

Fig. S1. Intrathecal administration of rapamycin does not reduce the number of capsaicin induced Fos-IR neurons in the dorsal horn. Effects of capsaicin injection in the hindpaw following intrathecal administration of rapamycin (or vehicle). There was no difference between rapamycin and vehicle pretreated animals in capsaicin induced c-Fos-IR neurons in any of the three ipsilateral domains studied. N=7 in each group.

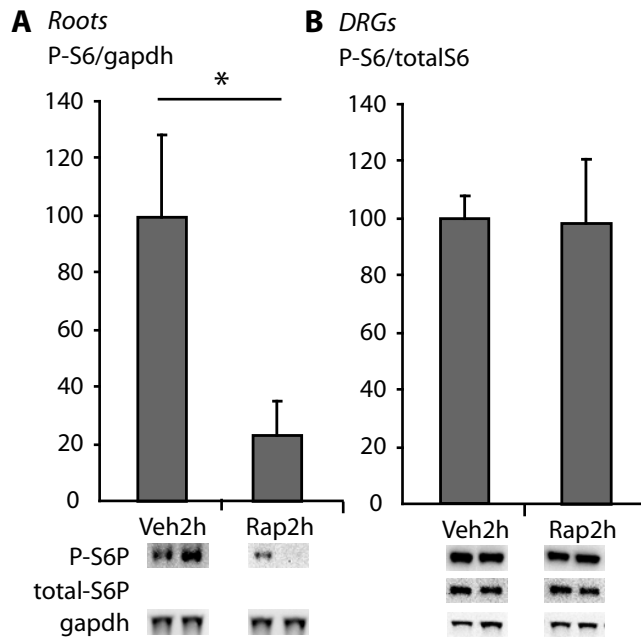


Fig. S2. Intrathecal administration of rapamycin decreases phosphorylation of the downstream target of mTORC1 S6 protein (S6) in the dorsal roots, but not in the DRGs. Immunoblots probed with anti-P-S6 antibody and anti-S6 antibody after gel electrophoresis of lysates from *A*, dorsal roots and *B*, L4-L6 DRGs. Animals received an intrathecal injection of rapamycin or vehicle 2 h before sacrifice. There was a significant reduction in S6 phosphorylation 2 h after rapamycin injection in the roots but not in the DRGs. N=3-4 in each condition. Mean \pm SEM is illustrated. * $P < 0.05$.

Spinal cord

P-4EBP/total4EBP

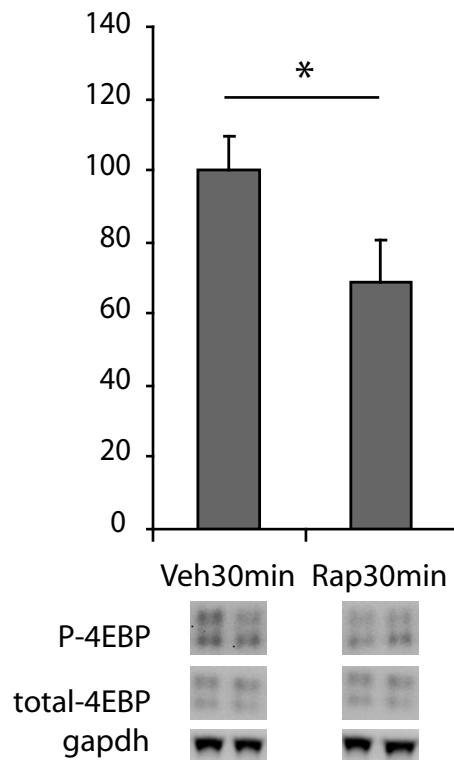


Fig. S3. Intrathecal administration of rapamycin decreases phosphorylation of 4E-BP1/2 in the spinal cord. Immunoblots probed with anti-P-4E-BP1/2 antibody after gel electrophoresis of lysates from spinal cord tissue. Animals received an intrathecal injection of rapamycin or vehicle 30 min before sacrifice. There was a significant reduction in 4E-BP1/2 phosphorylation 30 min after rapamycin injection in spinal cord tissue. N=3-4 in each condition. Mean \pm SEM is illustrated. * $P < 0.05$.

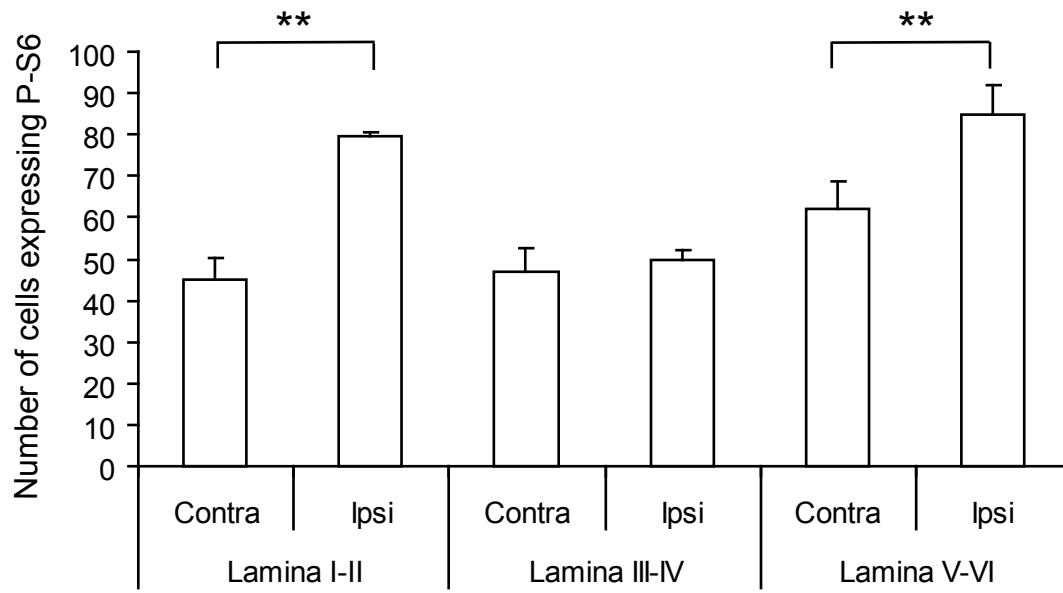


Fig. S4. Injection of capsaicin in the hindpaw increases the phosphorylation of S6 protein in the superficial dorsal horn. 2 h following capsaicin injection in the hindpaw, there was a significant increase in the number of cells expressing P-S6 in Lamina I and Lamina V to VI. Mean \pm SEM is illustrated. ** $P < 0.01$. N=4 in each group.

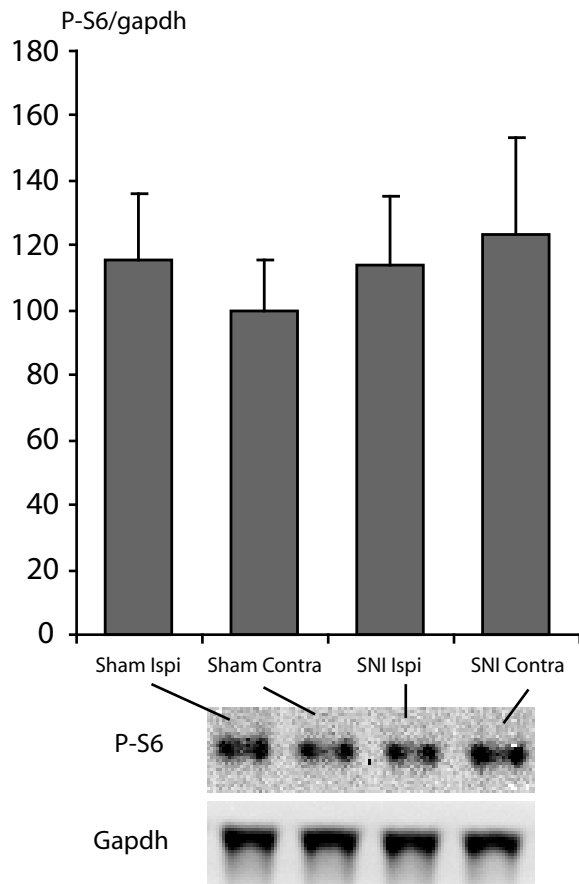
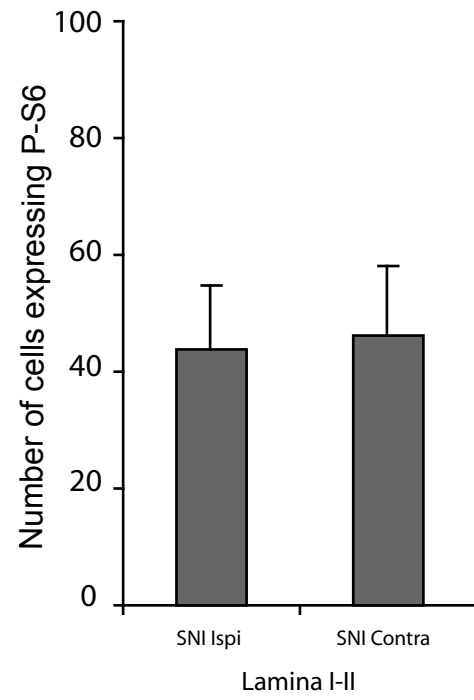
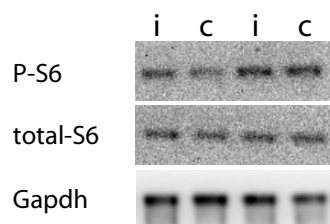
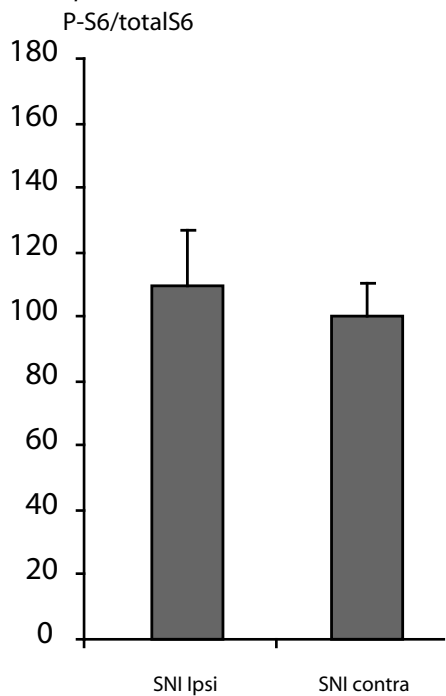
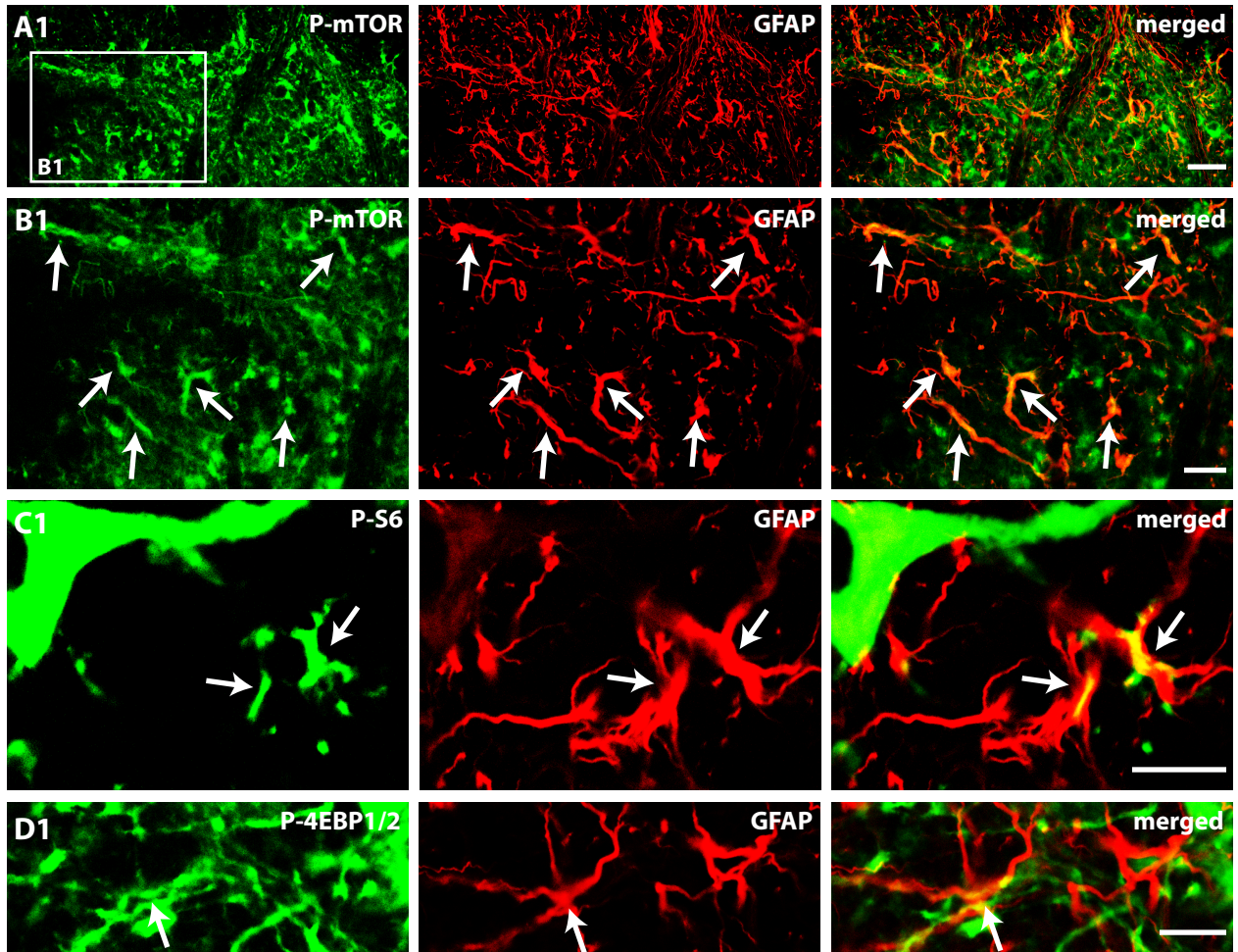
A *Roots***B** *Spinal cord***C** *Spinal cord*

Fig. S5. SNI surgery does not increase the phosphorylation of S6 protein in the dorsal roots and superficial dorsal horn. 7 days after SNI surgery, there was no difference in: *A/* the expression level of P-S6 in central roots, when compared to both sham and contralateral side (western blot analysis; data normalized to sham contra group; N = 4 in each group); *B/* the number of P-S6 expressing cells in the superficial dorsal horn of the spinal cord (specifically Lamina I-II; immunohistochemistry analysis; N = 3); *C/* the expression level of P-S6 in the ipsilateral dorsal horn when compared to the contralateral side (western blot analysis; N = 6). Mean \pm SEM is illustrated.

Ipsilateral to SNI surgery



Contralateral to SNI surgery

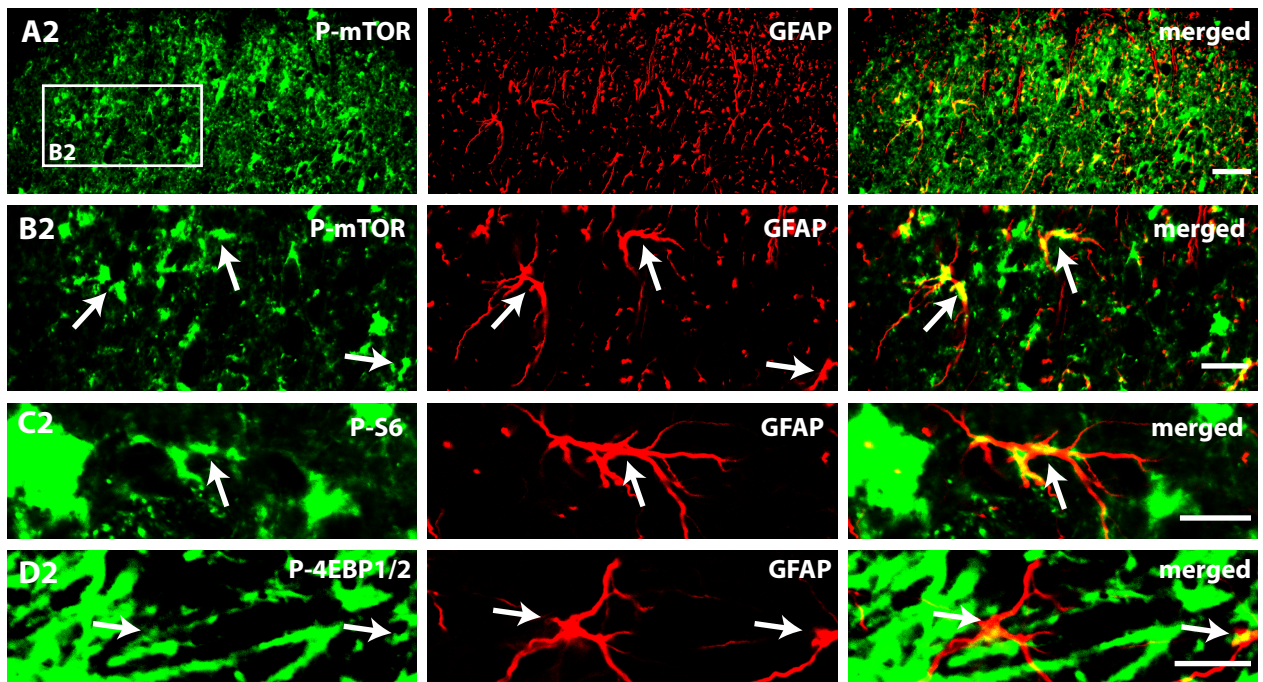


Fig. S6. P-mTOR, P-S6 and P-4EBP1/2 are expressed in astrocytes. 7 days after SNI surgery, animals were perfused and spinal cord tissue was cut in 40 μm sections and stained for P-mTOR, P-S6 or P-4EBP1/2, followed by GFAP (1:4000 O/N, Dako, Denmark). P-mTOR, P-S6 and P-4EBP1/2 were expressed in astrocytes both on the ipsilateral and the contralateral side of surgery. There was no evidence for increased glial expression of these translational markers 7 days following SNI surgery. Scale bars, *A1,2*: 100 μm ; *B1,2-D1,2*: 50 μm .