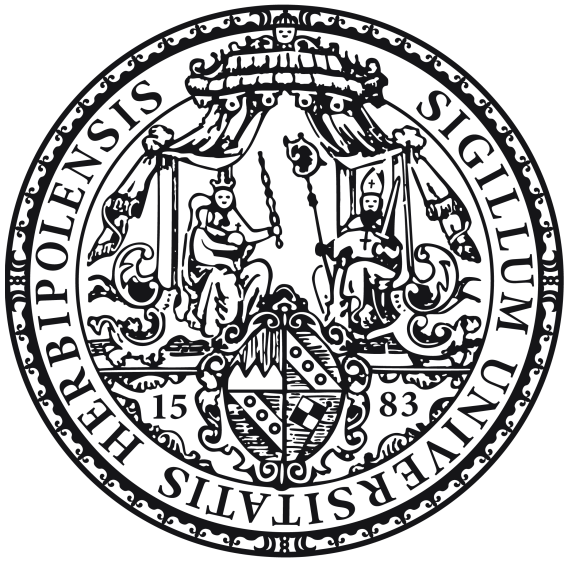

Aging and inflammation in the peripheral nervous system

Altern und Entzündung im peripheren Nervensystem

Dissertation for a doctoral degree
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The published manuscript and this thesis contain similar text passages in adapted form in some sections.

1. Abstract

Aging is known to be a risk factor for structural abnormalities and functional decline in the nervous system. Characterizing age-related changes is important to identify putative pathways to overcome deleterious effects and improve life quality for the elderly. In this study, the peripheral nervous system of 24-month-old aged C57BL/6 mice has been investigated and compared to 12-month-old adult mice. Aged mice showed pathological alterations in their peripheral nerves similar to nerve biopsies from elderly human individuals, with nerve fibers showing demyelination and axonal damage. Such changes were lacking in nerves of adult 12-month-old mice and adult, non-aged humans. Moreover, neuromuscular junctions of 24-month-old mice showed increased denervation compared to adult mice. These alterations were accompanied by elevated numbers of macrophages in the peripheral nerves of aged mice. The neuroinflammatory conditions were associated with impaired myelin integrity and with a decline of nerve conduction properties and muscle strength in aged mice.

To determine the pathological impact of macrophages in the aging mice, macrophage depletion was performed in mice by oral administration of CSF-1R specific kinase (c-FMS) inhibitor PLX5622 (300 mg/kg body weight), which reduced the number of macrophages in the peripheral nerves by 70%. The treated mice showed attenuated demyelination, less muscle denervation and preserved muscle strength. This indicates that macrophage-driven inflammation in the peripheral nerves is partially responsible for the age-related neuropathy in mice.

Based on previous observations that systemic inflammation can accelerate disease progression in mouse models of neurodegenerative diseases, it was hypothesized that systemic inflammation can exacerbate the peripheral neuropathy found in aged mice. To investigate this hypothesis, aged C57BL/6 mice were intraperitoneally injected with a single dose of lipopolysaccharide (LPS; 500 µg/kg body weight) to induce systemic inflammation by mimicking bacterial infection, mostly via activation of Toll-like receptors (TLRs). Altered endoneurial macrophage activation, highlighted by *Trem2* downregulation, was found in LPS-injected aged mice one month after injection. This was accompanied by a so far rarely observed form of axonal perturbation, i.e., the occurrence of “dark axons” characterized by a damaged cytoskeleton and an increased overall electron

density of the axoplasm. At the same time, however, LPS injection reduced demyelination and muscle denervation in aged mice. Interestingly, TREM2 deficiency in aged mice led to similar changes to LPS injection. This suggests that LPS injection likely mitigates aging-related demyelination and muscle denervation via *Trem2* downregulation.

Taken together, this study reveals the role of macrophage-driven inflammation as a pathogenic mediator in age-related peripheral neuropathy, and that targeting macrophages might be an option to mitigate peripheral neuropathies in aging individuals. Furthermore, this study shows that systemic inflammation may be an ambivalent modifier of age-related nerve damage, leading to a distinct type of axonal perturbation, but in addition to functionally counteracting, dampened demyelination and muscle denervation. Translationally, it is plausible to assume that tipping the balance of macrophage polarization to one direction or the other may determine the functional outcome in the aging peripheral nervous system of the elderly.

2. Zussamenfassung

Es ist bekannt, dass das Altern ein Risikofaktor für strukturelle Veränderungen und Funktionsstörungen des Nervensystems ist. Die Charakterisierung altersbedingter Veränderungen ist wichtig, um mögliche Wege zu identifizieren, um schädliche Auswirkungen zu überwinden und die Lebensqualität älterer Menschen zu verbessern. In dieser Studie wurde das periphere Nervensystem von 24 Monate alten gealterten C57BL/6-Mäusen untersucht und mit 12 Monate alten adulten Mäusen verglichen. Gealterte Mäuse zeigten ähnliche pathologische Veränderungen in ihren peripheren Nerven wie Nervenbiopsien älterer Menschen, wobei die Nervenfasern eine Demyelinisierung und axonale Schädigung zeigten. Bei den Nerven von adulten 12 Monate alten Mäusen und nicht gealterten Menschen fehlten solche Veränderungen. Darüber hinaus wiesen die neuromuskulären Endplatten von 24 Monate alten Mäusen im Vergleich zu adulten Mäusen eine erhöhte Denervation auf. Diese Veränderungen wurden von einer erhöhten Anzahl von Makrophagen in den peripheren Nerven gealterter Mäuse begleitet. Die neuroinflammatorischen Bedingungen waren mit einer Beeinträchtigung der Myelinintegrität, einer Abnahme der Nervenleitungseigenschaften und der Muskelkraft bei gealterten Mäusen verbunden.

Um den pathologischen Einfluss von Makrophagen bei alternden Mäusen zu bestimmen, wurde die Makrophagen-Depletion bei Mäusen durch orale Verabreichung des CSF-1R-spezifischen Kinase-Inhibitors (c-FMS) PLX5622 (300 mg/kg Körpergewicht) durchgeführt, welche die Anzahl der Makrophagen in den peripheren Nerven um 70% reduzierte. Die behandelten Mäuse zeigten eine verminderte Demyelinisierung, eine reduzierte Muskeldenervation und einen Erhalt der Muskelkraft. Dies deutet darauf hin, dass die durch Makrophagen verursachte Entzündung in den peripheren Nerven teilweise für die altersbedingte Neuropathie bei Mäusen verantwortlich ist.

Auf der Grundlage früherer Beobachtungen, dass systemische Entzündungen das Fortschreiten der Krankheit in Mausmodellen neurodegenerativer Erkrankungen beschleunigen können, wurde die Hypothese aufgestellt, dass systemische Entzündungen die periphere Neuropathie in gealterten Mäusen verschlimmern können. Um diese Hypothese zu untersuchen, wurde gealterten C57BL/6-Mäusen

eine Einzeldosis Lipopolysaccharid (LPS; 500 µg/kg Körpergewicht) intraperitonal injiziert, um eine systemische Entzündung durch Nachahmung einer bakteriellen Infektion, meist über die Aktivierung von Toll-like-Rezeptoren (TLRs), zu induzieren. Eine veränderte endoneuriale Makrophagenaktivierung, die durch eine reduzierte *Trem2*-Expression hervorgehoben wird, konnte bei LPS-injizierten gealterten Mäusen einen Monat nach der Injektion gefunden werden. Dies ging einher mit einer bisher selten beobachteten Form der axonalen Perturbation, d.h. dem Auftreten von "dunklen Axonen", die sich durch ein geschädigtes Zytoskelett und eine erhöhte Gesamtelektronendichte des Axoplasmas auszeichnen. Gleichzeitig verringerte die LPS-Injektion jedoch die Demyelinisierung und Muskeldenervation bei gealterten Mäusen. Interessanterweise führte die TREM2-Defizienz bei gealterten Mäusen zu vergleichbaren Veränderungen wie die LPSInjektion. Dies deutet darauf hin, dass die LPS-Injektion die alterungsbedingte Demyelinisierung und Muskeldenervierung über die *Trem2* Herunterregulation abschwächt.

Zusammenfassend zeigt diese Studie die Rolle der Makrophagen-getriebenen Entzündung als pathogener Mediator bei der altersbedingten peripheren Neuropathie. Zusätzlich deuten die Ergebnisse darauf hin, dass die gezielte Behandlung von Makrophagen eine Option zur Linderung peripherer Neuropathien bei alternden Menschen sein könnte. Darüber hinaus zeigt diese Studie, dass die systemische Entzündung ein ambivalenter Modifikator der altersbedingten Nervenschädigung sein kann, der zu einer bestimmten Art von axonaler Perturbation führt, aber zusätzlich zu einer funktionell entgegenwirkenden, weniger schweren Demyelinisierung und Muskeldenervation. Translatorisch ist es plausibel anzunehmen, dass eine Veränderung des Gleichgewichts der Makrophagenpolarisation in die eine oder andere Richtung das funktionelle Ergebnis im alternden peripheren Nervensystem der älteren Menschen bestimmen kann.

3. Introduction

3.1 Peripheral nerves in health and disease

The peripheral nervous system (PNS) transmits signals between the central nervous system (CNS) and the rest of the organism. It has been long considered as a simple and stable extension of the CNS. However, in contrast to this view, the PNS is a robust system with dynamic interactions among its cellular components. Structural and functional defects of the cellular components of the peripheral nerves can lead to disorders of the PNS.

PNS axons are the extensions of neurons located in the spinal cord or in the dorsal root ganglia (King, 2013). They transmit electrical signals between the neuronal soma and their terminals. The transmission of such signals, also known as action potentials, is achieved by the propagation of ion fluxes through respective channels. To increase the speed of conduction, some axons are insulated by myelin, leaving the node of Ranvier devoid of myelin. At these compartments, voltage-gated sodium channels are clustered, enabling the action potential to “jump” from one node of Ranvier to another. This is also known as the saltatory conduction (Huxley and Stämpfli, 1949). Demyelination, a process characterized by a loss of myelin, can lead to reduced nerve conduction velocities and even completely abolished nerve conduction, also known as conduction block (Ritchie, 1984).

In the PNS, myelination is achieved by Schwann cells. During development, neural crest cells give rise to Schwann cell precursors, which differentiate into immature Schwann cells. During the early postnatal stage, axonal signals, such as Neuregulin-1 (NRG-1), promote expression of different transcription factors (e.g. OCT-6) in immature Schwann cells, which drives the differentiation towards promyelinating Schwann cells (Taveggia et al., 2010; Jessen et al., 2015). These signals can further promote upregulation of transcription factor Krox20 (encoded by *Erg2*) (Pereira et al., 2012). As a key regulator of PNS myelination, Krox20 promotes differentiation of promyelinating Schwann cells to myelinating Schwann cells (Topilko et al., 1994; Salzer, 2008; Figlia et al., 2017). Myelinating Schwann cells subsequently synthesize lipids and proteins, the major components of myelin, and tightly wrap the axons to form myelin sheaths under the regulation of axonal

NRG-1 type III signal levels (Michailov et al., 2004; Taveggia et al., 2005) and the PI3K-Akt pathway (Domènech-Estévez et al., 2016; Figlia et al., 2017). Mutations in myelin protein genes or transcriptional factors that regulate myelination can cause hereditary motor and sensory neuropathies that are also referred to as Charcot-Marie-Tooth disease type 1 (CMT1) (Shy, 2004; Niemann et al., 2006). CMT1 is associated with reduction of nerve conduction velocities, but also axonal perturbations that cause muscle weakness in patients (Suter and Scherer, 2003).

Adult Schwann cells can also be associated with unmyelinated axons, forming structures known as Remak bundles (Salzer, 2008). The corresponding non-myelinating Schwann cells are also termed “Remak cells”. Remak cells are essential for maintaining axonal integrity, as functional perturbation of Remak cells results in loss of unmyelinated axons and can eventually lead to progressive peripheral sensory neuropathies (Chen et al., 2003; Griffin and Thompson, 2008; Nave, 2010). Following nerve injuries, Remak cells and myelinating Schwann cells both contribute to axon regeneration by differentiating into repair cells that facilitate myelin and axonal debris removal (Bosse et al., 2006; Napoli et al., 2012; Gomez-Sanchez et al., 2017; Stierli et al., 2018; Jessen and Mirsky, 2019). Also, as a result of macrophage-related endothelial attraction, repair cells migrate along connecting blood vessels, which helps the axons to bridge the gap ((Cattin et al., 2015), see below). Furthermore, Schwann cells can support axonal regrowth after nerve injuries by secreting trophic factors, such as brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF) (Meyer et al., 1992; Curtis et al., 1994). Additionally, there are reports indicating that metabolic constraints on Schwann cells impair axon survival (Viader et al., 2011; Fünfschilling et al., 2012; Beirowski et al., 2014), providing evidence for the Schwann cell function of providing metabolic support to axons. The metabolic support of Schwann cells has also been shown to promote axon survival after nerve trauma and in a mouse model of axonopathy (Babetto et al., 2020).

Although the majority of endoneurial cells are Schwann cells, peripheral nerves consist of more cell populations with distinct functions under different conditions, one of them being macrophages (Stierli et al., 2018; Wolbert et al., 2020). Macrophages are typically considered as immune cells present in the circulatory system or residing in tissues. They play an essential role in innate immune

response and maintenance of tissue homeostasis. In the CNS, a large number of studies have demonstrated the functions of resident myeloid cells in the CNS parenchyma, termed microglia, in development, homeostasis and during diseases (Prinz and Priller, 2014; Prinz et al., 2019; Amann and Prinz, 2020). Compared to microglia, PNS macrophages in healthy and uninjured peripheral nerves have attracted much less research interest, which is a shortcoming that needs to be addressed in future studies.

Earlier studies identified the presence of macrophages within the endoneurium of normal peripheral nerves in the late 1970s (Arvidson, 1977; Oldfors, 1980). The origin of these macrophages was first studied in rodents receiving bone marrow (BM) transplantation after lethal irradiation (Vass et al., 1993; Müller et al., 2010). These studies show replenishment of PNS macrophages from circulating monocytes after BM transplantation. However, the limitation of these studies is that irradiation without shielding nerves may cause local neural damage and attraction of immune cells in the circulation, leading to a misguided view of origin of the cells (Ajami et al., 2007; Mildner et al., 2007; Kierdorf et al., 2013; Klein and Martini, 2015; Amann and Prinz, 2020).

Recently, the development of transgenic mouse lines allows fate-mapping of PNS macrophages, a solid method avoiding artifacts (Amann and Prinz, 2020). Studies labelling hematopoietic stem cell (HSC)-derived cells confirm that circulating monocytes are not the main source of resident PNS macrophages (Wang et al., 2020; Ydens et al., 2020). BM transplantation after irradiation with partial shielding of the sciatic nerves and parabiosis in these two studies also revealed the high self-renewal capacity of PNS macrophages. Through Tamoxifen (TAM) injection in transgenic mouse lines at different embryonic and postnatal days, these studies confirm the late embryonic origin of most PNS macrophages, and – as opposed to resident microglia – only a small proportion of them are yolk sac (YS)-derived. During early postnatal development, most PNS macrophages are replaced by infiltrating BM-derived cells from the circulation, and mostly rely on self-renewal during adolescence and adulthood, while the contribution of BM to macrophage turnover is minimal (Ydens et al., 2020).

Although distinct regarding their ontogenetic origins, PNS macrophages share some similarities with microglia, their counterparts in the CNS parenchyma.

Transcriptomic characterization of PNS macrophages on the single-cell level by RNA sequencing (single-cell RNA sequencing, scRNA-seq) provides further insights into their transcriptional profiles. PNS macrophages and microglia show shared gene expressions, including *Cx3cr1*, *Hexb*, *Mef2c*, *Siglech* and *Trem2* (Amann and Prinz, 2020). Compared to microglia, PNS macrophages possess a unique transcription profile with typical upregulation of genes including *Cd209d*, *Mgl2*, *Mmp9*, and *Tslp* (Wang et al., 2020; Ydens et al., 2020). Moreover, microglia core signature genes such as *Lag3*, *Sall1* or *Tmem119* (Hammond et al., 2019; Jordão et al., 2019; Masuda et al., 2019), are not or only weakly expressed in PNS macrophages (Wang et al., 2020; Wolbert et al., 2020). Interestingly, PNS macrophages are also transcriptomically distinct from other tissue-resident macrophages and non-parenchymal border-associated macrophages (BAMs) at CNS interfaces (Mrdjen et al., 2018; Van Hove et al., 2019; Ydens et al., 2020), indicating the importance of tissue environment that shapes the transcriptional identities of macrophages. In addition, a previous study based on scRNA-seq revealed the heterogeneity of PNS macrophages and identified two spatially separate macrophage subsets: an endoneurial macrophage population and an epineurial macrophage population (Ydens et al., 2020).

To date, little has been known about the functions of PNS macrophages in homeostasis (Amann and Prinz, 2020). In peripheral nerve injuries, however, the role of PNS macrophages has been described in detail. After nerve trauma, axons distal to the injury undergo degeneration following an orchestrated multicellular process known as Wallerian degeneration (Waller, 1850; Stoll et al., 2002). Macrophages are recruited to the injury site by Schwann cell-secreted CCL2 in response to injury (Martini et al., 2008; Klein and Martini, 2015), and are alternatively activated to an “M2-like” phenotype (Ydens et al., 2012). These M2-like activated macrophages, characterized by upregulation of *Arg1*, *Ym1* and *Trem2*, are involved in myelin debris removal via phagocytosis (Stoll et al., 1989; Brück, 1997; Martini et al., 2008; Chen et al., 2015; Klein and Martini, 2015). Moreover, the successful regeneration in the peripheral nerves after Wallerian degeneration is also facilitated by PNS macrophages. In response to the hypoxia in nerves after cut injuries, macrophages can secrete VEGF-A in order to induce neovascularization, forming blood vessels to guide Schwann cell migration across

the injury site (see above, (Cattin et al., 2015; Cattin and Lloyd, 2016)). Additionally, macrophages regulate the differentiation of Schwann cells to repair cells after cut injuries, promoting the regenerative environment important for axonal regrowth (Barrette et al., 2008; Stratton et al., 2018). Macrophages are also known as important pathogenic mediators in inherited and non-inherited neuropathies, contributing to disease progression (Klein and Martini, 2015). This will be described in more detail in the following chapter of the Introduction. In summary, macrophages play a crucial role in nerve trauma and diseases, highlighting the impact of immune cells on peripheral nerves.

Another type of non-glial cells is the endoneurial fibroblast, a nerve-related cellular player that has so far attracted much less attention than Schwann cells and macrophages. Endoneurial fibroblasts are conventionally identified by labelling of CD34 and by their ultrastructure of extensive endoplasmic reticulum (Mäurer et al., 2003; Richard et al., 2012; Richard et al., 2014). A recent scRNA-seq study identified nerve-associated fibroblasts with the expression of marker genes *Fn1*, *Fgfr1*, *Col1a*, and *Col3a* (Wolbert et al., 2020). Another study defined fibroblasts immunohistochemically using a new set of markers, marking fibroblasts as NG2+ PDGFRβ+ p75+ αSMA- cells in the peripheral nerves (Stierli et al., 2018). The study also proposed a new term “tactocytes” for endoneurial fibroblasts due to their morphological nature of often being in contact with other cells (Stierli et al., 2018).

Fibroblasts represent approximately 12% of the nucleated cells in the peripheral nerves, but their function during homeostasis is poorly understood. After nerve injuries, fibroblasts are involved in nerve regeneration through ephrin-B/EphB signaling-mediated Schwann cell sorting (Parrinello et al., 2010; Cattin and Lloyd, 2016). In addition, in mouse models for CMT1, fibroblast-derived colony stimulating factor-1 (CSF-1) activates macrophages, leading to an amplified nerve pathology (Groh et al., 2012; Martini and Willison, 2016). As these CSF-1-expressing cells are frequently in contact with macrophages, they might at least partially comprise those cell types identified as tactocytes (Stierli et al., 2018).

Other endoneurial cells identified in healthy nerves, mostly endothelial cells and pericytes, are structurally important for the homeostasis of the peripheral nerves.

B- and T-lymphocytes can also be identified in healthy nerves, although in much fewer numbers. The functions of these cells are not yet known (Wolbert et al., 2020). In summary, the homeostasis of peripheral nerves requires proper functioning and interactions of different cell populations. Defects or malfunction of endoneurial cells can lead to diseases of the peripheral nerves or can mediate the pathology in distinct neurological conditions.

3.2 The role of inflammation in peripheral nervous system disorders

Disorders of the peripheral nervous system can result in various motor, sensory or autonomic dysfunction and can severely impair the quality of life of individuals. Among these PNS disorders, there are hereditary neuropathies, caused by genetic defects of axons or glial cells; and idiopathic neuropathies, with uncertain causes of diseases. Most PNS disorders do not have direct cures, and the treatments often rely on rehabilitation and drugs alleviating disease symptoms (Grandis and Shy, 2005; Carayannopoulos, 2017; Tosolini and Smith, 2018). Thus, the search for disease-mediating mechanisms is important for developing feasible treatment options for various neuropathies. So far, inflammation has been shown to be involved in the pathologies and is therefore a therapeutic target of high interest.

PNS disorders such as chronic inflammatory demyelinating polyneuropathy (CIDP) and its acute counterpart, Guillain-Barré-syndrome (GBS), are known to be associated with inflammation. Both CIDP and GBS are heterogenous and cover a wide spectrum of subforms. These diseases associated with primary inflammation, mostly caused by anti-ganglioside antibody reactivity and complement-mediated membrane attack (Goodfellow and Willison, 2016; Willison et al., 2016; Querol et al., 2017), will not be further addressed in this thesis.

Hereditary neuropathies are PNS disorders primarily caused by genetic mutations and can also be associated with inflammation. In the most common group of hereditary neuropathies, Charcot-Marie-Tooth disease (CMT), more than 100 causative genes have been identified to date (Rossor et al., 2016). During the recent years, studies have revealed the presence of low-grade secondary inflammation and its role as a disease amplifier in mouse models of CMT1, a demyelinating form of CMT (Martini and Willison, 2016). Moreover, macrophages

have been identified by immunohistochemical staining in sural nerve biopsies of CMT1 patients (Stoll et al., 1998). In distinct mouse models of CMT1, an age-dependent increase of numbers of endoneurial macrophages has also been shown compared to wild-type mice (Schmid et al., 2000; Carenini et al., 2001; Kobsar et al., 2002; Kobsar et al., 2005). In peripheral nerves of these CMT1 mutant mice, macrophages with a morphology resembling foam cells can be identified. Therefore, macrophages with such appearance are termed “foamy macrophages”. The foamy appearance of these cells is due to lipid-enriched myelin debris in these macrophages due to an increase of myelin phagocytosis (Martini et al., 2008).

The pathogenic role of macrophages in CMT1 models has been identified by targeting pivotal mediators of their activation. One of these mediators, colony stimulating factor-1 (CSF-1), is unexpectedly derived from fibroblasts and crucial for macrophage proliferation and activation. The upregulation of *Csf1* expression has been described in CMT1 mouse models (Groh et al., 2012). Crossbreeding CMT1 mutant mice with osteoporotic (op) mice deficient for CSF-1 prevents the increase of endoneurial macrophages in CMT1 mutant mice and leads to a substantial amelioration of the nerve pathology with reduced demyelination and improved muscle innervation (Carenini et al., 2001; Groh et al., 2012; Groh et al., 2015; Groh et al., 2016a). Moreover, macrophage depletion by pharmacological inhibition of CSF-1 receptor (CSF-1R) also leads to ameliorated axonal features and improved nerve conduction properties in CMT1 mouse models (Klein et al., 2015b). Another mediator of macrophage activation is Schwann cell-derived CCL2 (MCP-1, monocyte chemoattractant protein-1), which is important for macrophage recruitment. Previous studies have demonstrated the elevated *Ccl2* expression downstream of activated MEK/ERK pathway in Schwann cells of CMT1 mutant mice (Fischer et al., 2008b; Fischer et al., 2008a; Groh et al., 2010). By crossbreeding CMT1 mutant mice with mice deficient for CCL2, an attenuation of macrophage recruitment and improved nerve phenotype in CMT1 mutant mice could be observed when only half of the chemokine dose was expressed (Fischer et al., 2008b; Groh et al., 2010; Kohl et al., 2010a). However, the complete knockout of *Ccl2* in CMT1 mutant mice does not attenuate the demyelination and axonal damage. This is likely due to a compensatory pathogenic upregulation of

Csf-1 in the absence of *Ccl2* (Groh et al., 2010). In addition, in one mouse model for CMT1B, the absence of a macrophage-restricted adhesion molecule, sialoadhesin (Siglec-1), leads to reduction of numbers of endoneurial macrophages and an amelioration of the demyelinating nerve phenotype (Kobsar et al., 2006). Overall, it has been demonstrated that endoneurial macrophages contribute to the disease progression in different mouse models of CMT1.

Apart from the contribution of the innate immune system, the pathogenesis of CMT1 also involves the activation of the adaptive immune system, although the pathomechanisms behind this have not been as thoroughly investigated as for the innate immune system. In brief, T-lymphocytes have been found in the peripheral nerves in mouse models for CMT1. Crossbreeding CMT1 mutant mice with RAG1-deficient mice which lack mature T- and B-lymphocytes leads to an amelioration of the neuropathy of these CMT1 mutant mice (Schmid et al., 2000; Kobsar et al., 2005). However, RAG1 deficiency does not appear to rescue the nerve phenotype in PMP22 transgenic mice, a mouse model for CMT1A (Kohl et al., 2010b). Another study on a mouse model for CMT1B mice shows that deficiency of a co-inhibitory molecule expressed on activated T- and B-lymphocytes, programmed cell death protein 1 (PD-1), leads to a stronger increase in the number of T-lymphocytes and an aggravated histological phenotype (Kroner et al., 2009). These studies indicate a role of T-lymphocytes in mediating the pathology in CMT1 mouse models. Furthermore, deposition of endogenous antibodies has been shown to contribute to early macrophage-mediated demyelination and disease progression in a mouse model for CMT1B, possibly by providing recognition sites for macrophages via their Fc receptors (Klein et al., 2015a). This reflects the interplay of components of the innate immune system with the adaptive immune system in CMT1.

3.3 Aging of the peripheral nervous system

Aging is known to cause structural abnormalities and functional decline in the nervous system and is a risk factor for various neurodegenerative diseases. While most studies of the aging nervous system understandably focus on the CNS,

age-related changes in the aging PNS have not been thoroughly investigated, and studies remain mostly descriptive.

Functional impairment of the PNS has been detected in aging individuals. In elderly humans, a decline of motor and sensory nerve conduction velocities (NCVs) and of the amplitudes of compound muscle action potentials (CMAPs) compared to non-aged adults has been found in different nerves (Drechsler, 1975; Dorfman and Bosley, 1979; Bouche et al., 1993; Verdu et al., 2000; Ward et al., 2015; Ward et al., 2016). A similar reduction of NCV and the CMAP amplitude in aged mice (24 months to 30 months) compared to adult mice has also been revealed by electrophysiological measurements (Verdu et al., 1996; Canta et al., 2016; Hamilton et al., 2016). Additionally, muscle strength is reduced in elderly humans (Greig et al., 1993; Ling et al., 2009) and aged mice (Sheth et al., 2018), and is commonly associated with muscle weakness during aging (Yang et al., 1996).

Structural alterations in the nerves and at the neuromuscular junctions (NMJs) in aging PNS may account for the age-related functional decline. In sural nerve biopsies of elderly humans, axonal loss and a reduction of nerve fiber density can be observed (O'Sullivan and Swallow, 1968; Jacobs and Love, 1985). Distal axon degeneration is also found in aged rodents (Sharma et al., 1980; Grover-Johnson and Spencer, 1981; Ceballos et al., 1999) and other aged animals (Braund et al., 1982; Wheeler and Plummer, 1989). Interestingly, motor axons appear to be more affected than sensory axons during aging (Krishnan et al., 2016), but no loss of motoneuron cell bodies can be detected in the spinal cords of aged mice (Chai et al., 2011). The de- and regeneration of axons in aged nerves possibly account for the formation of regeneration clusters (Ceballos et al., 1999; Verdu et al., 2000), similar to those observed in mouse models for peripheral neuropathies (Martini et al., 1995; Martini, 1997; Scherer et al., 1998; Hube et al., 2017). Another indication of axonal damage, periaxonal vacuoles, has been detected in nerves of aged rats (Thomas, 1997). Moreover, axon diameter has been observed to decrease during aging, accompanied by reduced expressions of neurofilament genes (Chase et al., 1992; Parhad et al., 1995; Ceballos et al., 1999). Furthermore, the regeneration capacity of aged peripheral nerves after injury is reduced compared to adult nerves (Sharma et al., 1980; Verdu et al., 1995; Verdu et al., 2000; Scheib and

Höke, 2016b). This is likely due to the decrease of axonal transport in aging nerves (McQuarrie et al., 1989; Pannese, 2011; Milde et al., 2015) and the reduced ability of debris removal of aged Schwann cells and macrophages upon injury (Kang and Lichtman, 2013; Scheib and Höke, 2016a; Scheib and Höke, 2016b).

Signs of myelin alteration can also be detected in aging nerves. Supernumerary Schwann cells, a hallmark of demyelination, can be found together with axons with thin myelin in peripheral nerves of elderly humans and aged rodents (Grover-Johnson and Spencer, 1981; Jacobs and Love, 1985; Verdu et al., 2000; Canta et al., 2016; Hamilton et al., 2016). Demyelination is a possible cause of the reduced internodal length in sural nerve biopsies of elderly humans (Lascelles and Thomas, 1966) and the reorganization of voltage-sensitive sodium channels at the nodes of Ranvier of larger caliber axons (Adinolfi et al., 1991; Moldovan et al., 2016). Accompanying the morphological changes, expression of myelin protein genes also decreases in aged rodents (Melcangi et al., 1998a; Melcangi et al., 1999; Melcangi et al., 2000; Hamilton et al., 2016). In addition, earlier studies have reported other myelin alterations, such as myelin infoldings and outfoldings in the aged peripheral nerves (Knox et al., 1989; Ceballos et al., 1999; Verdu et al., 2000; Canta et al., 2016). These structural changes of myelin might be an early response of myelin sheaths of larger caliber axons to axonal dystrophy (Krinke et al., 1988).

Additionally, NMJs undergo structural alterations and functional disturbance during aging in elderly humans (Wokke et al., 1990; Jones et al., 2017; Taetzsch and Valdez, 2018) and aged mice (Valdez et al., 2010; Chai et al., 2011; Carnio et al., 2014; Gonzalez-Freire et al., 2014; Rudolf et al., 2014; Tintignac et al., 2015; Jones et al., 2017). It is likely that impairment of autophagy in the muscle, a loss of adult skeletal muscle stem cells and a loss of trophic factors contribute to the denervation in aged NMJs (Carnio et al., 2014; Tintignac et al., 2015; Liu et al., 2017). However, it is not clear yet whether NMJ dysfunction is a primary cause of muscle weakness or is secondary to the age-related muscle fiber degeneration (Tintignac et al., 2015; Willadt et al., 2018). The uncoupling of structural alteration of NMJ and the impairment of muscle function requires further research. Notably, results of studies on NMJs are dependent on the muscle and species of

observation. It has been shown that the rate of denervation varies among different muscles (Valdez et al., 2012), and that human NMJs are different from mouse NMJs both structurally and molecularly (Jones et al., 2017).

Phagocytosing macrophages and mast cells have been found in aging nerves (Ceballos et al., 1999; Verdu et al., 2000). This increase of immune cells correlates well with the general increase of inflammation in aging individuals, termed as “inflammaging” (Büttner et al., 2018). However, PNS macrophages in aged nerves have not been characterized, and their role in aging PNS is not yet known. This is in stark contrast to aged microglia, which have an increased burden of myelin clearance and show clear signs of activation in the aging CNS (Norden and Godbout, 2013; Safaiyan et al., 2016; Marschallinger et al., 2020).

Taken together, previous studies have shown age-related structural changes, functional declines and an increase in the number of immune cells in aging individuals of different species (summarized in Table 1). However, whether inflammation is pathogenic in an age-related neuropathy has not been studied yet. In the present study, the contribution of inflammation during the normal aging of peripheral nerves of mice is investigated.

Table 1 Structural, morphological and molecular changes in the PNS demonstrated by previous studies.

Age-related Changes in the PNS	Species	References
Reduced sensory and motor NCV, reduced CMAP amplitudes	Human	Drechsler, 1975 Dorfman and Bosley, 1979 Bouche et al., 1993 Ward et al., 2015 Ward et al., 2016
	Mouse	Verdu et al., 1996 Canta et al., 2016 Hamilton et al., 2016

(Table continues on the next page)

Reduced muscle strength	Human	Ling et al., 2009 Greig et al., 1993
	Mouse	Sheth et al., 2018
Reduced axonal transport	Rat	McQuarrie et al., 1989
	Mouse	Milde et al., 2015
Reduced regeneration capacity after injury	Rat	Sharma et al., 1980 Scheib and Höke, 2016
	Mouse	Verdu et al., 1995 Verdu et al., 2000
Reduced axon diameter	Cat	Chase et al., 1992
	Mouse	Ceballos et al., 1999
	Rat	Sharma et al., 1980
Axonal loss, reduced nerve fiber density	Human	O'Sullivan and Swallow, 1968 Jacobs and Love, 1985
	Horse	Wheeler and Plummer, 1989
	Dog	Braund et al., 1982
	Rat	Grover-Johnson and Spencer, 1981 Sharma et al., 1980
	Mouse	Ceballos et al., 1999 Krishnan et al., 2016
De- and remyelination	Human	Jacobs and Love, 1985
	Rat	Grover-Johnson and Spencer, 1981
	Mouse	Verdu et al., 2000 Canta et al., 2016 Hamilton et al., 2016
Shortened internodal length	Human	Lascelles and Thomas, 1966

(Table continues on the next page)

Regeneration clusters	Mouse	Ceballos et al., 1999 Verdu et al., 2000
Periaxonal vacuoles	Rat	Thomas, 1997
Reorganization of voltage-gated sodium channels	Cat	Adinolfi et al., 1991
	Mouse	Moldovan et al., 2016
Myelin abnormalities	Rat	Knox et al., 1989
	Mouse	Ceballos et al., 1999 Verdu et al., 2000 Canta et al., 2016
NMJ fragmentation, denervation and degeneration	Human	Wokke et al., 1990
	Mouse	Valdez et al., 2010 Chai et al., 2011 Carnio et al., 2014 Jones et al., 2017
Phagocytosing macrophages	Mouse	Ceballos et al., 1999 Verdu et al., 2000
Reduced gene expression of neurofilaments	Rat	Parhad et al., 1995
Reduced expression of myelin proteins and myelin genes	Rat	Melcangi et al., 1998 Melcangi et al., 1999 Melcangi et al., 2000
	Mouse	Hamilton et al., 2016

3.4 Aim of the study

The aim of the study was to determine the role of inflammation in aging peripheral nerves by addressing the following issues:

1) Analysis of nerve biopsies from adults and elderly humans for characterizing pathological alterations and inflammatory reactions occurring during aging.
2) Detailed characterization of pathological alterations, immune cells, inflammatory cytokines and functional analysis in aging peripheral nerves.
3) Investigating the pathogenic role and functional relevance of macrophages in peripheral nerves of aged mice by macrophage depletion.
4) Examining whether systemic inflammation acts as a risk factor for an aggravated neuropathy in aged mice by challenging aged mice with LPS and investigating the physiological, morphological and functional outcome after LPS injection.

4. Material and methods

4.1 Equipment, antibodies, buffers and solutions

Technical equipment (8.1), antibodies for immunohistochemistry (8.2), reagents (8.3), buffers and solutions (8.4) and primer sequences (8.5) are listed in detail in the appendices.

4.2 Analysis of human sural nerve biopsies and ethics statement

The nerve biopsy material was retrieved from the diagnostic archives of the Institute of Neuropathology, RWTH University Hospital, Aachen. The anonymous use of this material for scientific purposes is permitted according to the rulings of the Ethical Commission of the Medical Faculty, RWTH University with the approval number EK126/18 (applied by Prof. Dr. Joachim Weis, RWTH University, Aachen), and to the laws of the State of North Rhine-Westphalia without further individual ethical ruling. The biopsies had been obtained from a 39-year-old patient and 4 patients from 65 to 79 years of age (sexes not known) during examinations to determine the cause of neuromuscular symptoms. The patients included in the present study had undergone sural nerve biopsy surgery because of mild, unspecific sensory symptoms that had led to the suspicion of neuropathy. Their biopsies showed a healthy appearing nerve, and no signs of neuropathy were reported later. To the author's knowledge, no genetic testing was performed on these patients. The 65-year-old patient with chronic idiopathic axonal polyneuropathy (CIAP), whose biopsy is used as a disease control, was part of a previously published study (Hube et al., 2017). The selection of biopsies was performed by Prof. Dr. Joachim Weis, and the electron microscopic images were taken by Prof. Dr. Joachim Weis and Dr. Istvan Katona (RWTH University, Aachen; Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest).

4.3 Animals and genotyping

Wild-type (WT) mice of both sexes on a C57BL/6 background were investigated at 12, 18 and 24 months of age. *Trem2* knockout (*Trem2*-/-) mice on a C57BL/6

background were provided by Prof. Dr. Harald Neumann (Bonn) and Prof. Marco Colonna (St. Louis) and were investigated at 24 months of age. The genotypes of *Trem2*-/- mice were determined through polymerase chain reaction (PCR) on genomic DNA extracted and purified from ear biopsies using DNeasy blood & tissue kit (Qiagen) according to the manufacturer's guidelines (primer sequences are listed in Appendix 8.5).

Animals were kept in individually ventilated cages (IVC) under barrier conditions in the animal facility of the Department of Neurology, University Hospital of Würzburg with a 12h/12h day/night rhythm (<300 lux during day), or in the Center of Experimental Molecular Medicine, University of Würzburg with a 14h/10h day/night rhythm (<300 lux during day). All animal experiments were approved by the local authority, the Government of Lower Franconia, Germany.

4.4 Dissection and tissue processing

Animals were euthanized by asphyxiation with CO_2 (according to guidelines by the State Office of Health and Social Affairs, Berlin). Blood was rinsed with phosphate-buffered saline (PBS) containing heparin.

For immunohistochemistry of fresh frozen tissue, femoral quadriceps nerves and flexor digitorum brevis muscles were freshly dissected, embedded in Tissue-Tek® O.C.T.™ Compound (Sakura), and frozen in methylbutane cooled by liquid nitrogen. For isolation of total RNA and protein, tissue was snap frozen in liquid nitrogen and stored at -80°C until use.

For immunohistochemistry of fixed femoral nerves, mice were transcardially perfused with 2% paraformaldehyde (PFA) in PBS for 10 minutes. Femoral nerves were dissected and post-fixed in 2% PFA for 2 hours. After overnight dehydration in 30% sucrose in PBS, nerves were embedded in OCT medium and frozen in methylbutane cooled by liquid nitrogen. Fresh frozen and fixed tissue was cut into 10-µm-thick cross sections on a cryostat (Leica) and stored at -20°C.

For whole-mount preparations, flexor digitorum brevis muscles were dissected from euthanized mice, then squeezed between two glass slides with a drop of 4% PFA and post-fixed in 4% PFA for 2 hours before subsequent staining.

For electron microscopy, euthanized mice were transcardially perfused with 4% PFA and 2% glutaraldehyde in 0.1 M cacodylate buffer for 10 minutes. Dissected femoral nerves were subsequently post-fixed in the same solution overnight at 4°C. Osmification was performed with 2% osmium tetroxide in 0.1 M cacodylate buffer for 2 hours, followed by dehydration in ascending acetone concentrations. Nerves were embedded in Spurr's medium. Ultrathin sections (70 nm) were cut and mounted to copper grids and counterstained with lead citrate.

4.5 Immunohistochemistry

For detecting endoneurial macrophages in mouse peripheral nerves, cross sections of femoral nerves were first blocked with 5% BSA in PBS and then incubated at 4°C with rat anti-mouse F4/80 primary antibody (1:300, MCAP497, Serotec) in 1% BSA in PBS overnight. The primary antibody was omitted for negative controls. After washing with PBS, samples were incubated with Cy3-conjugated goat anti-rat IgG (1:300, 112-165-167, Dianova) secondary antibody for 1 hour at room temperature. Nuclei were stained with DAPI (1:500000, D9542, Sigma-Aldrich) for 10 minutes at room temperature.

For characterizing macrophage activation, immunohistochemical staining against CD206 was performed on 2% PFA fixed femoral nerves. After acetone fixation for 10 minutes at -20°C, sections were blocked with 5% BSA in PBS with 0.3% Triton X-100. Samples were then incubated with rat anti-mouse CD206 antibody (1:2000, MCA2235, Serotec) in 1% BSA in PBS with 0.3% Triton X-100 at 4°C overnight. CD206 was detected with Cy3-conjugated goat anti-rat IgG secondary antibody as described above. Nuclei were visualized with DAPI staining.

To detect T-lymphocytes, immunohistochemical stainings against CD4 or CD8 were applied on cross sections of fresh frozen femoral nerves as described above. Rat anti-mouse CD4 (1:1000, MCA1767, Serotec) or rat anti-mouse CD8 (1:500, MCA609G, Serotec) primary antibody was applied on sections overnight and was detected with Cy3-conjugated goat anti-rat IgG secondary antibody the next day. Nuclei were visualized with DAPI staining.

To determine muscle innervation, after blocking with 5% BSA with 0.3% Triton X-100, cross sections of the flexor digitorum brevis muscle were incubated with

guinea pig polyclonal primary antibody against synaptophysin (1:500, 101004, Synaptic Systems) in 1% BSA with 0.3% Triton X-100 at 4°C overnight, followed by visualization of presynaptic terminals using Cy3-conjugated donkey anti-guinea pig IgG secondary antibody (1:300, 706-165-148, Dianova). Postsynapses were labeled with AlexaFluor-488-conjugated α-bungarotoxin (1:300, B-13422, Invitrogen). At least 200 neuromuscular junctions (NMJs) were analyzed per animal. Innervated NMJs were identified by the complete overlap of synaptophysin with α-bungarotoxin labeling. Partially denervated NMJs were identified by an overlay of postsynaptic labeling with incomplete presynaptic staining. Denervated NMJs were identified by the absence of presynaptic staining. The latter two innervation patterns were collectively named as “abnormally innervated NMJs.”

To visualize NMJs from whole-mount preparations, after blocking with 5% BSA with 0.3% Triton X-100, flexor digitorum brevis muscles were incubated with primary antibodies against synaptophysin (guinea pig polyclonal, 1:500, 101004, Synaptic Systems) and βIII-tubulin (rabbit polyclonal, 1:500, AB18207, Abcam) at 4°C overnight. Primary antibodies were detected by Cy3-conjugated donkey anti-guinea pig IgG secondary antibody (1:300, 706-165-148, Dianova) and AlexaFluor-488-conjugated goat anti-rabbit IgG secondary antibody (1:300, A11008, Invitrogen). Postsynapses were labeled with AlexaFluor-647-conjugated α-bungarotoxin (1:300, B35450, Invitrogen).

To identify macrophages on human peripheral nerves, 3-4 µm paraffin sections of the formalin-fixed human sural nerve biopsy specimens were incubated for 1 hour with the FLEX monoclonal mouse anti-human CD68 antibody (1:50, GA609, Dako Omnis), followed by incubation with the EnVision FLEX$^+$ mouse DAB visualization kit (GV800, Dako Omnis). Staining of the human sural nerve biopsies were performed by Dr. Istvan Katona (Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest) and Prof. Joachim Weis (Institute of Neuropathology, RWTH Aachen University).

All samples were embedded after the final washing step and were mounted with Aqua-Poly/Mount® (Polysciences).

Digital fluorescence microscopic images of cross sections were acquired using an Axiophot 2 microscope (Zeiss) equipped with a CCD camera (Visitron Systems). Digital fluorescence microscopic images of muscle whole-mount preparations were acquired using an ApoTome 2 microscope (Zeiss). Images were processed with Photoshop CS6 (Adobe) or ImageJ (National Institutes of Health). For quantification of immune cells (CD4, CD8, CD86, CD206, F4/80), whole nerve cross sections were analyzed and the mean number of corresponding profiles per section in 7-10 consecutive sections per animal was calculated. Percentages of CD206+ profiles were calculated based on the total macrophage number shown by quantification of F4/80+ profiles.

4.6 RNA extraction and semi-quantitative real-time PCR

RNA extraction and semi-quantitative reverse transcription PCR (qRT-PCR) were performed as previously described (Fischer et al., 2008a). Snap frozen femoral quadriceps nerves were homogenized (ART-MICCRA D-8, ART Labortechnik) in TRIzol® reagent (Invitrogen). Total RNA was isolated through phenol-chloroform extraction, phase separation and subsequent precipitation. Before RNA precipitation, 20 µg of Glycogen (Roche Diagnostics) was added to each sample. Precipitated RNA was solubilized in 10 µl DEPC-H_2O and then incubated at 60°C for 10 minutes. Concentration and quality of RNA were measured using a photometer (Eppendorf).

Reverse transcription of 0.5 µg of RNA was performed in a 100 µl reaction tube using random hexamer primers and Moloney murine leukemia virus (M-MLV) reverse transcriptase (Applied Biosystems) according to the guidelines of manufacturer with the following cycler conditions: 10 minutes at 25°C; 60 minutes at 37°C; 5 minutes at 95°C. 1.5 µl of the generated complementary DNA (cDNA) was analyzed in duplicates by semi-quantitative real-time PCR using TaqMan universal PCR master mix (Applied Biosystems) and pre-developed TaqMan® assays (eukaryotic *18s rRNA*, 4319413E; *Adgre1*, Mm00802529_m1; *Arg1*, Mm00475988_m1; *Cd86*, Mm00444543; *Cd206*, Mm00485148_m1; *Ifnγ*, Mm01168134_m1; *Il-1β*, Mm00434228_m1; *Nos2*, Mm00440485_m1; *Itgam*, Mm00434455_m1; *Lif*, Mm00434761_m1; *Ccl2/Mcp-1*, Mm99999056_m1; *Tgf-β*,

Mm00441724_m1; *Tnf-α*, Mm00443258_m1; *Csf-1*, Mm00432686_m1; *Il-34*, Mm01243248_m1; *Trem-2*, Mm00451744_m1; *Gapdh*, Mm99999915_g1; *Rplp0*, Mm00725448_s1; *Igf1*, Mm00439560_m1) according to the instructions of the manufacturers (10 µl per sample). Data sets were analyzed with the $\Delta\Delta C_T$ method in relation to the corresponding endogenous controls and were compared to a 12M WT sample. *Igf1* expression was analyzed with *Gapdh* and *Rplp0* as endogenous controls. The endogenous control for other genes was *18s RNA*.

4.7 Protein extraction and western blot

Snap frozen femoral quadriceps nerves were sonicated (Sonoplus HD60, Bandelin electronic) and then solubilized in 100 µL RIPA lysis buffer per 10 mg tissue. Protein concentrations were measured by a Lowry assay (Sigma-Aldrich) using BSA as standard (Lowry et al., 1951). 6x Laemmli sample buffer (Laemmli, 1970) was added to individual samples and the samples were then denatured for 5 minutes at 95°C. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with 10% acrylamide was performed on equal amount of protein taken from each sample as well as a protein ladder (Thermo Fisher) using a Mini-PROTEAN3 module (Bio-RAD). Separated proteins were transferred from the gel to a nitrocellulose membrane (Hartenstein) using a Mini-Trans-Blot® module (Bio-RAD) at 400 mA with the addition of an ice-cooling unit. Protein transfer was confirmed with 0.1% Ponceau S staining (Carl Roth). Membranes were blocked with 5% skimmed milk in PBST and incubated with primary antibodies (chicken anti-P0, 1:3000, NB100-1607, Acris; mouse anti-Cx32, 1:500, 35-8900, Invitrogen; rabbit anti-ERK1/2, 1:10000, sc-94, Santa Cruz) in antibody solution overnight at 4°C. After three washing steps in PBST, the corresponding horseradish peroxidase (HRP) conjugated secondary antibodies (donkey anti-chicken, 1:10000, 703-035-155, Jackson ImmunoResearch; donkey anti-mouse, 1:10000, 715-035-150, Jackson ImmunoResearch; donkey anti-rabbit: 1:10000, 711-035-152, Jackson ImmunoResearch) were incubated for 1 hour at room temperature. After three further washing steps, immune reactions were examined by incubation with ECL reagents for 1 minute and the chemiluminescence was detected by ECL hyperfilm (GE Healthcare). Densitometric analyses were

performed with ImageJ (National Institutes of Health). Specific bands for P0, Cx32 and ERK1/2 were detectable at 28 kDa, 32 kDa and 41 kDa, respectively. ERK1/2 was used as a loading control.

4.8 Electron microscopy and morphological analysis of mouse peripheral nerves

Electron microscopic images were taken using a ProScan Slow Scan CCD camera mounted to a Leo 906E electron microscope (Carl Zeiss). Multiple images were aligned with iTEM software (Olympus Soft Imaging Solutions) to analyze complete nerve cross sections. Pathological alterations were quantified in relation to the total number of axons in corresponding cross sections of the femoral quadriceps nerve. Thinly myelinated axons, supernumerary Schwann cells (onion bulbs), fibers with undulated myelin, degenerating or degenerated axons, dark axons, Schwann cell protrusions, regeneration clusters, periaxonal vacuoles, and myelin abnormalities were determined and then quantified. Foamy macrophages were counted, and their numbers were given as relative values per 100 axons within the nerve. Fibers with undulated myelin were defined as fibers with 4 undulations of myelin. Myelin infoldings and myelin outfoldings were termed collectively as myelin abnormalities.

4.9 Nerve conduction studies

Mice were anesthetized by an intraperitoneal injection with a mixture of Ketavet (Pfizer) and Xylavet (CP-Pharma) (100 mg Ketavet and 6.67 mg Xylavet per kilogram bodyweight) and were placed under a heating lamp to avoid hypothermia. Body temperature of mice was controlled before and after measurements (34-36°C). Neurological properties of the left sciatic nerve were measured as described previously (Zielasek et al., 1996; Klein et al., 2015b). Shortly after supramaximal stimulation of the tibial nerve with monopolar needle electrodes at the ankle (distal) and the sciatic nerve at the sciatic notch (proximal), compound muscle action potentials (CMAPs) were recorded at the hind paw muscles using steel needle electrodes. Distal, proximal and F-wave latencies as well as the

distance between stimulation sites were measured. The corresponding nerve conduction velocity (NCV) was calculated by the distance between stimulation sites divided by the difference between proximal and distal latencies. All neurographic recordings were performed with a digital Neurosoft-Evidence 3102 electromyograph (Schreiber & Tholen Medizintechnik). Neurographic recordings were performed by Dr. Dennis Klein from Department of Neurology, University Hospital of Würzburg.

4.10 Macrophage depletion

Rodent chow containing 300 mg/kg CSF-1R specific kinase (c-FMS) inhibitor (PLX5622) was provided by Plexxikon Inc. (Berkeley, USA). Mice were treated with the chow *ad libitum* from 18 to 24 months of age and were dissected at 24 months of age. Untreated age-matched mice on normal chow were used as control. During experiments, animals were monitored weekly for weight, general health and behavior. No animals were required to be excluded from the study according to the standard criteria of University of Würzburg (https://www.uni-wuerzburg.de/verwaltung/agtu/aufgaben/tierschutz/ethik-tierversuch/).

4.11 Systemic inflammation challenge

To induce systemic inflammation, LPS (L5886, Sigma-Aldrich) was injected intraperitoneally into 11- or 23-month-old mice with a dose of 500 µg/kg body weight. 0.9% NaCl solution was injected intraperitoneally into control groups. General sickness behavior was monitored daily for the first week after injection, and then monitored weekly. Mice were euthanized and excluded if meeting the humane endpoints (Pettan-Brewer and M. Treuting, 2011; Burkholder et al., 2012).

4.12 Motor performance tests

Grip strength of the hindlimbs was measured using an automated Grip Strength Meter (Columbus Instruments) as described previously (Groh et al., 2010). With forelimbs supported by a metal grid, mice were trained to hold a grip bar properly

with hind paws. The maximum force (in newton) was measured when the mouse was pulled off the grip bar with constant strength. Ten measurements per day were performed on 3 consecutive days at approximately the same hour (before noon), and the mean of the measurements was calculated. Investigated mice were sex- and weight-matched.

Mice were placed on a rod from a RotaRod Advanced System (TSE Systems) with the starting speed of 5 rpm as described previously (Groh et al., 2016b) and were trained to walk on the accelerating rod (5-50 rpm; max latency: 300 seconds) until falling off. For the pre-injection measurements, mice were trained with 5 runs per day for 2 consecutive days and the latency to fall was measured for 5 runs on the third day.

4.13 Statistical analysis

All experiments were performed in a blinded manner, with the investigators not aware of the age or the treatment status of the analyzed mice. Statistical analyses were performed using PASW Statistics 18 (SPSS, IBM) software or Prism 7 (GraphPad). Data sets were controlled for normal distribution by the Shapiro-Wilk test, and variance homology was determined by Levene's test. Differences among different mouse groups with normally distributed data were tested by one-way ANOVA, followed by Tukey's post hoc test or Bonferroni-Holm correction. A two-way ANOVA test was applied to compare the grip strength and rotarod performance of different groups at different time points after injection. In the case of non-normally distributed datasets, the nonparametric Kruskal-Wallis test with Bonferroni-Holm correction was applied. Significance levels (* $p<0.05$; ** $p<0.01$; *** $p<0.001$) are indicated together with the applied statistical tests within the figure legends. Measurements and quantifications are shown as individual values and mean ± SD. Graphs were generated with Prism 7.

5. Results

5.1 Pathological alterations and inflammation in peripheral nerves of elderly humans

Previous studies have reported aged-related morphological and structural changes in peripheral nerves of both humans and mice (Jacobs and Love, 1985; Robertson et al., 1993; Ceballos et al., 1999; Verdu et al., 2000), but systematic studies concerning histopathology are rare and the underlying pathomechanisms are not known. To provide a human-related base for investigating possible pathomechanisms underlying aging-related neuropathy in this study, we became particularly interested in a systematic investigation of nerve biopsies of elderly people not primarily or secondarily suffering from peripheral neuropathies. As this is outside the scope of our expertise, we asked our colleagues, Prof. Joachim Weis (RWTH Aachen University) and Dr. Istvan Katona (Hungarian Academy of Sciences, Budapest), to investigate age-related changes in human peripheral nerves. Prof. Weis is a well-known and highly established neuropathologist working on neuromuscular disorders and managing one of the largest collections of human biopsies of peripheral nerves in the world.

Our colleagues found sural nerve biopsies of a 39-year-old (control) individual and of four elderly individuals (ages ranging from 65 to 77 years) in their diagnostic archives. As an important prerequisite mentioned in the Material and Methods, none of the selected individuals were diagnosed with a peripheral neuropathy. In contrast to the adult nerve showing normal small and large caliber axons (Fig. 1A, C), nerve biopsies of the elderly individuals showed features of demyelination (Fig. 1B, D), including onion bulbs and axons with thinner myelin. Phagocytosing macrophages, often myelin-laden (Fig. 1D), were identified in these nerve biopsies from elderly individuals on both the light microscopic (Fig. 1B) and electron microscopic (Fig. 1D) level, and their presence was confirmed through immunohistochemical staining of CD68, a lysosomal marker identifying phagocytosing macrophages (Fig. 1E). However, when compared to the sural nerve biopsy from a chronic idiopathic axonal polyneuropathy (CIAP) patient showing multiple regeneration clusters (Fig. 1F) (Hube et al., 2017), the sural nerve biopsies of elderly individuals showed only mild axon degeneration.

Taken together, pathological alterations of myelin and axons can be found in peripheral nerves of elderly humans, together with phagocytosing endoneurial macrophages.

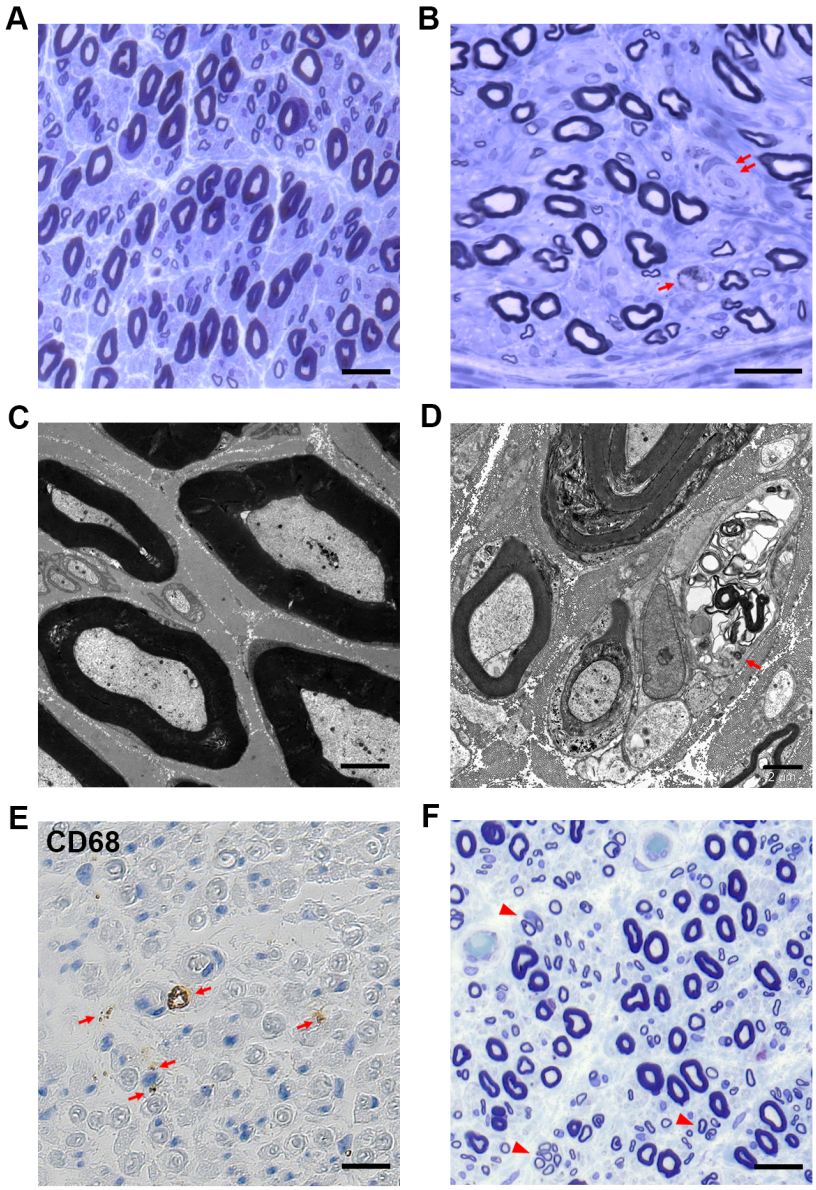

Figure 1 Increased pathological alterations in peripheral nerves of elderly humans. (**A**) Sural nerve biopsy of a 39-year-old subject showing normal large and small myelinated nerve fibers without pathological alterations. Semithin resin section, toluidine blue. Scale bar, 40 µm. (**B**) Myelinated large and small nerve fibers and a feature indicative of demyelination (double arrow) in a sural nerve biopsy from a 77-year-old patient. Note the presence of a macrophage (arrow). Semithin resin section, toluidine blue. Scale bar, 40 µm. (**C**) Electron micrograph of myelinated and unmyelinated axons of the nerve biopsy from the 39-year-old subject depicted in (**A**). Scale bar, 3 µm. (**D**) Macrophage profile (arrow) among several myelinated and unmyelinated axons in an electron micrograph of a nerve biopsy from a 70-year-old patient. Scale bar, 2 µm. (**E**) CD68+ endoneurial macrophages (arrows) in the sural nerve biopsy of a 65-year-old patient. Paraffin section. Scale bar, 40 µm. (**F**) Clusters of regenerated nerve fibers (arrowheads)

in a sural nerve biopsy of a 65-year-old patient with CIAP. Semithin resin section, toluidine blue. Scale bar, 40 µm.

5.2 Peripheral neuropathy and increased inflammation in the peripheral nervous system of aged mice

5.2.1 Increased pathological alterations in peripheral nerves of aged mice

To study the changes and possible mechanisms in the PNS during normal aging, WT C57BL/6 mice were selected as an animal model for their well-characterized lifespan (Fox et al., 2006; Graber et al., 2015b) and accessibility for neurobiological investigations. To histopathologically characterize peripheral nerves of aged mice, femoral quadriceps nerves of 12-month-old (adult) and 24-month-old (aged) WT C57BL/6 mice were analyzed by electron microscopy. The femoral quadricep nerve contains ~560 ± 40 myelinated axons, 40% - 50% of which are motor axons, allowing whole-nerve analyses for quantitative assessment of pathological alterations in mouse models of peripheral neuropathies (Frei et al., 1999; Kobsar et al., 2003). In cross sections of femoral quadriceps nerves of adult mice, myelinated axons showed the typical appearance with evenly bent myelin sheaths that are mostly near-circular (Fig. 2A). In comparison, in aged mice, myelin sheaths with an undulated appearance were identified (Fig. 2B), likely reflecting a shrinkage of corresponding axons. Quantification indeed confirmed that fibers with such undulated myelin were also significantly more frequent in aged mice than in adult mice (Fig. 3C). In addition, myelin inclusions, formed by myelin appearing to be protruding into intra-axonal space, were frequently detected (Fig. 2C). Other myelin abnormalities, such as myelin outfoldings, were also found in aged mice (data not shown), which is consistent with previous studies from other laboratories (Ceballos et al., 1999; Verdu et al., 2000). These myelin abnormalities were significantly more frequent in aged mice than in adult mice (Fig. 3H). Notably, onion bulbs, formed by supernumerary Schwann cells and typically considered as a hallmark of demyelinating neuropathies, were also detectable in aged mice (Fig. 2D), again corroborating previous studies (Ceballos et al., 1999; Verdu et al., 2000). Similar to the observation in peripheral nerves of aged humans (Fig. 1B), onion bulbs were often observed surrounding thinly myelinated axons (Fig. 2D).

Quantifications revealed that percentages of both onion bulbs and thinly myelinated axons substantially increased in aged mice compared to adult mice (Fig. 3A, B).

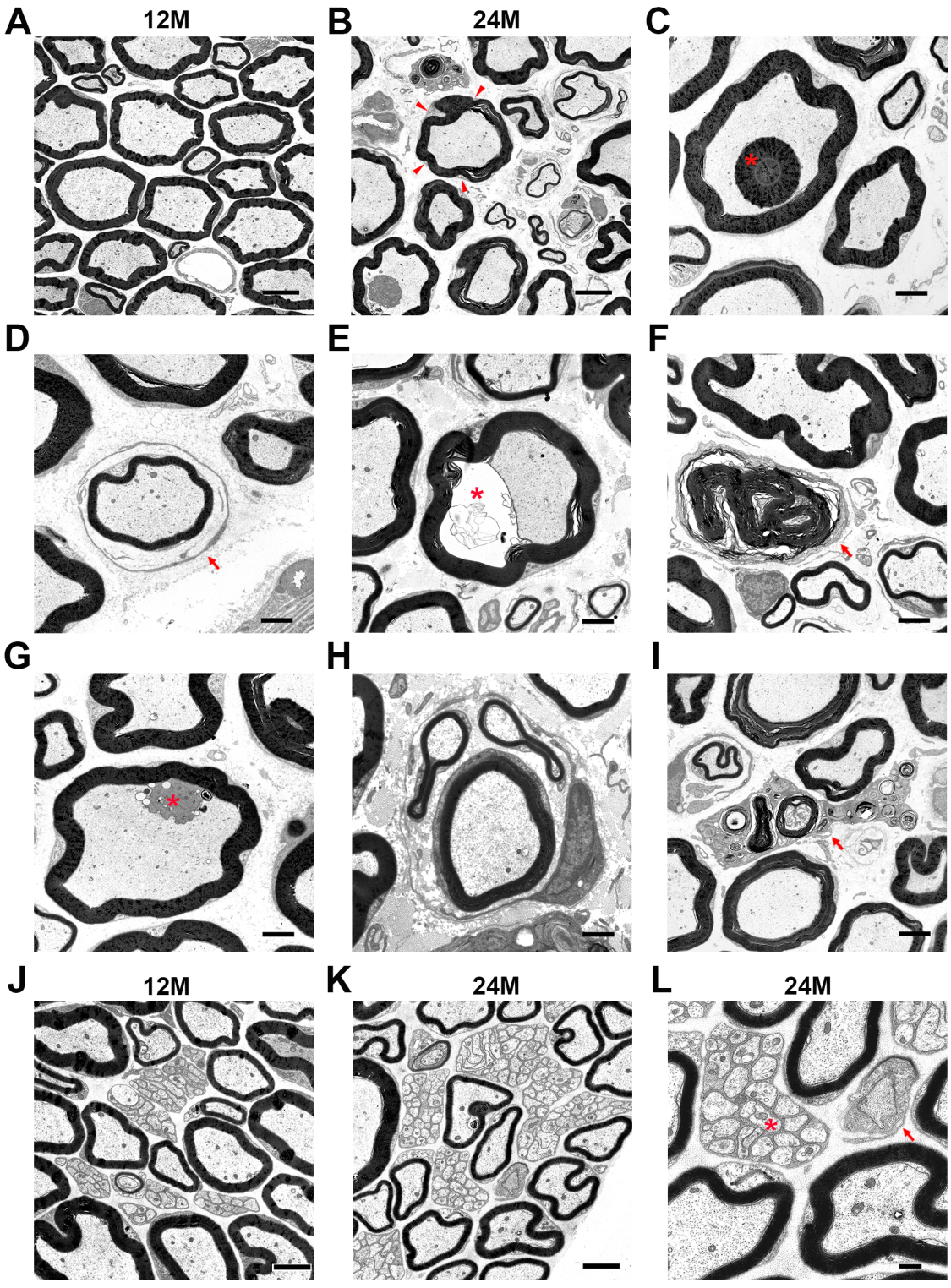

Figure 2 Pathological alterations in peripheral nerves of aged mice. (**A**, **B**) Electron microscopy of representative ultrathin sections of femoral quadriceps nerves from 12-month-old (12M) (**A**) and 24-month-old (24M) (**B**) mice. Note the undulated myelin

profiles in 24-month-old mice (arrowheads). This feature is lacking in 12M mice. (**C** – **I**) Representative electron micrographs of the following profiles: a myelin inclusion (**C**, asterisk), a thinly myelinated axon surrounded by a layer of supernumerary Schwann cells ("onion bulb"; **D**, arrow), a periaxonal vacuole (**E**, asterisk), a Schwann cell–myelin profile devoid of an axon (**F**, arrow), a Schwann cell protrusion (**G**, asterisk), a regeneration cluster (**H**), and a foamy macrophage (**I**, arrow). (**J** – **L**) Electron microscopy of representative ultrathin sections of femoral saphenous nerves from 12-month-old (**J**) and 24-month-old (**K**, **L**) mice. (**L**) Higher-magnification electron micrograph of (**K**) displaying a Remak bundle (asterisk) and a normal node of Ranvier (arrow). Scale bars: **A**, **B**, 5 µm; **C** – **K**, 2 µm; **L**, 0.5 µm.

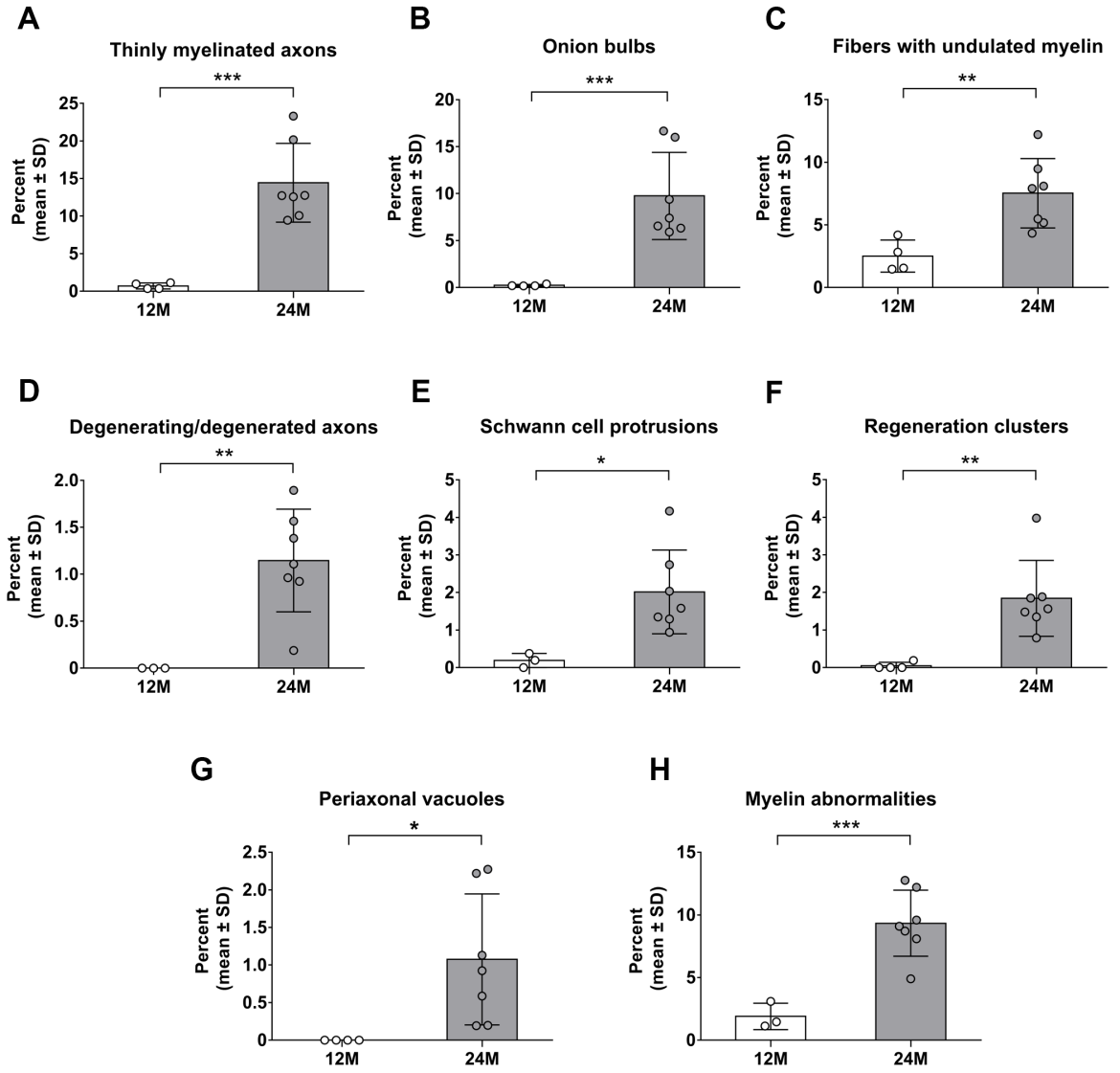

Figure 3 Increased pathological alterations in peripheral nerves of aged mice. (**A** – **H**) Quantifications of thinly myelinated axon (**A**), onion bulbs (**B**), fibers with undulated myelin (**C**), degenerating/degenerated axons (**D**), Schwann cell protrusions (**E**), regeneration clusters (**F**), periaxonal vacuoles (**G**) and myelin abnormalities (**H**) in 12-month-old (12M) and 24-month-old (24M) mice. Note different ranges of the size (y-axis) of different pathological features. Individual values and mean ± SD are shown. **A**, **B**, **D**, **G**: Mann-Whitney U test; **C**, **E**, **F**, **H**: student's t-test; * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

Additionally, profiles such as periaxonal vacuoles (Fig. 2E) and degenerated axons (Fig. 2F) were also found in 24-month-old mice, indicating axonal perturbation in these aged mice. Such profiles were not detected in 12-month-old mice (Fig. 3D, G). However, the axonal perturbation was mild in aged mice, and there was no significant axonal loss in peripheral nerves of aged mice (data not shown). Schwann cell protrusions (Fig. 2G) and regeneration clusters (Fig. 2H) were also found to be significantly higher in percentages in aged mice compared to adult mice (Fig. 3E, F). Interestingly, the femoral saphenous nerve, a sensory branch of the femoral nerve, did not show pathological alterations in aged mice (Fig. 2K, L) compared to adult mice (Fig. 2J).

5.2.2 Elevated numbers of macrophages and altered cytokine expressions in peripheral nerves of aged mice

Recent studies have demonstrated that inflammation amplifies disease progression in mouse models of hereditary peripheral neuropathies (Kobsar et al., 2003; Kobsar et al., 2005; Groh et al., 2010; Kohl et al., 2010a; Groh et al., 2012; Martini et al., 2013; Groh et al., 2015; Klein et al., 2015a; Klein and Martini, 2015; Klein et al., 2015b). Phagocytosing macrophages, also known as “foamy macrophages” due to incorporated myelin, were detected in femoral quadriceps nerves of 24-month-old mice (Fig. 2I). This was a histopathological feature comparable to the observations in sural nerve biopsies in elderly humans (Fig. 1D, E). The foamy macrophages were observed in peripheral nerves of 24-month-old mice but not in 12-month-old mice (Fig. 4C), reflecting increased levels of inflammation in peripheral nerves of aged mice. To characterize inflammation in the aging of mouse peripheral nerves, endoneurial macrophages in femoral quadriceps nerves of 12-month-old, 18-month-old and 24-month-old mice were investigated by immunohistochemical staining against a pan-macrophage marker, F4/80 (Fig. 4A). Numbers of macrophages in 24-month-old mice significantly higher compared to 12-month-old and 18-month-old mice (Fig. 4B). Quantification of foamy macrophages via electron microscopy also revealed significant increase of these cells in 24-month-old mice compared to 12-month-old and 18-month-old mice (Fig. 4C). There was no increase in the number of endoneurial macrophages

in the femoral saphenous nerves of 24-month-old mice (data not shown), which is in line with the lack of emerging pathological alterations in these nerves (Fig. 2K).

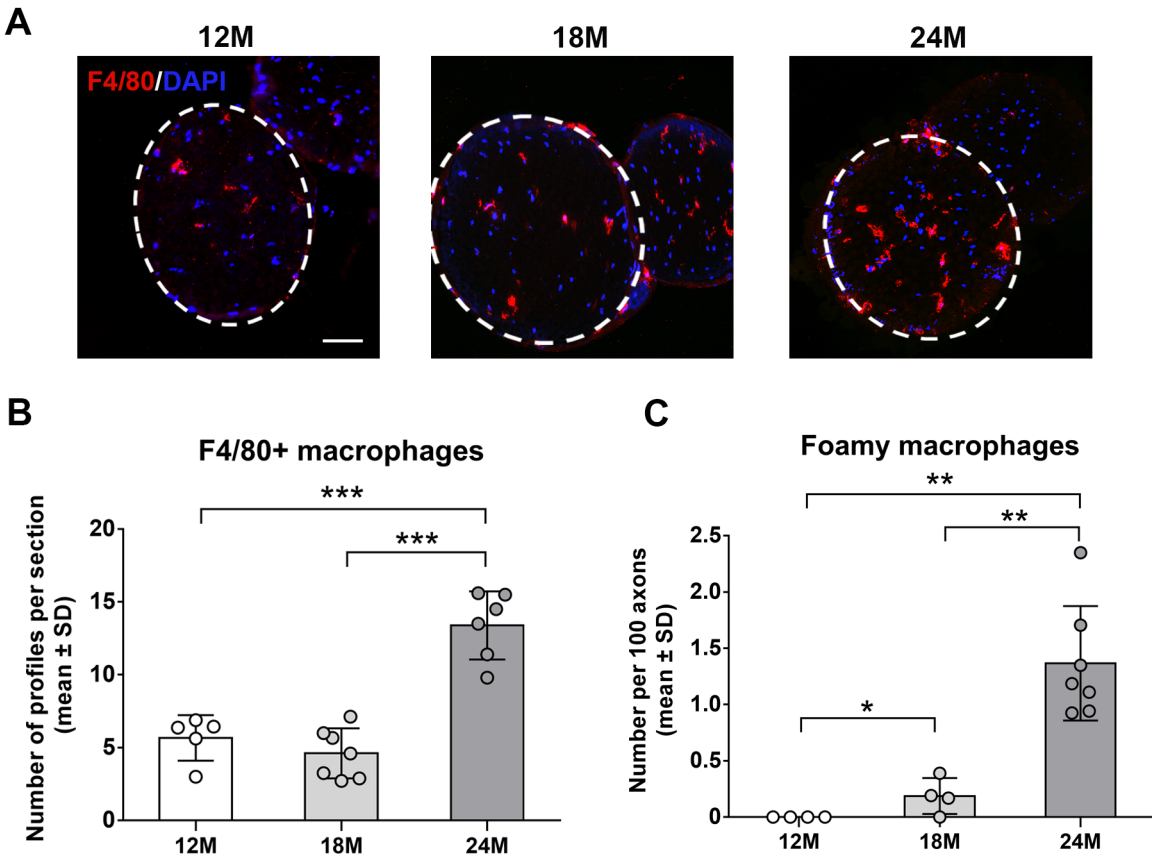

Figure 4 Elevated numbers of macrophages in peripheral nerves of aged mice. (**A**) Immunohistochemistry of F4/80+ macrophages (red) on cross sections of femoral quadriceps nerves from 12-month-old (12M), 18-month-old (18M) and 24-month-old (24M) mice. DAPI (blue) labels the cell nuclei. Dashed circles indicate the area of femoral quadriceps nerves. Scale bar, 50 µm. (**B**) Quantification of F4/80+ profiles in femoral quadriceps nerves in 12M, 18M and 24M mice. One-way ANOVA, *** $p<0.001$. (**C**) Quantification of foamy macrophages in femoral quadriceps nerves in 12M, 18M and 24M mice. Individual values and mean ± SD are shown. Kruskal-Wallis test, * $p<0.5$; ** $p<0.01$.

To investigate the activation of macrophages in peripheral nerves of aged mice, mRNA expressions of different macrophage polarization markers were measured in adult and aged mice. *Itgam* (encoding for CD11b), *Adgre1* (encoding for F4/80) and *Arg1* (encoding for Arginase) all showed a 2-fold to 3-fold increase during aging (Fig. 5A), which likely reflects the increase in the absolute number of macrophages (Fig. 4B). However, *Nos2* (encoding for inducible nitric oxide synthase, iNOS), a marker for the proinflammatory M1 macrophage phenotype, was not upregulated during aging, and was expressed at low levels in both adult

and aged mice (Fig. 5A). Interestingly, *Trem2* (encoding for triggering receptor expressed on myeloid cells 2, TREM2), a marker for M2 macrophage polarization, was 12-fold higher in expression in aged mice compared to adult mice (Fig. 5A). This upregulation was disproportionate to the increase of macrophage numbers during aging, suggesting that *Trem2* expression was strongly upregulated by individual endoneurial macrophages during aging.

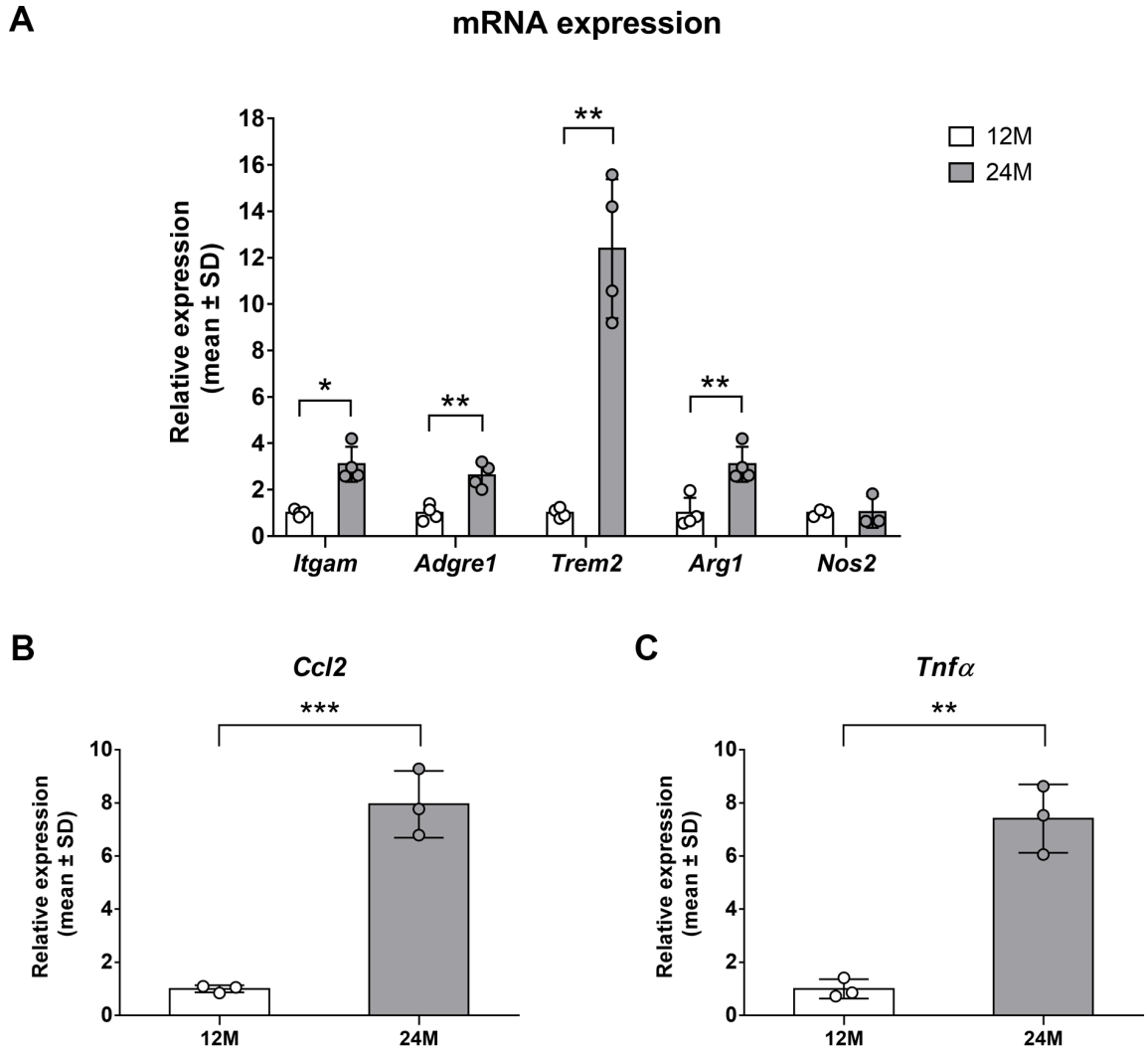

Figure 5 Altered mRNA expression of macrophage activation markers, cytokines and chemokines in peripheral nerves of aged mice. (**A**) Relative mRNA expression of macrophage polarization markers in femoral quadriceps nerves of 12M and 24M mice. *Itgam*, *Adgre1*, *Arg1*, *Nos2*: student's t-test, * p<0.5; ** p<0.01. *Trem2*: Welch's t-test, ** p<0.01. (**B**, **C**) Relative mRNA expression of *Ccl2* (**B**) and *Tnfα* (**C**) in femoral quadriceps nerves of 12M and 24M mice. Individual values and mean ± SD are shown. Student's t-test, ** p<0.01; *** p<0.001.

In addition, chemokines involved in macrophage recruitment and other inflammatory cytokines were also analyzed in femoral quadriceps nerves of adult and aged mice. As a chemokine shown to mediate macrophage-related neural damage in mouse models of hereditary peripheral neuropathies (Kobsar et al., 2005; Fischer et al., 2008b; Fischer et al., 2008a; Groh et al., 2010; Kohl et al., 2010a), *Ccl2* (encoding for CCL2/MCP-1) expression was significantly upregulated in aged mice compared to adult mice (Fig. 5B). This suggests a possible role of CCL2 in mediating nerve pathology in aged mice. Moreover, the mRNA expression of *Tnfα* (encoding for tumor necrosis factor alpha, TNF-α) was also upregulated in aged mice (Fig. 5C), reflecting the overall elevated level of inflammation in peripheral nerves during aging.

Components of the adaptive immune system were also characterized in peripheral nerves of 12-month-old and 24-month-old mice. Although generally rarely present, both CD8+ and CD4+ T-lymphocytes showed significantly higher numbers in femoral quadriceps nerves of 24-month-old mice compared to 12-month-old mice (Fig. 6A, B). Taken together, both innate immune responses and adaptive immune reactions increase in peripheral nerves during aging.

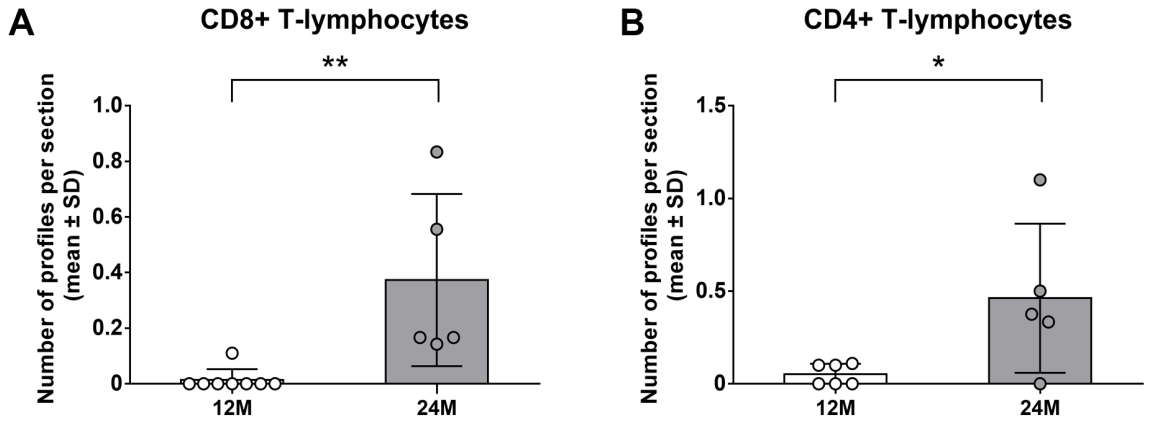

Figure 6 Higher numbers of T-lymphocytes in peripheral nerves of aged mice. (**A**, **B**) Quantifications of CD8+ T-lymphocytes (**A**) and CD4+ T-lymphocytes (**B**) on cross sections of femoral quadriceps nerves from 12M and 24M mice. Individual values and mean ± SD are shown. Mann-Whitney U test, * $p<0.5$; ** $p<0.01$.

5.2.3 Lack of altered myelin gene expressions in peripheral nerves of aged mice

Based on the increase in demyelination in peripheral nerves of aged mice (Fig. 3A, B), mRNA expression and protein expression of myelin genes was analyzed in femoral quadriceps nerves of 12-month-old and 24-month-old mice. Cx32 (encoded by *Gjb1*) did not show altered expression in aged mice on either the mRNA (Fig. 7A) or protein (Fig. 7C) level. Similarly, the gene expression of another myelin protein, MBP (encoded by *Mbp*), did not show downregulation in aged mice compared to adult mice (Fig. 7B). Moreover, protein expression of P0 also did not show reduction in aged mice (Fig. 7D). In summary, a lack of altered myelin gene expressions and myelin protein expressions could be identified in peripheral nerves of aged mice.

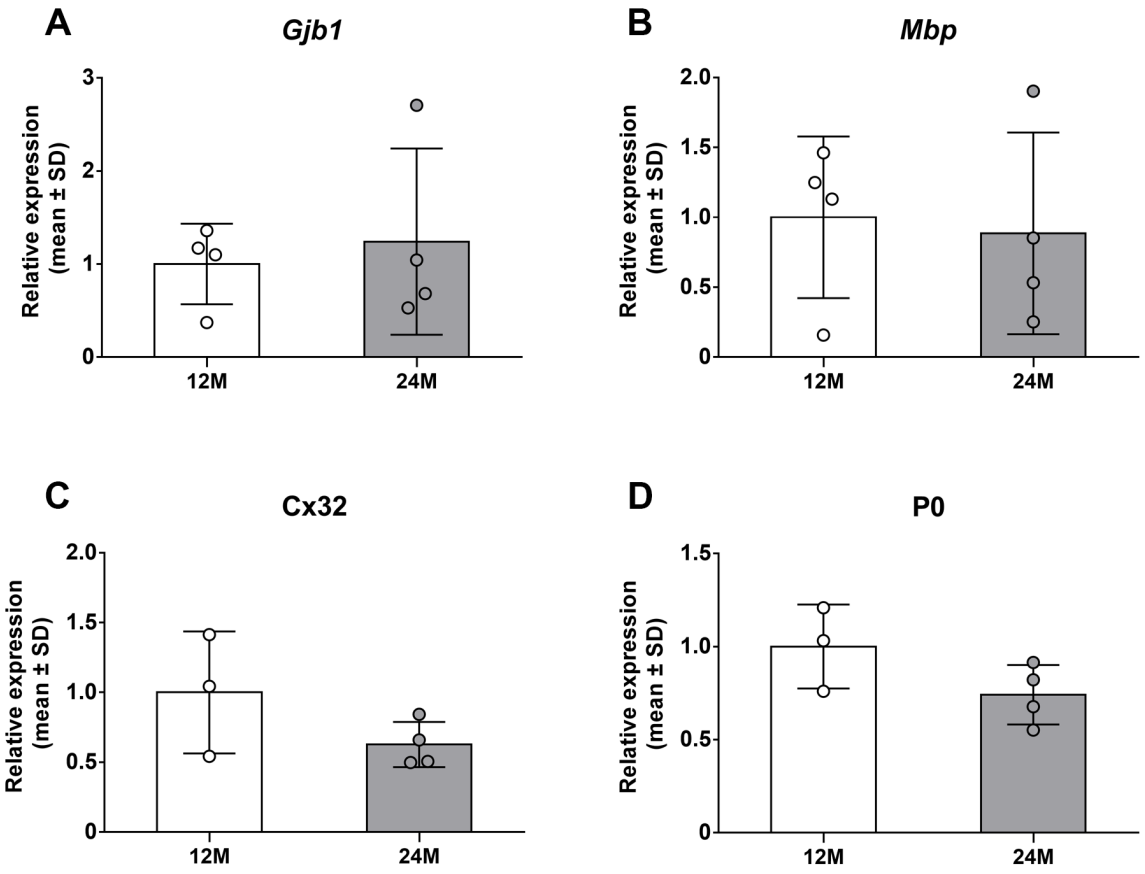

Figure 7 Lack of altered myelin gene expressions and myelin protein expressions in peripheral nerves of aged mice. (**A**, **B**) Relative mRNA expression of *Gjb1* (**A**) and *Mbp* (**B**) in femoral quadriceps nerves of 12M and 24M mice. (**C**, **D**) Densitometric analysis for Western blots of Cx32 (**C**) and P0 (**D**) from femoral quadriceps nerve lysates of 12M and

24M mice. ERK1/2 was used as a loading control. Individual values and mean ± SD are shown. Student's t-test.

5.2.4 Nerve conduction properties are mildly impaired in aged mice

Previous studies have shown deterioration of nerve conduction properties in peripheral nerves during aging (Drechsler, 1975; Dorfman and Bosley, 1979; Bouche et al., 1993; Verdu et al., 1996; Verdu et al., 2000). In addition, studies on mouse models of distinct peripheral neuropathies correlated demyelination and axonal damage with different patterns of neurological impairment, such as reduced motor nerve conduction velocities and prolonged distal motor latencies (Huxley et al., 1998; Tankisi et al., 2007). To investigate whether the pathological alterations observed in aged mice affected their nerve conduction properties, neurographic recordings were performed in 12-month-old and 24-month-old mice. Distal and proximal compound muscle action potentials (CMAPs) were significantly lower in aged mice compared to adult mice (Fig. 8A, B), whereas F wave latencies of aged mice were not significantly prolonged (Fig. 8C). This is consistent with previous findings (Verdu et al., 1996). Nerve conduction velocities (NCV) of aged mice were also not significantly lower compared to adult mice (Fig. 8D). Contrary to studies done in humans of different age groups showing the sex difference in NCV (Robinson et al., 1993; Thakur et al., 2010b), NCV did not show significant differences between aged male and female mice (Fig. 8E).

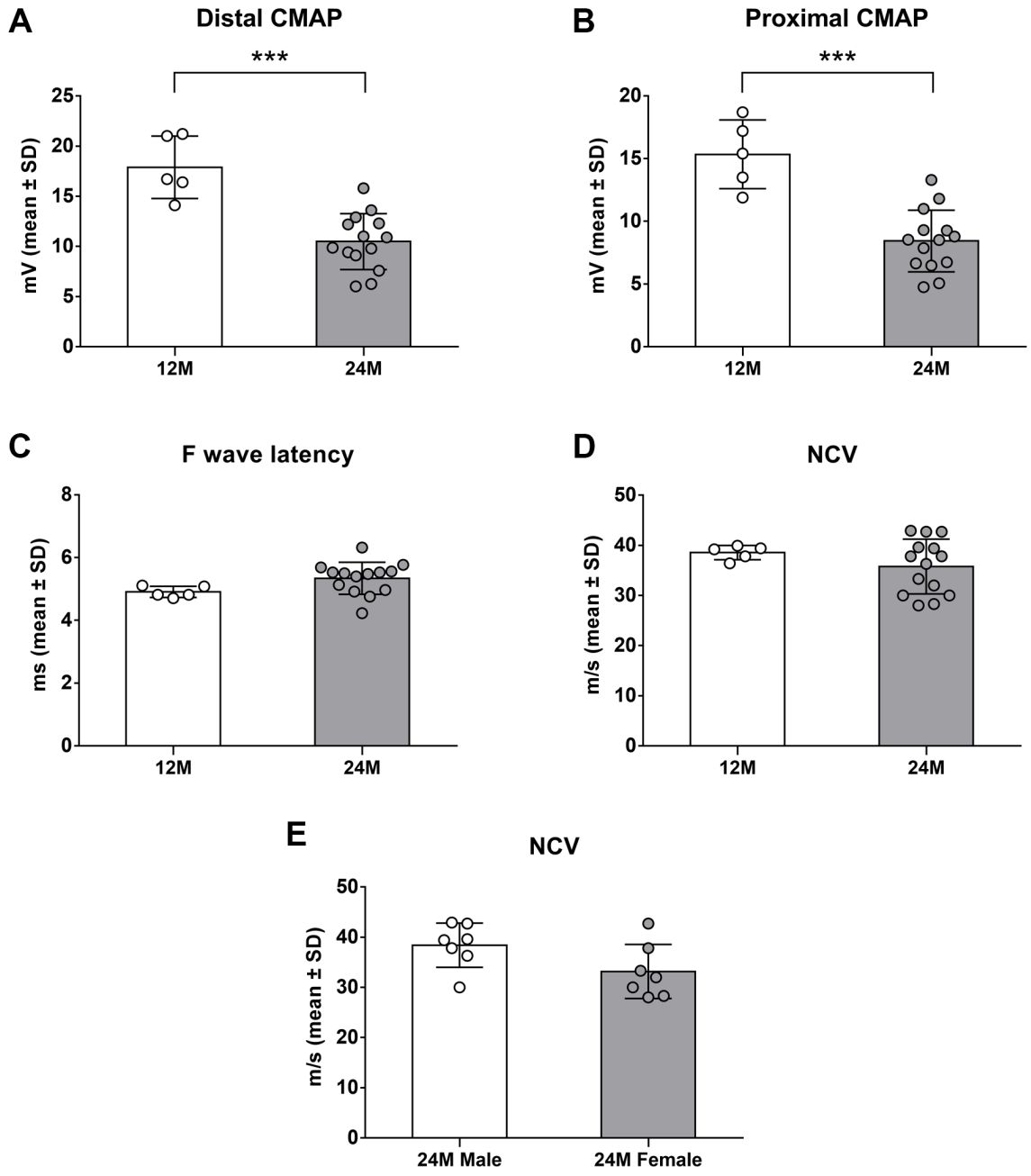

Figure 8 Mildly impaired nerve conduction properties in aged mice. (**A**, **B**) Distal (**A**) and proximal (**B**) compound muscle action potential (CMAP) amplitudes were lower in 24M mice compared to 12M mice. (**C**) F wave latency did not show prolongation in 24M mice compared to 12M mice. (**D**) Nerve conduction velocity (NCV) of 24M mice did not reduce compared to 12M mice. (**E**) No sex difference of NCV of 24M mice shown in (**D**). Individual values and mean ± SD are shown. Student's t-test, *** $p<0.001$.

5.2.5 Increased muscle denervation in aged mice

As a further possible functional outcome reflecting nerve function, muscle innervation was investigated in 12-month-old and 24-month-old mice by analyzing the overlay of presynaptic (synaptophysin) and postsynaptic (α-Bungarotoxin)

markers on cross-sections of flexor digitorum brevis muscles (Fig. 9A). The numbers of denervated and partially denervated neuromuscular junctions (NMJs), collectively named as “abnormally innervated NMJs”, were substantially higher in aged mice than adult mice (Fig. 9D), indicating increased muscle denervation during aging.

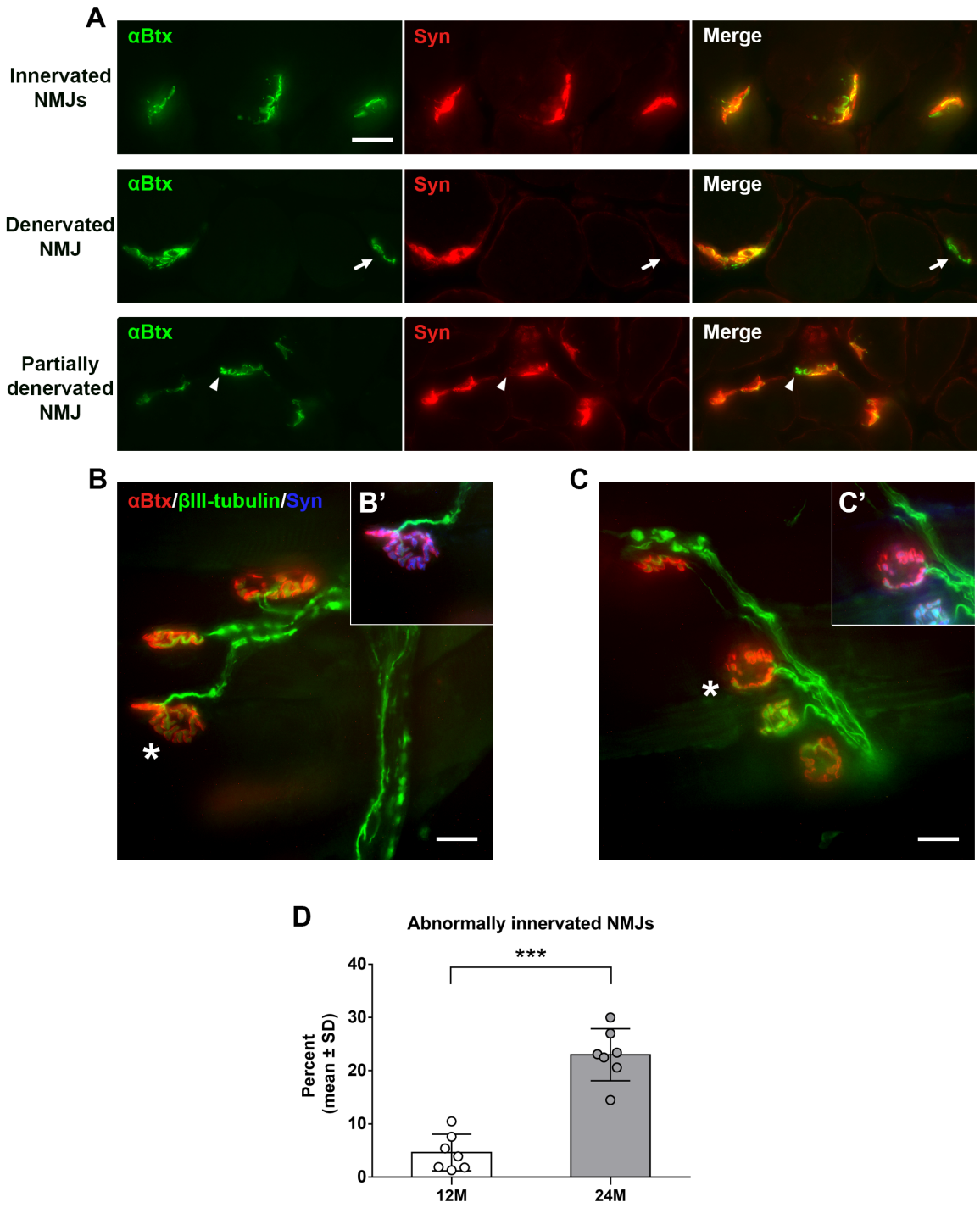

Figure 9 Increased NMJ denervation in aged mice. (**A**) Examples of completely innervated NMJs (top row), completely denervated NMJs (arrows, middle row), and

partially denervated NMJs (arrowheads, bottom row) as identified by double-immunolabeling with presynaptic synaptophysin (red) and postsynaptic α-bungarotoxin (Fried et al.) in cross sections of flexor digitorum brevis muscle. Right column represents merged double immunofluorescence. Scale bar, 20 µm. (**B**, **C**) Examples of whole-mount preparations of flexor digitorum brevis muscles of 12M (**B**) and 24M (**C**) mice showing completely innervated and partially denervated NMJs, respectively, as identified by merged α-bungarotoxin (red) and axonal βIII-tubulin (Fried et al.) labeling. Scale bar, 20 µm. (**B'**), (**C'**): the same junctions as indicated in (**B**) and (**C**) (asterisks), with synaptophysin (blue) as an additional presynaptic marker. There is complete NMJ innervation in (**B**), (**B'**) and partial denervation in (**C**), (**C'**). (**D**) Quantification of abnormally innervated NMJs from cross sections of flexor digitorum brevis muscle from 12M and 24M mice. Individual values and mean ± SD are shown. Student's t-test, *** $p<0.001$.

Muscle denervation was also observed on whole-mount preparations of flexor digitorum brevis muscles of aged mice (Fig. 9C), represented by loss of both the presynaptic marker synaptophysin (Fig. 9C') and an axonal marker, βIII-tubulin (Fig. 9C). By contrast, signs indicative of denervation were rarely observed in adult mice (Fig. 9B) with most neuromuscular junctions exhibiting a pretzel-like shape labeled by both pre- and postsynaptic markers (Fig. 9B') and the axonal marker (Fig. 9B). This confirms findings from a recent study (Gonzalez-Freire et al., 2014). Additionally, on whole-mount preparations of muscles from aged mice, βIII-tubulin revealed axonal swellings (Fig. 9C), confirming the axonal perturbation at the distal ends of peripheral nerves in aged mice.

5.3 Macrophage depletion ameliorates peripheral neuropathy in aged mice

5.3.1 Reduced macrophage numbers in aged mice after macrophage depletion

Having demonstrated that pathological alterations and the number of endoneurial macrophages increase in peripheral nerves of aged mice, macrophage depletion was performed to test the hypothesis that macrophages amplify the age-related changes in mouse peripheral nerves. Macrophages were targeted selectively with a CSF-1R (c-FMS) inhibitor (CSF-1Ri) PLX5622 with a dose of 300 mg/kg in chow, which was previously established in mouse models of hereditary peripheral neuropathies (Klein et al., 2015b). The treatment started in mice at 18 months of age, when their endoneurial macrophage numbers were not yet elevated (Fig. 4B), and lasted for 6 months until the mice reached 24 months of age.

CSF-1Ri treatment led to a ~70% reduction of F4/80+ macrophage numbers in femoral quadriceps nerves of mice by the end of the treatment (Fig. 10A, B). Furthermore, on the electron microscopic level, numbers of foamy macrophages were also significantly reduced in CSF-1Ri treated mice (Fig. 10C).

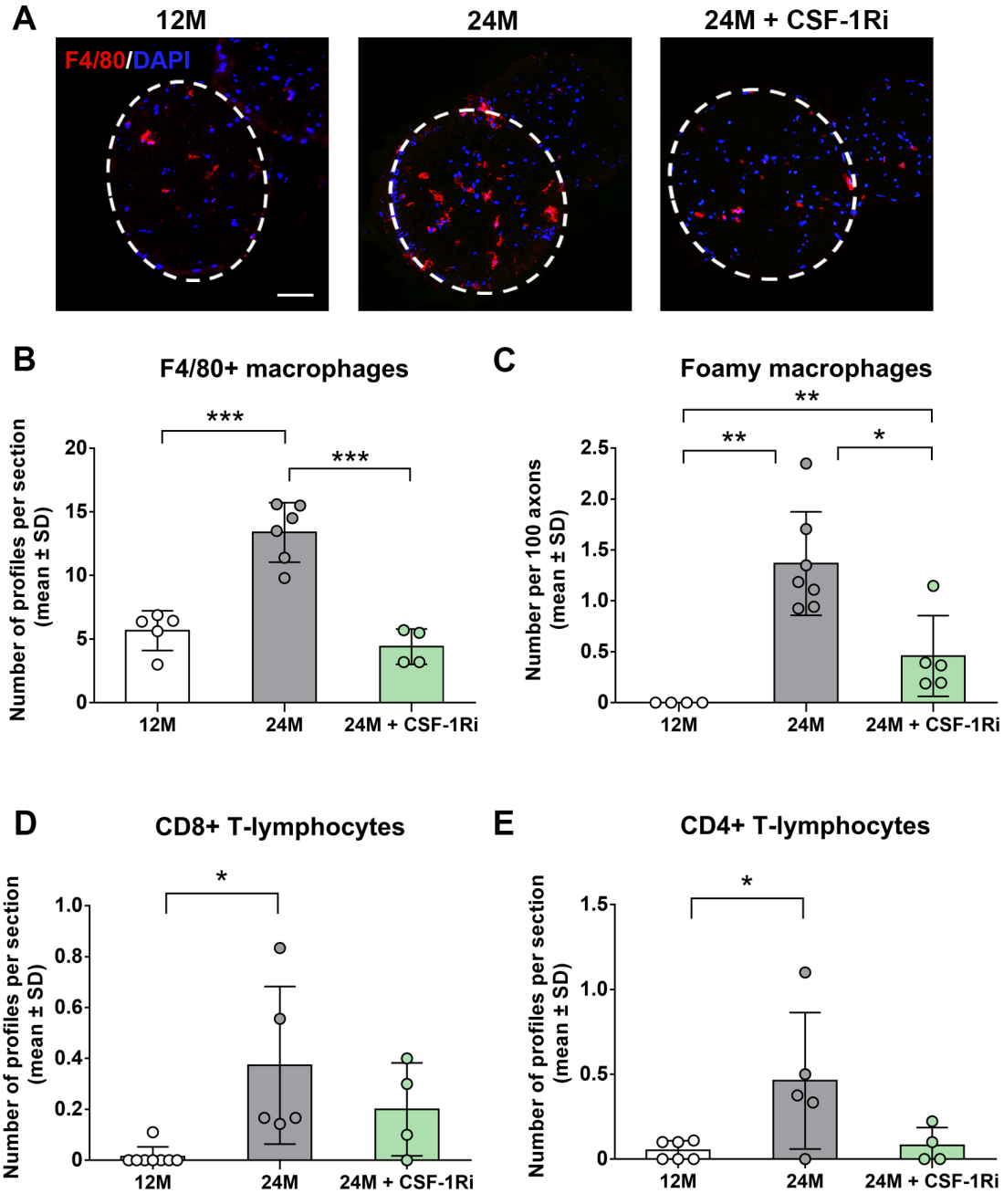

Figure 10 Reduced macrophage numbers in aged mice after CSF-1Ri treatment. (**A**) Immunohistochemistry of F4/80+ macrophages (red) on cross sections of femoral quadriceps nerves of 12M, 24M mice, and 24M mice treated with CSF-1Ri (24M + CSF-1Ri). DAPI (blue) labels the cell nuclei. Dashed circles indicate the area of femoral quadriceps nerves. Scale bar, 50 µm. (**B**) Quantification of F4/80+ profiles in femoral quadriceps nerves in 12M, 24M and 24M + CSF-1Ri mice. One-way ANOVA, *** $p<0.001$. (**C**) Quantification of foamy macrophages in femoral quadriceps nerves in 12M, 24M and

24M + CSF-1Ri mice. Kruskal-Wallis test, * p<0.5; ** p<0.01. (**D**, **E**) Quantifications of CD8+ T-lymphocytes (**D**) and CD4+ T-lymphocytes (**E**) on cross sections of femoral quadriceps nerves from 12M, 24M and 24M + CSF-1Ri mice. Kruskal-Wallis test, * p<0.05. Individual values and mean ± SD are shown. For comparison, data from 12M and 24M mice, as shown in **Figure 4** and **Figure 6,** is presented here again.

By contrast, numbers of CD8+ and CD4+ T-lymphocytes only showed a mild trend of reduction after macrophage depletion (Fig. 10D, E). T-lymphocytes do not express CSF-1R (Pridans et al., 2014; Stanley and Chitu, 2014) and are therefore not targeted via CSF-1R inhibition directly. However, the results in the present study do not provide sufficient evidence for a secondary reduction of T-lymphocyte numbers caused by interactions between endoneurial macrophages and T-lymphocytes in peripheral nerves of aged mice.

5.3.2 Attenuated pathological alterations characteristic for demyelination in aged mice after macrophage depletion

As a next step, femoral quadriceps nerves of CSF-1Ri treated aged mice were analyzed on the electron microscopic level for quantifying pathological alterations (Fig. 11A). Thinly myelinated axons and onion bulbs, the two major pathological alterations in peripheral nerves during aging, substantially decreased in aged mice treated with CSF-1Ri compared to untreated aged mice (Fig. 11B, C).

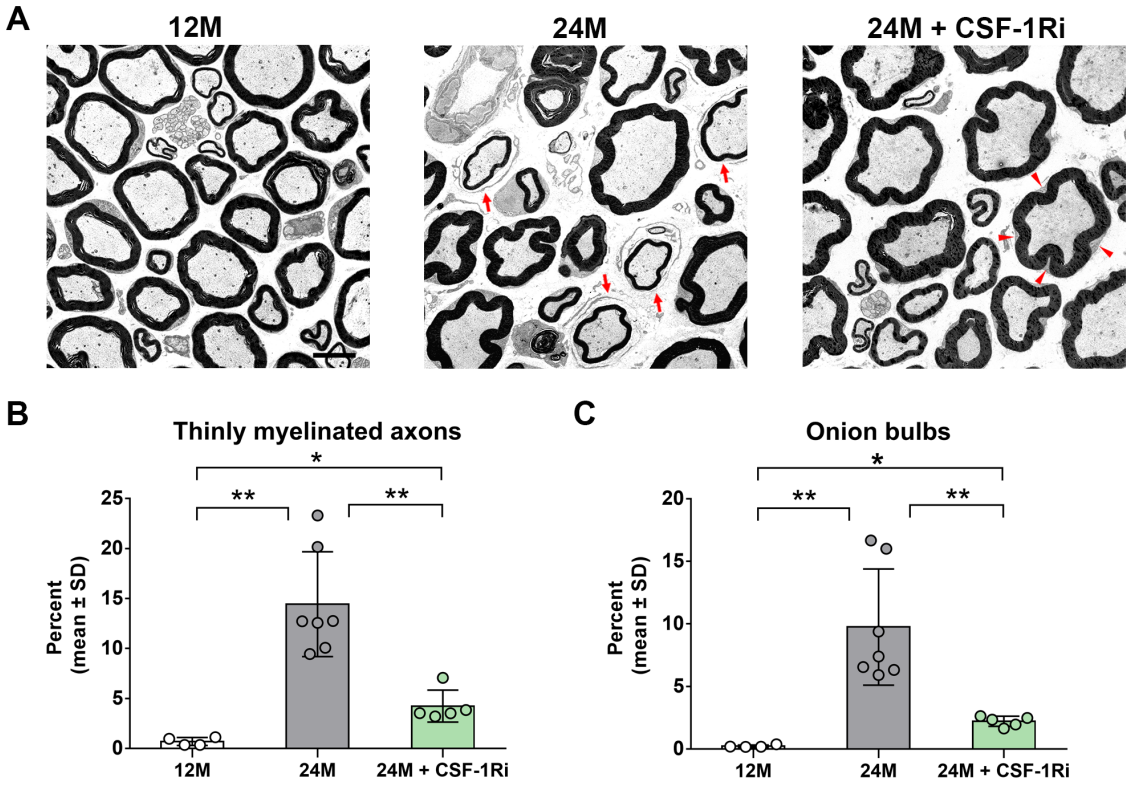

Figure 11 Pathological alterations characteristic for demyelination in peripheral nerves of aged mice were attenuated after CSF-1Ri treatment. (**A**) Electron microscopy of representative ultrathin sections of femoral quadriceps nerves from 12M, 24M, and 24M + CSF-1Ri mice. Features indicative of demyelination, as exemplified by thin myelin and onion bulbs (arrows, 24M), are lacking on this micrograph after CSF-1Ri treatment (24M + CSF-1Ri) (see **B**, **C**), whereas the undulating appearance of the myelin sheaths (arrowheads) is not changed in 24M + CSF-1Ri mice. Scale bar, 5 µm. (**B**, **C**) Quantifications of thinly myelinated axons (**B**) and onion bulbs (**C**) reflect a substantial attenuation of demyelination after CSF-1Ri treatment. Individual values and mean ± SD are shown. Kruskal-Wallis test, * $p<0.05$; ** $p<0.01$. For comparison, data from 12M and 24M mice, as shown in **Figure 3**, is presented here again.

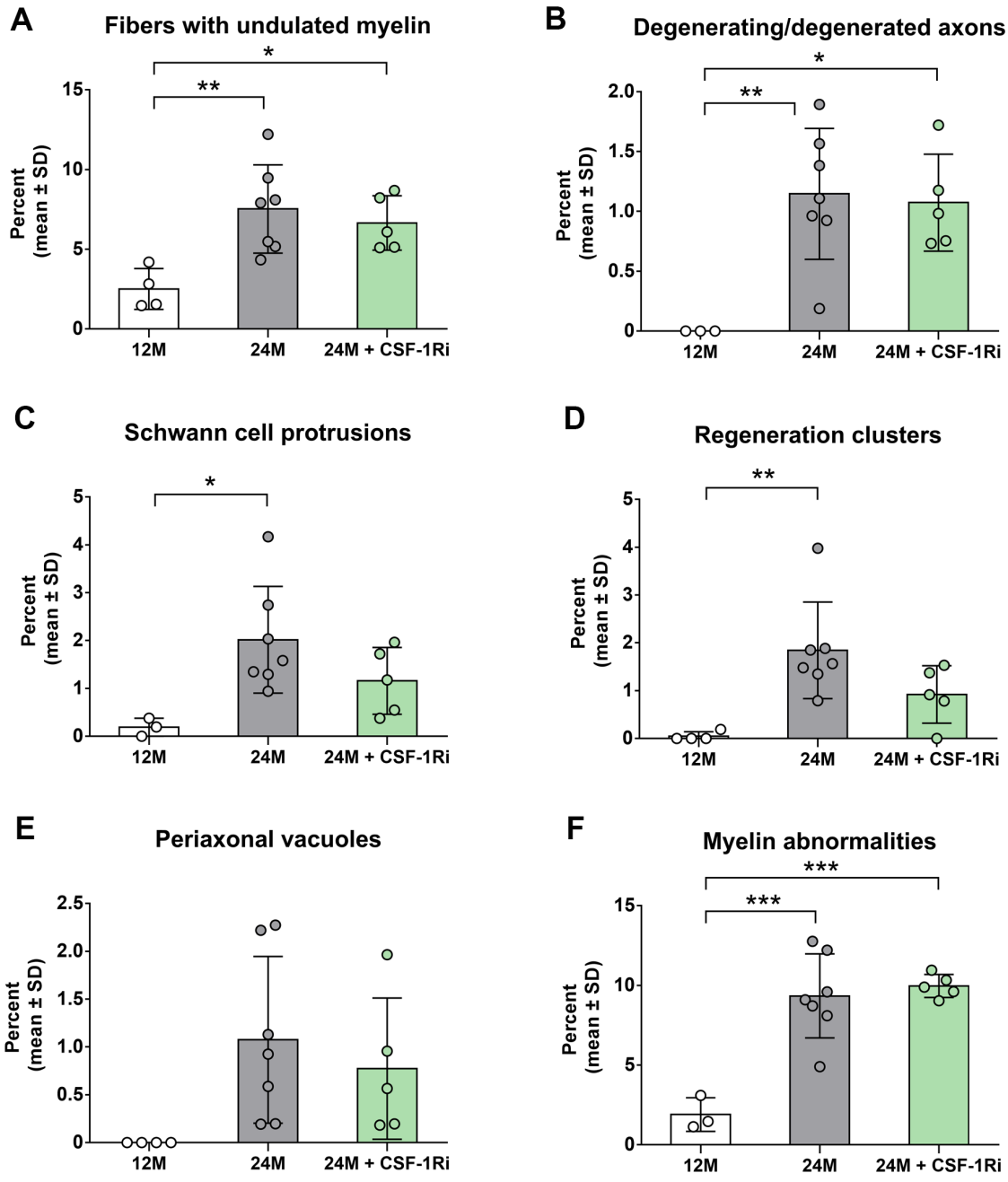

Figure 12 Lack of a macrophage depletion effect on distinct pathological alterations in peripheral nerves of aged mice. (**A** – **F**) EM-based quantifications reveal that fibers with

undulated myelin (**A**), degenerating/degenerated axons (**B**), Schwann cell protrusions (**C**), regeneration clusters (**D**), periaxonal vacuoles (**E**) and myelin abnormalities (**F**) were not significantly affected in 24M + CSF-1Ri mice compared to untreated 24M mice. Individual values and mean ± SD are shown. **A**, **C**, **F**: One-way ANOVA, * $p<0.05$; ** $p<0.01$; *** $p<0.001$. **B**, **D**, **E**: Kruskal-Wallis test, * $p<0.05$; ** $p<0.01$. For comparison, data from 12M and 24M mice, as shown in **Figure 3**, is presented here again.

By contrast, the percentage of fibers with undulated myelin was not reduced after CSF-1Ri treatment in aged mice (Fig. 11A, 12A). Similarly, degenerated axons, Schwann cell protrusions, and myelin abnormalities were not significantly altered after CSF-1Ri treatment (Fig. 12B, C, F). In addition, regeneration clusters and periaxonal vacuoles showed a non-significant trend of decrease in aged mice following CSF-1Ri treatment (Fig. 12D, E).

In summary, macrophage depletion prevented the increase of pathological alterations indicative of demyelination in aged mice, while other age-related pathological features were not altered by macrophage depletion.

5.3.3 Less muscle denervation and preserved muscle strength in aged mice after macrophage depletion

Neuromuscular junctions in 24-month-old mice treated with CSF-1Ri were investigated to analyze the effects of macrophage depletion on muscle innervation. Compared to untreated aged mice, aged mice treated with CSF-1Ri showed significantly less muscle denervation (Fig. 13A). Moreover, CSF-1Ri treated aged mice also showed higher grip strength compared to untreated aged mice, whereas the untreated aged mice showed a trend towards lower grip strength compared to untreated adult mice (Fig. 13B). This indicates the preserved muscle function of aged mice after macrophage depletion. However, distal and proximal CAMP did not show significant differences in aged mice treated with CSF-1Ri compared to untreated aged mice. This suggest that the impaired nerve conduction properties of aged mice were not prevented by macrophage depletion (Fig. 13C, D).

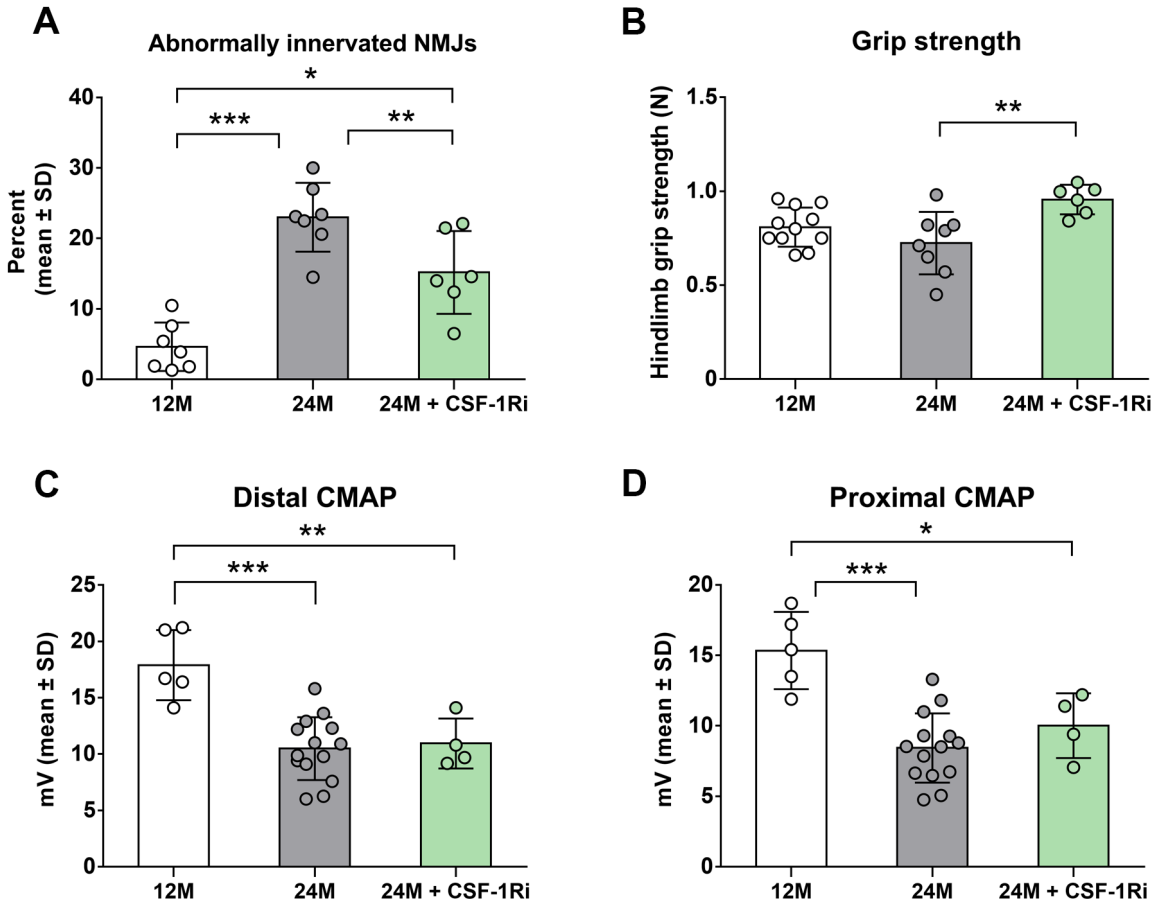

Figure 13 CSF-1Ri treatment ameliorates NMJ denervation and preserves muscle strength but has no effect on nerve conduction properties in aged mice. (**A**) Quantification of abnormally innervated NMJs from cross sections of flexor digitorum brevis muscle from 12M and 24M and 24M + CSF-1Ri mice. Muscle denervation was partially prevented in 24M mice treated with CSF-1Ri. (**B**) Grip strength of 12M, 24M, and 24M + CSF-1Ri mice. (**C**, **D**) Distal (**C**) and proximal (**D**) CMAP amplitudes in 12M, 24M and 24M + CSF-1Ri mice. Individual values and mean ± SD are shown. One-way ANOVA, * $p<0.5$; ** $p<0.01$; *** $p<0.001$. For comparison, data from 12M and 24M mice, as shown in **Figure 8** and **Figure 9**, is presented here again.

5.4 Systemic inflammation alters macrophage activation and ameliorates the demyelinating phenotype in peripheral nerves of aged mice

5.4.1 Transient physiological and behavioral changes in mice after LPS injection

The present study has demonstrated that macrophage-driven inflammation mediates the peripheral neuropathy in aged mice, since morphological and functional alterations found in peripheral nerves of aged mice can be ameliorated by macrophage depletion. Based on previous studies in the CNS (Huxley et al.,

1998; Cunningham et al., 2005; Perry et al., 2007; Henry et al., 2009), the question emerged whether an experimental increase in inflammation exacerbates disease outcomes. To test this hypothesis, lipopolysaccharide (LPS) was injected intraperitoneally into 12-month-old and 24-month-old mice to mimic bacterial infection. Single dose injection (500 µg/kg body weight) was performed to prevent a possible innate immune memory-induced tolerance (Wendeln et al., 2018).

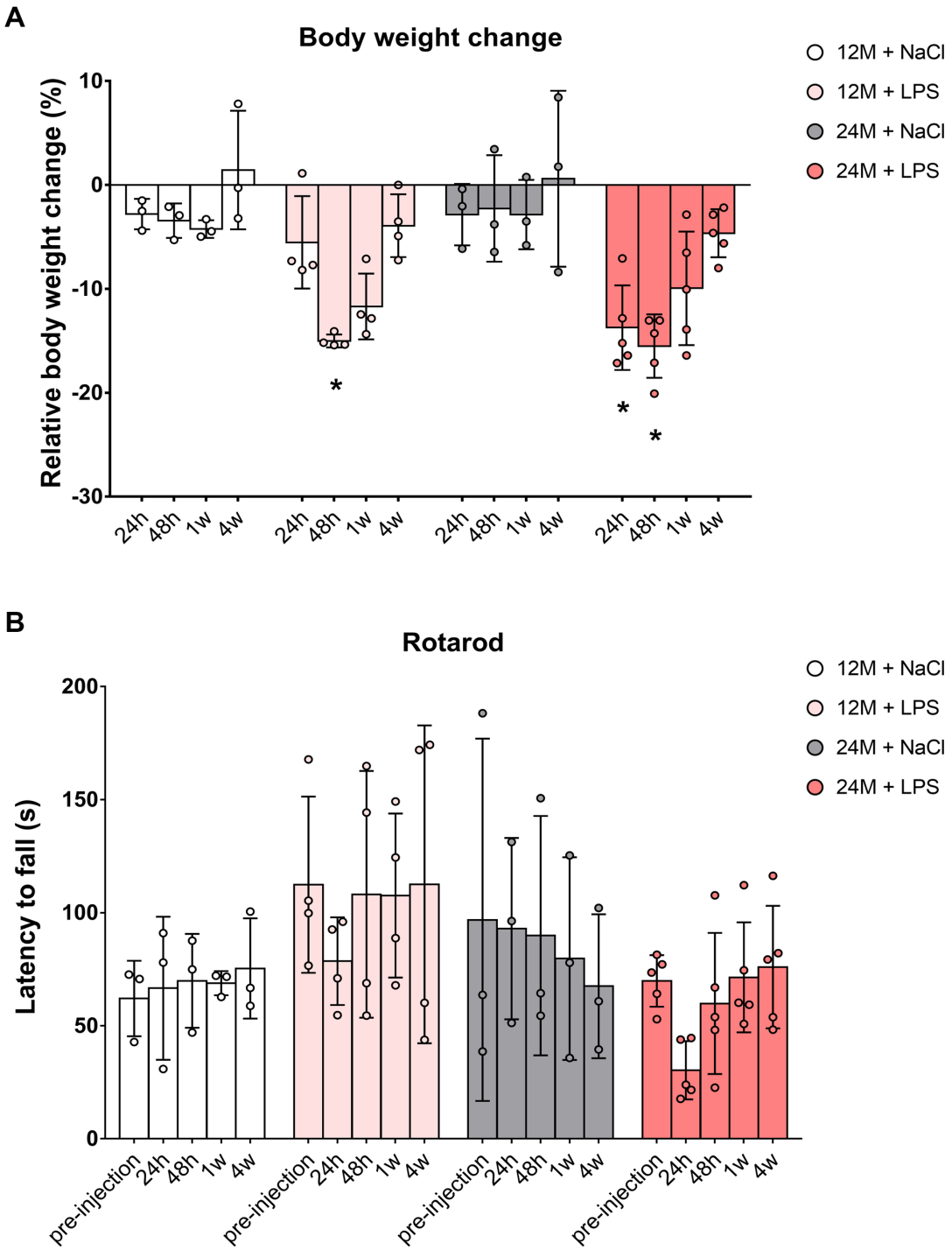

Figure 14 Transient physiological and behavioral changes in LPS-injected mice. (**A**) Relative body weight change of 12M and 24M mice injected with either NaCl or LPS. (**B**)

rotarod performance of 12M and 24M mice injected with either NaCl or LPS. Individual values and mean ± SD are shown. Two-way ANOVA, * $p<0.05$. * marks the significant differences between experimental groups (NaCl vs LPS) of a distinct age (12M or 24M).

After LPS injection, well-known changes in general behavior and physiology were observed in both adult mice and aged mice, including reduced locomotor activity, ruffled fur and diarrhea (Hart, 1988; Biesmans et al., 2013; Szentirmai and Krueger, 2014). LPS-injected adult and aged mice showed a trend towards body weight loss between 24 and 48 hours after injection compared to age-matched NaCl-injected mice (Fig. 14A). LPS-injected mice started to recover from the body weight loss one week after injection and their body weight was restored to pre-injection values by the end of observation (4 weeks after injection). Impaired rotarod performance was shown in adult and aged mice injected with LPS at 24 hours after injection (Fig. 14B). As the LPS-injected mice recovered from the transient acute sickness behavior, their rotarod performance were also restored to pre-injection levels 1 week after injection, with almost full restoration to pre-injection levels 48 hours after injection already (Fig. 14B).

5.4.2 Altered macrophage activation in aged mice injected with LPS

To investigate whether the neuroinflammation in peripheral nerves of aged mice is altered by systemic inflammation, endoneurial macrophages in mice injected with either LPS or NaCl were labeled with the pan-macrophage marker F4/80 4 weeks after the injection (Fig. 15A). LPS injection also did not alter the number of macrophages in the femoral quadriceps nerves of adult mice and aged mice (Fig. 15B). In femoral saphenous nerves, where the number of macrophages did not increase during aging, the number of macrophages was also not altered by LPS injection (Fig. 15C).

As a next step, activation of endoneurial macrophages was also investigated. Immunohistochemical staining against the mannose receptor CD206, a marker for M2 activation of macrophages (Kigerl et al., 2009), was performed on femoral quadriceps nerves of 12-month-old and 24-month-old mice injected with either LPS or NaCl (Fig. 16A). Both the absolute number and percentage of CD206+ macrophages in aged mice injected with LPS were significantly lower compared to

aged mice injected with NaCl (Fig. 16B, C), suggesting a shift away from M2 polarization of macrophages in aged mice after LPS injection. The lower number and percentage of CD206+ macrophages were not observed in adult mice injected with LPS (Fig. 16B, C). Notably, in aged mice injected with NaCl, a proportion of CD206+ ring-like profiles were observed (Fig. 16D, E). Interestingly, these ring-like CD206+ profiles were rarely found in aged mice injected with LPS (Fig. 16E).

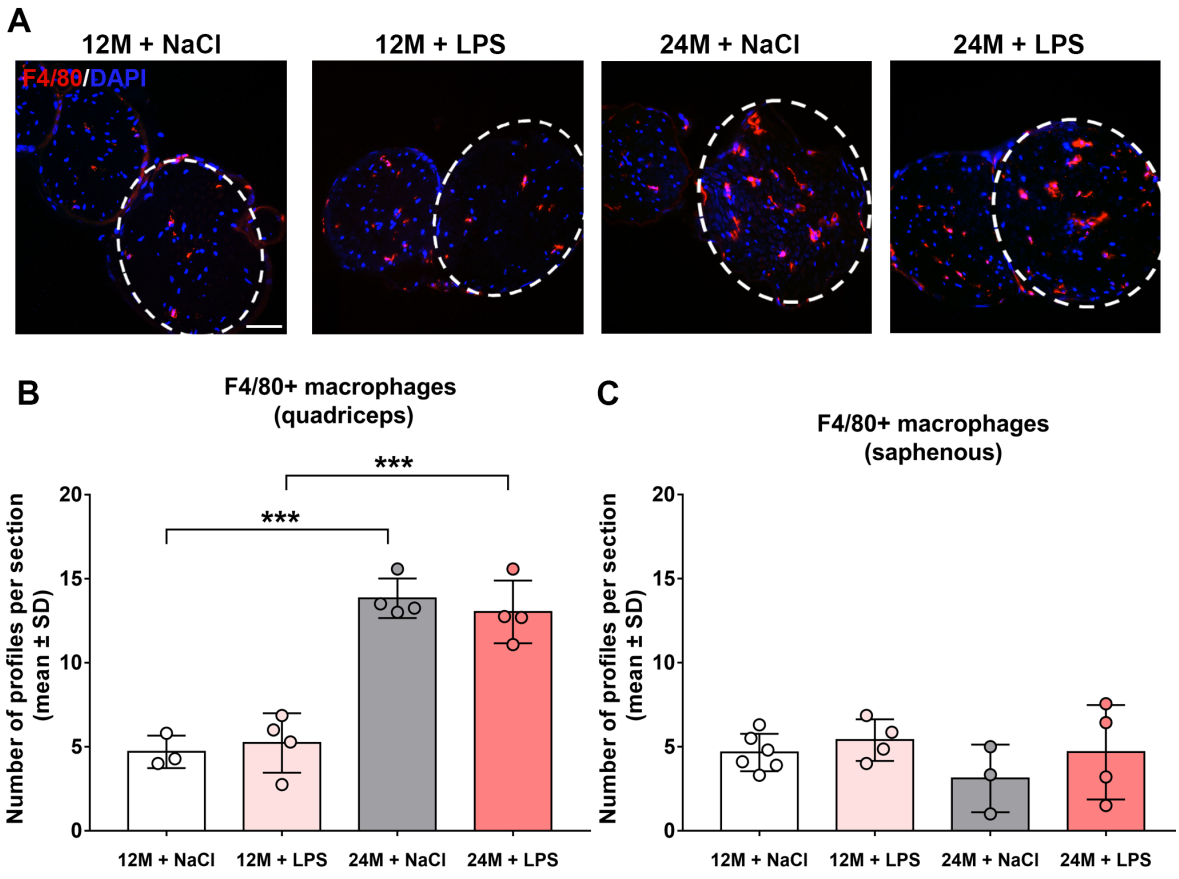

Figure 15 Macrophage numbers remain unchanged in the peripheral nerves of aged mice after LPS injection. (**A**) Immunohistochemical micrographs of F4/80+ macrophages (red) on cross sections of femoral quadriceps nerves in 12M mice injected with either NaCl or LPS (12M + NaCl, 12M + LPS) and 24M mice injected with either NaCl or LPS (24M + NaCl, 24M + LPS) mice. DAPI (blue) labels the cell nuclei. Dashed circles indicate the area of femoral quadriceps nerves. Scale bar, 50 µm. (**B**, **C**) Quantifications of F4/80+ profiles on cross sections of femoral quadriceps nerves (**B**) or femoral saphenous nerves (**C**) from 12M and 24M mice injected with either NaCl or LPS. Individual values and mean ± SD are shown. One-way ANOVA, *** $p<0.001$.

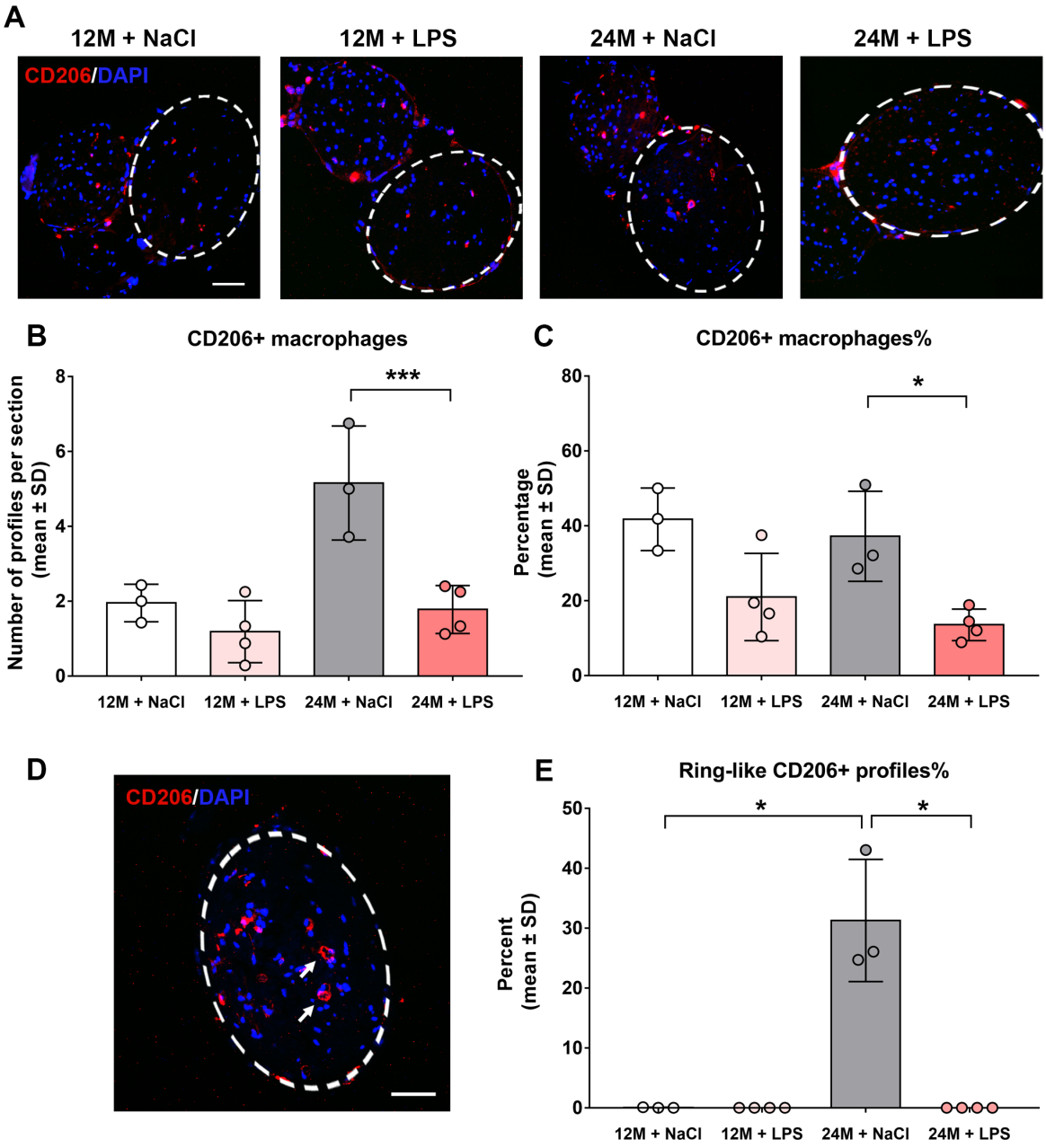

Figure 16 Altered macrophage polarization in the peripheral nerves of aged mice after LPS injection. (**A**) Immunohistochemical micrographs of CD206+ macrophages (red) on cross sections of femoral quadriceps nerves in 12M and 24M mice injected with either NaCl or LPS. DAPI (blue) labels the cell nuclei. Dashed circles indicate the area of femoral quadriceps nerves. Scale bar, 50 µm. (**B**, **C**) Absolute number (**B**) and percentage (**C**) of CD206+ macrophages on cross sections of femoral quadriceps nerves of 12M and 24M mice injected with either NaCl or LPS. The number and percentage of CD206+ macrophages in 24M + LPS mice were significantly lower compared to 24M + NaCl mice, most likely reflecting a reduced M2-polarization. One-way ANOVA, * $p<0.05$; *** $p<0.001$. (**D**) Ring-like CD206+ profiles (arrows) in 24M mice injected with NaCl. Scale bar, 50 µm. (**E**) Quantification of ring-like CD206+ profiles in 12M and 24M mice injected with either NaCl or LPS. Individual values and mean ± SD are shown. Kruskal-Wallis test, * $p<0.05$.

Previous studies on LPS injection in aged mice and mouse models of neurodegenerative diseases revealed the release of proinflammatory cytokines in the CNS and microglial hyperactivation after LPS injection (Henry et al., 2009; Field et al., 2010). Therefore, to investigate changes in cytokine release and macrophage alteration in the peripheral nerves after LPS injection, gene expressions of inflammatory cytokines and markers for macrophage activation were analyzed in femoral quadriceps nerves from another cohort of aged and adult mice 1 month after LPS injection. Complementary DNA (cDNA) of femoral quadriceps nerves of uninjected mice (shown in Fig. 5, 7) were used as controls due to the limited numbers of aged mice. *Trem2*, an M2 activation marker that showed a strong upregulation during aging (Fig. 5A), was significantly downregulated in aged mice one month after LPS injection (Fig. 17A). As TREM2 plays a critical role in phagocytosis (Takahashi et al., 2007; Hsieh et al., 2009; N'Diaye et al., 2009; Kleinberger et al., 2014; Kawabori et al., 2015), the result of the present study implies that LPS injection leads to a shift in macrophage polarization towards a less phagocytic phenotype in aged mice (Fig. 16). In contrast, *Trem2* downregulation was not observed in adult mice one month after LPS injection (Fig. 17A). Gene expression of *Arg1*, another M2 polarization marker, showed a non-significant trend of downregulation in aged mice injected with LPS compared to uninjected aged mice (Fig. 17B). These results suggest that LPS injection does not cause a change in the number of macrophages but instead alters the macrophage activation in aged mice, resulting in a less M2-like polarization of macrophages.

In addition, mRNA expression of *Cd86*, a marker for M1 polarization of macrophages, did not show changes 4 weeks after injection in aged mice injected with LPS compared to uninjected aged mice (Fig 17D). The mRNA expressions of *Irf5* (Fig. 17C) and *Tgfβ* (Fig. 17E) in peripheral nerves of aged mice 4 weeks after LPS injection were not significantly altered compared to uninjected aged mice. Of note, in peripheral nerves of adult mice injected with LPS, expression of *Tgfβ* was downregulated compared to uninjected adult mice (Fig. 17E). Interestingly, *Igf1* (encoding for insulin-like growth factor 1) showed significant downregulation in LPS-injected adult mice compared to uninjected adult mice (Fig. 17F). Moreover, *Igf1* expression in uninjected aged mice was significantly lower compared to adult

mice. A trend towards further downregulation of *Igf1* was observed in LPS-injected aged mice compared to uninjected aged mice (Fig. 17F).

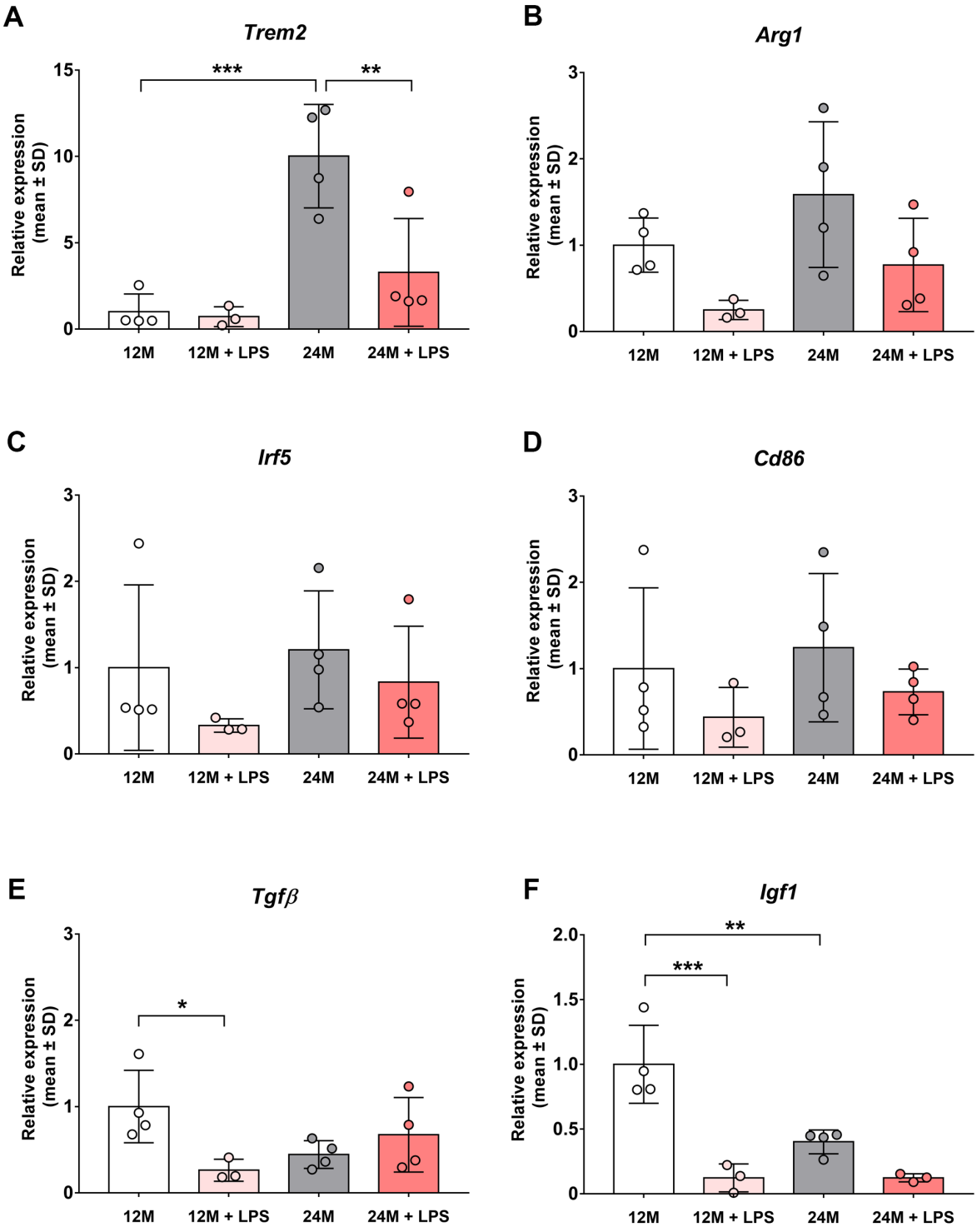

Figure 17 *Trem2* downregulation in peripheral nerves of aged mice after LPS injection. (**A** – **F**) mRNA expression of *Trem2* (**A**), *Arg1* (**B**), *Irf5* (**C**), *Cd86* (**D**), *Tgfβ* (**E**) and *Igf1* (**F**) in femoral quadriceps nerve lysates of uninjected 12M and 24M mice, and 12M and 24M mice injected with LPS. Individual values and mean ± SD are shown. One-way ANOVA, * $p<0.05$; ** $p<0.01$; *** $p<0.001$.

Studies of the CNS have demonstrated upregulation of pro-inflammatory cytokine genes, such as *Tnfα* and *Il-1β*, as early as 6 hours after LPS injection (Cunningham et al., 2005; Shemer et al., 2020). In peripheral nerves, however, LPS injection did not lead to significant changes of *Tnfα* expression in aged or adult mice 1 month after injection, whereas *Ifnɣ*, *Il-1β* and *Nos2* showed low mRNA expression, and thus could not be detected by qPCR in all groups investigated (data not shown). Taken together, these results provide evidence for the altered macrophage polarization in peripheral nerves of aged mice one month after LPS injection.

5.4.3 Dampened demyelination in aged mice injected with LPS

To examine whether the neuropathy in aged mice is aggravated by LPS injection, femoral quadriceps nerves of 12-month-old and 24-month-old mice were investigated by electron microscopy (Fig. 18A). Uninjected adult and aged mice were selected as controls due to the limited numbers of NaCl-injected aged mice for electron microscopic investigation. Surprisingly, percentages of thinly myelinated axons and onion bulbs were significantly lower in aged mice injected with LPS compared to uninjected aged mice (Fig. 18B, C). Quantifications also revealed significantly lower percentages of myelin abnormalities in aged mice injected with LPS (Fig. 18D). Furthermore, the number of foamy macrophages was lower in aged mice injected with LPS compared to uninjected aged mice (Fig. 18E). Collectively, these observations indicate dampened demyelination and less myelin phagocytosis in aged mice after LPS injection (Fig. 16, 17, 18).

Accelerated neurodegeneration after LPS injection has been reported in studies on rodent models of neurodegenerative diseases in the CNS (Deng et al., 2014; Noailles et al., 2018). Therefore, degenerated and degenerating axons were quantified in femoral quadriceps nerves of 12-month-old and 24-month-old mice injected with LPS. Quantification revealed that LPS injection did not lead to a significant change in axon degeneration in aged mice injected with LPS (Fig. 19A). Interestingly, a significantly lower percentage of regeneration clusters could be observed in aged mice after LPS injection (Fig. 19B). This can either reflect less axonal damage (meaning fewer damaged axons giving rise to regenerative clusters) or an impaired regeneration capacity in aged mice after LPS injection.

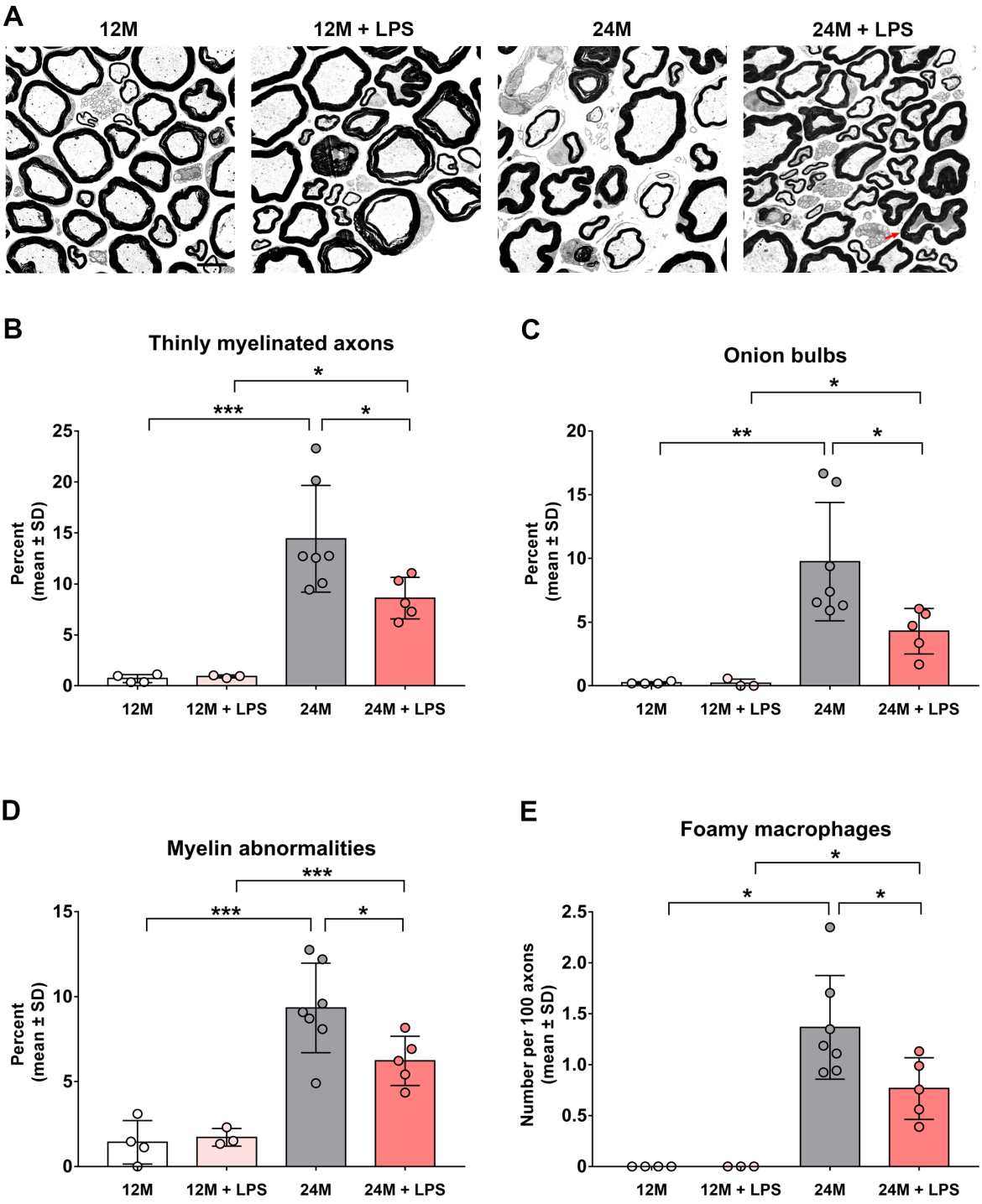

Figure 18 Decreased features indicative of demyelination in peripheral nerves of aged mice injected with LPS. (**A**) Electron micrographs of femoral quadriceps nerves of uninjected 12M and 24M mice, and 12M and 24M mice injected with LPS. Note the dark axon (arrow) from a 24M + LPS mouse. Scale bar, 5 µm. (**B** – **E**) Quantifications of thinly myelinated axons (**B**), onion bulbs (**C**), myelin abnormalities (**D**) and foamy macrophages (**E**) in uninjected 12M and 24M mice, and 12M and 24M mice injected with LPS. Individual values and mean ± SD are shown. Kruskal-Wallis test, * $p<0.05$; ** $p<0.01$; *** $p<0.001$. For comparison, data from 12M and 24M mice, as shown in **Figure 3**, **Figure 4** and **Figure 11**, is presented here again.

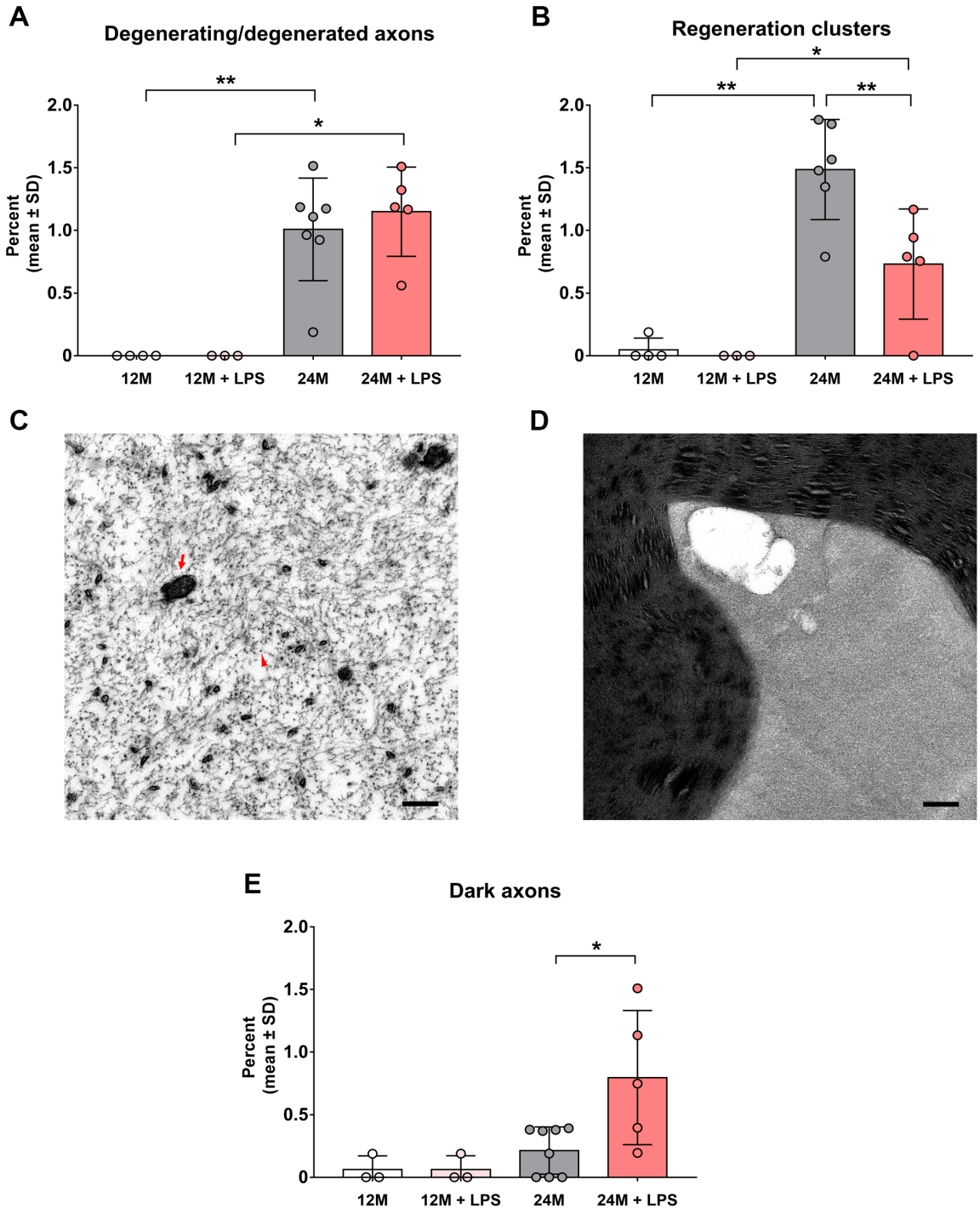

Figure 19 Lower percentages of regeneration clusters and higher percentages of dark axons in peripheral nerves of aged mice injected with LPS. (**A**, **B**) Quantifications of degenerating/degenerated axons (**A**) and regeneration clusters (**B**) in femoral quadriceps nerves of uninjected 12M and 24M mice, and 12M and 24M mice injected with LPS. (**C**, **D**) Representative electron micrographs of a normal axon from femoral quadriceps nerve of a 24M mouse (**C**) and a dark axon from femoral quadriceps nerve of a 24M + LPS mouse (**D**). Note mitochondria and preserved axonal cytoskeleton mostly consisting of neurofilaments (arrowhead, **C**), while the dark axon (**D**) lacks structural preserved cytoskeleton and mitochondria (arrow, **C**). Scale bar, 0.2 µm. (**E**) Quantification of dark axons in femoral quadriceps nerves of uninjected 12M and 24M mice, and 12M and 24M

mice injected with LPS. Kruskal-Wallis test, * p<0.05. For comparison, data from 12M and 24M mice, as shown in **Figure 3**, is presented here again.

Notably, some axons with dense axoplasm were observed in aged mice injected with LPS (Fig. 18A, 19D). These axons, termed “dark axons”, lack structurally preserved cytoskeleton and mitochondria (Fig. 19D) compared to normal axons (Fig. 19C) and were only scarcely seen in normal aging mice (Fig. 19E). Dark axons in the peripheral nerves of aged mice injected with LPS resemble the axons undergoing dark degeneration during Wallerian degeneration in optic nerves of distinct animal models (Cook et al., 1974; Carroll et al., 1992; Narciso et al., 2001; Marques et al., 2003; Saggu et al., 2010). Moreover, in brains of aged monkeys, dark axons have also been identified (Peters, 2009), reflecting the increase of such profiles during aging. Dark axons showed a significantly higher percentage in aged mice injected with LPS compared to uninjected aged mice (Fig. 19E) and might represent a form of axonal perturbation that is rarely observed in the PNS.

The increase of axonal perturbation in LPS-injected aged mice, in the form of dark axons, did not lead to increased NMJ denervation. In contrast, LPS-injected aged ice showed significantly less NMJ denervation (Fig. 20). In summary, these results suggest that LPS injection leads to improved myelin integrity and reduced muscle denervation in aged mice.

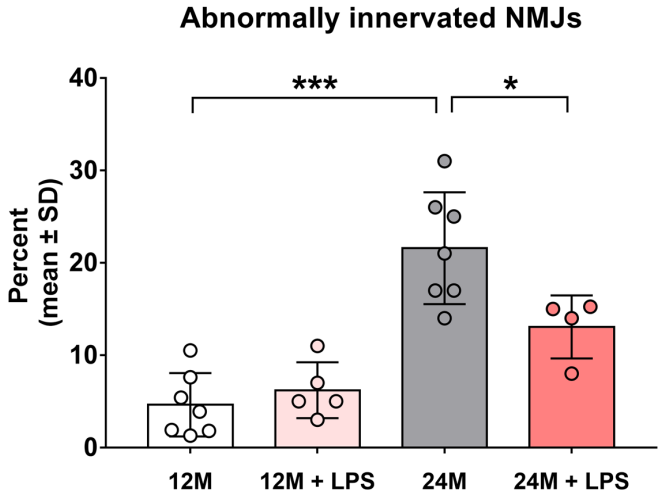

Figure 20 Reduced muscle denervation in peripheral nerves of aged mice injected with LPS. Quantification of abnormally innervated NMJs in 12M and 24M mice injected with LPS, and in uninjected or NaCl-injected 12 and 24M mice. Individual values and mean ±

SD are shown. One-way ANOVA, * p<0.05; *** p<0.001. For comparison, data from 12M mice, as shown in **Figure 13**, is presented here again.

5.4.4 Similarities between systemic inflammation and TREM2 deficiency in aged peripheral nerves

The present study has shown that LPS injection is associated with dampened demyelination and an increase of dark axons in peripheral nerves of aged WT mice. Additionally, *Trem2* downregulation was found in peripheral nerves of aged mice after LPS injection, similar to the results in recent studies on neurodegenerative diseases in the CNS (Kleinberger et al., 2014; Zhou et al., 2019). Previous studies have also reported that TREM2 deficiency or loss of function mutation impair the phagocytosis function of microglia (Takahashi et al., 2007; Hsieh et al., 2009; Kleinberger et al., 2014). Therefore, it was hypothesized that *Trem2* downregulation is involved in the attenuation of demyelination in peripheral nerves of LPS-injected aged mice via reducing myelin phagocytosis.

To investigate the possible role of *Trem2* downregulation in modifying changes in peripheral nerves of aged mice after LPS injection, femoral quadriceps nerves of 24-month-old *Trem2*-/- mice were analyzed by electron microscopy (Fig. 21A). Interestingly, quantifications of pathological profiles revealed similarities between aged *Trem2*-/- mice and aged WT mice injected with LPS. Thinly myelinated axons and onion bulbs, features indicative of demyelination, were less frequent in aged *Trem2*-/- mice compared to aged WT mice (Fig. 21B, C). Similarly, myelin abnormalities were less frequent in aged *Trem2*-/- mice compared to aged WT mice (Fig. 21D), comparable to the observation in aged mice injected with LPS (Fig. 18D, 21D). Interestingly, both LPS injection and TREM2 deficiency led to fewer regeneration clusters in aged mice (Fig. 21F). In addition, a higher prevelance of dark axons was also shown in aged *Trem2*-/- mice (Fig. 21G). Of note, despite a critical role of TREM2 in phagocytosis (Takahashi et al., 2005; Hsieh et al., 2009; N'Diaye et al., 2009), aged *Trem2*-/- mice did not show a reduction in the number of foamy macrophages in their peripheral nerves (Fig. 21E).

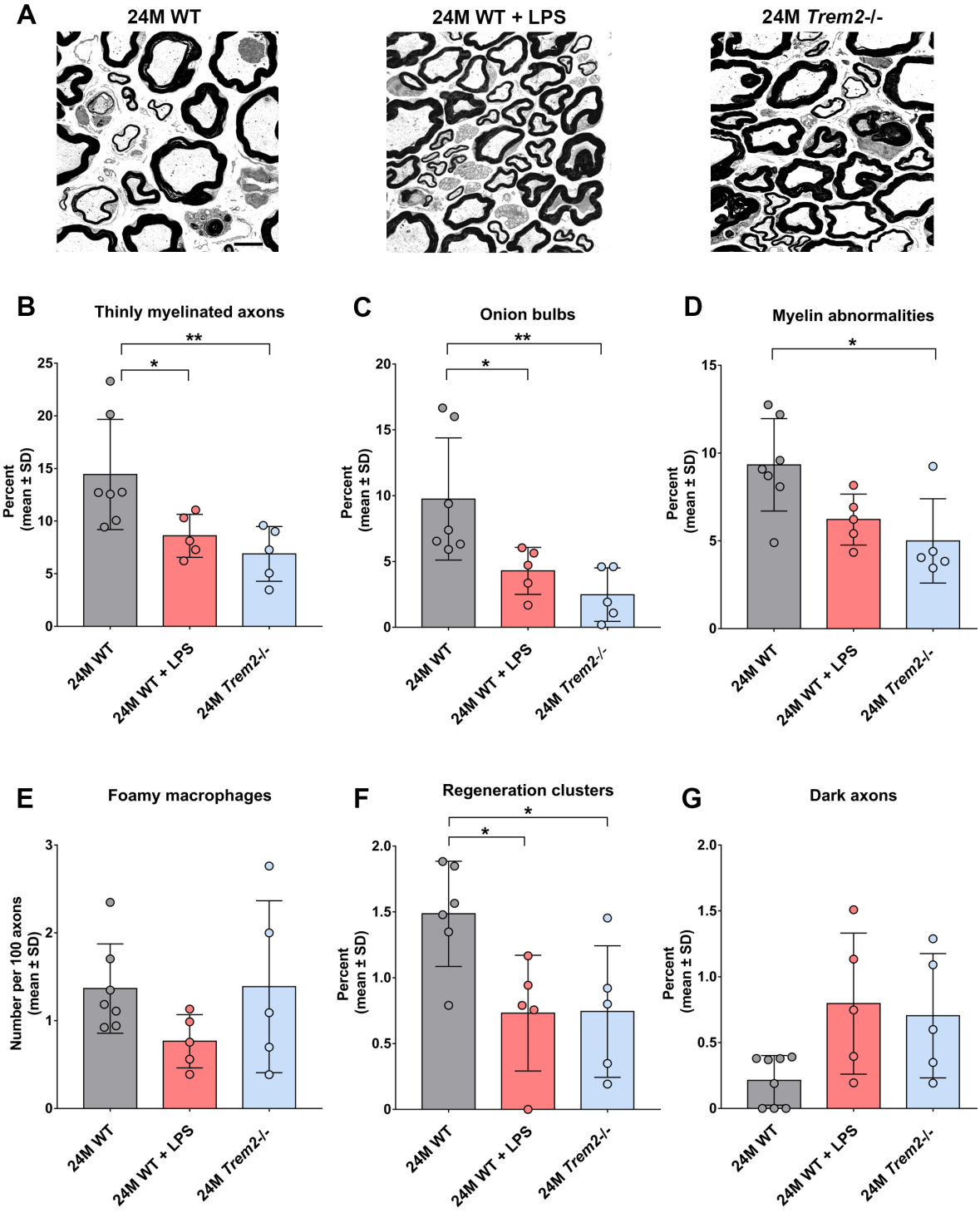

Figure 21 Decreased features indicative of demyelination in peripheral nerves of aged *Trem2*-/- mice. (**A**) Electron micrographs of femoral quadriceps nerves of uninjected 24M wild-type (WT) and 24M *Trem2*-/- mice, and 24M WT mice injected with LPS. Scale bar, 5 µm. (**B** – **G**) Quantifications of thinly myelinated axons (**B**), onion bulbs (**C**), myelin abnormalities (**D**), foamy macrophages (**E**), regeneration clusters (**F**) and dark axons (**G**) in uninjected 24M WT and 24M *Trem2*-/- mice, and 24M WT mice injected with LPS. Individual values and mean ± SD are shown. **B** – **F**: One-way ANOVA, * $p<0.05$; ** $p<0.01$. **G**: Kruskal-Wallis test. For comparison, data from 24M WT (referred to as 24M in previous figures) and 24M WT + LPS (referred to as 24M + LPS in previous figures) mice, as shown in **Figure 18** and **Figure 19**, is presented here again.

Interestingly, aged mice deficient for TREM2 showed less muscle denervation compared to aged WT mice, which is similar to the observations of LPS-injected aged WT mice (Fig. 22A). This was accompanied by a significantly higher grip strength in aged *Trem2*-/- mice compared to aged WT mice (Fig. 22B). A trend towards increased grip strength was also be observed in aged WT mice injected with LPS. Overall, similar to LPS injection, TREM2 deficiency in aged mice leads to dampened demyelination and less muscle denervation, accompanied by improved muscle function.

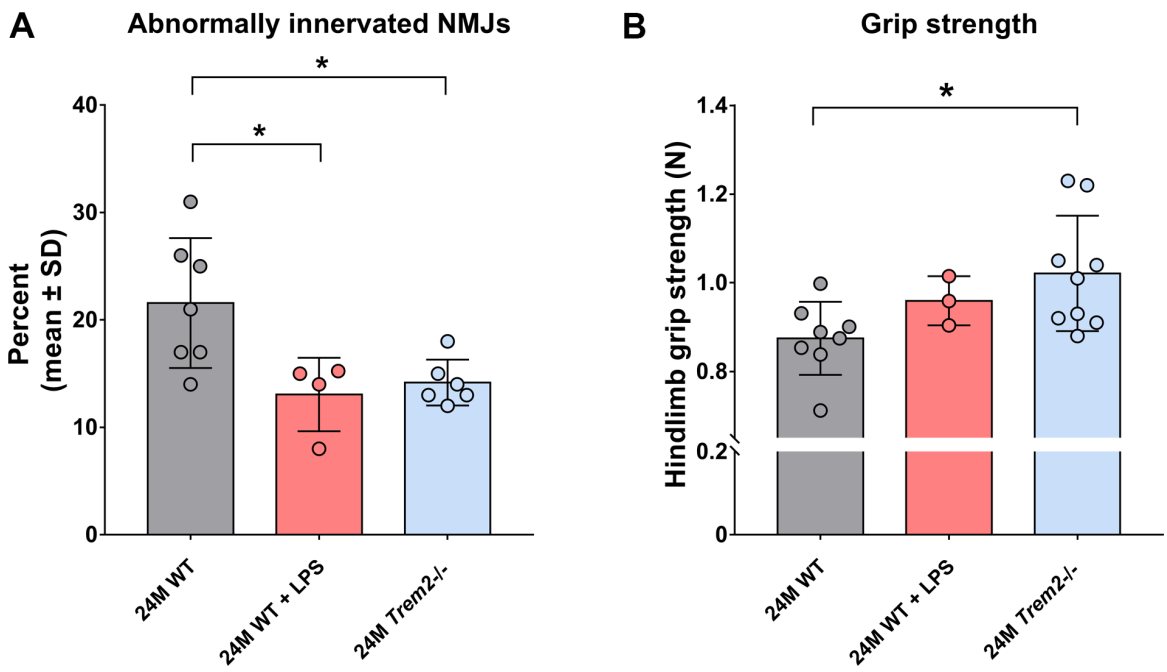

Fig 22 Improved muscle function in aged *Trem2*-/- mice. (**A**) Quantification of abnormally innervated NMJs in uninjected or NaCl-injected 24M WT mice, 24M WT mice injected with LPS (24M WT + LPS) and 24M *Trem2*-/- mice. For comparison, data of 24M WT mice, as shown in **Figure 13** and **Figure 20**, is presented here again. (**B**) Hindlimb grip strength of 24M WT, 24M WT + LPS and 24M *Trem2*-/- mice. Individual values and mean ± SD are shown. One-way ANOVA, * $p<0.05$.

5.4.5 Lack of evidence for a TREM2-independent mechanism for altered nerve phenotypes in aged mice after LPS injection

As LPS injection and TREM2 deficiency in aged mice are associated with similar nerve phenotypes, it is plausible that these two factors act via a common pathway implicating TREM2. However, the possibility that LPS injection in aged mice alters

nerve phenotypes via a TREM2-independent mechanism cannot be excluded. To investigate if the mechanism modifying the changes in peripheral nerves after LPS injection in aged mice is predominantly TREM2-dependent, LPS injection was performed in 24-month-old mice deficient for TREM2 (*Trem2*-/- mice).

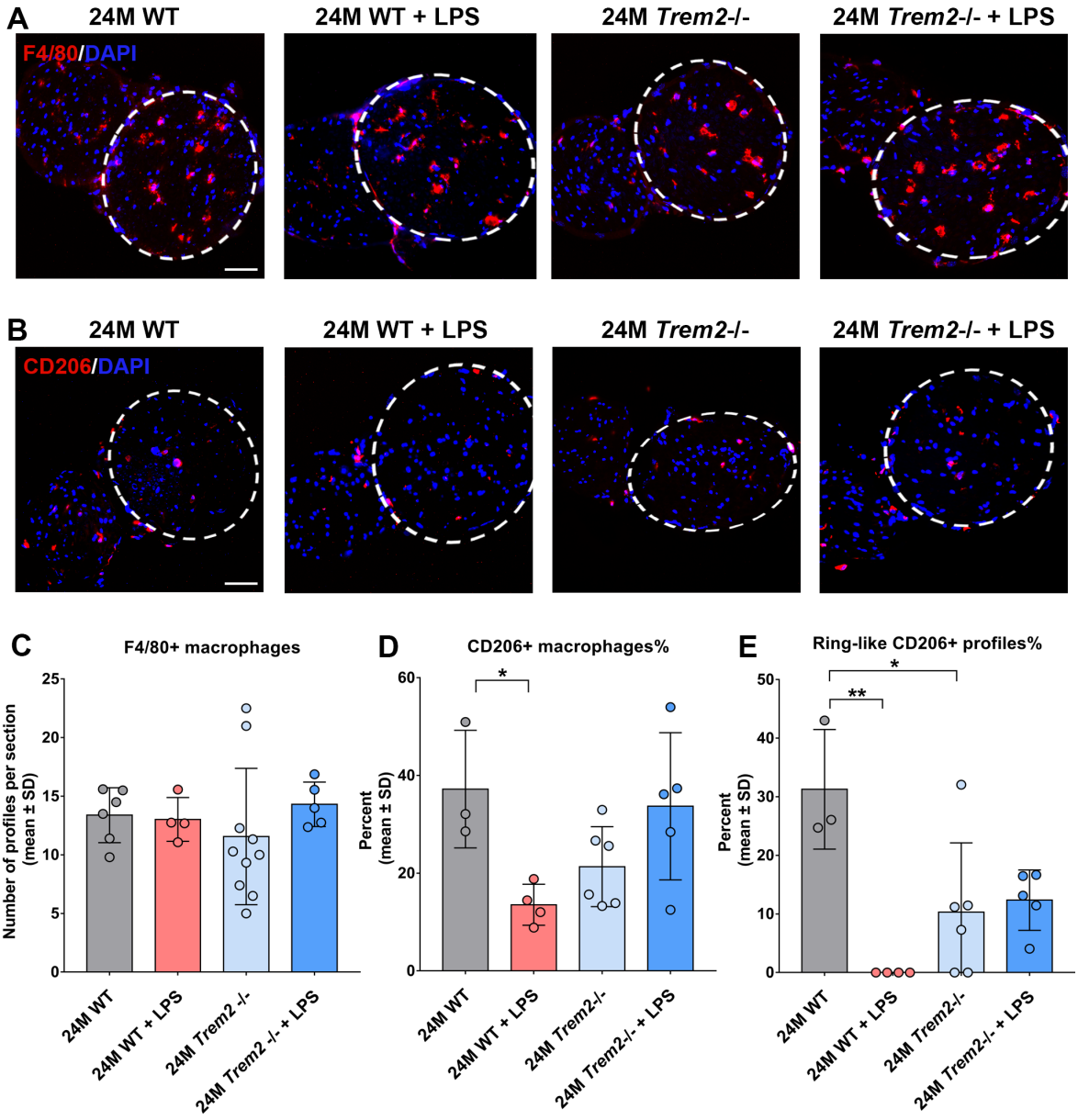

Figure 23 Mild alterations in macrophage activation in peripheral nerves of *Trem2*-/- mice after LPS injection. (**A**) Immunohistochemical micrographs of F4/80+ macrophages (red) on cross sections of femoral quadriceps nerves in uninjected 24M WT and *Trem2*-/- mice, and 24M WT and 24M *Trem2*-/- mice injected with LPS (24M WT + LPS, 24M *Trem2*-/- + LPS). Dashed circles indicate the area of femoral quadriceps nerves. Scale bar, 50 µm. (**B**) Immunohistochemical micrographs of CD206+ macrophages (red) on cross sections of femoral quadriceps nerves in 24M WT, 24M WT + LPS, 24M *Trem2*-/- and 24M *Trem2*-/- + LPS mice. Scale bar, 50 µm. (**C**) Quantification of F4/80+ profiles in femoral quadriceps nerves in 24M WT, 24M WT + LPS, 24M *Trem2*-/- and 24M *Trem2*-/- + LPS

mice. (**D**) Percentage of CD206+ macrophages on cross sections of femoral quadriceps nerves in 24M WT, 24M WT + LPS, 24M *Trem2*-/- and 24M *Trem2*-/- + LPS mice. (**E**) Quantification of ring-like CD206+ profiles on cross sections of femoral quadriceps nerves in 24M WT, 24M WT + LPS, 24M *Trem2*-/- and 24M *Trem2*-/- + LPS mice. Individual values and mean ± SD are shown. **C**, **D**: One-way ANOVA, * $p<0.05$. **E**: Kruskal-Wallis test, * $p<0.05$; ** $p<0.01$. For comparison, data of 24M WT and 24M WT + LPS mice, as shown in **Figure 4**, **Figure 15** and **Figure 16**, is presented here again.

LPS injection did not lead to a significant change in the number of endoneurial macrophages in femoral quadriceps nerves of aged *Trem2*-/- mice (Fig. 23A, C). A non-significant trend of higher percentages of CD206+ macrophages was observed in aged *Trem2*-/- mice injected with LPS compared to uninjected aged *Trem2*-/- mice (Fig. 23B, D), whereas in aged WT mice, LPS injection led to lower percentages of CD206+ macrophages (Fig. 23D). In addition, in aged *Trem2*-/- mice, LPS injection did not lead to a change in percentages of ring-like CD206+ profiles, while in aged WT mice, LPS injection led to a substantial reduction in the percentages of ring-like CD206+ profiles (Fig. 23E). These results indicate only a mild change in macrophage polarization in aged *Trem2*-/- mice after LPS injection.

To examine whether LPS injection in aged *Trem2*-/- mice leads to further changes of pathological alterations, femoral quadriceps nerves of aged WT mice and aged *Trem2*-/- mice were investigated by electron microscopy (Fig. 24A). LPS injection in aged *Trem2*-/- mice did not result in changes in axonal degeneration (Fig. 24B). Furthermore, LPS-injected aged *Trem2*-/- mice did not show higher numbers of dark axons compared to uninjected aged *Trem2*-/- mice (Fig. 24C). These results suggest that the mechanism underlying the mild increase of axonal perturbation in aged mice after LPS injection is likely TREM2-dependent.

Quantifications also revealed that features indicative of demyelination, such as thinly myelinated axons and onions bulbs, did not show changes of percentages in LPS-injected aged *Trem2*-/- mice compared to uninjected aged *Trem2*-/- mice (Fig. 25A, B). LPS injection did not lead to a change in numbers of myelin abnormalities and foamy macrophages in aged *Trem2*-/- mice (Fig. 25C, D). These results imply a TREM2-dependent mechanism for the improvement of myelin integrity in aged mice after LPS injection. Additionally, LPS injection in aged *Trem2*-/- mice did not alter the percentages of regeneration clusters in their femoral quadriceps nerves (Fig. 25E). Fibers with undulated myelin, a feature not

significantly altered by TREM2 deficiency in aged mice, showed a mild trend of lower percentages in LPS-injected aged *Trem2*-/- mice compared to uninjected aged *Trem2*-/- mice (Fig. 25F). In summary, these results do not provide evidence for a TREM2-independent mechanism for the altered nerve phenotypes in aged WT mice after LPS injection.

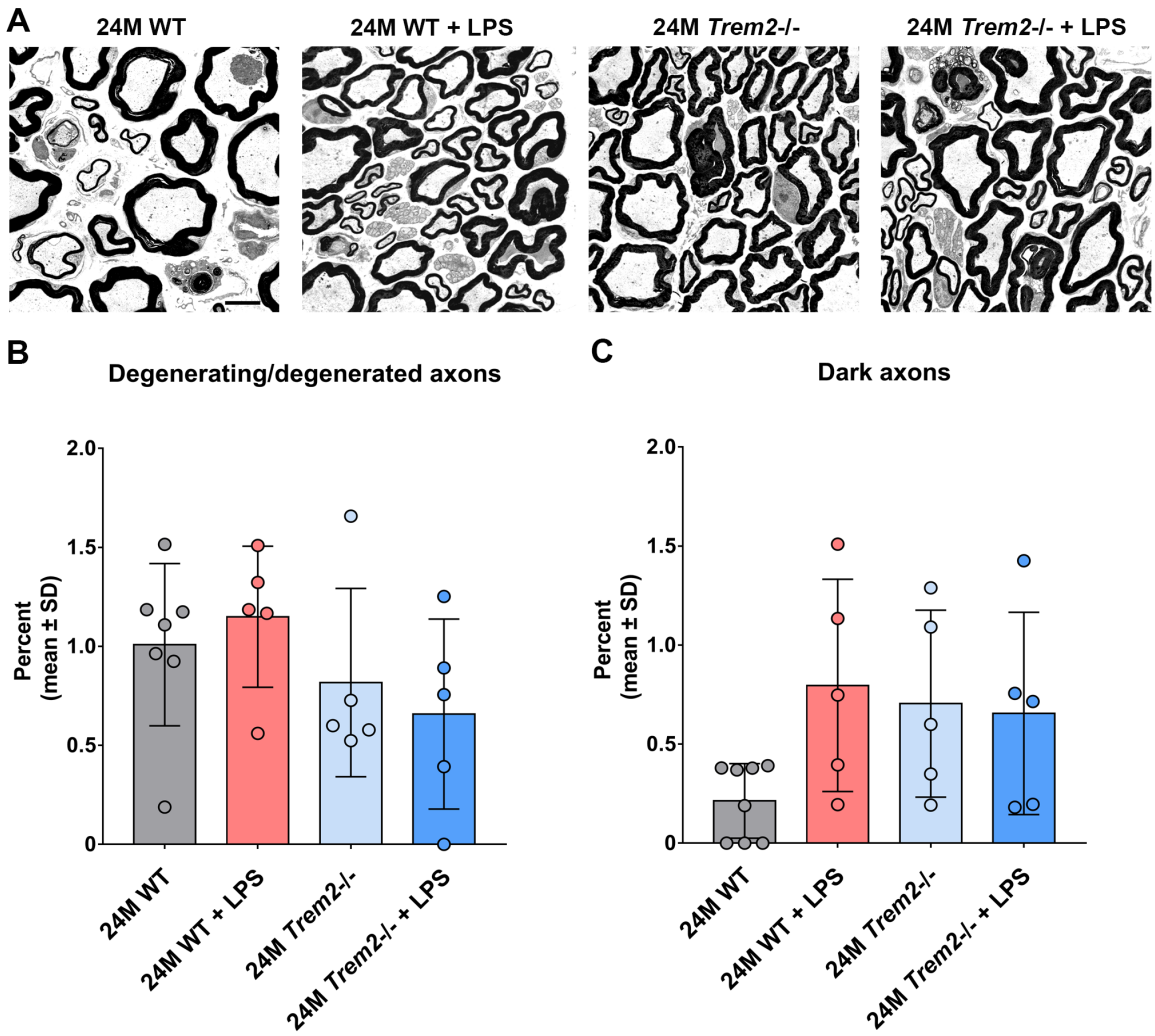

Figure 24 Absence of increased axonal perturbation in peripheral nerves of *Trem2*-/- mice after LPS injection. (**A**) Electron micrographs of femoral quadriceps nerves of uninjected 24M WT and *Trem2*-/- mice, and LPS-injected 24M WT and *Trem2*-/- mice. Scale bar, 5 µm. (**B**, **C**) Quantifications of degenerating/degenerated axons (**B**) and dark axons (**C**) in femoral quadriceps nerves of 24M WT, 24M WT + LPS, 24M *Trem2*-/- and 24M *Trem2*-/- + LPS mice. Individual values and mean ± SD are shown. Kruskal-Wallis test, p>0.05. For comparison, data of 24M WT, 24M WT + LPS and 24M *Trem2*-/- mice, as shown in **Figure 18** and **Figure 21**, is presented here again.

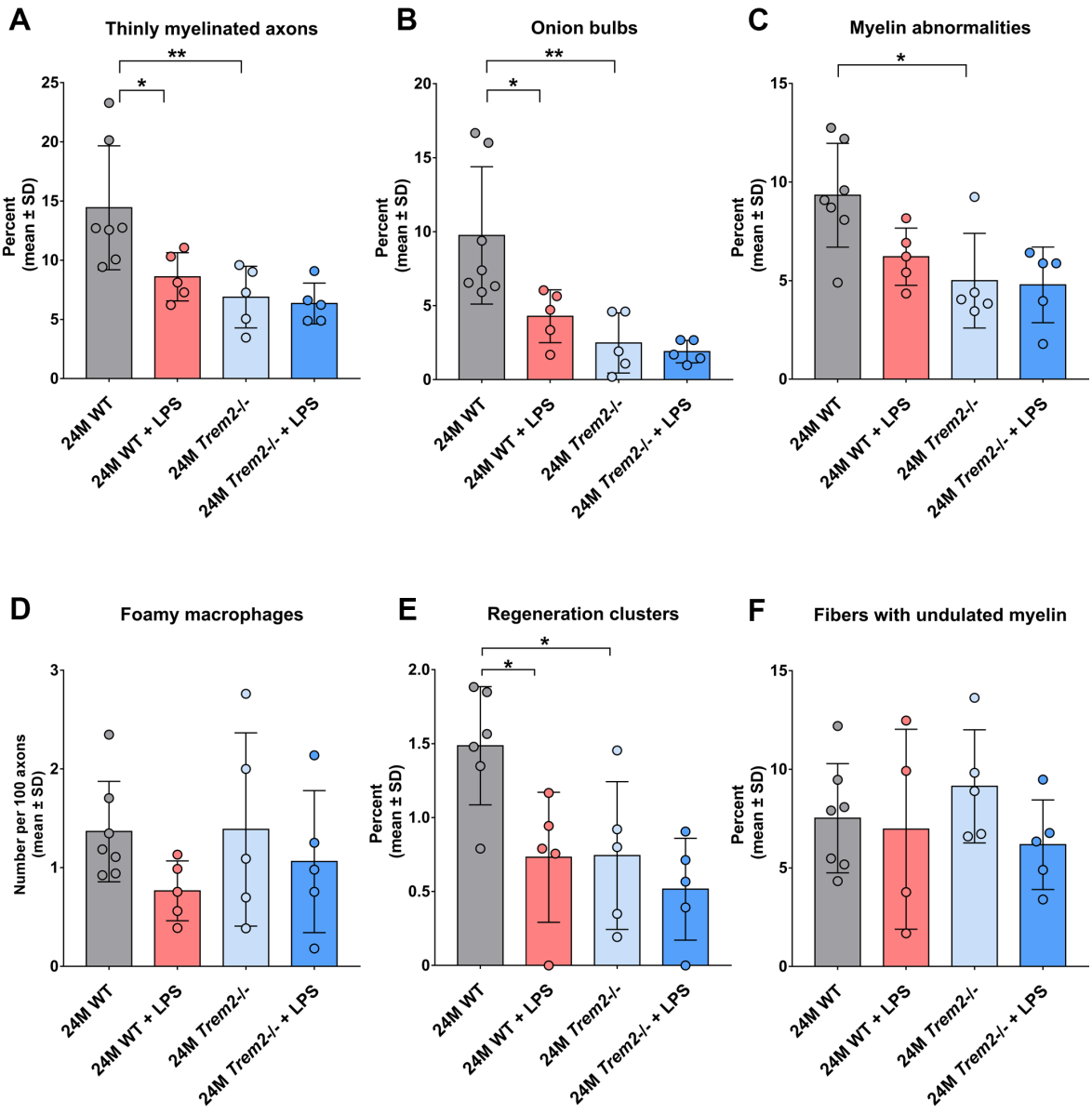

Figure 25 Lack of changes in myelin integrity and other pathological alterations in peripheral nerves of *Trem2*-/- mice after LPS injection. (**A** – **F**) Quantifications of thinly myelinated axons (**A**), onion bulbs (**B**), myelin abnormalities (**C**), foamy macrophages (**D**), regeneration clusters (**E**) and fibers with undulated myelin (**F**) in femoral quadriceps nerves of uninjected 24M WT and 24M *Trem2*-/- mice, and 24M WT and 24M *Trem2*-/- mice injected with LPS. Individual values and mean ± SD are shown. **A** – **D**, **F**: One-way ANOVA, * $p<0.05$; ** $p<0.01$. **E**: Kruskal-Wallis test, * $p<0.05$. For comparison, data of 24M WT, 24M WT + LPS and 24M *Trem2*-/- mice, as shown in **Figure 3** and **Figure 21**, is presented here again.

Taken together, these results suggest that LPS injection reduces demyelination in aged mice via *Trem2* downregulation. This study highlights a possible role for TREM2 as a key molecule for altered macrophage activation, dampened

demyelination and reduced muscle denervation in the PNS of aged mice after systemic inflammation. Systemic inflammation can ambivalently modify age-related nerve damage, and may lead to an increase of a distinct form of axonal perturbation, but also a more functionally relevant reduction of demyelination and less muscle denervation in aged mice. Overall, it can be inferred that modulating macrophage activation can affect functional outcome in the PNS during aging.

6. Discussion

6.1 Age-related changes in peripheral nerves of mice and humans

This study described and characterized similar pathological alterations in peripheral nerves of normal aging humans and aged wild-type (WT) mice. As described in an earlier study of aged Swiss OF1 mice (Ceballos et al., 1999), during adulthood of mice, peripheral nerves show structural stability. Although mild morphological changes can be found in peripheral nerves of 18-month-old mice, progressive degenerative changes, such as demyelination and axonal degeneration, occur between 20 months and 22 months of age in mouse peripheral nerves (Yuan and Klein, unpublished observation), leading to an “aging phenotype” in 24-month-old mice.

The increase of thinly myelinated axons in peripheral nerves of aged mice shown in the present study is in line with the decrease of myelin thickness reported by two previous studies in aged mice (Canta et al., 2016; Hamilton et al., 2016). The present study also shows the increase of onion bulbs, a hallmark of demyelinating neuropathies, in peripheral nerves of aged mice. In addition, regeneration clusters, representing regenerating sprouts, are also found in peripheral nerves of aged mice. These features observed in aged mice are typically found in distinct mouse models for the demyelinating hereditary neuropathy, CMT1 (Martini et al., 1995; Martini, 1997; Scherer et al., 1998), indicating a resemblance of nerve morphology between aged mice and CMT1 mouse models. Although the underlying cause of such demyelination is not clear yet, it is possible that activated macrophages in peripheral nerves of aged mice acquire a myelin-phagocytosing phenotype.

The demyelination observed in aged mice has raised speculations on the downregulation of myelin protein expressions, as the increase in oxidative stress during aging might cause damage to myelin proteins and affect their expressions (Opalach et al., 2010; Hamilton et al., 2016). It is also likely that increased oxidative stress induces cellular stress response, such as the unfolded protein response (Domènech-Estévez et al.), which consequently activates the low-grade neuroinflammation in the nerves (Ydens et al., 2013). Indeed, an elevated level of cellular stress was observed in peripheral nerves of aged mice (Klein, unpublished observation). In the present study, however, no downregulation of myelin genes in

aged mice compared to adult mice was detected on either the mRNA or protein level, as opposed to previous findings in aged rats (Melcangi et al., 1998a; Melcangi et al., 2000; Melcangi et al., 2003). However, in the previous studies, expressions of myelin protein genes in aged rats were compared to 3-month-old young adults, of which higher expression of myelin protein genes can be detected compared to older non-aged adults (Ahn et al., 2017) investigated in the present study. The findings in the present study argue against the hypothesis that oxidative stress is the primary cause for the demyelination in aged mice. Based on the observations that foamy macrophages take up myelin and consequently contribute to the demyelination, it is likely that the thinner myelin in aged mice results from myelin phagocytosis instead of reduced myelin gene transcription and translation due to oxidative stress, and that the phagocytosed myelin components can still be detected on the protein level.

Abnormalities of myelin, such as myelin infoldings and outfoldings, also increase with age in mouse peripheral nerves. Observed in different mouse models for peripheral neuropathies (Schenone et al., 1994; Lee et al., 2013), myelin infoldings and outfoldings might reflect the myelin redundancy also observed in the aging CNS (Peters, 2009; Safaiyan et al., 2016). The cause of these myelin abnormalities, however, is not clear yet. The formation of myelin infoldings is likely an early myelinic response of large caliber axons to axonal dystrophy (Krinke et al., 1988), whereas the formation of myelin outfoldings might occur due to disrupted compact myelin structure maintenance (Ceballos et al., 1999).

Another important feature of the peripheral nerves of aged mice is that they show lower axon sphericity compared to adult mice, reflected by an increase in numbers of fibers with undulated myelin. The undulation of myelin is likely a result of axonal shrinkage with a stable myelin sheath. The axonal shrinkage in aged mice can be explained by a reduction of neurofilament gene expression during aging (Parhad et al., 1995; Ceballos et al., 1999) and might lead to a progressive decrease of axon diameters in aged mice (Chase et al., 1992; Ceballos et al., 1999; Hamilton et al., 2016). In addition, the collapse of the cytoskeleton following axonal shrinkage might also cause axonal perturbation and degeneration observed in aged mice and other species (O'Sullivan and Swallow, 1968; Jacobs and Love, 1985; Knox et al., 1989; Wheeler and Plummer, 1989; Verdu et al., 1995). The

increase of axonal perturbation and degeneration in aged nerves can also be due to a reduction of growth factors which offer trophic support during regeneration and protect the nerves from oxidative stress (Maggio et al., 2006; Maggio et al., 2013). Although axonal degeneration is mild and does not result in significant axonal loss in peripheral nerves of mice, it contributes to the distinct phenotype of aged nerves from adult nerves.

Interestingly, large caliber axons are more vulnerable during aging, as degenerative changes observed in aged nerves predominantly occur in large caliber axons. This is likely due to the higher abundance of neurofilament in large caliber axons and their susceptibility to oxidative stress (Cleveland, 1999; Nguyen et al., 2000). Additionally, most degenerative changes identified in femoral quadriceps are not found in aged femoral saphenous nerves, which are a purely sensory branch of the femoral nerve. This might reflect the perseverance of the femoral saphenous nerves during aging, which resembles the observations in mouse models of hereditary peripheral neuropathies (Martini et al., 1995; Scherer et al., 1998; Wrabetz et al., 2000). The reason for such nerve difference is not clear yet. Further transcriptomic and proteomic analyses could address the underlying mechanism for the selective vulnerability and perseverance in peripheral nerves during aging.

Accompanying the structural changes in peripheral nerves of aged humans and mice, myelin-laden phagocytosing macrophages are identified in these aged individuals. Furthermore, endoneurial macrophages in aged mice adopt a typical phenotype characterized by a substantial, disproportionate upregulation of *Trem2*, a molecule associated with a phagocytosing, regenerative phenotype (Ydens et al., 2012; Colonna and Butovsky, 2017; Meilandt et al., 2020). It can thus be inferred that the activation of endoneurial macrophages in aged mice leads to more myelin phagocytosis and consequently demyelination.

The reason for the increase of macrophage numbers in aged peripheral nerves remains enigmatic. Proliferation of resident macrophages and recruitment of macrophages from the circulation are two possible sources for the increase of macrophage numbers. The proliferation of resident macrophages relies on the activation of CSF-1R by binding to its ligands CSF-1 or IL-34 (Pixley and Stanley, 2004; Guillonneau et al., 2017). In aged mice, *Csf-1* and *Il-34* only show a mild

and insignificant trend of upregulation compared to adult mice (Yuan, unpublished observation). Therefore, it is likely that macrophage recruitment from the circulation mainly contributes to the increase of macrophage numbers during aging, but not proliferation of resident macrophages. In Wallerian degeneration and in mouse models of hereditary peripheral neuropathies, where macrophages also increase in number and present a similar phenotype to the aging phenotype, MEK-ERK activation in Schwann cells leads to an upregulated gene expression of CCL2, an important chemokine for macrophage recruitment (Fischer et al., 2008a; Groh et al., 2010; Kohl et al., 2010a; Napoli et al., 2012; Martini et al., 2013). In aged mice, shrinking axons might lead to a Wallerian-like neuroinflammatory response with upregulations of cytokines that similarly recruit macrophages via the MEK-ERK signaling pathway upstream of *Ccl2* upregulation. However, despite the upregulation of *Ccl2* expression, no increase of ERK phosphorylation could be detected in peripheral nerves of aged mice (Yuan, unpublished observation). This suggests that the MEK-ERK signaling pathway might not be responsible for the macrophage recruitment in aging mice. Therefore, in order to determine the pathway responsible for macrophage recruitment in aged mice, candidate signaling molecules upstream of CCL2 remain a topic for investigation. Of note, CCL2 deficiency in aged *Ccl2*-/- mice does not lead to lower endoneurial macrophage numbers (Yuan, unpublished observation). However, this is not evidence against a CCL2-mediated macrophage recruitment mechanism in aged mice. It is likely that in aged *Ccl2*-/- mice, the increase of macrophage numbers occurs due to the proliferation of resident macrophages in the presence of a compensatory *Csf-1* upregulation, as observed in mouse models for CMT1 (Fischer et al., 2008b; Groh et al., 2010). Future experiments examining *Csf-1* expression and CSF-1 secretion in aged *Ccl2*-/- mice are needed to confirm the hypothesis. Moreover, single-cell RNA sequencing and recently developed mouse models for lineage tracing of PNS macrophages can be utilized for identifying the origin of the increase of endoneurial macrophages in aged mice (Wang et al., 2020; Ydens et al., 2020).

Along with prominent changes observed in the innate immune system during aging, the adaptive immune system also shows progressive changes in aged mice. T-lymphocyte infiltration into the CNS has been reported in a recent study

(Dulken et al., 2019) and it is associated with axonal degeneration (Groh, unpublished observation). Although the number of T-lymphocytes in peripheral nerves also increases during aging, the role of T-lymphocytes in aged peripheral nerves is not clear yet, as genetic studies on *Rag1*-/- mice without mature T- or B-lymphocytes demonstrate a worsened nerve pathology and higher macrophage numbers in peripheral nerves of aged mice (Yuan and Klein, unpublished observation). One possible explanation for this observation is that the lack of regulatory T-lymphocyte caused by RAG1 deficiency leads to hyperactivation of macrophages. Molecular profiling is necessary to elucidate the role of T-lymphocytes in aged peripheral nerves in more detail.

6.2 Macrophages mediate pathological changes in aged mice

In this study, it is shown for the first time that macrophage depletion in aged mice ameliorates the peripheral neuropathy, highlighting a role of endoneurial macrophages in mediating age-related changes in peripheral nerves. Macrophage depletion is achieved by pharmacological inhibition of CSF-1R, leading to a substantial attenuation of demyelination. Additionally, aged *Cx3cr1* gfp/gfp mice, in which fractalkine receptor gene *Cx3cr1* is replaced by green fluorescent protein (GFP) reporter gene (Jung et al., 2000), have been investigated as a genetic approach for targeting endoneurial macrophages. CX3CR1 deficiency in aged mice is associated with lower macrophage numbers and dampened demyelination in their peripheral nerves compared to aged WT mice (Yuan and Klein, unpublished observation). The similarity of nerve phenotypes between aged mice deficient for CX3CR1 and aged mice treated with CSF-1R inhibitor further demonstrates the role of endoneurial macrophages as a mediator for the peripheral neuropathy in aged mice.

Functions of the remaining macrophages in aged mice after the depletion are not yet thoroughly investigated. They are numerically equal to the macrophages in adult mice which might also have physiological functions. Microglia, the CNS counterparts of these endoneurial macrophages, are involved in myelin degradation under homeostatic conditions (Safaiyan et al., 2016). The impairment of myelin degradation and cholesterol clearance in microglia during aging burdens

the CNS and leads to a maladaptive immune response that consequently limits remyelination in aged mice (Cantuti-Castelvetri et al., 2018). Similarly, defects in myelin debris clearance of macrophages in aged peripheral nerves also contribute to an impaired regeneration in aged PNS after injury (Scheib and Höke, 2016a; Scheib and Höke, 2016b). Therefore, it is likely that endoneurial macrophages are associated with myelin turnover, and that such function is also dysregulated in uninjured aged mice. Further experiments testing this hypothesis can be performed by applying a long-term macrophage depletion method with a higher depletion rate in aged mice.

In addition, this study demonstrated for the first time that macrophage depletion ameliorates the denervation of neuromuscular junctions (NMJs) in aged mice and preserves muscle strength. NMJs develop structural and functional changes during aging in both humans (Wokke et al., 1990; Jones et al., 2017; Taetzsch and Valdez, 2018) and mice (Gonzalez-Freire et al., 2014; Rudolf et al., 2014; Tintignac et al., 2015). The amelioration can be due to the improvement of the nerve structure which affects the synapses, or due to the depletion of macrophages associated with the synapses (synaptic macrophages). However, observations have shown that only 0.3% and 1% of the synapses are associated with macrophages in 12-month-old and 24-month-old mice, respectively (Yuan et al., 2018), arguing against the possibility that synaptic macrophages mediate the denervation of NMJs in aged mice. These findings provide evidence for a neurogenic cause of NMJ denervation in aged mice. On the other hand, studies have shown that NMJ degeneration can occur due to loss of skeletal muscle stem cells (Liu et al., 2017) and an accumulation of muscle fiber degeneration after dynamic muscle fiber de- and regeneration at the synapses over time (Li et al., 2011). Muscle-derived molecules can also regulate pre-synaptic development in a retrograde manner (Kummer et al., 2006; Yumoto et al., 2012; Rudolf et al., 2014). Therefore, it is likely that in aging, NMJ maintenance is regulated bidirectionally. To date, no correlation between structural alterations of the NMJs and the sarcopenia observed in aging has been established (Tintignac et al., 2015; Krishnan et al., 2016; Liu et al., 2017; Willadt et al., 2018), and this remains to be investigated. Of note, studies in mouse NMJs during aging do not recapitulate the

situation in aging humans, as human NMJs are stable across adult lifespan, and are structurally and molecularly different from mouse NMJs (Jones et al., 2017).

When discussing the functional preservation caused by macrophage depletion, the possible impact of microglia in the spinal cords on motor units should not be excluded. A recent study shows that CSF-1R inhibition leads to depletion of microglia in the spinal cords of aged mice and prevents the loss of functional motor units (Giorgetti et al., 2019). Although this study does not cover the effect of improved nerve structure in the peripheral nerves, it provides evidence for an effect of altered microglia activation profiles on age-related motor unit changes. Indeed, CSF-1Ri depletion by PLX5622 in the present study leads to depletion of microglia in the CNS (Groh, unpublished observation), which can consequently affect motor neurons. As specific pharmacological targeting of macrophages in the peripheral nerves is currently technically difficult to achieve, a genetic approach of lineage-specific conditional depletion should be considered in order to further elucidate the influence of microglia and macrophages on motor units during aging.

Nerve conduction properties that show impairment in aged mice are not rescued by macrophage depletion. In the present study, amplitudes of compound muscle action potentials (CMAPs) of aged mice are significantly lower compared to adult mice. This finding is in line with previous studies reporting the reduction of CMAP amplitudes in both aged mice (Walsh et al., 2015) (Verdu et al., 1995, 1996) and elderly humans (Drechsler, 1975; Bouche et al., 1993; Thakur et al., 2010a). This likely reflects the axonal perturbation and NMJ alterations in the PNS during aging. Since macrophage depletion does not ameliorate the axonal perturbation in aged mice, it does not lead to a rescue of the CMAP amplitudes. Furthermore, nerve conduction velocities (NCVs) are also not altered after macrophage depletion in aged mice. Notably, NCVs of 24-month-old mice were not significantly lower compared to 12-month-old adult mice, as opposed to results in humans showing a decrease of both motor and sensory NCV during aging (Drechsler, 1975; Dorfman and Bosley, 1979; Bouche et al., 1993; Ward et al., 2015). This discrepancy of age-related NCV changes between mice and humans remains to be resolved. According to previous studies, demyelination can lead to disorganization of voltage-gated sodium channels in aged animals (Adinolfi et al., 1991; Moldovan et al., 2016), which consequently affects nerve conduction properties. The decrease

in axon caliber can also have negatively impacts on NCVs (Chase et al., 1992; Verdu et al., 2000). Despite a high intragroup variance, one possible reason for the relatively stable NCV in aged mice in the present study is that the structural changes in the nerve have not yet reached the threshold for a significant decline of NCV. This hypothesis is supported by the reduced NCV in mice older than 27 months of age (Klein and Yuan, unpublished observation). Importantly, motor NCV is measured in sciatic nerves in this study, whereas morphological analyses are performed in femoral quadriceps nerves. As different peripheral nerves are affected at different rates in aged mice (Yuan and Klein, unpublished observation), electrophysiological measurements of femoral nerves are required to directly assess the effect of structural changes in nerves and inflammation on nerve conduction properties.

6.3 Systemic inflammation as an ambivalent modifier of age-related nerve damage in aged mice

Previous studies on distinct neurodegenerative diseases in the CNS have reported that systemic inflammation induced by LPS injection can cause ER stress and hyperactivation of microglia, which consequently lead to aggravation of diseases (Henry et al., 2009; Wynne et al., 2010; Deng et al., 2014; Olympiou et al., 2016; Wendeln et al., 2018). In the present study, it is shown for the first time that systemic inflammation induced by LPS injection alters macrophage activation and likely leads to mitigation of a demyelinating phenotype in nerves of aged mice.

The mechanisms through which peripheral LPS affects the nerves is currently unclear. LPS might cross the blood-nerve barrier and directly target toll-like receptor 4 (TLR4)-expressing macrophages or other endoneurial cells that might express TLR4, such as Schwann cells (Takeuchi et al., 1999; Beutler, 2000; Lee et al., 2006; Goethals et al., 2010; Tzekova et al., 2014; Zhang et al., 2018). Importantly, endothelial cells in peripheral nerves can also express TLR4 and secrete inflammatory cytokines upon activation (Maiuolo et al., 2019), which can subsequently alter the nerve microenvironment. However, it is also likely that the change of inflammatory cytokine levels in the blood after LPS injection (Wynne et al., 2010; Olympiou et al., 2016; Wendeln et al., 2018) shapes the nerve

microenvironment and alters endoneurial macrophage activation. The reduction of ring-like CD206+ macrophages, possibly phagocytosing macrophages, reflects altered macrophage activation/polarization in aged mice after LPS injection. These profiles are not observed in adult WT mice before or after LPS injection, suggesting that these changes require already primed immune cells.

Previous studies have reported upregulation of *Tnfα, Irf5* and *Tgfβ* in tissue-resident/monocyte-derived macrophages after LPS injection (Chen et al., 2008; Schneider et al., 2018). In the present study, no significant changes of *Irf5* and *Tgfβ* expression were detected in peripheral nerves of aged mice one month after LPS injection. A mild trend of *Tnfα* downregulation was found in peripheral nerves of mice after LPS injection (Yuan, unpublished observation). However, these results do not provide evidence for a lack of macrophage activation in peripheral nerves after LPS injection. This is partially due to the different time points investigated in the present study versus earlier studies. Previous reports focus on gene expressions 24 hours after LPS injection, instead of the 1-month post-injection time point in the present study. Moreover, the cellular source of these cytokines and transcription factors has not been identified in the present study. It is likely that the tissue microenvironment is also shaped by altered macrophage activation in peripheral nerves of aged mice one month after LPS injection. Detailed characterization, particularly single-cell analysis, of the transcriptomes of macrophages after LPS injection can provide more insights into the molecular atlas of altered macrophage activation in peripheral nerves in LPS-injected aged mice.

Notably, *Tgfβ* expression is reduced in peripheral nerves of 12-month-old mice one month after LPS injection. This is in line with the results of a previous study of brains of LPS-injected 12-month-old mice (Wynne et al., 2010) and might reflect mild changes in tissue microenvironment of peripheral nerves of adult mice after LPS injection. The decreased expression in LPS-injected aged mice of another growth factor gene, *Igf1*, points to a less regenerative microenvironment in aged nerves after LPS injection (Apel et al., 2010; Liu et al., 2020), consistent with the lower numbers of regeneration clusters in these mice. This suggests either less axonal damage, or an impairment of regenerative capacity in peripheral nerves of aged mice after LPS injection. To address this issue, further analysis on the

dynamics of the LPS-induced axonal changes at earlier time points (e.g. one week after injection) is needed.

In the present study, *Trem2* expression in aged peripheral nerves is associated with demyelination. Upregulation of *Trem2* in aged WT mice is associated with the increase of demyelination, and downregulation of *Trem2* in aged WT mice after LPS injection is associated with dampened demyelination in the PNS. Similarly, TREM2 deficiency in aged mice leads to attenuated demyelination in the PNS. Furthermore, LPS injection in aged mice deficient for TREM2 does not further reduce the demyelination. These results suggest that the reduced demyelination in aged mice injected with LPS is very likely due to the downregulation of *Trem2*. As *Trem2* is exclusively expressed on myeloid cells (Kawabori et al., 2015), the changes of *Trem2* expression and deficiency of TREM2 in the PNS predominantly affect PNS macrophages. Since TREM2 plays a crucial role in phagocytosis (Takahashi et al., 2005; Hsieh et al., 2009; N'Diaye et al., 2009), it can be inferred that *Trem2* downregulation in LPS-injected aged WT mice might negatively affect myelin phagocytosis in PNS macrophages, resulting in less demyelination. Similar to LPS-injected aged WT mice, aged *Trem2*-/- mice show fewer hallmarks of demyelination, but their numbers of foamy macrophages, indicating myelin phagocytosis, are comparable to those in aged WT mice. It is possible that TREM2 deficiency in aged mice affects the digestion and not the engulfment of myelin in endoneurial macrophages, causing lipid accumulation in the foamy macrophages, similar to a study on loss of function of TREM2 in microglia (Nugent et al., 2020). How TREM2 deficiency affects different steps of phagocytosis in PNS macrophages is still an open question and requires further investigation on possible molecular machineries downstream of *Trem2* (Deczkowska et al., 2020). However, the quantification of foamy macrophages is only performed on ultrathin sections of nerves and might only reflect a small part of the nerves. Future investigations on the whole-nerve level are necessary in order to analyze the foamy macrophages in further detail. Furthermore, LPS injection in aged *Trem2*-/- mice does not further alter their nerve phenotypes, suggesting that the mechanisms dampening demyelination in peripheral nerves after LPS-injection are most likely TREM2-dependent. Additionally, myelin abnormalities in both LPS-injected aged mice and aged *Trem2*-/- mice are less frequent compared to

aged WT mice, suggesting a possible role of *Trem2* in maintaining structural integrity of myelin. Interestingly, unlike demyelination, myelin abnormalities are not altered by macrophage depletion in aged mice. This likely indicates that the formation of myelin abnormalities is regulated by a different inflammation-associated mechanism during aging. Further experiments on the transcriptomic profile of macrophages in aged mice after LPS injection or with TREM2 deficiency can be performed in order to elucidate the role of macrophages in mediating these changes in peripheral nerves of aged mice.

The increase of numbers of dark axons in aged mice after LPS injection or with TREM2 deficiency in their peripheral nerves represent a form of axonal perturbation induced by systemic inflammation. These dark axons, also observed at a lower number in aged WT mice, resemble the axons undergoing dark degeneration in mouse models of optic nerve injuries (Narciso et al., 2001; Marques et al., 2003) and in mice with iron-mediated damage (Mukherjee et al., 2020). Compared to healthy appearing axons, dark axons lack distinguishable cytoskeletons and organelles, and are often associated with the breakdown of neurofilaments (Marques et al., 2003). The dark axons might result from oxidative damage initiated by reactive oxygen species (ROS), a major contributor to the cytotoxicity observed in aging (Rouault, 2013). Neurons are particularly vulnerable to oxidative stress due to their high oxygen demand, and are therefore susceptible to ROS-mediated damage (Wang and Michaelis, 2010; Wang et al., 2013; Salim, 2017; Stefanatos and Sanz, 2018). Moreover, previous studies have shown that LPS can activate macrophages and microglia, induce *Trem2* downregulation, and trigger ROS production *in vitro* and *in vivo* (Hsu and Wen, 2002; Li et al., 2010; Park et al., 2015; Khan et al., 2016; Zhong et al., 2017). Thus, it is likely that LPS-induced ROS production by macrophages leads to increased oxidative stress in peripheral nerves of aged mice after LPS injection, which contributes to the increase of dark axons. TREM2 deficiency in the PNS of aged mice might trigger a similar ROS production, which might lead to the similar increase of dark axons in aged *Trem2*-/- mice. Future experiments on ROS production at different time points after LPS injection are necessary for more insights into this hypothesis, particularly in the context of TREM2 expression. Additionally, iron-related damage can also be a source of the oxidative stress that might cause dark axons

(Mukherjee et al., 2020). Studies in the brains of both mice and humans have shown that iron accumulation during aging can be due to deficiency of neurofilaments (Buijs et al., 2017; Stockwell et al., 2017; Vickers et al., 2019). Since aged nerves show reduced gene expressions of neurofilaments (Parhad et al., 1995; Ceballos et al., 1999) and appear to be damaged in aged mice after LPS injection or with TREM2 deficiency, these conditions might also lead to increased iron-related damages in the peripheral nerves. Of note, these dark axons only represent a relatively small proportion of axons in the whole nerve. It is also not clear if dark axons will eventually degenerate in peripheral nerves of aged mice. This might explain why the increase of dark axons does not appear to dictate PNS functions in aged mice after LPS injection, particularly since their rare appearance is likely counteracted by the more robust mitigation of demyelination. The fate and potential functional relevance of dark axons requires further investigation at later time points and novel non-invasive functional readouts. Interestingly, axon sphericity reduces in LPS-injected aged mice and in aged *Trem2*-/- mice compared to aged WT mice (Yuan, Klein, Wilhelm, Schmidt and Martini, unpublished observation). This might be an additional indicator for mild axonal alterations. Detailed whole-nerve characterization on axon sphericity including data segmentation might help to resolve the current methodological limitation in characterizing mild axonal changes in aged mice under different conditions.

The shifted nerve phenotypes in aged mice after LPS injection or with TREM2 deficiency are associated with less muscle denervation. The reduction of demyelination in these two groups resembles that from aged mice after macrophage depletion, where muscle denervation is also reduced. This suggests that dampened demyelination may reduce muscle denervation in aged mice. Interestingly, the reduced muscle denervation in LPS-injected aged mice and aged *Trem2*-/- mice does not reflect the increase of dark axons observed in these two groups. The reason for this remains unclear. It is likely that a substantial proportion of the dark axons are afferent fibers, whose damage cannot be reflected by NMJ innervation. Future experiments on molecular changes in aged mice after LPS injection or with TREM2 deficiency might help to elucidate the cause of the improvement of muscle innervation in these mice.

Despite causing similar nerve phenotypes, LPS injection in aged WT mice only mildly preserves muscle function, as opposed to TREM2 deficiency, which significantly improves grip strength in aged mice. This might be due to a relatively short time window (4 weeks) in LPS-injected aged mice for improvement of muscle function, whereas in aged *Trem2*-/- mice, TREM2 deficiency might indirectly modulate muscle innervation at younger ages. Further experiments can focus on a later time point for investigation in aged mice after LPS injection to examine if a longer time window is sufficient for significant changes in muscle functions to develop.

To summarize, these results indicate that macrophages likely mediate the shift of nerve phenotypes away from a demyelinating nerve phenotype in aged mice after systemic inflammation via *Trem2* downregulation, leading to improvement of muscle innervation and preservation of muscle function.

6.4 Possible mitigations of age-related functional decline in the peripheral nervous system

This study demonstrates that macrophage-driven inflammation in the peripheral nerves can ambivalently mediate age-related changes in the PNS of mice. Macrophage depletion ameliorates the neuropathy in aged mice and leads to functional improvement. As a principle, macrophage depletion in aged peripheral nerves has functional benefits; however, mitigation of age-related functional decline in the PNS with macrophage depletion should be tissue specific. This is due to possible side effects of microglia depletion in the CNS and depletion of resident macrophages in other tissues. On a practical level, tissue-specific pharmacological macrophage depletion is currently difficult to apply in humans in a manner that does not cause tissue damage, which is a concern with intraneural injections (Stratton et al., 2018). Thus, developing non-invasive treatment options specifically targeting PNS macrophages is highly attractive for ameliorating age-related peripheral neuropathies in the elderly. Additionally, this study reveals the ambivalent role of systemic inflammation in modifying age-related damage in the PNS, leading to partially restored PNS functions. This does not imply a therapeutic value of systemic inflammation in improving PNS functions in aging, as

systemic inflammation exacerbates age-related damage in the CNS (Groh et al., in revision). However, the present study highlights the importance of macrophage activation on the functional outcome in an aging PNS. Therefore, modulating macrophage activation in the PNS might have positive therapeutic implications and is an interesting subject for future research.

Another treatment option for mitigating the age-related neuropathy is to apply neurosteroids. Neurosteroids have certain neuroprotective functions and some show declined expressions with age (Schumacher et al., 2003). One of these neuroactive steroids, progesterone, has been implicated to rescue demyelination in aged rodents (Melcangi et al., 1998a; Melcangi et al., 1998b; Magnaghi et al., 1999; Melcangi et al., 2000). One possible reason for this rescue is that progesterone plays an important role in myelination (Koenig et al., 1995; Schumacher et al., 2001) and can increase the expression of myelin protein P0 and PMP22 in aged nerves (Magnaghi et al., 1999; Melcangi et al., 2000). However, the reduced expression of myelin protein genes was not observed in the present study, arguing against this possibility. A more likely reason for why progesterone can ameliorate the nerve phenotype in aging nerves is that along with other neurosteroids, progesterone has anti-inflammatory effects (Giatti et al., 2012; Yilmaz et al., 2019) that can reduce neuroinflammation-related alterations by dampening inflammation. In addition, estrogen, a neurosteroid that can be synthesized in the nervous system and decreases during aging (Schumacher et al., 2003), is also seen as a possible option for the hormone therapy in the elderly. Studies on estrogen replacement treatment in elderly humans mostly focus on the effects of estrogen in the CNS, such as cognitive improvement and rescuing age-related memory decline (Phillips and Sherwin, 1992; Brenner et al., 1994; Tang et al., 1996; Jacobs et al., 1998; Resnick et al., 1998), but the possible effect on the aged nerves remains to be investigated. Nevertheless, estrogen has been shown to promote Schwann cell proliferation and differentiation and can accelerate remyelination upon nerve injuries (Olsen and Kovacs, 1996; Chen et al., 2016; Vacca et al., 2016). This makes estrogen a therapeutic option for ameliorating the demyelinating nerve phenotype in aging.

Physical exercise has been considered to exert various beneficial effects on the nervous system in aging and disease (Leveille et al., 1999; Abbott et al., 2004;

Larson et al., 2006; Handschin and Spiegelman, 2008; Richter et al., 2014). It is known that in peripheral nerves and neuromuscular junctions (NMJs), physical exercise accelerates recovery from injuries and degenerative changes (Panenic and Gardiner, 1998; Wilson and Deschenes, 2005; English et al., 2011; Li and Thompson, 2011). Additionally, previous studies on aged mice show that physical exercise attenuates age-related changes in the NMJs and improves the motor function of mice (Valdez et al., 2010; Graber et al., 2015a). Some of these protective effects are likely achieved by the increase of active zone proteins (Gordon et al., 2012; Cheng et al., 2013; Nishimune et al., 2014), NMJ hypertrophy via increase of muscle fiber diameter (Handschin and Spiegelman, 2008), induced expression of neurotrophic factors and other growth factors, such as *Gdnf* and *Igf1* (Yang et al., 1996; Hameed et al., 2003; Dupont‐Versteegden et al., 2004; Dobrowolny et al., 2005). NMJ innervation can be enhanced by both endurance exercises on treadmills (Panenic and Gardiner, 1998; Nishimune et al., 2014) and motor adaptation tasks such as rotarod (Yuan, unpublished observation), suggesting that the benefits of exercise are not restricted to a certain form of exercise, but to the general increase in physical activity. Importantly, physical exercise can attenuate the low-grade inflammation in aged individuals (Greiwe et al., 2001; Beavers et al., 2010; Woods et al., 2012; Zheng et al., 2019), including the macrophage-driven inflammation in the peripheral nerves, leading to amelioration of the peripheral neuropathy in the elderly. One possible reason why exercise can attenuate neuroinflammation is that the adipose tissue in the proximity of peripheral nerves, which likely contributes to the inflammation, is reduced after physical exercises (Fried et al., 1998; Harkins et al., 2004). Moreover, macrophages in the adipose tissue are shifted towards M2 activation by physical exercise, suggesting a transition to the regenerative macrophage phenotype (Kawanishi et al., 2010; Goh et al., 2016).

Interestingly, caloric restrictions in aging alleviates oxidative damage in peripheral nerves and attenuates structural changes NMJ in aged rodents (Huffman, 2010; Opalach et al., 2010; Valdez et al., 2010). Similar to physical exercise, caloric restriction leads to less adipose tissue and a shift towards M2 macrophage activation (Galván-Peña and O'Neill, 2014; Fabbiano et al., 2016). Furthermore, a previous study has reported that caloric restriction supports the molecular

architecture and maintenance of Schwann cell phenotype in aging by promoting the expression of protein chaperons, which prevents the loss of myelin proteins during aging (Rangaraju et al., 2009). It can be inferred that physical exercise and dietary intervention are two promising options for ameliorating the peripheral neuropathy in the elderly. Of note, excessive exercise can cause microstructural damage in the muscles, infiltration of immune cells as well as induced addiction (Woods et al., 2012; Richter et al., 2014). Taken together, balancing exercise with rest and modulating macrophage activation might be promising targets for future research in mitigating the age-related functional decline in aging individuals.

6.5 Synopsis

- During aging, endoneurial macrophages increase in number, express higher levels of *Trem2* and show increased myelin phagocytosis. This is associated with demyelination and muscle denervation of aged mice.
- Macrophage depletion by CSF-1R inhibition in aged mice reduces number of macrophages, resulting in attenuated demyelination, less muscle denervation and preserved muscle function.
- Systemic inflammation induced by LPS injection in aged mice alters macrophage activation (highlighted by *Trem2* downregulation), and is associated with dampened demyelination and less muscle denervation. A small portion of axons is also perturbed in LPS-injected aged mice (shown by dark axons), possibly due to increased ROS production.
- Aged mice deficient for TREM2 present show a nerve phenotype similar to LPS-injected aged WT mice. Additionally, TREM2 deficiency in aged mice leads to reduced muscle denervation and preserved muscle function.
- Overall, the findings of the present study (summarized in Fig. 26) demonstrate that macrophage-driven inflammation mediates pathological alterations aged in mice. Systemic inflammation ambivalently modifies the age-related nerve damage in aged mice, possibly via downregulation of *Trem2*. It can be inferred that modifying macrophage activation can have therapeutic implications for improving functional outcome in the aging PNS.

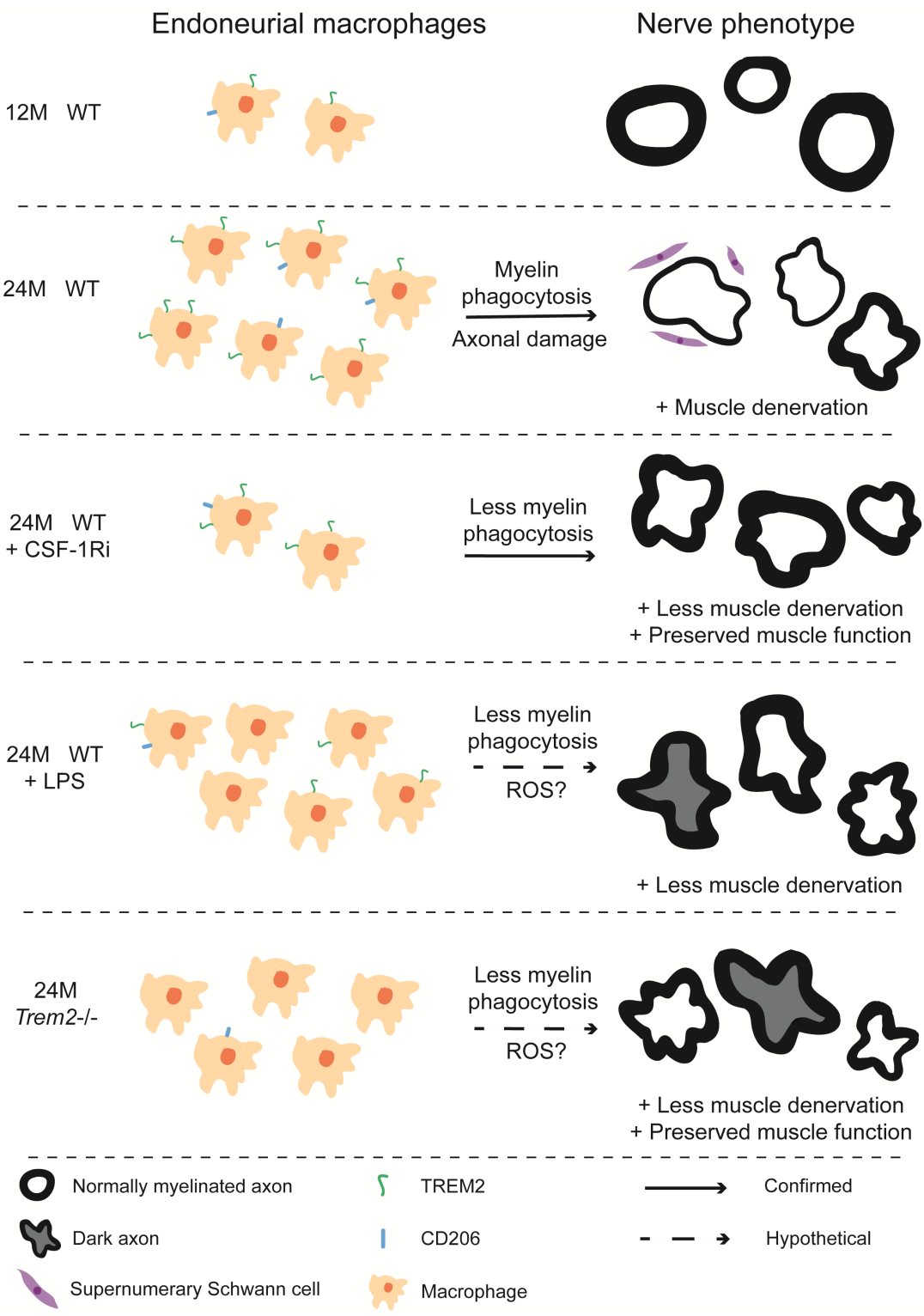

Figure 26 Synoptic view on endoneurial macrophages and nerve phenotypes in aged mice.

7. Reference

Abbott RD, White LR, Ross GW, Masaki KH, Curb JD, Petrovitch H (2004) Walking and dementia in physically capable elderly men. Jama 292:1447-1453.

Adinolfi AM, Yamuy J, Morales FR, Chase MH (1991) Segmental demyelination in peripheral nerves of old cats. Neurobiology of aging 12:175-179.

Ahn JH, Lee T-K, Park JH, Cho JH, Kim IH, Lee JC, Hong S, Jeon YH, Kang IJ, Lee YJ (2017) Age-dependent differences in myelin basic protein expression in the hippocampus of young, adult and aged gerbils. Laboratory animal research 33:237-243.

Ajami B, Bennett JL, Krieger C, Tetzlaff W, Rossi FMV (2007) Local self-renewal can sustain CNS microglia maintenance and function throughout adult life. Nature neuroscience 10:1538-1543.

Amann L, Prinz M (2020) The origin, fate and function of macrophages in the peripheral nervous system—an update. International Immunology.

Apel PJ, Ma J, Callahan M, Northam CN, Alton TB, Sonntag WE, Li Z (2010) Effect of locally delivered IGF-1 on nerve regeneration during aging: an experimental study in rats. Muscle & Nerve: Official Journal of the American Association of Electrodiagnostic Medicine 41:335-341.

Arvidson B (1977) Cellular uptake of exogenous horseradish peroxidase in mouse peripheral nerve. Acta neuropathologica 37:35-41.

Babetto E, Wong KM, Beirowski B (2020) A glycolytic shift in Schwann cells supports injured axons. Nature Neuroscience 23:1215-1228.

Barrette B, Hébert M-A, Filali M, Lafortune K, Vallieres N, Gowing G, Julien J-P, Lacroix S (2008) Requirement of myeloid cells for axon regeneration. Journal of Neuroscience 28:9363-9376.

Beavers KM, Brinkley TE, Nicklas BJ (2010) Effect of exercise training on chronic inflammation. Clinica chimica acta 411:785-793.

Beirowski B, Babetto E, Golden JP, Chen Y, Jr., Yang K, Gross RW, Patti GJ, Milbrandt J (2014) Metabolic regulator LKB1 is crucial for Schwann cell–mediated axon maintenance. Nature neuroscience 17:1351-1361.

Beutler B (2000) Tlr4: central component of the sole mammalian LPS sensor. Current opinion in immunology 12:20-26.

Biesmans S, Meert TF, Bouwknecht JA, Acton PD, Davoodi N, De Haes P, Kuijlaars J, Langlois X, Matthews LJR, Ver Donck L (2013) Systemic immune activation leads to neuroinflammation and sickness behavior in mice. Mediators of inflammation 2013.

Bosse F, Hasenpusch-Theil K, Küry P, Müller HW (2006) Gene expression profiling reveals that peripheral nerve regeneration is a consequence of both novel injury-dependent and reactivated developmental processes. Journal of neurochemistry 96:1441-1457.

Bouche P, Cattelin F, Saint-Jean O, Leger JM, Queslati S, Guez D, Moulonguet A, Brault Y, Aquino JP, Simunek P (1993) Clinical and electrophysiological study of the peripheral nervous system in the elderly. Journal of neurology 240:263-268.

Braund KG, McGuire JA, Lincoln CE (1982) Age-related changes in peripheral nerves of the dog. II. A morphologic and morphometric study of cross-sectional nerve. Veterinary pathology 19:379-398.

Brenner DE, Kukull WA, Stergachis A, van Belle G, Bowen JD, McCormick WC, Ten L, Larson EB (1994) Postmenopausal estrogen replacement therapy and the risk of Alzheimer's disease: a population-based case-control study. American journal of epidemiology 140:262-267.

Brück W (1997) The role of macrophages in Wallerian degeneration. Brain pathology 7:741-752.

Buijs M, Doan NT, van Rooden S, Versluis MJ, van Lew B, Milles J, van der Grond J, van Buchem MA (2017) In vivo assessment of iron content of the cerebral cortex in healthy aging using 7-Tesla T2*-weighted phase imaging. Neurobiology of aging 53:20-26.

Burkholder T, Foltz C, Karlsson E, Linton CG, Smith JM (2012) Health evaluation of experimental laboratory mice. Current protocols in mouse biology 2:145-165.

Büttner R, Schulz A, Reuter M, Akula AK, Mindos T, Carlstedt A, Riecken LB, Baader SL, Bauer R, Morrison H (2018) Inflammaging impairs peripheral nerve maintenance and regeneration. Aging cell 17:e12833.

Canta A, Chiorazzi A, Carozzi VA, Meregalli C, Oggioni N, Bossi M, Rodriguez-Menendez V, Avezza F, Crippa L, Lombardi R (2016) Age-related changes in the function and structure of the peripheral sensory pathway in mice. Neurobiology of aging 45:136-148.

Cantuti-Castelvetri L, Fitzner D, Bosch-Queralt M, Weil M-T, Su M, Sen P, Ruhwedel T, Mitkovski M, Trendelenburg G, Lütjohann D (2018) Defective cholesterol clearance limits remyelination in the aged central nervous system. Science 359:684-688.

Carayannopoulos AG (2017) Peripheral Nerve Stimulation (PNS): New Therapies. In: Treatment of Chronic Pain Conditions, pp 233-234: Springer.

Carenini S, Maurer M, Werner A, Blazyca H, Toyka KV, Schmid CD, Raivich G, Martini R (2001) The role of macrophages in demyelinating peripheral nervous system of mice heterozygously deficient in p0. J Cell Biol 152:301-308.

Carnio S, LoVerso F, Baraibar MA, Longa E, Khan MM, Maffei M, Reischl M, Canepari M, Loefler S, Kern H (2014) Autophagy impairment in muscle induces neuromuscular junction degeneration and precocious aging. Cell reports 8:1509-1521.

Carroll EW, Curtis RL, Sullivan DA (1992) Wallerian degeneration in the optic nerve of the wabbler-lethal (wl/wl) mouse. Brain research bulletin 29:411-418.

Cattin A-L, Lloyd AC (2016) The multicellular complexity of peripheral nerve regeneration. Current opinion in neurobiology 39:38-46.

Cattin A-L, Burden JJ, Van Emmenis L, Mackenzie FE, Hoving JJA, Calavia NG, Guo Y, McLaughlin M, Rosenberg LH, Quereda V (2015) Macrophage-induced blood vessels guide Schwann cell-mediated regeneration of peripheral nerves. Cell 162:1127-1139.

Ceballos D, Cuadras J, Verdú E, Navarro X (1999) Morphometric and ultrastructural changes with ageing in mouse peripheral nerve. Journal of anatomy 195:563-576.

Chai RJ, Vukovic J, Dunlop S, Grounds MD, Shavlakadze T (2011) Striking denervation of neuromuscular junctions without lumbar motoneuron loss in geriatric mouse muscle. PloS one 6.

Chase MH, Engelhardt JK, Adinolfi AM, Chirwa SS (1992) Age-dependent changes in cat masseter nerve: an electrophysiological and morphological study. Brain research 586:279-288.

Chen P, Piao X, Bonaldo P (2015) Role of macrophages in Wallerian degeneration and axonal regeneration after peripheral nerve injury. Acta neuropathologica 130:605-618.

Chen S, Rio C, Ji R-R, Dikkes P, Coggeshall RE, Woolf CJ, Corfas G (2003) Disruption of ErbB receptor signaling in adult non-myelinating Schwann cells causes progressive sensory loss. Nature neuroscience 6:1186-1193.

Chen Y, Kam CSK, Liu FQ, Liu Y, Lui VCH, Lamb JR, Tam PKH (2008) LPS-induced up-regulation of TGF-β receptor 1 is associated with TNF-α expression in human monocyte-derived macrophages. Journal of leukocyte biology 83:1165-1173.

Chen Y, Guo W, Xu L, Li W, Cheng M, Hu Y, Xu W (2016) 17β-Estradiol promotes Schwann cell proliferation and differentiation, accelerating early remyelination in a mouse peripheral nerve injury model. BioMed research international 2016.

Cheng A, Morsch M, Murata Y, Ghazanfari N, Reddel SW, Phillips WD (2013) Sequence of age-associated changes to the mouse neuromuscular junction and the protective effects of voluntary exercise. PloS one 8.

Cleveland DW (1999) From Charcot to SOD1: mechanisms of selective motor neuron death in ALS. Neuron 24:515-520.

Colonna M, Butovsky O (2017) Microglia function in the central nervous system during health and neurodegeneration. Annual review of immunology 35:441-468.

Cook RD, Ghetti B, Wiśniewski HM (1974) The pattern of Wallerian degeneration in the optic nerve of newborn kittens: an ultrastructural study. Brain research 75:261-275.

Cunningham C, Wilcockson DC, Campion S, Lunnon K, Perry VH (2005) Central and systemic endotoxin challenges exacerbate the local inflammatory response and increase neuronal death during chronic neurodegeneration. Journal of Neuroscience 25:9275-9284.

Curtis R, Scherer SS, Somogyi R, Adryan KM, Ip NY, Zhu Y, Lindsay RM, DiStefano PS (1994) Retrograde axonal transport of LIF is increased by peripheral nerve injury: correlation with increased LIF expression in distal nerve. Neuron 12:191-204.

Deczkowska A, Weiner A, Amit I (2020) The Physiology, Pathology, and Potential Therapeutic Applications of the TREM2 Signaling Pathway. Cell 181:1207-1217.

Deng X, Li M, Ai W, He L, Lu D, Patrylo PR, Cai H, Luo X, Li Z, Yan X (2014) Lipolysaccharide-induced neuroinflammation is associated with Alzheimer-like amyloidogenic axonal pathology and dendritic degeneration in rats. Advances in Alzheimer's disease 3:78.

Dobrowolny G, Giacinti C, Pelosi L, Nicoletti C, Winn N, Barberi L, Molinaro M, Rosenthal N, Musarò A (2005) Muscle expression of a local Igf-1 isoform protects motor neurons in an ALS mouse model. The Journal of cell biology 168:193-199.

Domènech-Estévez E, Baloui H, Meng X, Zhang Y, Deinhardt K, Dupree JL, Einheber S, Chrast R, Salzer JL (2016) Akt regulates axon wrapping and myelin sheath thickness in the PNS. Journal of Neuroscience 36:4506-4521.

Dorfman LJ, Bosley TM (1979) Age-related changes in peripheral and central nerve conduction in man. Neurology 29:38-38.

Drechsler F (1975) Sensory action potentials of the median and ulnar nerves in aged persons. In: Studies on neuromuscular diseases, pp 232-235: Karger Publishers.

Dulken BW, Buckley MT, Negredo PN, Saligrama N, Cayrol R, Leeman DS, George BM, Boutet SC, Hebestreit K, Pluvinage JV (2019) Single-cell analysis reveals T cell infiltration in old neurogenic niches. Nature 571:205-210.

Dupont-Versteegden EE, Houlé JD, Dennis RA, Zhang J, Knox M, Wagoner G, Peterson CA (2004) Exercise-induced gene expression in soleus muscle is dependent on time after spinal cord injury in rats. Muscle & Nerve: Official Journal of the American Association of Electrodiagnostic Medicine 29:73-81.

English AW, Wilhelm JC, Sabatier MJ (2011) Enhancing recovery from peripheral nerve injury using treadmill training. Annals of Anatomy-Anatomischer Anzeiger 193:354-361.

Fabbiano S, Suárez-Zamorano N, Rigo D, Veyrat-Durebex C, Dokic AS, Colin DJ, Trajkovski M (2016) Caloric restriction leads to browning of white adipose tissue through type 2 immune signaling. Cell metabolism 24:434-446.

Field R, Campion S, Warren C, Murray C, Cunningham C (2010) Systemic challenge with the TLR3 agonist poly I: C induces amplified IFNα/β and IL-1β responses in the diseased brain and exacerbates chronic neurodegeneration. Brain, behavior, and immunity 24:996-1007.

Figlia G, Norrmén C, Pereira JA, Gerber D, Suter U (2017) Dual function of the PI3K-Akt-mTORC1 axis in myelination of the peripheral nervous system. Elife 6:e29241.

Fischer S, Weishaupt A, Troppmair J, Martini R (2008a) Increase of MCP-1 (CCL2) in myelin mutant Schwann cells is mediated by MEK-ERK signaling pathway. Glia 56:836-843.

Fischer S, Kleinschnitz C, Müller M, Kobsar I, Ip CW, Rollins BJ, Martini R (2008b) Monocyte chemoattractant protein-1 is a pathogenic component in a model for a hereditary peripheral neuropathy. Molecular and Cellular Neuroscience 37:359-366.

Fox JG, Barthold S, Davisson M, Newcomer CE, Quimby FW, Smith A (2006) The mouse in biomedical research: normative biology, husbandry, and models: Elsevier.

Frei R, Motzing S, Kinkelin I, Schachner M, Koltzenburg M, Martini R (1999) Loss of distal axons and sensory Merkel cells and features indicative of muscle denervation in hindlimbs of P0-deficient mice. J Neurosci 19:6058-6067.

Fried SK, Bunkin DA, Greenberg AS (1998) Omental and subcutaneous adipose tissues of obese subjects release interleukin-6: depot difference and regulation by glucocorticoid. The Journal of Clinical Endocrinology & Metabolism 83:847-850.

Fünfschilling U, Supplie LM, Mahad D, Boretius S, Saab AS, Edgar J, Brinkmann BG, Kassmann CM, Tzvetanova ID, Möbius W (2012) Glycolytic oligodendrocytes maintain myelin and long-term axonal integrity. Nature 485:517-521.

Galván-Peña S, O'Neill LAJ (2014) Metabolic reprograming in macrophage polarization. Frontiers in immunology 5:420.

Giatti S, Boraso M, Melcangi RC, Viviani B (2012) Neuroactive steroids, their metabolites, and neuroinflammation. Journal of molecular endocrinology 49:R125-R134.

Giorgetti E, Panesar M, Zhang Y, Joller S, Ronco M, Obrecht M, Lambert C, Accart N, Beckmann N, Doelemeyer A (2019) Modulation of microglia by voluntary exercise or CSF1R inhibition prevents age-related loss of functional motor units. Cell reports 29:1539-1554.

Goethals S, Ydens E, Timmerman V, Janssens S (2010) Toll-like receptor expression in the peripheral nerve. Glia 58:1701-1709.

Goh J, Goh KP, Abbasi A (2016) Exercise and adipose tissue macrophages: new frontiers in obesity research? Frontiers in endocrinology 7:65.

Gomez-Sanchez JA, Pilch KS, van der Lans M, Fazal SV, Benito C, Wagstaff LJ, Mirsky R, Jessen KR (2017) After nerve injury, lineage tracing shows that myelin and Remak Schwann cells elongate extensively and branch to form repair Schwann cells, which shorten radically on remyelination. Journal of Neuroscience 37:9086-9099.

Gonzalez-Freire M, de Cabo R, Studenski SA, Ferrucci L (2014) The Neuromuscular Junction: Aging at the Crossroad between Nerves and Muscle. Frontiers in Aging Neuroscience 6:208.

Goodfellow JA, Willison HJ (2016) Guillain–Barré syndrome: a century of progress. Nature Reviews Neurology 12:723-731.

Gordon PM, Liu D, Sartor MA, IglayReger HB, Pistilli EE, Gutmann L, Nader GA, Hoffman EP (2012) Resistance exercise training influences skeletal muscle immune activation: a microarray analysis. Journal of applied physiology 112:443-453.

Graber TG, Ferguson-Stegall L, Liu H, Thompson LV (2015a) Voluntary aerobic exercise reverses frailty in old mice. Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences 70:1045-1058.

Graber TG, Kim J-H, Grange RW, McLoon LK, Thompson LV (2015b) C57BL/6 life span study: age-related declines in muscle power production and contractile velocity. Age 37:36.

Grandis M, Shy ME (2005) Current therapy for Charcot-Marie-Tooth disease. Current treatment options in neurology 7:23-31.

Greig CA, Botella J, Young A (1993) The quadriceps strength of healthy elderly people remeasured after eight years. Muscle & Nerve: Official Journal of the American Association of Electrodiagnostic Medicine 16:6-10.

Greiwe JS, Cheng BO, Rubin DC, Yarasheski KE, Semenkovich CF (2001) Resistance exercise decreases skeletal muscle tumor necrosis factor α in frail elderly humans. The FASEB Journal 15:475-482.

Griffin JW, Thompson WJ (2008) Biology and pathology of nonmyelinating Schwann cells. Glia 56:1518-1531.

Groh J, Basu R, Stanley ER, Martini R (2016a) Cell-surface and secreted isoforms of CSF-1 exert opposing roles in macrophage-mediated neural damage in Cx32-deficient mice. Journal of Neuroscience 36:1890-1901.

Groh J, Weis J, Zieger H, Stanley ER, Heuer H, Martini R (2012) Colony-stimulating factor-1 mediates macrophage-related neural damage in a model for Charcot-Marie-Tooth disease type 1X. Brain 135:88-104.

Groh J, Klein I, Hollmann C, Wettmarshausen J, Klein D, Martini R (2015) CSF-1-activated macrophages are target-directed and essential mediators of Schwann cell dedifferentiation and dysfunction in Cx32-deficient mice. Glia 63:977-986.

Groh J, Heinl K, Kohl B, Wessig C, Greeske J, Fischer S, Martini R (2010) Attenuation of MCP-1/CCL2 expression ameliorates neuropathy in a mouse model for Charcot-Marie-Tooth 1X. Hum Mol Genet 19:3530-3543.

Groh J, Friedman HC, Orel N, Ip CW, Fischer S, Spahn I, Schaffner E, Horner M, Stadler D, Buttmann M, Varallyay C, Solymosi L, Sendtner M, Peterson AC, Martini R (2016b) Pathogenic inflammation in the CNS of mice carrying human PLP1 mutations. Hum Mol Genet 25:4686-4702.

Grover-Johnson N, Spencer PS (1981) Peripheral nerve abnormalities in aging rats. Journal of neuropathology and experimental neurology 40:155-165.

Guillonneau C, Bézie S, Anegon I (2017) Immunoregulatory properties of the cytokine IL-34. Cellular and Molecular Life Sciences 74:2569-2586.

Hameed M, Orrell RW, Cobbold M, Goldspink G, Harridge SDR (2003) Expression of IGF-I splice variants in young and old human skeletal muscle after high resistance exercise. The Journal of physiology 547:247-254.

Hamilton R, Walsh M, Singh R, Rodriguez K, Gao X, Rahman MM, Chaudhuri A, Bhattacharya A (2016) Oxidative damage to myelin proteins accompanies peripheral nerve motor dysfunction in aging C57BL/6 male mice. Journal of the neurological sciences 370:47-52.

Hammond TR, Dufort C, Dissing-Olesen L, Giera S, Young A, Wysoker A, Walker AJ, Gergits F, Segel M, Nemesh J (2019) Single-cell RNA sequencing of microglia throughout the mouse lifespan and in the injured brain reveals complex cell-state changes. Immunity 50:253-271.

Handschin C, Spiegelman BM (2008) The role of exercise and PGC1α in inflammation and chronic disease. Nature 454:463-469.

Harkins JM, Moustaid-Moussa N, Chung Y-J, Penner KM, Pestka JJ, North CM, Claycombe KJ (2004) Expression of interleukin-6 is greater in preadipocytes than in adipocytes of 3T3-L1 cells and C57BL/6J and ob/ob mice. The Journal of nutrition 134:2673-2677.

Hart BL (1988) Biological basis of the behavior of sick animals. Neuroscience & Biobehavioral Reviews 12:123-137.

Henry CJ, Huang Y, Wynne AM, Godbout JP (2009) Peripheral lipopolysaccharide (LPS) challenge promotes microglial hyperactivity in aged mice that is associated with

exaggerated induction of both pro-inflammatory IL-1beta and anti-inflammatory IL-10 cytokines. Brain Behav Immun 23:309-317.

Hsieh CL, Koike M, Spusta SC, Niemi EC, Yenari M, Nakamura MC, Seaman WE (2009) A role for TREM2 ligands in the phagocytosis of apoptotic neuronal cells by microglia. Journal of neurochemistry 109:1144-1156.

Hsu H-Y, Wen M-H (2002) Lipopolysaccharide-mediated reactive oxygen species and signal transduction in the regulation of interleukin-1 gene expression. Journal of Biological Chemistry 277:22131-22139.

Hube L, Dohrn MF, Karsai G, Hirshman S, Van Damme P, Schulz JB, Weis J, Hornemann T, Claeys KG (2017) Metabolic Syndrome, Neurotoxic 1-Deoxysphingolipids and Nervous Tissue Inflammation in Chronic Idiopathic Axonal Polyneuropathy (CIAP). PLoS One 12:e0170583.

Huffman DM (2010) Exercise as a calorie restriction mimetic: implications for improving healthy aging and longevity. In: Body Composition and Aging, pp 157-174: Karger Publishers.

Huxley AF, Stämpfli R (1949) Evidence for saltatory conduction in peripheral myelinated nerve fibres. The Journal of physiology 108:315.

Huxley C, Passage E, Robertson AM, Youl B, Huston S, Manson A, Sabéran-Djoniedi D, Figarella-Branger D, Pellissier JF, Thomas PK, Fontés M (1998) Correlation Between Varying Levels of PMP22 Expression and the Degree of Demyelination and Reduction in Nerve Conduction Velocity in Transgenic Mice. Human Molecular Genetics 7:449-458.

Jacobs DM, Tang MX, Stern Y, Sano M, Marder K, Bell KL, Schofield P, Dooneief G, Gurland B, Mayeux R (1998) Cognitive function in nondemented older women who took estrogen after menopause. Neurology 50:368-373.

Jacobs JM, Love S (1985) Qualitative and quantitative morphology of human sural nerve at different ages. Brain 108 (Pt 4):897-924.

Jessen KR, Mirsky R (2019) The success and failure of the Schwann cell response to nerve injury. Frontiers in cellular neuroscience 13:33.

Jessen KR, Mirsky R, Lloyd AC (2015) Schwann cells: development and role in nerve repair. Cold Spring Harbor perspectives in biology 7:a020487.

Jones RA, Harrison C, Eaton SL, Hurtado ML, Graham LC, Alkhammash L, Oladiran OA, Gale A, Lamont DJ, Simpson H (2017) Cellular and molecular anatomy of the human neuromuscular junction. Cell reports 21:2348-2356.

Jordão MJC, Sankowski R, Brendecke SM, Locatelli G, Tai Y-H, Tay TL, Schramm E, Armbruster S, Hagemeyer N, Groß O (2019) Single-cell profiling identifies myeloid cell subsets with distinct fates during neuroinflammation. Science 363.

Jung S, Aliberti J, Graemmel P, Sunshine MJ, Kreutzberg GW, Sher A, Littman DR (2000) Analysis of fractalkine receptor CX3CR1 function by targeted deletion and green fluorescent protein reporter gene insertion. Molecular and cellular biology 20:4106-4114.

Kang H, Lichtman JW (2013) Motor axon regeneration and muscle reinnervation in young adult and aged animals. Journal of Neuroscience 33:19480-19491.

Kawabori M, Kacimi R, Kauppinen T, Calosing C, Kim JY, Hsieh CL, Nakamura MC, Yenari MA (2015) Triggering receptor expressed on myeloid cells 2 (TREM2) deficiency attenuates phagocytic activities of microglia and exacerbates ischemic damage in experimental stroke. Journal of Neuroscience 35:3384-3396.

Kawanishi N, Yano H, Yokogawa Y, Suzuki K (2010) Exercise training inhibits inflammation in adipose tissue via both suppression of macrophage infiltration and acceleration of phenotypic switching from M1 to M2 macrophages in high-fat-diet-induced obese mice. Exercise immunology review 16.

Khan MS, Ali T, Kim MW, Jo MH, Jo MG, Badshah H, Kim MO (2016) Anthocyanins protect against LPS-induced oxidative stress-mediated neuroinflammation and neurodegeneration in the adult mouse cortex. Neurochemistry international 100:1-10.

Kierdorf K, Katzmarski N, Haas CA, Prinz M (2013) Bone marrow cell recruitment to the brain in the absence of irradiation or parabiosis bias. PloS one 8:e58544.

Kigerl KA, Gensel JC, Ankeny DP, Alexander JK, Donnelly DJ, Popovich PG (2009) Identification of two distinct macrophage subsets with divergent effects causing either neurotoxicity or regeneration in the injured mouse spinal cord. Journal of Neuroscience 29:13435-13444.

King R (2013) Microscopic anatomy: normal structure. In: Handbook of clinical neurology, pp 7-27: Elsevier.

Klein D, Martini R (2015) Myelin and macrophages in the PNS: an intimate relationship in trauma and disease. Brain research.

Klein D, Groh J, Weishaupt A, Martini R (2015a) Endogenous antibodies contribute to macrophage-mediated demyelination in a mouse model for CMT1B. J Neuroinflammation 12:49.

Klein D, Patzko A, Schreiber D, van Hauwermeiren A, Baier M, Groh J, West BL, Martini R (2015b) Targeting the colony stimulating factor 1 receptor alleviates two forms of Charcot-Marie-Tooth disease in mice. Brain 138:3193-3205.

Kleinberger G, Yamanishi Y, Suárez-Calvet M, Czirr E, Lohmann E, Cuyvers E, Struyfs H, Pettkus N, Wenninger-Weinzierl A, Mazaheri F (2014) TREM2 mutations implicated in neurodegeneration impair cell surface transport and phagocytosis. Science translational medicine 6:243ra286-243ra286.

Knox CA, Kokmen E, Dyck PJ (1989) Morphometric alteration of rat myelinated fibers with aging. Journal of neuropathology and experimental neurology 48:119-139.

Kobsar I, Maurer M, Ott T, Martini R (2002) Macrophage-related demyelination in peripheral nerves of mice deficient in the gap junction protein connexin 32. Journal of the Peripheral Nervous System 7:210-210.

Kobsar I, Hasenpusch-Theil K, Wessig C, Muller HW, Martini R (2005) Evidence for macrophage-mediated myelin disruption in an animal model for Charcot-Marie-Tooth neuropathy type 1A. J Neurosci Res 81:857-864.

Kobsar I, Oetke C, Kroner A, Wessig C, Crocker P, Martini R (2006) Attenuated demyelination in the absence of the macrophage-restricted adhesion molecule sialoadhesin (Siglec-1) in mice heterozygously deficient in P0. Molecular and Cellular Neuroscience 31:685-691.

Kobsar I, Berghoff M, Samsam M, Wessig C, Maurer M, Toyka KV, Martini R (2003) Preserved myelin integrity and reduced axonopathy in connexin32-deficient mice lacking the recombination activating gene-1. Brain 126:804-813.

Koenig HL, Schumacher M, Ferzaz B, Thi AN, Ressouches A, Guennoun R, Jung-Testas I, Robel P, Akwa Y, Baulieu E-E (1995) Progesterone synthesis and myelin formation by Schwann cells. Science 268:1500-1503.

Kohl B, Fischer S, Groh J, Wessig C, Martini R (2010a) MCP-1/CCL2 modifies axon properties in a PMP22-overexpressing mouse model for Charcot-Marie-tooth 1A neuropathy. Am J Pathol 176:1390-1399.

Kohl B, Groh J, Wessig C, Wiendl H, Kroner A, Martini R (2010b) Lack of evidence for a pathogenic role of T-lymphocytes in an animal model for Charcot-Marie-Tooth disease 1A. Neurobiology of disease 38:78-84.

Krinke G, Froehlich E, Herrmann M, Schnider K, Da Suva F, Suter J, Traber K (1988) Adjustment of the myelin sheath to axonal atrophy in the rat spinal root by the formation of infolded myelin loops. Cells Tissues Organs 131:182-187.

Krishnan VS, White Z, McMahon CD, Hodgetts SI, Fitzgerald M, Shavlakadze T, Harvey AR, Grounds MD (2016) A neurogenic perspective of sarcopenia: time course study of sciatic nerves from aging mice. Journal of Neuropathology & Experimental Neurology 75:464-478.

Kroner A, Schwab N, Ip CW, Sommer C, Wessig C, Wiendl H, Martini R (2009) The co-inhibitory molecule PD-1 modulates disease severity in a model for an inherited, demyelinating neuropathy. Neurobiology of disease 33:96-103.

Kummer TT, Misgeld T, Sanes JR (2006) Assembly of the postsynaptic membrane at the neuromuscular junction: paradigm lost. Current opinion in neurobiology 16:74-82.

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680-685.

Larson EB, Wang L, Bowen JD, McCormick WC, Teri L, Crane P, Kukull W (2006) Exercise is associated with reduced risk for incident dementia among persons 65 years of age and older. Annals of internal medicine 144:73-81.

Lascelles RG, Thomas PK (1966) Changes due to age in internodal length in the sural nerve in man. J Neurol Neurosurg Psychiatry 29:40-44.

Lee H, Jo E-K, Choi S-Y, Oh SB, Park K, Kim JS, Lee SJ (2006) Necrotic neuronal cells induce inflammatory Schwann cell activation via TLR2 and TLR3: implication in Wallerian degeneration. Biochemical and biophysical research communications 350:742-747.

Lee SM, Sha D, Mohammed AA, Asress S, Glass JD, Chin L-S, Li L (2013) Motor and sensory neuropathy due to myelin infolding and paranodal damage in a transgenic mouse model of Charcot–Marie–Tooth disease type 1C. Human molecular genetics 22:1755-1770.

Leveille SG, Guralnik JM, Ferrucci L, Langlois JA (1999) Aging successfully until death in old age: opportunities for increasing active life expectancy. American Journal of Epidemiology 149:654-664.

Li L, Maitra U, Singh N, Gan L (2010) Molecular mechanism underlying LPS-induced generation of reactive oxygen species in macrophages. The FASEB Journal 24:422-423.

Li Y, Thompson WJ (2011) Nerve terminal growth remodels neuromuscular synapses in mice following regeneration of the postsynaptic muscle fiber. Journal of Neuroscience 31:13191-13203.

Li Y, il Lee Y, Thompson WJ (2011) Changes in aging mouse neuromuscular junctions are explained by degeneration and regeneration of muscle fiber segments at the synapse. Journal of Neuroscience 31:14910-14919.

Ling SM, Conwit RA, Ferrucci L, Metter EJ (2009) Age-associated changes in motor unit physiology: observations from the Baltimore Longitudinal Study of Aging. Archives of physical medicine and rehabilitation 90:1237-1240.

Liu W, Klose A, Forman S, Paris ND, Wei-LaPierre L, Cortes-Lopez M, Tan A, Flaherty M, Miura P, Dirksen RT (2017) Loss of adult skeletal muscle stem cells drives age-related neuromuscular junction degeneration. Elife 6:e26464.

Liu W, Taso O, Wang R, Bayram S, Graham AC, Garcia-Reitboeck P, Mallach A, Andrews WD, Piers TM, Botia JA, Pocock JM, Cummings DM, Hardy J, Edwards FA, Salih DA (2020) Trem2 promotes anti-inflammatory responses in microglia and is suppressed under pro-inflammatory conditions. Human Molecular Genetics.

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265-275.

Maggio M, Ble A, Ceda GP, Metter EJ (2006) Decline in insulin-like growth factor-I levels across adult life span in two large population studies. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences 61:182-183.

Maggio M, De Vita F, Lauretani F, Buttò V, Bondi G, Cattabiani C, Nouvenne A, Meschi T, Dall'Aglio E, Ceda GP (2013) IGF-1, the cross road of the nutritional, inflammatory and hormonal pathways to frailty. Nutrients 5:4184-4205.

Magnaghi V, Cavarretta I, Zucchi I, Susani L, Rupprecht R, Hermann B, Martini L, Melcangi RC (1999) Po gene expression is modulated by androgens in the sciatic nerve of adult male rats. Brain Res Mol Brain Res 70:36-44.

Maiuolo J, Gliozzi M, Musolino V, Carresi C, Nucera S, Macrì R, Scicchitano M, Bosco F, Scarano F, Ruga S (2019) The role of endothelial dysfunction in peripheral blood nerve barrier: molecular mechanisms and pathophysiological implications. International journal of molecular sciences 20:3022.

Marques SA, Taffarel Ml, Martinez AMB (2003) Participation of neurofilament proteins in axonal dark degeneration of rat's optic nerves. Brain research 969:1-13.

Marschallinger J, Iram T, Zardeneta M, Lee SE, Lehallier B, Haney MS, Pluvinage JV, Mathur V, Hahn O, Morgens DW (2020) Lipid-droplet-accumulating microglia represent a dysfunctional and proinflammatory state in the aging brain. Nature Neuroscience 23:194-208.

Martini R (1997) Animal models for inherited peripheral neuropathies. Journal of anatomy 191 (Pt 3):321-336.

Martini R, Willison H (2016) Neuroinflammation in the peripheral nerve: Cause, modulator, or bystander in peripheral neuropathies? Glia 64:475-486.

Martini R, Klein D, Groh J (2013) Similarities between inherited demyelinating neuropathies and Wallerian degeneration: an old repair program may cause myelin and axon perturbation under nonlesion conditions. Am J Pathol 183:655-660.

Martini R, Fischer S, López-Vales R, David S (2008) Interactions between Schwann cells and macrophages in injury and inherited demyelinating disease. Glia 56:1566-1577.

Martini R, Zielasek J, Toyka KV, Giese KP, Schachner M (1995) Protein zero (P0)-deficient mice show myelin degeneration in peripheral nerves characteristic of inherited human neuropathies. Nat Genet 11:281-286.

Masuda T, Sankowski R, Staszewski O, Böttcher C, Amann L, Scheiwe C, Nessler S, Kunz P, van Loo G, Coenen VA (2019) Spatial and temporal heterogeneity of mouse and human microglia at single-cell resolution. Nature 566:388-392.

Mäurer M, Müller M, Kobsar I, Leonhard C, Martini R, Kiefer R (2003) Origin of pathogenic macrophages and endoneurial fibroblast-like cells in an animal model of inherited neuropathy. Molecular and Cellular Neuroscience 23:351-359.

McQuarrie IG, Brady ST, Lasek RJ (1989) Retardation in the slow axonal transport of cytoskeletal elements during maturation and aging. Neurobiology of aging 10:359-365.

Meilandt WJ, Ngu H, Gogineni A, Lalehzadeh G, Lee S-H, Srinivasan K, Imperio J, Wu T, Weber M, Kruse AJ (2020) Trem2 deletion reduces late-stage amyloid plaque accumulation, elevates the Aβ42: Aβ40 ratio, and exacerbates axonal dystrophy and dendritic spine loss in the PS2APP Alzheimer's mouse model. Journal of Neuroscience 40:1956-1974.

Melcangi RC, Magnaghi V, Martini L (2000) Aging in peripheral nerves: regulation of myelin protein genes by steroid hormones. Progress in neurobiology 60:291-308.

Melcangi RC, Magnaghi V, Cavarretta I, Martini L, Piva F (1998a) Age-induced decrease of glycoprotein Po and myelin basic protein gene expression in the rat sciatic nerve. Repair by steroid derivatives. Neuroscience 85:569-578.

Melcangi RC, Magnaghi V, Cavarretta I, Riva MA, Piva F, Martini L (1998b) Effects of steroid hormones on gene expression of glial markers in the central and peripheral nervous system: variations induced by aging. Exp Gerontol 33:827-836.

Melcangi RC, Magnaghi V, Cavarretta I, Zucchi I, Bovolin P, D'Urso D, Martini L (1999) Progesterone derivatives are able to influence peripheral myelin protein 22 and P0 gene expression: possible mechanisms of action. J Neurosci Res 56:349-357.

Melcangi RC, Ballabio M, Cavarretta I, Gonzalez LC, Leonelli E, Veiga S, Martini L, Magnaghi V (2003) Effects of neuroactive steroids on myelin of peripheral nervous system. The Journal of steroid biochemistry and molecular biology 85:323-327.

Meyer M, Matsuoka I, Wetmore C, Olson L, Thoenen H (1992) Enhanced synthesis of brain-derived neurotrophic factor in the lesioned peripheral nerve: different mechanisms are responsible for the regulation of BDNF and NGF mRNA. The Journal of cell biology 119:45-54.

Michailov GV, Sereda MW, Brinkmann BG, Fischer TM, Haug B, Birchmeier C, Role L, Lai C, Schwab MH, Nave K-A (2004) Axonal neuregulin-1 regulates myelin sheath thickness. Science 304:700-703.

Milde S, Adalbert R, Elaman MH, Coleman MP (2015) Axonal transport declines with age in two distinct phases separated by a period of relative stability. Neurobiology of aging 36:971-981.

Mildner A, Schmidt H, Nitsche M, Merkler D, Hanisch U-K, Mack M, Heikenwalder M, Brück W, Priller J, Prinz M (2007) Microglia in the adult brain arise from Ly-6C hi CCR2+ monocytes only under defined host conditions. Nature neuroscience 10:1544-1553.

Moldovan M, Rosberg MR, Alvarez S, Klein D, Martini R, Krarup C (2016) Aging-associated changes in motor axon voltage-gated Na+ channel function in mice. Neurobiology of aging 39:128-139.

Mrdjen D, Pavlovic A, Hartmann FJ, Schreiner B, Utz SG, Leung BP, Lelios I, Heppner FL, Kipnis J, Merkler D (2018) High-dimensional single-cell mapping of central nervous system immune cells reveals distinct myeloid subsets in health, aging, and disease. Immunity 48:380-395.

Mukherjee C, Kling T, Russo B, Miebach K, Kess E, Schifferer M, Pedro LD, Weikert U, Fard MK, Kannaiyan N (2020) Oligodendrocytes Provide Antioxidant Defense Function for Neurons by Secreting Ferritin Heavy Chain. Cell Metabolism.

Müller M, Leonhard C, Krauthausen M, Wacker K, Kiefer R (2010) On the longevity of resident endoneurial macrophages in the peripheral nervous system: a study of physiological macrophage turnover in bone marrow chimeric mice. Journal of the Peripheral Nervous System 15:357-365.

N'Diaye E-N, Branda CS, Branda SS, Nevarez L, Colonna M, Lowell C, Hamerman JA, Seaman WE (2009) TREM-2 (triggering receptor expressed on myeloid cells 2) is a phagocytic receptor for bacteria. Journal of Cell Biology 184:215-223.

Napoli I, Noon LA, Ribeiro S, Kerai AP, Parrinello S, Rosenberg LH, Collins MJ, Harrisingh MC, White IJ, Woodhoo A (2012) A central role for the ERK-signaling pathway in controlling Schwann cell plasticity and peripheral nerve regeneration in vivo. Neuron 73:729-742.

Narciso MS, Hokoç JN, Martinez A (2001) Watery and dark axons in Wallerian degeneration of the opossum's optic nerve: different patterns of cytoskeletal breakdown? Anais da Academia Brasileira de Ciências 73:231-243.

Nave K-A (2010) Myelination and support of axonal integrity by glia. Nature 468:244-252.

Nguyen MD, Lariviere RC, Julien J-P (2000) Reduction of axonal caliber does not alleviate motor neuron disease caused by mutant superoxide dismutase 1. Proceedings of the National Academy of Sciences 97:12306-12311.

Niemann A, Berger P, Suter U (2006) Pathomechanisms of mutant proteins in Charcot-Marie-Tooth disease. Neuromolecular medicine 8:217-241.

Nishimune H, Stanford JA, Mori Y (2014) Role of exercise in maintaining the integrity of the neuromuscular junction. Muscle & nerve 49:315-324.

Noailles A, Maneu V, Campello L, Lax P, Cuenca N (2018) Systemic inflammation induced by lipopolysaccharide aggravates inherited retinal dystrophy. Cell death & disease 9:1-18.

Norden DM, Godbout JP (2013) Microglia of the aged brain: primed to be activated and resistant to regulation. Neuropathology and applied neurobiology 39:19-34.

Nugent AA, Lin K, Van Lengerich B, Lianoglou S, Przybyla L, Davis SS, Llapashtica C, Wang J, Xia D, Lucas A (2020) TREM2 regulates microglial cholesterol metabolism upon chronic phagocytic challenge. Neuron 105:837-854.

O'Sullivan DJ, Swallow M (1968) The fibre size and content of the radial and sural nerves. Journal of neurology, neurosurgery, and psychiatry 31:464.

Oldfors A (1980) Macrophages in peripheral nerves. Acta neuropathologica 49:43-49.

Olsen NJ, Kovacs WJ (1996) Gonadal steroids and immunity. Endocrine reviews 17:369-384.

Olympiou M, Sargiannidou I, Markoullis K, Karaiskos C, Kagiava A, Kyriakoudi S, Abrams CK, Kleopa KA (2016) Systemic inflammation disrupts oligodendrocyte gap junctions and induces ER stress in a model of CNS manifestations of X-linked Charcot-Marie-Tooth disease. Acta neuropathologica communications 4:95.

Opalach K, Rangaraju S, Madorsky I, Leeuwenburgh C, Notterpek L (2010) Lifelong calorie restriction alleviates age-related oxidative damage in peripheral nerves. Rejuvenation research 13:65-74.

Panenic R, Gardiner PF (1998) The case for adaptability of the neuromuscular junction to endurance exercise training. Canadian journal of applied physiology 23:339-360.

Pannese E (2011) Morphological changes in nerve cells during normal aging. Brain Structure and Function 216:85-89.

Parhad IM, Scott JN, Cellars LA, Bains JS, Krekoski CA, Clark AW (1995) Axonal atrophy in aging is associated with a decline in neurofilament gene expression. Journal of neuroscience research 41:355-366.

Park J, Min J-S, Kim B, Chae U-B, Yun JW, Choi M-S, Kong I-K, Chang K-T, Lee D-S (2015) Mitochondrial ROS govern the LPS-induced pro-inflammatory response in microglia cells by regulating MAPK and NF-κB pathways. Neuroscience letters 584:191-196.

Parrinello S, Napoli I, Ribeiro S, Digby PW, Fedorova M, Parkinson DB, Doddrell RDS, Nakayama M, Adams RH, Lloyd AC (2010) EphB signaling directs peripheral nerve regeneration through Sox2-dependent Schwann cell sorting. Cell 143:145-155.

Pereira JA, Lebrun-Julien F, Suter U (2012) Molecular mechanisms regulating myelination in the peripheral nervous system. Trends in neurosciences 35:123-134.

Perry VH, Cunningham C, Holmes C (2007) Systemic infections and inflammation affect chronic neurodegeneration. Nature reviews Immunology 7:161-167.

Peters A (2009) The effects of normal aging on myelinated nerve fibers in monkey central nervous system. Frontiers in neuroanatomy 3:11.

Pettan-Brewer C, M. Treuting P (2011) Practical pathology of aging mice. Pathobiology of Aging & Age-related Diseases 1:7202.

Phillips SM, Sherwin BB (1992) Effects of estrogen on memory function in surgically menopausal women. Psychoneuroendocrinology 17:485-495.

Pixley FJ, Stanley ER (2004) CSF-1 regulation of the wandering macrophage: complexity in action. Trends in cell biology 14:628-638.

Pridans C, Lillico S, Whitelaw B, Hume DA (2014) Lentiviral vectors containing mouse Csf1r control elements direct macrophage-restricted expression in multiple species of birds and mammals. Molecular Therapy-Methods & Clinical Development 1:14010.

Prinz M, Priller J (2014) Microglia and brain macrophages in the molecular age: from origin to neuropsychiatric disease. Nature Reviews Neuroscience 15:300-312.

Prinz M, Jung S, Priller J (2019) Microglia biology: one century of evolving concepts. Cell 179:292-311.

Querol L, Siles AM, Alba-Rovira R, Jáuregui A, Devaux J, Faivre-Sarrailh C, Araque J, Rojas-Garcia R, Diaz-Manera J, Cortés-Vicente E (2017) Antibodies against peripheral nerve antigens in chronic inflammatory demyelinating polyradiculoneuropathy. Scientific reports 7:1-9.

Rangaraju S, Hankins D, Madorsky I, Madorsky E, Lee WH, Carter CS, Leeuwenburgh C, Notterpek L (2009) Molecular architecture of myelinated peripheral nerves is supported by calorie restriction with aging. Aging cell 8:178-191.

Resnick SM, Maki PM, Golski S, Kraut MA, Zonderman AB (1998) Effects of estrogen replacement therapy on PET cerebral blood flow and neuropsychological performance. Hormones and behavior 34:171-182.

Richard L, Védrenne N, Vallat J-M, Funalot B (2014) Characterization of endoneurial fibroblast-like cells from human and rat peripheral nerves. Journal of Histochemistry & Cytochemistry 62:424-435.

Richard L, Topilko P, Magy L, Decouvelaere A-V, Charnay P, Funalot B, Vallat J-M (2012) Endoneurial fibroblast-like cells. Journal of neuropathology and experimental neurology 71:938-947.

Richter SH, Gass P, Fuss J (2014) Resting is rusting: a critical view on rodent wheel-running behavior. The Neuroscientist 20:313-325.

Ritchie JM (1984) Pathophysiology of conduction in demyelinated nerve fibers. In: Myelin, pp 337-367: Springer.

Ritzel RM, Crapser J, Patel AR, Verma R, Grenier JM, Chauhan A, Jellison ER, McCullough LD (2016) Age-associated resident memory CD8 T cells in the central nervous system are primed to potentiate inflammation after ischemic brain injury. The Journal of Immunology 196:3318-3330.

Robertson A, Day B, Pollock M, Collier P (1993) The neuropathy of elderly mice. Acta neuropathologica 86:163-171.

Robinson LR, Rubner DE, Wahl PW, Fujimoto WY, Stolov WC (1993) Influences of height and gender on normal nerve conduction studies. Archives of physical medicine and rehabilitation 74:1134-1138.

Rossor AM, Tomaselli PJ, Reilly MM (2016) Recent advances in the genetic neuropathies. Current opinion in neurology 29:537.

Rouault TA (2013) Iron metabolism in the CNS: implications for neurodegenerative diseases. Nature Reviews Neuroscience 14:551-564.

Rudolf R, Khan MM, Labeit S, Deschenes MR (2014) Degeneration of neuromuscular junction in age and dystrophy. Frontiers in aging neuroscience 6:99.

Safaiyan S, Kannaiyan N, Snaidero N, Brioschi S, Biber K, Yona S, Edinger AL, Jung S, Rossner MJ, Simons M (2016) Age-related myelin degradation burdens the clearance function of microglia during aging. Nature neuroscience 19:995.

Saggu SK, Chotaliya HP, Blumbergs PC, Casson RJ (2010) Wallerian-like axonal degeneration in the optic nerve after excitotoxic retinal insult: an ultrastructural study. BMC neuroscience 11:97.

Salim S (2017) Oxidative Stress and the Central Nervous System. J Pharmacol Exp Ther 360:201-205.

Salzer JL (2008) Switching myelination on and off. The Journal of Cell Biology 181:575-577.

Scheib J, Höke A (2016a) Impaired regeneration in aged nerves: Clearing out the old to make way for the new. Experimental neurology 284:79-83.

Scheib JL, Höke A (2016b) An attenuated immune response by Schwann cells and macrophages inhibits nerve regeneration in aged rats. Neurobiology of aging 45:1-9.

Schenone A, Abbruzzese M, Uccelli A, Mandich R, James R, Bellone E, Giunchedi M, Rolando S, Capello E, Mancardi GL (1994) Hereditary motor and sensory neuropathy with myelin outfolding: clinical, genetic and neuropathological study of three cases. Journal of the neurological sciences 122:20-27.

Scherer SS, Xu Y-T, Nelles E, Fischbeck K, Willecke K, Bone LJ (1998) Connexin32-null mice develop demyelinating peripheral neuropathy. Glia 24:8-20.

Schmid CD, Stienekemeier M, Oehen S, Bootz F, Zielasek J, Gold R, Toyka KV, Schachner M, Martini R (2000) Immune deficiency in mouse models for inherited peripheral neuropathies leads to improved myelin maintenance. Journal of Neuroscience 20:729-735.

Schneider A, Weier M, Herderschee J, Perreau M, Calandra T, Roger T, Giannoni E (2018) IRF5 is a key regulator of macrophage response to lipopolysaccharide in newborns. Frontiers in immunology 9:1597.

Schumacher M, Guennoun R, Mercier G, Désarnaud F, Lacor P, Bénavides J, Ferzaz B, Robert F, Baulieu EE (2001) Progesterone synthesis and myelin formation in peripheral nerves. Brain research reviews 37:343-359.

Schumacher M, Weill-Engerer S, Liere P, Robert F, Franklin RJM, Garcia-Segura LM, Lambert JJ, Mayo W, Melcangi RC, Parducz A (2003) Steroid hormones and neurosteroids in normal and pathological aging of the nervous system. Progress in neurobiology 71:3-29.

Sharma AK, Bajada S, Thomas PK (1980) Age changes in the tibial and plantar nerves of the rat. Journal of anatomy 130:417-428.

Shemer A, Scheyltjens I, Frumer GR, Kim J-S, Grozovski J, Ayanaw S, Dassa B, Van Hove H, Chappell-Maor L, Boura-Halfon S (2020) Interleukin-10 prevents

pathological microglia hyperactivation following peripheral endotoxin challenge. Immunity.

Sheth KA, Iyer CC, Wier CG, Crum AE, Bratasz A, Kolb SJ, Clark BC, Burghes AHM, Arnold WD (2018) Muscle strength and size are associated with motor unit connectivity in aged mice. Neurobiology of aging 67:128-136.

Shy ME (2004) Charcot-Marie-Tooth disease: an update. Current opinion in neurology 17:579-585.

Stanley ER, Chitu V (2014) CSF-1 receptor signaling in myeloid cells. Cold Spring Harbor perspectives in biology 6:a021857.

Stefanatos R, Sanz A (2018) The role of mitochondrial ROS in the aging brain. Febs Letters 592:743-758.

Stierli S, Napoli I, White IJ, Cattin A-L, Cabrejos AM, Calavia NG, Malong L, Ribeiro S, Nihouarn J, Williams R (2018) The regulation of the homeostasis and regeneration of peripheral nerve is distinct from the CNS and independent of a stem cell population. Development 145.

Stockwell BR, Angeli JPF, Bayir H, Bush AI, Conrad M, Dixon SJ, Fulda S, Gascón S, Hatzios SK, Kagan VE (2017) Ferroptosis: a regulated cell death nexus linking metabolism, redox biology, and disease. Cell 171:273-285.

Stoll G, Jander S, Myers RR (2002) Degeneration and regeneration of the peripheral nervous system: from Augustus Waller's observations to neuroinflammation. Journal of the Peripheral Nervous System 7:13-27.

Stoll G, Griffin JW, Li CY, Trapp BD (1989) Wallerian degeneration in the peripheral nervous system: participation of both Schwann cells and macrophages in myelin degradation. Journal of neurocytology 18:671-683.

Stoll G, Gabreëls-Festen AAWM, Jander S, Müller HW, Hanemann CO (1998) Major histocompatibility complex class II expression and macrophage responses in genetically proven Charcot–Marie–Tooth type 1 and hereditary neuropathy with liability to pressure palsies. Muscle & Nerve: Official Journal of the American Association of Electrodiagnostic Medicine 21:1419-1427.

Stratton JA, Holmes A, Rosin NL, Sinha S, Vohra M, Burma NE, Trang T, Midha R, Biernaskie J (2018) Macrophages regulate Schwann cell maturation after nerve injury. Cell reports 24:2561-2572.

Suter U, Scherer SS (2003) Disease mechanisms in inherited neuropathies. Nature reviews neuroscience 4:714-726.

Szentirmai É, Krueger JM (2014) Sickness behaviour after lipopolysaccharide treatment in ghrelin deficient mice. Brain, behavior, and immunity 36:200-206.

Taetzsch T, Valdez G (2018) NMJ maintenance and repair in aging. Current opinion in physiology 4:57-64.

Takahashi K, Rochford CDP, Neumann H (2005) Clearance of apoptotic neurons without inflammation by microglial triggering receptor expressed on myeloid cells-2. The Journal of experimental medicine 201:647-657.

Takahashi K, Prinz M, Stagi M, Chechneva O, Neumann H (2007) TREM2-transduced myeloid precursors mediate nervous tissue debris clearance and facilitate recovery in an animal model of multiple sclerosis. PLoS medicine 4.

Takeuchi O, Hoshino K, Kawai T, Sanjo H, Takada H, Ogawa T, Takeda K, Akira S (1999) Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components. Immunity 11:443-451.

Tang M-X, Jacobs D, Stern Y, Marder K, Schofield P, Gurland B, Andrews H, Mayeux R (1996) Effect of oestrogen during menopause on risk and age at onset of Alzheimer's disease. The Lancet 348:429-432.

Tankisi H, Pugdahl K, Johnsen B, Fuglsang-Frederiksen A (2007) Correlations of nerve conduction measures in axonal and demyelinating polyneuropathies. Clinical neurophysiology 118:2383-2392.

Taveggia C, Feltri ML, Wrabetz L (2010) Signals to promote myelin formation and repair. Nature Reviews Neurology 6:276-287.

Taveggia C, Zanazzi G, Petrylak A, Yano H, Rosenbluth J, Einheber S, Xu X, Esper RM, Loeb JA, Shrager P (2005) Neuregulin-1 type III determines the ensheathment fate of axons. Neuron 47:681-694.

Thakur D, Paudel BH, Jha CB (2010a) Nerve Conduction study in healthy individuals, a preliminary age based study. Kathmandu University Medical Journal 8:311-316.

Thakur D, Paudel BH, Bajaj BK, Jha CB (2010b) Nerve conduction study in healthy individuals: a gender based study. Health Renaissance 8:169-175.

Thomas PK (1997) Electrophysiological and morphological changes in the peripheral nervous system with ageing. Electroencephalography and Clinical Neurophysiology 1:8.

Tintignac LA, Brenner H-R, Rüegg MA (2015) Mechanisms regulating neuromuscular junction development and function and causes of muscle wasting. Physiological reviews 95:809-852.

Topilko P, Schneider-Maunoury S, Levi G, Baron-Van Evercooren A, Chennoufi ABY, Seitanidou T, Babinet C, Charnay P (1994) Krox-20 controls myelination in the peripheral nervous system. Nature 371:796-799.

Tosolini AP, Smith GM (2018) Gene Therapy for the Central and Peripheral Nervous System. Frontiers in molecular neuroscience 11:54.

Tzekova N, Heinen A, Küry P (2014) Molecules involved in the crosstalk between immune-and peripheral nerve Schwann cells. Journal of clinical immunology 34:86-104.

Vacca V, Marinelli S, Pieroni L, Urbani A, Luvisetto S, Pavone F (2016) 17beta-estradiol counteracts neuropathic pain: a behavioural, immunohistochemical, and proteomic investigation on sex-related differences in mice. Scientific reports 6:18980.

Valdez G, Tapia JC, Lichtman JW, Fox MA, Sanes JR (2012) Shared resistance to aging and ALS in neuromuscular junctions of specific muscles. PloS one 7.

Valdez G, Tapia JC, Kang H, Clemenson GD, Gage FH, Lichtman JW, Sanes JR (2010) Attenuation of age-related changes in mouse neuromuscular synapses by caloric

restriction and exercise. Proceedings of the National Academy of Sciences 107:14863-14868.

Van Hove H, Martens L, Scheyltjens I, De Vlaminck K, Antunes ARP, De Prijck S, Vandamme N, De Schepper S, Van Isterdael G, Scott CL (2019) A single-cell atlas of mouse brain macrophages reveals unique transcriptional identities shaped by ontogeny and tissue environment. Nature neuroscience 22:1021-1035.

Vass K, Hickey WF, Schmidt RE, Lassmann H (1993) Bone marrow-derived elements in the peripheral nervous system. An immunohistochemical and ultrastructural investigation in chimeric rats. Laboratory investigation; a journal of technical methods and pathology 69:275-282.

Verdu E, Buti M, Navarro X (1995) The effect of aging on efferent nerve fibers regeneration in mice. Brain research 696:76-82.

Verdu E, Buti M, Navarro X (1996) Functional changes of the peripheral nervous system with aging in the mouse. Neurobiology of aging 17:73-77.

Verdu E, Ceballos D, Vilches JJ, Navarro X (2000) Influence of aging on peripheral nerve function and regeneration. Journal of the peripheral nervous system : JPNS 5:191-208.

Viader A, Golden JP, Baloh RH, Schmidt RE, Hunter DA, Milbrandt J (2011) Schwann cell mitochondrial metabolism supports long-term axonal survival and peripheral nerve function. Journal of Neuroscience 31:10128-10140.

Vickers JC, King AE, McCormack GH, Bindoff AD, Adlard PA (2019) Iron is increased in the brains of ageing mice lacking the neurofilament light gene. PloS one 14:e0224169.

Waller A (1850) Experiments on the Section of the Glossopharyngeal and Hypoglossal Nerves of the Frog, and Observations of the Alterations Produced Thereby in the Structure of Their Primitive Fibres. Philosophical Transactions of the Royal Society of London 140:423-429.

Walsh ME, Sloane LB, Fischer KE, Austad SN, Richardson A, Van Remmen H (2015) Use of nerve conduction velocity to assess peripheral nerve health in aging mice. Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences 70:1312-1319.

Wang C-H, Wu S-B, Wu Y-T, Wei Y-H (2013) Oxidative stress response elicited by mitochondrial dysfunction: implication in the pathophysiology of aging. Experimental biology and medicine 238:450-460.

Wang PL, Yim AKY, Kim K-W, Avey D, Czepielewski RS, Colonna M, Milbrandt J, Randolph GJ (2020) Peripheral nerve resident macrophages share tissue-specific programming and features of activated microglia. In: Nature communications, p 2552.

Wang X, Michaelis EK (2010) Selective neuronal vulnerability to oxidative stress in the brain. Frontiers in aging neuroscience 2:12-12.

Ward RE, Caserotti P, Cauley JA, Boudreau RM, Goodpaster BH, Vinik AI, Newman AB, Strotmeyer ES (2016) Mobility-related consequences of reduced lower-extremity peripheral nerve function with age: a systematic review. Aging and disease 7:466.

Ward RE, Boudreau RM, Caserotti P, Harris TB, Zivkovic S, Goodpaster BH, Satterfield S, Kritchevsky S, Schwartz AV, Vinik AI (2015) Sensory and motor peripheral nerve function and longitudinal changes in quadriceps strength. Journals of Gerontology Series A: Biomedical Sciences and Medical Sciences 70:464-470.

Wendeln A-C, Degenhardt K, Kaurani L, Gertig M, Ulas T, Jain G, Wagner J, Häsler LM, Wild K, Skodras A (2018) Innate immune memory in the brain shapes neurological disease hallmarks. Nature 556:332-338.

Wheeler SJ, Plummer JM (1989) Age-related changes in the fibre composition of equine peripheral nerve. Journal of the neurological sciences 90:53-66.

Willadt S, Nash M, Slater C (2018) Age-related changes in the structure and function of mammalian neuromuscular junctions. Annals of the New York Academy of Sciences 1412:41-53.

Willison HJ, Jacobs BC, Van Doorn PA (2016) Guillain-barre syndrome. The Lancet 388:717-727.

Wilson MH, Deschenes MR (2005) The neuromuscular junction: anatomical features and adaptations to various forms of increased, or decreased neuromuscular activity. International journal of neuroscience 115:803-828.

Wokke J, Jennekens F, Van den Oord C, Veldman H, Smit L, Leppink G (1990) Morphological changes in the human end plate with age. Journal of the neurological sciences 95:291-310.

Wolbert J, Li X, Heming M, Mausberg AK, Akkermann D, Frydrychowicz C, Fledrich R, Groeneweg L, Schulz C, Stettner M (2020) Redefining the heterogeneity of peripheral nerve cells in health and autoimmunity. Proceedings of the National Academy of Sciences 117:9466-9476.

Woods JA, Wilund KR, Martin SA, Kistler BM (2012) Exercise, inflammation and aging. Aging and disease 3:130.

Wrabetz L, Feltri ML, Quattrini A, Imperiale D, Previtali S, D'Antonio M, Martini R, Yin X, Trapp BD, Zhou L (2000) P0 glycoprotein overexpression causes congenital hypomyelination of peripheral nerves. The Journal of cell biology 148:1021-1034.

Wynne AM, Henry CJ, Huang Y, Cleland A, Godbout JP (2010) Protracted downregulation of CX3CR1 on microglia of aged mice after lipopolysaccharide challenge. Brain, behavior, and immunity 24:1190-1201.

Yang S, Alnaqeeb M, Simpson H, Goldspink G (1996) Cloning and characterization of an IGF-1 isoform expressed in skeletal muscle subjected to stretch. Journal of Muscle Research & Cell Motility 17:487-495.

Ydens E, Lornet G, Smits V, Goethals S, Timmerman V, Janssens S (2013) The neuroinflammatory role of Schwann cells in disease. Neurobiology of disease 55:95-103.

Ydens E, Cauwels A, Asselbergh B, Goethals S, Peeraer L, Lornet G, Almeida-Souza L, Van Ginderachter JA, Timmerman V, Janssens S (2012) Acute injury in the peripheral nervous system triggers an alternative macrophage response. J Neuroinflammation 9:176.

Ydens E, Amann L, Asselbergh B, Scott CL, Martens L, Sichien D, Mossad O, Blank T, De Prijck S, Low D (2020) Profiling peripheral nerve macrophages reveals two

macrophage subsets with distinct localization, transcriptome and response to injury. Nature neuroscience 23:676-689.

Yilmaz C, Karali K, Fodelianaki G, Gravanis A, Chavakis T, Charalampopoulos I, Alexaki VI (2019) Neurosteroids as regulators of neuroinflammation. Frontiers in Neuroendocrinology 55:100788.

Yuan X, Klein D, Kerscher S, West BL, Weis J, Katona I, Martini R (2018) Macrophage Depletion Ameliorates Peripheral Neuropathy in Aging Mice. J Neurosci 38:4610-4620.

Yumoto N, Kim N, Burden SJ (2012) Lrp4 is a retrograde signal for presynaptic differentiation at neuromuscular synapses. Nature 489:438-442.

Zhang H, Shao Z, Zhu Y, Shi L, Li Z, Hou R, Zhang C, Yao D (2018) Toll-like receptor 4 (TLR4) expression affects schwann cell behavior in vitro. Scientific reports 8:1-12.

Zheng G, Qiu P, Xia R, Lin H, Ye B, Tao J, Chen L (2019) Effect of aerobic exercise on inflammatory markers in healthy middle-aged and older adults: a systematic review and meta-analysis of randomized controlled trials. Frontiers in aging neuroscience 11:98.

Zhong L, Zhang Z-L, Li X, Liao C, Mou P, Wang T, Wang Z, Wang Z, Wei M, Xu H (2017) TREM2/DAP12 complex regulates inflammatory responses in microglia via the JNK signaling pathway. Frontiers in Aging Neuroscience 9:204.

Zhou J, Yu W, Zhang M, Tian X, Li Y, Lü Y (2019) Imbalance of microglial TLR4/TREM2 in LPS-treated APP/PS1 transgenic mice: a potential link between Alzheimer's disease and systemic inflammation. Neurochemical research 44:1138-1151.

Zielasek J, Martini R, Toyka KV (1996) Functional abnormalities in P0-deficient mice resemble human hereditary neuropathies linked to P0 gene mutations. Muscle & nerve 19:946-952.

8. Appendices

8.1 Technical equipment

BioPhotometer 6131	Eppendorf (Hamburg, Germany)
Biosphere Filter Tips	Sarstedt (Nümbrecht, Germany)
Centrifuges	
Biofuge 15R	Heraeus (Hanau, Germany)
Biofuge Pico	Heraeus (Hanau, Germany)
Centrifuge 5424	Eppendorf (Hamburg, Germany)
Cryostat CM 3050S	Leica (Wetzlar, Germany)
Discardit II™ Syringe 10 mL	Becton Dickinson (Franklin Lakes, USA)
Dry block thermostat TDB-120	Hartenstein (Würzburg, Germany)
Dry block thermostat TDB-100	Hartenstein (Würzburg, Germany)
Eclipse™ Needle (0.4 mm x 13 mm)	Becton Dickinson (Franklin Lakes, USA)
ELISA reader Original Multiskan EX	Labsystems (Helsinki, Finland)
Freezer	Liebherr (Biberach, Germany)
Gel chamber Mini-Trans-Blot® cell	Bio-Rad (Munich, Germany)
Grip Strength Meter	Columbus Instruments (Columbus, USA)
Heating plate	Medax (Neumünster, Germany)
Homogenizer MICCRA D-8	ART (Mühlheim, Germany)
Hotplate Stirrer L-81	Hartenstein (Würzburg, Germany)
Hyperfilm ECL	GE Healthcare (Buckinghamshire, UK)
MicroAmp® Fast 96-well reaction plate	Applied Biosystems (Darmstadt, Germany)

Microscopes	
ApoTome 2	Zeiss (Oberkochen, Germany)
Axiophot 2	Zeiss (Oberkochen, Germany)
CM10	Philips (Hamburg, Germany)
LEO 906 E	Zeiss (Oberkochen, Germany)
Neurosoft-Evidence 3102 electromyograph	Schreiber & Tholen Medizintechnik (Stade, Germany)
Object slides superfrost	Langenbrinck (Teningen, Germany)
Olympus Veleta camera system	Olympus (Hamburg, Germany)
Optical adhesive covers	Applied Biosystems (Darmstadt, Germany)
PapPen	SCI (Munich, Germany)
PCR tubes	Hartenstein (Würzburg, Germany)
Perfusion pump Reglo	Ismatec (Glattbrugg, Switzerland)
ProScan Slow Scan CCD camera Pro Scan	(Lagerlechfeld, Germany)
Pipettes	Abimed (Berlin, Germany)
	Eppendorf (Hamburg, Germany)
	Gilson (Bad Camberg, Germany)
Power supply	Bio-RAD (Munich, Germany)
PROTRAN® Nitrocellulose Transfer Membrane	Hartenstein (Würzburg, Germany)
RotaRod Advanced	TSE Systems (Bad Homburg, Germany)
Sonication device Sonoplus HD60	Bandelin Electronic (Berlin, Germany)
Step One Plus Real Time PCR System	Applied Biosystems (Darmstadt, Germany)
Ultracut	Leica (Wetzlar, Germany)

Software

Adobe Photoshop CS6	Adobe (San Jose, USA)
Evidence	Schreiber & Tholen Medizintechnik (Stade, Germany)
iTEM	Olympus (Hamburg, Germany)
ImageJ	National Institutes of Health (Bethesda, USA)
Office 2019	Microsoft (Redmond, USA)
Prism 7	GraphPad Software (San Diego, USA)
ZEN 3.0 (blue edition)	Zeiss (Oberkochen, Germany)

8.2 Antibodies for immunohistochemistry

Primary Antibodies

Reactivity	Company	Host	Dilution	Fixation	Additives
βIII-tubulin	Abcam	Rabbit	1:500	None	0.3% Triton
CD206	Serotec	Rat	1:2000	Acetone	0.3% Triton
CD4	Serotec	Rat	1:1000	Acetone	None
CD68	Dako Omnis	Mouse	1:50	None	None
CD8	Serotec	Rat	1:500	Acetone	None
Cx32	Invitrogen	Mouse	1:500	None	None
ERK1/2	Santa Cruz	Rabbit	1:1000	None	None
F4/80	Serotec	Rat	1:300	None	None
pERK	Cell Signaling	Rabbit	1:1000	None	None
P0	Acris	Chicken	1:3000	None	None
Synaptophysin	Synaptic Systems	Guinea pig	1:500	None	0.3% Triton

Secondary antibodies

Reactivity	Company	Host	Dilution	Conjugation
Rat IgG	Dianova	Goat	1:300	Cy3
Guinea pig IgG	Dianova	Goat	1:300	Cy3
Rabbit IgG	Invitrogen	Goat	1:300	AlexaFluor 488
Chicken IgG	Jackson ImmunoResearch	Donkey	1:10000	Horseradish peroxidase
Mouse IgG	Jackson ImmunoResearch	Donkey	1:10000	Horseradish peroxidase
Rabbit IgG	Jackson ImmunoResearch	Donkey	1:10000	Horseradish peroxidase

8.3 Reagents

Acetone	Invitrogen (Karlsruhe, Germany)
Agarose	Carl Roth (Karlsruhe, Deutschland)
α-Bungarotoxin, AF488 conjugated	Thermo Fisher Scientific (Darmstadt, Germany)
α-Bungarotoxin, AF647 conjugated	Thermo Fisher Scientific (Darmstadt, Germany)
Ammonium persulfate (Ritzel et al.)	Carl Roth (Karlsruhe, Deutschland)
AmpliTaq DNA Polymerase	Applied Biosystems (Darmstadt, Germany)
Aqua-Poly/Mount®	Polysciences (Eppelheim, Germany)
Boric acid	Merck (Darmstadt, Germany)
Bovine serum albumin 96%	Sigma-Aldrich (Munich, Germany)
Bromphenol blue	Sigma-Aldrich (Munich, Germany)

Cacodylic acid	Serva (Heidelberg, Germany)
Chloroform	Sigma-Aldrich (Munich, Germany)
DAPI	Sigma-Aldrich (Munich, Germany)
Diethyl pyrocarbonate (DEPC)	Sigma-Aldrich (Munich, Germany)
1, 4-Dithiothreitol (DTT)	Sigma-Aldrich (Munich, Germany)
ECL detection reagents	GE Healthcare (Munich, Germany)
Ethanol	Sigma-Aldrich (Munich, Germany)
Ethylenediaminetetraacetic acid (EDTA)	Merck (Darmstadt, Germany)
Gel Star DNA-Dye	Lonza (Basel, Switzerland)
Glycine	Sigma-Aldrich (Munich, Germany)
Glycerol	Merck (Darmstadt, Germany)
Glycogen	Roche (Mannheim, Germany)
HD Green DNA-Dye	Intas (Göttingen, Germany)
Heparin	Ratiopharm (Ulm, Germany)
HEPES	Carl Roth (Karlsruhe, Germany)
Ketavet	Pfizer (Berlin, Germany)
Lowry reagent	Sigma-Aldrich (Munich, Germany)
LPS	Sigma-Aldrich (Munich, Germany)
2-Mercaptoethanol	Thermo Scientific (Darmstadt, Germany)
Methanol	Sigma-Aldrich (Munich, Germany)
2-Methylbutane	Carl Roth (Karlsruhe, Germany)
Nonidet P-40 substitute (NP-40)	Fluka (Buchs, Switzerland)
Orange DNA Loading Dye (6X)	Thermo Fisher Scientific (Darmstadt, Germany)
O'RangeRuler™ 100+500 bp DNA Ladder	Thermo Fisher Scientific (Darmstadt, Germany)

Page Ruler Plus prestained protein ladder	Thermo Fisher Scientific (Darmstadt, Germany)
Polyacrylamid	Carl Roth (Karlsruhe, Germany)
Ponceau S	Carl Roth (Karlsruhe, Germany)
Primers	Sigma-Aldrich (Munich, Germany)
Roti®-Block (10x)	Carl Roth (Karlsruhe, Germany)
Potassium chloride	Merck (Darmstadt, Germany)
Potassium di-hydrogen phosphate	Merck (Darmstadt, Germany)
Protease inhibitor cocktail set	Calbiochem (Darmstadt, Germany)
Sodium chloride	Merck (Darmstadt, Germany)
Sodium dodecyl sulfate (SDS)	Carl Roth (Karlsruhe, Germany)
Sucrose	Carl Roth (Karlsruhe, Germany)
Tetramethylethylenediamine (TEMED)	Sigma-Aldrich (Munich, Germany)
Tissue-Tek® O.C.T.™ Compound	Sakura (Alphen aan den Rijn, Netherlands)
Tris(hydroxymethyl)aminomethane (Tris)	Merck (Darmstadt, Germany)
Tris-HCl	Merck (Darmstadt, Germany)
TritonX-100	Carl Roth (Karlsruhe, Germany)
Tween-20	Carl Roth (Karlsruhe, Germany)
Vinyl/ERL4221D	ServaElectrophoresis (Heidelberg, Germany)
Xylavet	CP-Pharma (Burgdorf, Germany)

8.4 Buffers and solutions

Anesthetic	1.2% Ketavet
	0.11% Xylavet
	0.9% NaCl

Cacodylate buffer	0.1 M cacodylic acid Dissolved in distilled water pH 7.4
DEPC-H_2O	0.01% diethyl pyrocarbonate (DEPC) Dissolved in distilled water and autoclaved
Gel running buffer (10x)	0.25 M Tris 1.92 M Glycin 1% SDS
Laemmli (6x)	12% Tris-HCl 12% SDS 2.4% EDTA 20% Glycerol 0.1% Bromphenol blue 4.75% 2-Mercaptoethanol
PBS (1x)	137 mM NaCl 2.7 mM KCl 1.5 mM KH_2PO_4 8.1 mM Na_2HPO_4 pH 7.4
PBST (1x)	0.1% Tween-20 in PBS
Ponceau S	1% Trichloroacetic acid 0.1% Ponceau S
RIPA lysis buffer	25 mM Tris pH 8

	10 mM HEPES pH 4.4
	150 mM NaCl
	5 mM $MgCl_2$
	145 mM KCl
	0.4% EDTA
	0.1% SDS
	1% NP-40
	10% Glycerol
SDS PAGE buffer (10x)	0.25 M Tris
	1.92 M Glycine
	1% SDS
Separating gel buffer (4x)	1.5 M Tris
	0.4% SDS
	0.4% TEMED
	pH 8.8
Spurr's medium	10 g Vinyl/ERL
	6 g D.E.R® 736
	26 g NSA
	0.4 g DMAE
TBE	89 mM Tris
	89 mM Borate
	2 mM EDTA
	pH 8.0
Transfer buffer (10x)	0.25 M Tris
	1.92 M Glycine

Transfer buffer (1x)	20% Methanol Dissolved in 1x Transfer buffer

8.5 Primer sequences

Trem2	*Trem2* 25393	5´-TCTGACCACAGGTGTTCCCG 3´
	Trem2 24558	5´-CCAGCAGTGGCCTAAATGGA 3´
	Trem2 25057	5´-CTTATGTGGAAGAGCTCATCGGG 3´

9. Abbreviations

Af	Alexa fluor
BAM	Border-associated macrophages
BDNF	Brain-derived neurotrophic factor
BSA	Bovine serum albumin
CCL2/MCP-1	Chemokine (C-C motif) ligand 2/Monocyte chemoattractant protein-1
CD	Cluster of differentiation
cDNA	Complementary DNA
CIAP	Chronic idiopathic axonal polyneuropathy
CIDP	Chronic inflammatory demyelinating polyneuropathy
CMAP	Compound muscle action potential
CNS	Central nervous system
CSF-1	Colony stimulating factor 1
CSF-1R	CSF-1 receptor
Cx32/Gjb1	Connexin 32 / Gap junction protein beta 1
Cy	Cyanine
DAPI	4',6-Diamidino-2-phenylindole
DEPC	Diethyl pyrocarbonate
DNA	Deoxyribonucleic acid
ECL	Enhanced chemiluminescence
EDTA	Ethylenediaminetetraacetic acid
ERK	Extracellular-signal regulated kinase
GBS	Guillain-Barré-syndrome
GDNF	Glial cell line-derived neurotrophic factor
HRP	Horseradish peroxidase
HSC	Hematopoietic stem cell
IFNγ	Interferon gamma
IGF-1	Insulin-like growth factor

IL-1β	Interleukin-1 beta
iNOS	Inducible nitric oxide synthase
IRF5	Interferon regulatory factor 5
kDa	Kilo Dalton
MBP	Myelin basic protein
M-MLV	Moloney murine leukemia virus
mRNA	Messenger RNA
NCV	Nerve conduction velocity
NMJ	Neuromuscular junction
NRG	Neuregulin
P0	Myelin protein zero
PBS/PBST	Phosphate buffered saline/ PBS with Tween-20
PCR	Polymerase chain reaction
PFA	Paraformaldehyde
PNS	Peripheral nervous system
qRT-PCR	Semi-quantitative reverse transcription PCR
RIPA	Radioimmunoprecipitation assay
RNA	Ribonucleic acid
RNA-seq	RNA sequencing
ROS	Reactive oxygen species
scRNA-seq	Single-cell RNA sequencing
SD	Standard deviation
SDS	Sodium dodecyl sulfate
SDS-PAGE	SDS polyacrylamide gel electrophoresis
TAM	Tamoxifen
TEMED	Tetramethylethylenediamine
TGF-β	Transforming growth factor beta
TNF-α	Tumor necrosis factor alpha
TREM-2	Triggering receptor expressed on myeloid cells-2

Tris	Tris(hydroxymethyl)aminomethane
WT	Wild-type
YS	Yolk sac

10. CV

11. List of publications

Original articles in peer-reviewed international journals

Yuan X, Klein D, Kerscher S, West BL, Weis J, Katona I, Martini R. Macrophage Depletion Ameliorates Peripheral Neuropathy in Aging Mice. J Neurosci. 2018; 38(19):4610-4620.

Shashi V, Magiera MM, Klein D, Zaki M, Schoch K, Rudnik-Schöneborn S, Norman A, Lopes Abath Neto O, Dusl M, **Yuan X**, Bartesaghi L, De Marco P, Alfares AA, Marom R, Arold ST, Guzmán-Vega FJ, Pena LD, Smith EC, Steinlin M, Babiker MO, Mohassel P, Foley AR, Donkervoort S, Kaur R, Ghosh PS, Stanley V, Musaev D, Nava C, Mignot C, Keren B, Scala M, Tassano E, Picco P, Doneda P, Fiorillo C, Issa MY, Alassiri A, Alahmad A, Gerard A, Liu P, Yang Y, Ertl-Wagner B, Kranz PG, Wentzensen IM, Stucka R, Stong N, Allen AS, Goldstein DB; Undiagnosed Diseases Network, Schoser B, Rösler KM, Alfadhel M, Capra V, Chrast R, Strom TM, Kamsteeg EJ, Bönnemann CG, Gleeson JG, Martini R, Janke C, Senderek J. Loss of tubulin deglutamylase CCP1 causes infantile-onset neurodegeneration. EMBO J. 2018; 37(23).

Oral Presentations

Yuan X et al., (2019) GSLS retreat, Waldmünchen, Germany: “The effect of inflammation on peripheral nerves in aging mice”

Yuan X et al., (2017) Arbeitstagung der Bayerischen Neuromuskulären Zentren, Würzburg, Germany:

“Impact of low-grade inflammation by macrophages during aging of the peripheral nervous system”

Poster presentations

Yuan X et al., (2019) Current Topics in Myelin Research, Kassel, Germany: “Impact of systemic inflammation on age-related peripheral neuropathy in mice”

Yuan X et al., (2019) EUREKA! Symposium 2019, Würzburg, Germany: “Impact of systemic inflammation on age-related peripheral neuropathy in mice”

Yuan X et al., (2018) EUREKA! Symposium 2018, Würzburg, Germany: “Macrophage depletion ameliorates peripheral neuropathy in aging mice”

Yuan X et al., (2018) GSLS retreat, Bad Kissingen, Würzburg, Germany: “Macrophage depletion ameliorates peripheral neuropathy in aging mice”

Yuan X et al., (2017) XXVI AINI Congress and 16th ESNI Course, Venice, Italy: “Macrophage depletion ameliorates peripheral neuropathy in aging mice”

Yuan X et al., (2017) EUREKA! Symposium 2017, Würzburg, Germany: “Macrophage depletion ameliorates peripheral neuropathy in aging mice”

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