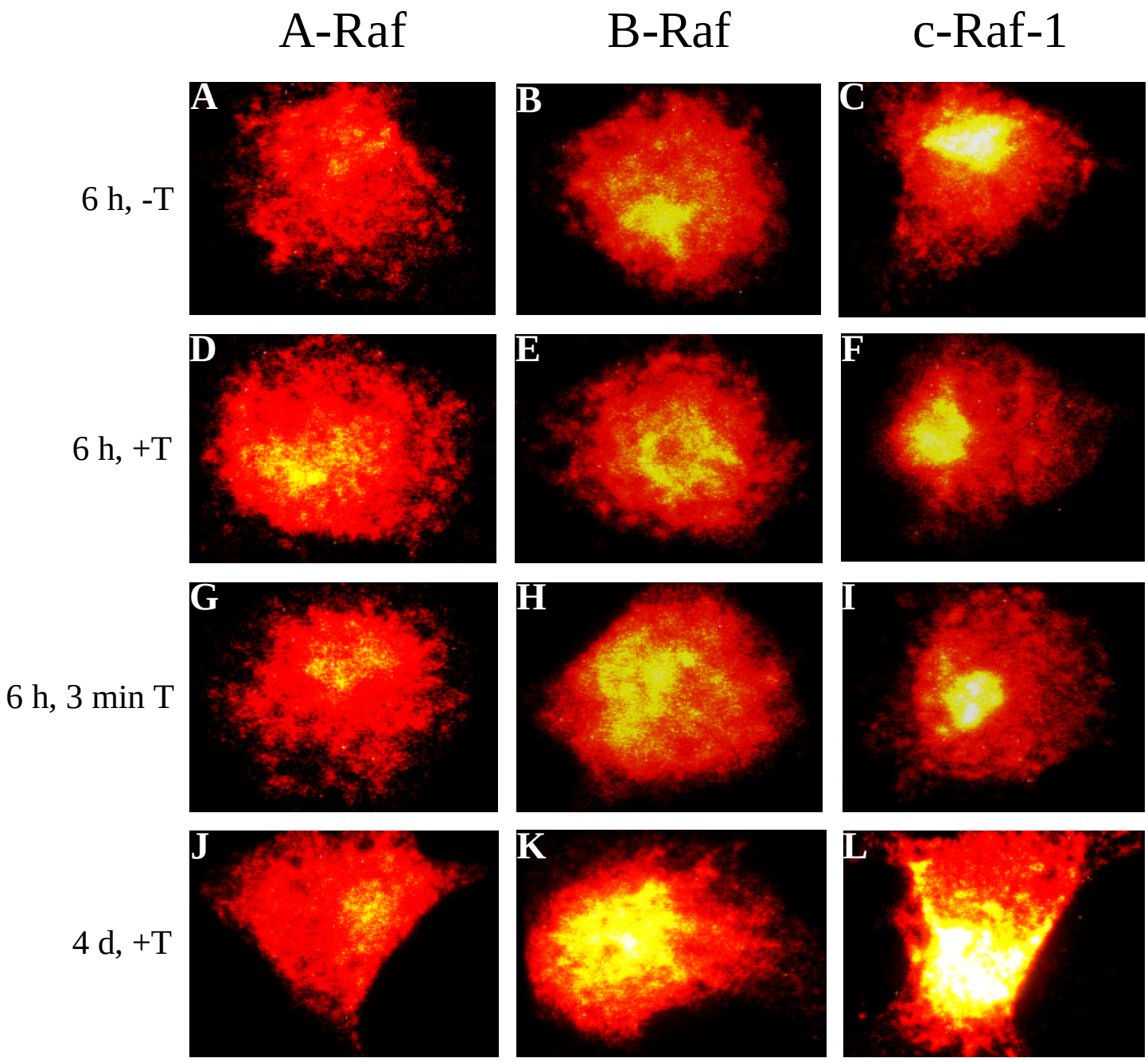


Fig. 14. Photomicrographs of immuofluorescence of cultured E14 mouse motoneurons: (A,B,C) 6 h culture without treatment, (D,E,F) 6 h culture with addition of both CNTF and BDNF for induction of Raf activation, (G,H,I) 3 min treatment with both CNTF and BDNF after 6 h culture, (J,K,L) 4 d culture with treatment with both CNTF and BDNF.

The cells were immunostained with specific antibodies against A-Raf (A,D,G,J), B-Raf (B,E,H,K) and c-Raf-1 (C,F,I,L) protein kinases by using Cy3 fluorochrome-labelled Streptavidin for binding secondary biotinlated antibody for final visualization. Single motoneurons were chosen from each group and analyzed under the confocal laser scanning microscope (50 X). Note that most of the A-Raf and B-Raf immunoreactivities are located in the perinuclear space and in the nuclei in the motoneurons, especially after activation by addition of CNTF and BDNF *in vitro*. Interestingly, after 6 h culture, irrespective of addition of neurotrophic factors, c-Raf-1 distinctly translocated from cytoplasmic region into the nucleus of the motoneurons. The same result was also obtained with cultures from E13 mouse embryos (data not shown).