Q  S  L  L  I  G  V
gccacctccagtctggccctgcacgcgccgtcgcaaattgtcgcggcgattaaatctcgc
 A  T  S  S  L  A  L  H  A  P  S  Q  I  V  A  A  I  K  S  R
gccgatcaactgggtgccagcgtggtggtgtcgatggtagaacgaagcggcgtcgaagcc
 A  D  Q  L  G  A  S  V  V  V  S  M  V  E  R  S  G  V  E  A
tgtaaagcggcggtgcacaatcttctcgcgcaacgcgtcagtgggctgatcattaactat
 C  K  A  A  V  H  N  L  L  A  Q  R  V  S  G  L  I  I  N  Y
ccgctggatgaccaggatgccattgctgtggaagctgcctgcactaatgttccggcgtta
 P  L  D  D  Q  D  A  I  A  V  E  A  A  C  T  N  V  P  A  L
tttcttgatgtctctgaccagacacccatcaacagtattattttctcccatgaagacggt
 F  L  D  V  S  D  Q  T  P  I  N  S  I  I  F  S  H  E  D  G
acgcgactgggcgtggagcatctggtcgcattgggtcaccagcaaatcgcgctgttagcg
 T  R  L  G  V  E  H  L  V  A  L  G  H  Q  Q  I  A  L  L  A
ggcccattaagttctgtctcggcgcgtctgcgtctggctggctggcataaatatctcact
 G  P  L  S  S  V  S  A  R  L  R  L  A  G  W  H  K  Y  L  T
cgcaatcaaattcagccgatagcggaacgggaaggcgactggagtgccatgtccggtttt
 R  N  Q  I  Q  P  I  A  E  R  E  G  D  W  S  A  M  S  G  F
caacaaaccatgcaaatgctgaatgagggcatcgttcccactgcgatgctggttgccaac
 Q  Q  T  M  Q  M  L  N  E  G  I  V  P  T  A  M  L  V  A  N
gatcagatggcgctgggcgcaatgcgcgccattaccgagtccgggctgcgcgttggtgcg
 D  Q  M  A  L  G  A  M  R  A  I  T  E  S  G  L  R  V  G  A
gatatctcggtagtgggatacgacgataccgaagacagctcatgttatatcccgccgtca
 D  I  S  V  V  G  Y  D  D  T  E  D  S  S  C  Y  I  P  P  S
accaccatcaaacaggattttcgcctgctggggcaaaccagcgtggaccgcttgctgcaa
 T  T  I  K  Q  D  F  R  L  L  G  Q  T  S  V  D  R  L  L  Q
ctctctcagggccaggcggtgaagggcaatcagctgttgcccgtctcactggtgaaaaga
 L  S  Q  G  Q  A  V  K  G  N  Q  L  L  P  V  S  L  V  K  R
```

```
aaaaccaccctggcgcccaatacgcaaaccgcctctccccgcgcgttggccgattcatta
 K  T  T  L  A  P  N  T  Q  T  A  S  P  R  A  L  A  D  S  L
atgcagctggcacgacaggtttcccgactggaaagcgggcagtgagcgcaacgcaattaa
 M  Q  L  A  R  Q  V  S  R  L  E  S  G  Q  -  A  Q  R  N  -
tgtgagttagctcactcattaggcaccccaggctttacactttatgcttccggctcgtat
 C  E  L  A  H  S  L  G  T  P  G  F  T  L  Y  A  S  G  S  Y
gttgtgtggaattgtgagcggataacaatttcacacaggaaacagctatgaccatgatta
 V  V  W  N  C  E  R  I  T  I  S  H  R  K  Q  L  -  P  -  L
cggattcactggccgtcgttttacaacgtcgtgactgggaaaaccctggcgttacccaac
 R  I  H  W  P  S  F  Y  N  V  V  T  G  K  T  L  A  L  P  N
ttaatcgccttgcagcacatccccctttcgccagctggcgtaatagcgaagaggcccgca
 L  I  A  L  Q  H  I  P  L  S  P  A  G  V  I  A  K  R  P  A
ccgatcgcccttcccaacagttgcgcagcctgaatggcgaatggcgctttgcctggtttc
 P  I  A  L  P  N  S  C  A  A  -  M  A  N  G  A  L  P  G  F
cggcaccagaagcggtgccggaaagctggctggagtgcgatcttcctgaggccgatactg
 R  H  Q  K  R  C  R  K  A  G  W  S  A  I  F  L  R  P  I  L
tcgtcgtcccctcaaactggcagatgcacggttacgatgcgcccatctacaccaacgtaa
 S  S  S  P  Q  T  G  R  C  T  V  T  M  R  P  S  T  P  T  -
cctatcccattacggtcaatccgccgtttgttcccacggagaatccgacgggttgttact
 P  I  P  L  R  S  I  R  R  L  F  P  R  R  I  R  R  V  V  T
cgctcacatttaatgttgatgaaagctggctacaggaaggccagacgcgaattatttttg
 R  S  H  L  M  L  M  K  A  G  Y  R  K  A  R  R  E  L  F  L
atggcgttggaatt
 M  A  L  E
```

```
MSPILGYWKIKGLVQPTRLLLEYLEEKYEEHLYERDEGDKWRNKKFELGLEFPNLPYYIDGDVKLTQSMAIIRYI
ADKHNMLGGCPKERAEISMLEGAVLDIRYGVSRIAYSKDFETLKVDFLSKLPEMLKMFEDRLCHKTYLNGDHVTH
PDFMLYDALDVVLYMDPMCLDAFPKLVCFKKRIEAIPQIDKYLKSSKYIAWPLQGWQATFGGGDHPPKSDLEVLF
QGPLGSPGIPDNFDYLTRDWSILGPHHLDEFKRIWAEYDPEAKGRIKHLDVVTLLRRIQPPLGFGKLCPHRVACK
RLVSMNMPLNSDGTVMFNATLFALVRTALRIKTEGNLEQANEELRAIIKKIWKRTSMKLLDQVVPPAGDDEVTVG
KFYATFLIQEYFRKFKKRKEQGLVGKPSQRNALSLQAGLRTLHDIGPEIRRAISGDLTAEEELDKAMKEAVSAAS
EDDIFRRAGGLFGNHVSYYQSDSRSAFPQTFTTQRPLHISKAGNNQGDTESPSHEKLVDSTFTPSSYSSTGSNAN
INNANNTALGRLPRPAGYPSTVSTVEGHGSPLSPAVRAQEAAWKLSSKRCHSQESQIAMACQEGASQDDNYDVRI
GEDAECCSEPSLLSTEMLSYQDDENRQLAPPEEEKRDIRLSPKKGFLRSASLGRRASFHLECLKRQKNQGGDISQ
KTVLPLHLVHHQALAVAGLSPLLQRSHSPTSLPRPCATPPATPGSRGWPPQPIPTLRLEGADSSEKLNSSFPSIH
CGSWSGENSPCRGDSSAARRARPVSLTVPSQAGAQGRQFHGSASSLVEAVLISEGLGQFAQDPKFIEVTTQELAD
ACDLTIEEMENAADDILSGGARQSPNGTLLPFVNRRDPGRDRAGQNEQDASGACAPGCGQSEEALADRRAGVSSL
■ Stop
```

Expasy/ProtParam (allows computation of physical and chemical parameters for a given protein sequence)

User-provided sequence:

```
        10         20         30         40         50         60
MSPILGYWKI KGLVQPTRLL LEYLEEKYEE HLYERDEGDK WRNKKFELGL EFPNLPYYID

        70         80         90        100        110        120
GDVKLTQSMA IIRYIADKHN MLGGCPKERA EISMLEGAVL DIRYGVSRIA YSKDFETLKV

       130        140        150        160        170        180
DFLSKLPEML KMFEDRLCHK TYLNGDHVTH PDFMLYDALD VVLYMDPMCL DAFPKLVCFK

       190        200        210        220        230        240
KRIEAIPQID KYLKSSKYIA WPLQGWQATF GGGDHPPKSD LEVLFQGPLG SPGIPDNFDY

       250        260        270        280        290        300
LTRDWSILGP HHLDEFKRIW AEYDPEAKGR IKHLDVVTLL RRIQPPLGFG KLCPHRVACK

       310        320        330        340        350        360
RLVSMNMPLN SDGTVMFNAT LFALVRTALR IKTEGNLEQA NEELRAIIKK IWKRTSMKLL

       370        380        390        400        410        420
DQVVPPAGDD EVTVGKFYAT FLIQEYFRKF KKRKEQGLVG KPSQRNALSL QAGLRTLHDI
```

```
       430        440        450        460        470        480
GPEIRRAISG DLTAEEELDK AMKEAVSAAS EDDIFRRAGG LFGNHVSYYQ SDSRSAFPQT

       490        500        510        520        530        540
FTTQRPLHIS KAGNNQGDTE SPSHEKLVDS TFTPSSYSST GSNANINNAN NTALGRLPRP

       550        560        570        580        590        600
AGYPSTVSTV EGHGSPLSPA VRAQEAAWKL SSKRCHSQES QIAMACQEGA SQDDNYDVRI

       610        620        630        640        650        660
GEDAECCSEP SLLSTEMLSY QDDENRQLAP PEEEKRDIRL SPKKGFLRSA SLGRRASFHL

       670        680        690        700        710        720
ECLKRQKNQG GDISQKTVLP LHLVHHQALA VAGLSPLLQR SHSPTSLPRP CATPPATPGS

       730        740        750        760        770        780
RGWPPQPIPT LRLEGADSSE KLNSSFPSIH CGSWSGENSP CRGDSSAARR ARPVSLTVPS

       790        800        810        820        830        840
QAGAQGRQFH GSASSLVEAV LISEGLGQFA QDPKFIEVTT QELADACDLT IEEMENAADD

       850        860        870        880        890        900
ILSGGARQSP NGTLLPFVNR RDPGRDRAGQ NEQDASGACA PGCGQSEEAL ADRRAGVSSL
```

Number of amino acids: 900

Molecular weight: 99750.8

Theoretical pI: 6.40

```
ACGTTATCGACTGCACGGTGCACCAATGCTTCTGGCGTCAGGCAGCCATCGGAAGCTGTGGTATGGCTGTGCAGG
TCGTAAATCACTGCATAATTCGTGTCGCTCAAGGCGCACTCCCGTTCTGGATAATGTTTTTTGCGCCGACATCAT
AACGGTTCTGGCAAATATTCTGAAATGAGCTGTTGACAATTAATCATCGGCTCGTATAATGTGTGGAATTGTGAG
CGGATAACAATTTCACACAGGAAACAGTATTCATGTCCCCTATACTAGGTTATTGGAAAATTAAGGGCCTTGTGC
AACCCACTCGACTTCTTTTGGAATATCTTGAAGAAAAATATGAAGAGCATTTGTATGAGCGCGATGAAGGTGATA
AATGGCGAAACAAAAAGTTTGAATTGGGTTTGGAGTTTCCCAATCTTCCTTATTATATTGATGGTGATGTTAAAT
TAACACAGTCTATGGCCATCATACGTTATATAGCTGACAAGCACAACATGTTGGGTGGTTGTCCAAAAGAGCGTG
CAGAGATTTCAATGCTTGAAGGAGCGGTTTTGGATATTAGATACGGTGTTTCGAGAATTGCATATAGTAAAGACT
TTGAAACTCTCAAAGTTGATTTTCTTAGCAAGCTACCTGAAATGCTGAAAATGTTCGAAGATCGTTTATGTCATA
AAACATATTTAAATGGTGATCATGTAACCCATCCTGACTTCATGTTGTATGACGCTCTTGATGTTGTTTTATACA
TGGACCCAATGTGCCTGGATGCGTTCCCAAAATTAGTTTGTTTTAAAAAACGTATTGAAGCTATCCCACAAATTG
ATAAGTACTTGAAATCCAGCAAGTATATAGCATGGCCTTTGCAGGGCTGGCAAGCCACGTTTGGTGGTGGCGACC
ATCCTCCAAAATCGGATCTGGAAGTTCTGTTCCAGGGGCCCCTGGGATCCCCAGGAATTCCCAGGCAGCATGGAA
GCTCAGCTCCAAGAGATGCCACTCCCAGGAGAGCCAGATAGCCATGGCGTGTCAGGAGGGCGCATCCCAGGACGA
CAACTACGACGTGAGGATCGGTGAAGATGCAGAGTGCTGCAGTGAGCCCAGCCTGCTCTCCACAGAGATGCTCTC
CTACCAGGATGACGAAAACCGACAACTGGCGCCCCCGGAGGAGGAGAAGCGGGACATCAGGCTGTCTCCAAAGAA
GGGTTTCCTGCGCTCCGCATCACTGGGTCGAAGGGCTTCCTTCCACCTGGAGTGTCTGAAGCGGCAGAAGAATCA
AGGGGGAGACATCTCTCAGAAGACAGTCCTGCCCCTGCATCTGGTCCACCACCAGGCATTGGCAGTGGCGGGCCT
GAGTCCCCTCCTGCAGAGAAGCCATTCCCCCACCTCGCTCCCTAGGCCCTGTGCCACGCCCCCTGCCACACCGGG
CAGCCGAGGCTGGCCCCCACAGCCCATCCCCACCCTGCGGCTGGAGGGGGCCGACTCCAGTGAGAAACTCAACAG
CAGCTTCCCGTCCATCCACTGCGGCTCATGGTCTGGGGAGAACAGCCCCTGCAGAGGGGACAGCAGCGCCGCCCG
GAGAGCCCGGCCCGTCTCCCTCACTGTGCCCAGCCAGGCTGGGGCCCAGGGGAGACAGTTCCATGGCAGCGCCAG
CAGCCTGGTGGAAGCGGTCTTGATTTCCGAAGGACTGGGGCAGTTTGCTCAAGATCCCAAGTTCATCGAGGTCAC
GACCCAGGAGCTGGCTGACGCCTGCGATCTGACCATAGAGGAGATGGAGAACGCGGCCGACGACATTCTCAGCGG
GGGCGCCCGGCAGAGCCCCAATGGCACCCTGTTACCCTTTGTGAACCGCAGGGACCCGGGCCGGGACAGAGCGGG
GCAGAACGAGCAGGACGCGAGCGGCGCATGCGCCCCAGGGTGCGGGCAGAGCGAGGAGGCCCTCGCGGACCGCAG
GGCCGGCGTCAGCAGCCTGTAGCTCGAGCGGCCGCATCGTGACTGACTGACGATCTGCCTCGCGCGTTTCGGTGA
TGACGGTGAAAACCTCTGACACATGCAGCTCCCGGAGACGGTCACAGCTTGTCTGTAAGCGGATGCCGGGAGCAG
ACAAGCCCGTCAGGGCGCGTCAGCGGGTGTTGGCGGGTGTCGGGGCGCAGCCATGACCCAGTCACGTAGCGATAG
```

GAATTCCC = EcoRI site; CTCGAG = XhoI site; TAG = stop codon

~ half of C-terminal cytoplasmic tail

IQ-Motiv lack

```
CGGAGTGTATAATTCTTGAAGACGAAAGGGCCTCGTGATACGCCTATTTTTATAGGTTAATGTCATGATAATAAT
GGTTTCTTAGACGTCAGGTGGCACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACA
TTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGGAAGAGTATGAG
TATTCAACATTTCCGTGTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTTTTGCTCACCCAGAAAC
GCTGGTGAAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGG
TAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGC
GGTATTATCCCGTGTTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGA
GTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCAT
GAGTGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAA
CATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGA
CACCACGATGCCTGCAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCCG
GCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTGGCTG
GTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAA
GCCCTCCCGTATCGTAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATCGCTGA
GATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAAGTTTACTCATATATACTTTAGATTGATTTAAA
ACTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAATCCCTTAACGTGA
GTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGT
AATCTGCTGCTTGCAAACAAAAAAACCACCGCTACCAGCGGTGGTTTGTTTGCCGGATCAAGAGCTACCAACTCT
TTTTCCGAAGGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAGCCGTAGTTAGGCCA
CCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTGCCAGTGG
CGATAAGTCGTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCGGGCTGAACGGG
GGGTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGTGAGCTATGAGA
AAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGCAC
GAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGTCCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCG
ATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGGTTCCTGGC
CTTTTGCTGGCCTTTTGCTCACATGTTCTTTCCTGCGTTATCCCCTGATTCTGTGGATAACCGTATTACCGCCTT
TGAGTGAGCTGATACCGCTCGCCGCAGCCGAACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCG
CCTGATGCGGTATTTTCTCCTTACGCATCTGTGCGGTATTTCACACCGCATAAATTCCGACACCATCGAATGGTG
CAAAACCTTTCGCGGTATGGCATGATAGCGCCCGGAAGAGAGTCAATTCAGGGTGGTGAATGTGAAACCAGTAAC
GTTATACGATGTCGCAGAGTATGCCGGTGTCTCTTATCAGACCGTTTCCCGCGTGGTGAACCAGGCCAGCCACGT
TTCTGCGAAAACGCGGGAAAAAGTGGAAGCGGCGATGGCGGAGCTGAATTACATTCCCAACCGCGTGGCACAACA
ACTGGCGGGCAAACAGTCGTTGCTGATTGGCGTTGCCACCTCCAGTCTGGCCCTGCACGCGCCGTCGCAAATTGT
CGCGGCGATTAAATCTCGCGCCGATCAACTGGGTGCCAGCGTGGTGGTGTCGATGGTAGAACGAAGCGGCGTCGA
AGCCTGTAAAGCGGCGGTGCACAATCTTCTCGCGCAACGCGTCAGTGGGCTGATCATTAACTATCCGCTGGATGA
CCAGGATGCCATTGCTGTGGAAGCTGCCTGCACTAATGTTCCGGCGTTATTTCTTGATGTCTCTGACCAGACACC
CATCAACAGTATTATTTTCTCCCATGAAGACGGTACGCGACTGGGCGTGGAGCATCTGGTCGCATTGGGTCACCA
GCAAATCGCGCTGTTAGCGGGCCCATTAAGTTCTGTCTCGGCGCGTCTGCGTCTGGCTGGCTGGCATAAATATCT
CACTCGCAATCAAATTCAGCCGATAGCGGAACGGGAAGGCGACTGGAGTGCCATGTCCGGTTTTCAACAAACCAT
GCAAATGCTGAATGAGGGCATCGTTCCCACTGCGATGCTGGTTGCCAACGATCAGATGGCGCTGGGCGCAATGCG
CGCCATTACCGAGTCCGGGCTGCGCGTTGGTGCGGATATCTCGGTAGTGGGATACGACGATACCGAAGACAGCTC
ATGTTATATCCCGCCGTCAACCACCATCAAACAGGATTTTCGCCTGCTGGGGCAAACCAGCGTGGACCGCTTGCT
GCAACTCTCTCAGGGCCAGGCGGTGAAGGGCAATCAGCTGTTGCCCGTCTCACTGGTGAAAAGAAAAACCACCCT
GGCGCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTTTCCCG
ACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTTACACT
TTATGCTTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGGAAACAGCTATGACCATG
ATTACGGATTCACTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTT
GCAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGC
AGCCTGAATGGCGAATGGCGCTTTGCCTGGTTTCCGGCACCAGAAGCGGTGCCGGAAAGCTGGCTGGAGTGCGAT
CTTCCTGAGGCCGATACTGTCGTCGTCCCCTCAAACTGGCAGATGCACGGTTACGATGCGCCCATCTACACCAAC
GTAACCTATCCCATTACGGTCAATCCGCCGTTTGTTCCCACGGAGAATCCGACGGGTTGTTACTCGCTCACATTT
AATGTTGATGAAAGCTGGCTACAGGAAGGCCAGACGCGAATTATTTTTGATGGCGTTGGAATT

acgttatcgactgcacggtgcaccaatgcttctggcgtcaggcagccatcggaagctgtgg
  R  Y  R  L  H  G  A  P  M  L  L  A  S  G  S  H  R  K  L  W
tatggctgtgcaggtcgtaaatcactgcataattcgtgtcgctcaaggcgcactcccgtt
 Y  G  C  A  G  R  K  S  L  H  N  S  C  R  S  R  R  T  P  V
ctggataatgtttttgcgccgacatcataacggttctggcaaatattctgaaatgagct
 L  D  N  V  F  C  A  D  I  I  T  V  L  A  N  I  L  K  -  A
gttgacaattaatcatcggctcgtataatgtgtggaattgtgagcggataacaatttcac
 V  D  N  -  S  S  A  R  I  M  C  G  I  V  S  G  -  Q  F  H
acaggaaacagtattcatgtcccctatactaggttattggaaaattaagggccttgtgca
 T  G  N  S  I  H  V  P  Y  T  R  L  L  E  N  -  G  P  C  A
acccactcgacttcttttggaatatcttgaagaaaaatatgaagagcatttgtatgagcg
```

```
 T  H  S  T  S  F  G  I  S  -  R  K  I  -  R  A  F  V  -  A
cgatgaaggtgataaatggcgaaacaaaaagtttgaattgggtttggagtttcccaatct
 R  -  R  -  -  M  A  K  Q  K  V  -  I  G  F  G  V  S  Q  S
tccttattatattgatggtgatgttaaattaacacagtctatggccatcatacgttatat
 S  L  L  Y  -  W  -  C  -  I  N  T  V  Y  G  H  H  T  L  Y
agctgacaagcacaacatgttgggtggttgtccaaaagagcgtgcagagatttcaatgct
 S  -  Q  A  Q  H  V  G  W  L  S  K  R  A  C  R  D  F  N  A
tgaaggagcggttttggatattagatacggtgtttcgagaattgcatatagtaaagactt
 -  R  S  G  F  G  Y  -  I  R  C  F  E  N  C  I  -  -  R  L
tgaaactctcaaagttgattttcttagcaagctacctgaaatgctgaaaatgttcgaaga
 -  N  S  Q  S  -  F  S  -  Q  A  T  -  N  A  E  N  V  R  R
tcgtttatgtcataaaacatatttaaatggtgatcatgtaacccatcctgacttcatgtt
 S  F  M  S  -  N  I  F  K  W  -  S  C  N  P  S  -  L  H  V
gtatgacgctcttgatgttgttttatacatggacccaatgtgcctggatgcgttcccaaa
 V  -  R  S  -  C  C  F  I  H  G  P  N  V  P  G  C  V  P  K
attagtttgttttaaaaaacgtattgaagctatcccacaaattgataagtacttgaaatc
 I  S  L  F  -  K  T  Y  -  S  Y  P  T  N  -  -  V  L  E  I
cagcaagtatatagcatggcctttgcagggctggcaagccacgtttggtggtggcgacca
 Q  Q  V  Y  S  M  A  F  A  G  L  A  S  H  V  W  W  W  R  P
tcctccaaaatcggatctggaagttctgttccaggggcccctgggatccccaggaattcc
 S  S  K  I  G  S  G  S  S  V  P  G  A  P  G  I  P  R  N  S
caggcagcatggaagctcagctccaagagatgccactcccaggagagccagatagccatg
 Q  A  A  W  K  L  S  S  K  R  C  H  S  Q  E  S  Q  I  A  M
gcgtgtcaggagggcgcatcccaggacgacaactacgacgtgaggatcggtgaagatgca
 A  C  Q  E  G  A  S  Q  D  D  N  Y  D  V  R  I  G  E  D  A
gagtgctgcagtgagcccagcctgctctccacagagatgctctcctaccaggatgacgaa
 E  C  C  S  E  P  S  L  L  S  T  E  M  L  S  Y  Q  D  D  E
aaccgacaactggcgcccccggaggaggagaagcgggacatcaggctgtctccaaagaag
 N  R  Q  L  A  P  P  E  E  E  K  R  D  I  R  L  S  P  K  K
ggtttcctgcgctccgcatcactgggtcgaagggcttccttccacctggagtgtctgaag
 G  F  L  R  S  A  S  L  G  R  R  A  S  F  H  L  E  C  L  K
cggcagaagaatcaaggggagacatctctcagaagacagtcctgcccctgcatctggtc
 R  Q  K  N  Q  G  G  D  I  S  Q  K  T  V  L  P  L  H  L  V
caccaccaggcattggcagtggcgggcctgagtcccctcctgcagagaagccattccccc
 H  H  Q  A  L  A  V  A  G  L  S  P  L  L  Q  R  S  H  S  P
acctcgctccctaggccctgtgccacgccccctgccacaccgggcagccgaggctggccc
 T  S  L  P  R  P  C  A  T  P  P  A  T  P  G  S  R  G  W  P
ccacagcccatccccaccctgcggctggaggggccgactccagtgagaaactcaacagc
 P  Q  P  I  P  T  L  R  L  E  G  A  D  S  S  E  K  L  N  S
agcttcccgtccatccactgcggctcatggtctggggagaacagcccctgcagaggggac
 S  F  P  S  I  H  C  G  S  W  S  G  E  N  S  P  C  R  G  D
agcagcgccgcccggagagcccggcccgtctccctcactgtgcccagccaggctggggcc
 S  S  A  A  R  R  A  R  P  V  S  L  T  V  P  S  Q  A  G  A
caggggagacagttccatggcagcgccagcagcctggtggaagcggtcttgatttccgaa
 Q  G  R  Q  F  H  G  S  A  S  S  L  V  E  A  V  L  I  S  E
ggactggggcagtttgctcaagatcccaagttcatcgaggtcacgacccaggagctggct
 G  L  G  Q  F  A  Q  D  P  K  F  I  E  V  T  T  Q  E  L  A
gacgcctgcgatctgaccatagaggagatggagaacgcggccgacgacattctcagcggg
 D  A  C  D  L  T  I  E  E  M  E  N  A  A  D  D  I  L  S  G
ggcgcccggcagagccccaatggcaccctgttacccttttgtgaaccgcagggacccgggc
 G  A  R  Q  S  P  N  G  T  L  L  P  F  V  N  R  R  D  P  G
cgggacagagcggggcagaacgagcaggacgcgagcggcgcatgcgccccagggtgcggg
 R  D  R  A  G  Q  N  E  Q  D  A  S  G  A  C  A  P  G  C  G
cagagcgaggaggccctcgcggaccgcagggccggcgtcagcagcctgtagctcgagcgg
 Q  S  E  E  A  L  A  D  R  R  A  G  V  S  S  L  -  L  E  R
ccgcatcgtgactgactgacgatctgcctcgcgcgtttcggtgatgacggtgaaaacctc
 P  H  R  D  -  L  T  I  C  L  A  R  F  G  D  D  G  E  N  L
tgacacatgcagctcccggagacggtcacagcttgtctgtaagcggatgccgggagcaga
 -  H  M  Q  L  P  E  T  V  T  A  C  L  -  A  D  A  G  S  R
caagcccgtcagggcgcgtcagcgggtgttggcgggtgtcggggcgcagccatgacccag
 Q  A  R  Q  G  A  S  A  G  V  G  G  C  R  G  A  A  M  T  Q
tcacgtagcgatagcggagtgtataattcttgaagacgaaagggcctcgtgatacgccta
 S  R  S  D  S  G  V  Y  N  S  -  R  R  K  G  L  V  I  R  L
```

```
ttttttaggttaatgtcatgataataatggtttcttagacgtcaggtggcacttttcgg
 F  L  -  V  N  V  M  I  I  M  V  S  -  T  S  G  G  T  F  R
ggaaatgtgcgcggaacccctatttgtttatttttctaaatacattcaaatatgtatccg
 G  N  V  R  G  T  P  I  C  L  F  F  -  I  H  S  N  M  Y  P
ctcatgagacaataaccctgataaatgcttcaataatattgaaaaaggaagagtatgagt
 L  M  R  Q  -  P  -  -  M  L  Q  -  Y  -  K  R  K  S  M  S
attcaacatttccgtgtcgcccttattccctttttgcggcattttgccttcctgttttt
 I  Q  H  F  R  V  A  L  I  P  F  F  A  A  F  C  L  P  V  F
gctcacccagaaacgctggtgaaagtaaaagatgctgaagatcagttgggtgcacgagtg
 A  H  P  E  T  L  V  K  V  K  D  A  E  D  Q  L  G  A  R  V
ggttacatcgaactggatctcaacagcggtaagatccttgagagttttcgccccgaagaa
 G  Y  I  E  L  D  L  N  S  G  K  I  L  E  S  F  R  P  E  E
cgttttccaatgatgagcacttttaaagttctgctatgtggcgcggtattatcccgtgtt
 R  F  P  M  M  S  T  F  K  V  L  L  C  G  A  V  L  S  R  V
gacgccgggcaagagcaactcggtcgccgcatacactattctcagaatgacttggttgag
 D  A  G  Q  E  Q  L  G  R  R  I  H  Y  S  Q  N  D  L  V  E
tactcaccagtcacagaaaagcatcttacggatggcatgacagtaagagaattatgcagt
 Y  S  P  V  T  E  K  H  L  T  D  G  M  T  V  R  E  L  C  S
gctgccataaccatgagtgataacactgcggccaacttacttctgacaacgatcggagga
 A  A  I  T  M  S  D  N  T  A  A  N  L  L  L  T  T  I  G  G
ccgaaggagctaaccgcttttttgcacaacatgggggatcatgtaactcgccttgatcgt
 P  K  E  L  T  A  F  L  H  N  M  G  D  H  V  T  R  L  D  R
tgggaaccggagctgaatgaagccataccaaacgacgagcgtgacaccacgatgcctgca
 W  E  P  E  L  N  E  A  I  P  N  D  E  R  D  T  T  M  P  A
gcaatggcaacaacgttgcgcaaactattaactggcgaactacttactctagcttcccgg
 A  M  A  T  T  L  R  K  L  L  T  G  E  L  L  T  L  A  S  R
caacaattaatagactggatggaggcggataaagttgcaggaccacttctgcgctcggcc
 Q  Q  L  I  D  W  M  E  A  D  K  V  A  G  P  L  L  R  S  A
cttccggctggctggtttattgctgataaatctggagccggtgagcgtgggtctcgcggt
 L  P  A  G  W  F  I  A  D  K  S  G  A  G  E  R  G  S  R  G
atcattgcagcactggggccagatggtaagccctcccgtatcgtagttatctacacgacg
 I  I  A  A  L  G  P  D  G  K  P  S  R  I  V  V  I  Y  T  T
gggagtcaggcaactatggatgaacgaaatagacagatcgctgagataggtgcctcactg
 G  S  Q  A  T  M  D  E  R  N  R  Q  I  A  E  I  G  A  S  L
attaagcattggtaactgtcagaccaagtttactcatatatactttagattgatttaaaa
 I  K  H  W  -  L  S  D  Q  V  Y  S  Y  I  L  -  I  D  L  K
cttcatttttaatttaaaaggatctaggtgaagatccttttttgataatctcatgaccaaa
 L  H  F  -  F  K  R  I  -  V  K  I  L  F  D  N  L  M  T  K
atcccttaacgtgagttttcgttccactgagcgtcagaccccgtagaaaagatcaaagga
 I  P  -  R  E  F  S  F  H  -  A  S  D  P  V  E  K  I  K  G
tcttcttgagatccttttttttctgcgcgtaatctgctgcttgcaaacaaaaaaaccaccg
 S  S  -  D  P  F  F  L  R  V  I  C  C  L  Q  T  K  K  P  P
ctaccagcggtggtttgtttgccggatcaagagctaccaactctttttccgaaggtaact
 L  P  A  V  V  C  L  P  D  Q  E  L  P  T  L  F  P  K  V  T
ggcttcagcagagcgcagataccaaatactgtccttctagtgtagccgtagttaggccac
 G  F  S  R  A  Q  I  P  N  T  V  L  L  V  -  P  -  L  G  H
cacttcaagaactctgtagcaccgcctacatacctcgctctgctaatcctgttaccagtg
 H  F  K  N  S  V  A  P  P  T  Y  L  A  L  L  I  L  L  P  V
gctgctgccagtggcgataagtcgtgtcttaccgggttggactcaagacgatagttaccg
 A  A  A  S  G  D  K  S  C  L  T  G  L  D  S  R  R  -  L  P
gataaggcgcagcggtcgggctgaacgggggttcgtgcacacagcccagcttggagcga
 D  K  A  Q  R  S  G  -  T  G  G  S  C  T  Q  P  S  L  E  R
acgacctacaccgaactgagatacctacagcgtgagctatgagaaagcgccacgcttccc
 T  T  Y  T  E  L  R  Y  L  Q  R  E  L  -  E  S  A  T  L  P
gaagggagaaaggcggacaggtatccggtaagcggcagggtcggaacaggagagcgcacg
 E  G  R  K  A  D  R  Y  P  V  S  G  R  V  G  T  G  E  R  T
agggagcttccagggggaaacgcctggtatctttatagtcctgtcgggtttcgccacctc
 R  E  L  P  G  G  N  A  W  Y  L  Y  S  P  V  G  F  R  H  L
tgacttgagcgtcgatttttgtgatgctcgtcaggggggcggagcctatggaaaaacgcc
 -  L  E  R  R  F  L  -  C  S  S  G  G  R  S  L  W  K  N  A
agcaacgcggccttttacggttcctggccttttgctggccttttgctcacatgttcttt
 S  N  A  A  F  L  R  F  L  A  F  C  W  P  F  A  H  M  F  F
cctgcgttatcccctgattctgtggataaccgtattaccgcctttgagtgagctgatacc
```

```
 P  A  L  S  P  D  S  V  D  N  R  I  T  A  F  E  -  A  D  T
gctcgccgcagccgaacgaccgagcgcagcgagtcagtgagcgaggaagcggaagagcgc
 A  R  R  S  R  T  T  E  R  S  E  S  V  S  E  E  A  E  E  R
ctgatgcggtattttctccttacgcatctgtgcggtatttcacaccgcataaattccgac
 L  M  R  Y  F  L  L  T  H  L  C  G  I  S  H  R  I  N  S  D
accatcgaatggtgcaaaacctttcgcggtatggcatgatagcgcccggaagagagtcaa
 T  I  E  W  C  K  T  F  R  G  M  A  -  -  R  P  E  E  S  Q
ttcagggtggtgaatgtgaaaccagtaacgttatacgatgtcgcagagtatgccggtgtc
 F  R  V  V  N  V  K  P  V  T  L  Y  D  V  A  E  Y  A  G  V
tcttatcagaccgtttcccgcgtggtgaaccaggccagccacgtttctgcgaaaacgcgg
 S  Y  Q  T  V  S  R  V  V  N  Q  A  S  H  V  S  A  K  T  R
gaaaaagtggaagcggcgatggcggagctgaattacattcccaaccgcgtggcacaacaa
 E  K  V  E  A  A  M  A  E  L  N  Y  I  P  N  R  V  A  Q  Q
ctggcgggcaaacagtcgttgctgattggcgttgccacctccagtctggccctgcacgcg
 L  A  G  K  Q  S  L  L  I  G  V  A  T  S  S  L  A  L  H  A
ccgtcgcaaattgtcgcggcgattaaatctcgcgccgatcaactgggtgccagcgtggtg
 P  S  Q  I  V  A  A  I  K  S  R  A  D  Q  L  G  A  S  V  V
gtgtcgatggtagaacgaagcggcgtcgaagcctgtaaagcggcggtgcacaatcttctc
 V  S  M  V  E  R  S  G  V  E  A  C  K  A  A  V  H  N  L  L
gcgcaacgcgtcagtgggctgatcattaactatccgctggatgaccaggatgccattgct
 A  Q  R  V  S  G  L  I  I  N  Y  P  L  D  D  Q  D  A  I  A
gtggaagctgcctgcactaatgttccggcgttatttcttgatgtctctgaccagacaccc
 V  E  A  A  C  T  N  V  P  A  L  F  L  D  V  S  D  Q  T  P
atcaacagtattattttctcccatgaagacggtacgcgactgggcgtggagcatctggtc
 I  N  S  I  I  F  S  H  E  D  G  T  R  L  G  V  E  H  L  V
gcattgggtcaccagcaaatcgcgctgttagcgggcccattaagttctgtctcggcgcgt
 A  L  G  H  Q  Q  I  A  L  L  A  G  P  L  S  S  V  S  A  R
ctgcgtctggctggctggcataaatatctcactcgcaatcaaattcagccgatagcggaa
 L  R  L  A  G  W  H  K  Y  L  T  R  N  Q  I  Q  P  I  A  E
cgggaaggcgactggagtgccatgtccggttttcaacaaaccatgcaaatgctgaatgag
 R  E  G  D  W  S  A  M  S  G  F  Q  Q  T  M  Q  M  L  N  E
ggcatcgttcccactgcgatgctggttgccaacgatcagatggcgctgggcgcaatgcgc
 G  I  V  P  T  A  M  L  V  A  N  D  Q  M  A  L  G  A  M  R
gccattaccgagtccgggctgcgcgttggtgcggatatctcggtagtgggatacgacgat
 A  I  T  E  S  G  L  R  V  G  A  D  I  S  V  V  G  Y  D  D
accgaagacagctcatgttatatcccgccgtcaaccaccatcaaacaggattttcgcctg
 T  E  D  S  S  C  Y  I  P  P  S  T  T  I  K  Q  D  F  R  L
ctggggcaaaccagcgtggaccgcttgctgcaactctctcagggccaggcggtgaagggc
 L  G  Q  T  S  V  D  R  L  L  Q  L  S  Q  G  Q  A  V  K  G
aatcagctgttgcccgtctcactggtgaaaagaaaaaccaccctggcgcccaatacgcaa
 N  Q  L  L  P  V  S  L  V  K  R  K  T  T  L  A  P  N  T  Q
accgcctctccccgcgcgttggccgattcattaatgcagctggcacgacaggtttcccga
 T  A  S  P  R  A  L  A  D  S  L  M  Q  L  A  R  Q  V  S  R
ctggaaagcgggcagtgagcgcaacgcaattaatgtgagttagctcactcattaggcacc
 L  E  S  G  Q  -  A  Q  R  N  -  C  E  L  A  H  S  L  G  T
ccaggctttacactttatgcttccggctcgtatgttgtgtggaattgtgagcggataaca
 P  G  F  T  L  Y  A  S  G  S  Y  V  V  W  N  C  E  R  I  T
atttcacacaggaaacagctatgaccatgattacggattcactggccgtcgttttacaac
 I  S  H  R  K  Q  L  -  P  -  L  R  I  H  W  P  S  F  Y  N
gtcgtgactgggaaaaccctggcgttacccaacttaatcgccttgcagcacatccccctt
 V  V  T  G  K  T  L  A  L  P  N  L  I  A  L  Q  H  I  P  L
tcgccagctggcgtaatagcgaagaggcccgcaccgatcgcccttcccaacagttgcgca
 S  P  A  G  V  I  A  K  R  P  A  P  I  A  L  P  N  S  C  A
gcctgaatggcgaatggcgctttgcctggtttccggcaccagaagcggtgccggaaagct
 A  -  M  A  N  G  A  L  P  G  F  R  H  Q  K  R  C  R  K  A
ggctggagtgcgatcttcctgaggccgatactgtcgtcgtcccctcaaactggcagatgc
 G  W  S  A  I  F  L  R  P  I  L  S  S  S  P  Q  T  G  R  C
acggttacgatgcgcccatctacaccaacgtaacctatcccattacggtcaatccgccgt
 T  V  T  M  R  P  S  T  P  T  -  P  I  P  L  R  S  I  R  R
ttgttcccacggagaatccgacgggttgttactcgctcacatttaatgttgatgaaagct
 L  F  P  R  R  I  R  R  V  V  T  R  S  H  L  M  L  M  K  A
ggctacaggaaggccagacgcgaattatttttgatggcgttggaatt
 G  Y  R  K  A  R  R  E  L  F  L  M  A  L  E
```

ABBREVIATIONS

~	approximately
$[Ca^{2+}]$	Ca^{2+} concentration
AID	alpha interaction domain
BID	beta interaction domain
Ca^{2+}	calcium
CDI	Ca^{2+} dependent inactivation
cDNA	complementary DNA
CT	C-terminal tail
DHP	Dihydropyridine
DNA	Desoxyribonucleid acid
EC	excitation-contraction
ER	endoplasmic reticulum
E. coli	Escherichia coli
HEK	human embryonic kidney
IQ	isoleucine-glutamine
NO	nitric oxide
OD	optical density
PAGE	polyacrylamide gel electrophoresis
PCR	polymerase chain reaction
Pos	positive
RNA	Ribonucleid acid
SH3	Src homology 3
SR	sarcoplasmic reticulum
VGCC	voltage-gated calcium channel
WW	domain with conserved tryptophans

Abbreviations: Proteins

AJ	adherens junction protein
$Ca_v1.2$	$Ca_v1.2\alpha1c$
CaM	calmodulin
CaM-BD	calmodulin binding domain
CASK	Ca^{2+}/calmodulin-dependent membrane-associated kinase
CFTR	cystic fibrosis transmembrane conductance regulator
cGMP	cyclic-guanosin cyclise
GUK	guanylate kinase
DLG	Drosophila discs large
eNOS	endothelial nitric oxide synthase
GST	glutathione S-transferase

HRP	horse radish peroxidase
IgG	immunoglobulin G
iNOS	inducible nitric oxide synthase
IP_3R	inositol triphophate receptor
LTCC	L-type calcium channel
MAGI	multi-PDZ-containing protein membrane associated guanylate kinase inverted
MAGUK	membrane-associated guanylate kinase
MUPP	multi-PDZ domain protein
NCX	sodium/calcium exchanger
NHERF	sodium-hydrogen antiporter 3 regulator 1
NMDA-R	N-methyl-D-aspartic acid receptor
nNOS	neuronal nitric oxide synthase
PKA	protein kinase A
PKC	protein kinase C
PLC	phospholipase C
PMCA	plasma membrane calcium ATPase
PSD	postsynaptic density protein
RyR	ryanodine receptor
SERCA	sarcoplasmic reticulum Ca^{2+} ATPase
sGC	soluble guanylate cyclase
TJ	tight junction protein
ZO	zonula occludens

Abbreviations: Chemicals

APS	Ammonium persulphate
BCA	Bicinchoninic acis
BSA	Bovine serum albumin
DMEM	Dulbecco/modified Eagle's minimal essential medium
EDTA	ethylenediaminetetraacetic acid
FCS	Foetal calf serum
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
IPTG	isopropyl-1-thio-β-D-galactopyranoside
LB	Luria Bertani
LB-Amp	LB-medium supplemented with Ampicillin
PBS	Phosphate buffered saline
SDS	sodium dodecyl sulphate
TBS	Tris buffered saline

Abbreviations: units of measurement

µ	micro
x g	G-force
A	Ampere
bp	base pairs
kDa	kilodalton
g	gram
h	hour
kg	kilogram
l	litre
m	mili
M	molar
mg/ml	miligram per mililitre
min	minutes
n	nano
nm	nanometers
rpm	revolutions per minute
s	second
V	Volt

Abbreviations: amino acid residues

*	free carboxyl group
Φ	hydrophobic residue
Ψ	aromatic residue
X	any residue
A	Alanine
C	Cysteine
D	Aspartic acid
E	Glutamic acid
F	Phenylalanine
G	Glycine
H	Histidine
I	Isoleucine
K	Lysine
L	Leucine
M	Methionine
N	Asparagine
P	Proline
Q	Glutamine
R	Arginine

S	Serine
T	Threonine
V	Valine
W	Tryptophan
Y	Tyrosine

Affidavit

I hereby declare that my thesis entitled "Novel $Ca_v1.2$ and PMCA4b interacting PDZ domain containing proteins" is the result of my own work. I did not receive any help or support from commercial consultants. All sources and / or materials applied are listed and specified in the thesis.

Furthermore, I verify that this thesis has not yet been submitted as part of another examination process neither in identical nor in similar form.

Würzburg, September 2011 (Doreen Fetting)

Eidesstattliche Erklärung

Hiermit erkläre ich an Eides statt, die Dissertation „Novel $Ca_v1.2$ and PMCA4b interacting PDZ domain containing proteins" eigenständig, d.h. insbesondere selbstständig und ohne Hilfe eines komerziellen Promotionsberaters, angefertigt und keine anderen als die von mir angegebenen Quellen und Hilfsmittel verwendet zu haben.

Ich erkläre außerdem, dass die Dissertation weder in gleicher noch in ähnlicher Form bereits in einem anderen Prüfungsverfahren vorgelegen hat.

Würzburg, September 2011 (Doreen Fetting)