Figure S3
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Figure S3. Motoneuron death in a model of nerve axotomy is accompanied by increased LIGHT levels in the spinal cord. (A,B) Peripheral sciatic nerve axotomy in neonates induces death of motoneurons. (A) An unilateral axotomy of the sciatic nerve was performed in P3 mice, 10 days after the spinal cords were processed for VACHT immunostaining. (B) Motoneuron survival was determined by counting the number of VACHT+ motoneurons in the ipsi- and contralateral side of the spinal cord. Neuron survival is expressed relative to the control condition. (C,D) Levels of IFNγ increased in the spinal cord following axotomy. (C) A neonatal sciatic nerve axotomy was performed. Ten days after, total protein extracts of lumbar spinal cord from control and axotomized mice were assessed by Western blot analysis for IFNγ and actin immunoreactivity. (D) IFNγ signals was expressed relative to their respective actin signals, values were obtained from scanning densitometry of immunoblots (arbitrary units). (E,F) Levels of LT-βR are not modified following axotomy. The sciatic nerve from P3 mice was bilaterally sectioned. (E) Thirty microgram of total proteins from spinal cord of indicated groups were resolved by SDS-PAGE and immunoblotted with anti-LT-βR antibodies. Actin was used as a loading control. (F) Histogram of densitometric analysis of LT-βR levels normalized to actin levels. (G,H) Increased levels of LIGHT in the spinal cord following axotomy of the sciatic nerve. (G) Representative Western blot showing the expression of LIGHT in the spinal cord of sham-operated control and axotomized mice (10 days post-lesion). (H) The intensity of LIGHT signal was quantified by densitometric scanning and normalized to actin levels. Results shown in (B), (D), (F) and (H) are mean values ± SEM, n = 3, unpaired two-tailed t test, n.s., not significant. (I) An unilateral sciatic nerve crush was performed in 50-day-old mice. Ten 10 days after, the lumbar spinal cord was processed for VACHT immunolabeling. Scale bar, 50 µm. (J) Histogram represents the percentage of surviving motoneurons in the control and lesioned side (crush). The sham-operated side is taken as 100%. Values are means ± SEM, n = 4, unpaired two-tailed t test.