Activation of the Unfolded Protein Response enhances motor recovery after spinal cord injury.

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Supporting Information

Supplementary Figure legends

Figure S1 ATF4 expression and accumulation of ubiquitinated proteins in the spinal cord after hemisection. (**a**) Wild type mice were spinal cord hemisected at the T12 vertebral level. 1, 3 and 6 hours after surgery, spinal cord tissue from the operated region was extracted and processed. ATF4 protein levels were analyzed by Western blot and semi-quantified by normalizing to HSP90 protein levels. Blot at 1, 3 and 6 hours after spinal cord hemisection from the operated zone is shown. (**b**) Protein ubiquitination was studied by Western blot in tissue samples from spinal cord hemisected or sham operated wild type mice at the T12 vertebral level.

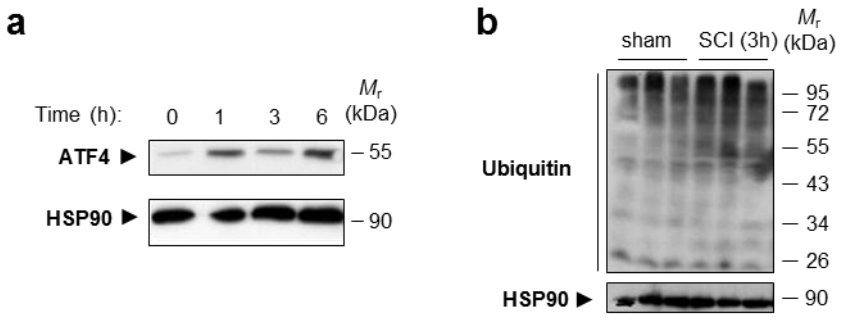
Figure S2 Altered cellular environment at 14 days post-SCI in UPR deficient mice. (**a**) Non-injured *atf4*$^{+/+}$ and *atf4*$^{-/-}$ mice were trained and scored by the RotaRod test (ramp mode). (**b**) *atf4*$^{+/+}$ and *atf4*$^{-/-}$ mice were spinal cord hemisected or sham operated at the T12 vertebral level. 14 days after surgery, spinal cord tissue processed for immunofluorescence, to study oligodendrocytes (OL) and OL progenitors (red); nuclei were counterstained using Hoechst (blue). Olig2-positive particles colocalizing with Hoechst were quantified every 50 µm starting at the injury site. (**c**) Spinal cord neurons were analyzed by immunofluorescence using an antibody against NeuN. Neuronal density

was analyzed in *atf4*$^{+/+}$ and *atf4*$^{-/-}$ mice 14 days after SCI in both contralateral and ipsilateral sides. (**c**) *atf4*$^{+/+}$ and *atf4*$^{-/-}$ mice were spinal cord hemisected or sham operated at the T12 vertebral level. 14 days after surgery, spinal cord tissue processed for immunofluorescence using an antibody against GFAP to study astrocytes (green); nuclei were counterstained using Hoechst (blue). GFAP-positive cells were quantified every 50 µm starting at the injury site (right panel). (**d**) Nuclear density in the injured region was analyzed by averaging Hoechst-positive nuclei intensity from 3 mice for each condition and displayed as a surface plot. The Z-axis represents average intensity in a pseudo-colored map. Mean +/- SEM. *, $p<0.05$; **, $p<0.005$; Student's *t*-test; n=3 animals per group for immunofluorescence analysis; n=10 for RotaRod test. Scale bars, 100 µm in (**a**), 200 µm in (**b**), 20 µm in (**c**), 300 µm in (**d**).

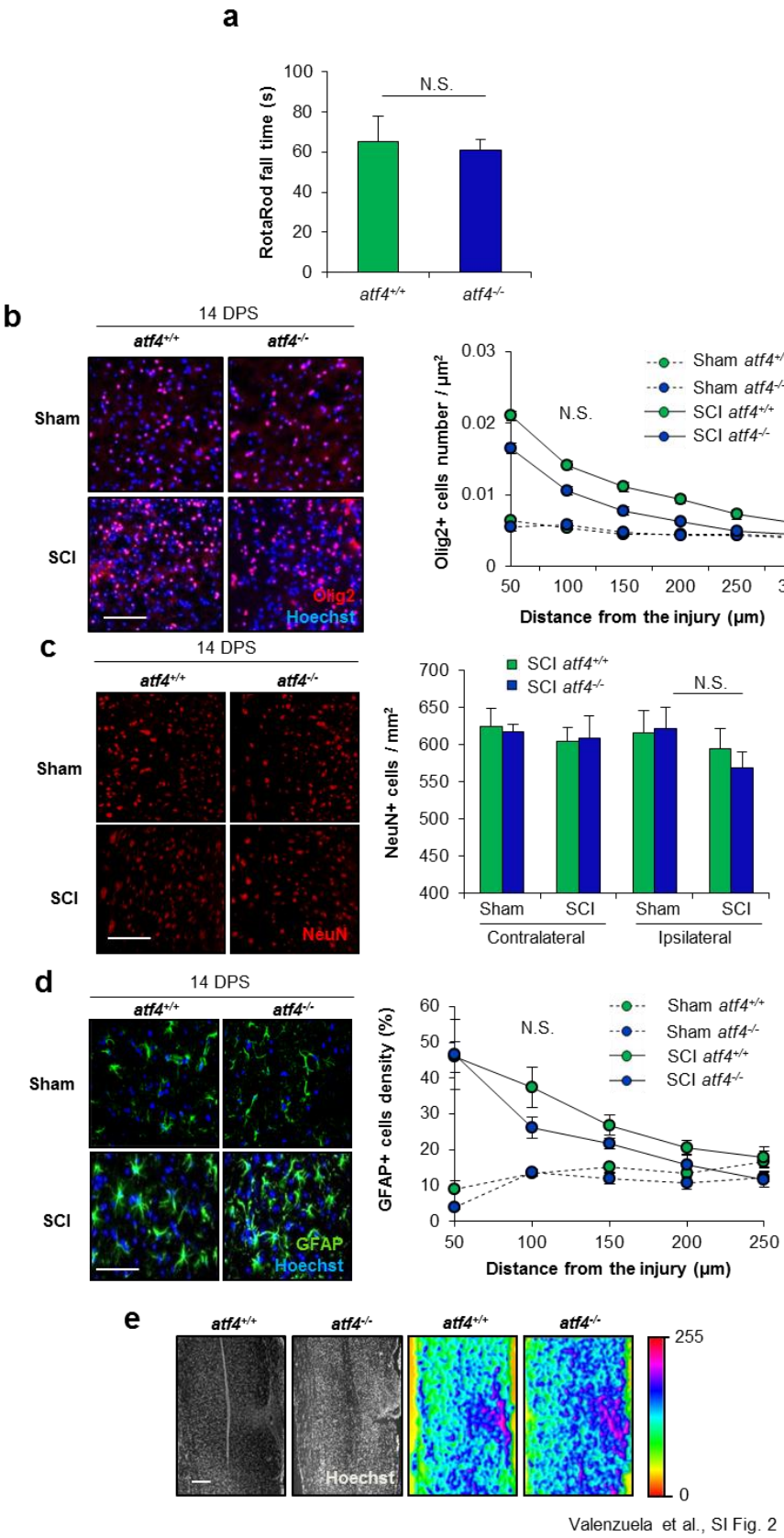
Figure S3 *Xbp1s* gene transfer with AAVs enhances locomotion recovery after SCI. (**a**) Wild type mice were hemisected at the T12 vertebral level and immediately injected into the injury site with 2 µl (10^{12} DRP/mL) of AAV-GFP or AAV-Xbp1s/GFP. Locomotion recovery pattern was monitored before (0d) and after spinal cord hemisection and viral transduction with AAV-GFP or AAV Xbp1s/GFP using the Basso Mouse Scale (BMS) open field test. The BMS subscore, which assess fine locomotor capabilities was scored and individual parameters of this subscore are presented at 28 and 35 days post-SCI. Mean +/- SEM. *, $p<0.05$; **, $p<0.005$. n=8 animals per group. Student's *t*-test. (**b**) Transduction levels were analyzed 35 days post-injection by GFP expression (green), immunolabelled with an Olig2 antibody (red) and counterstained with Hoechst (blue) in the AAV-Xbp1s/GFP injections. Higher exposure of GFP fluorescence reveal AAV transduction of Olid2-positive cells (arrows) in addition to neurons (arrowheads). Scale bar, 50 µm.

Figure S4 Analysis of UPR markers from the microarray GDS2159 in the T8 spinal cord segment up to 28 days after moderate contusion injury. (**a**) Expression levels of the

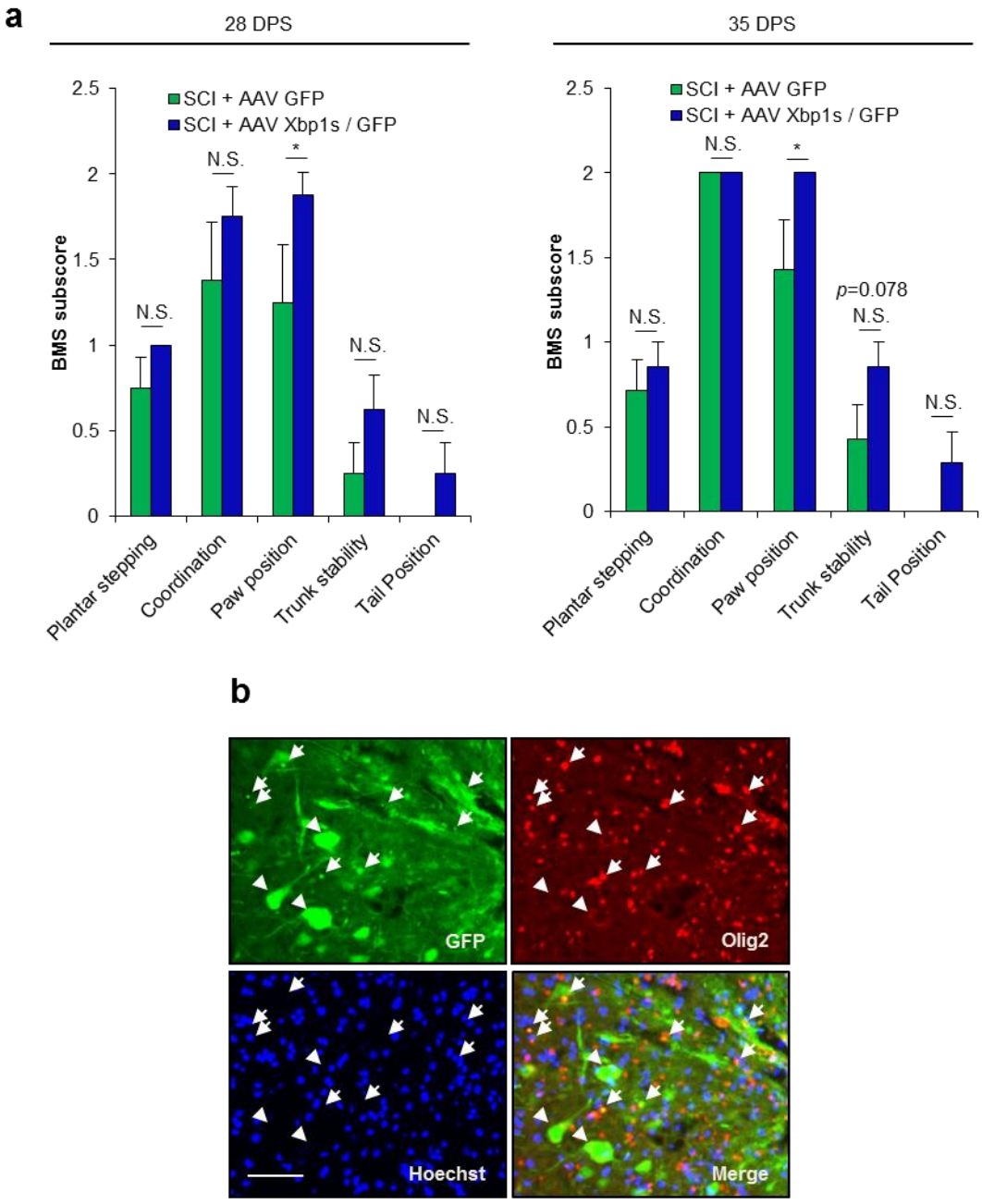
indicated genes at different times after moderate spinal cord contusion in wild type mice obtained from the GDS2159 microarray data. Relative expression levels were normalized with actin mRNA levels and then sham normalized. (**b**) Table showing relative fold-induction values of UPR markers after moderate spinal cord contusion in wild type mice relative to control values (non-injured). Data was obtained from the microarray GDS2159 available at GEO dataset browser (http://www.ncbi.nlm.nih.gov/sites/GDSbrowser?acc=GDS2159).



Valenzuela et al., SI Fig. 1

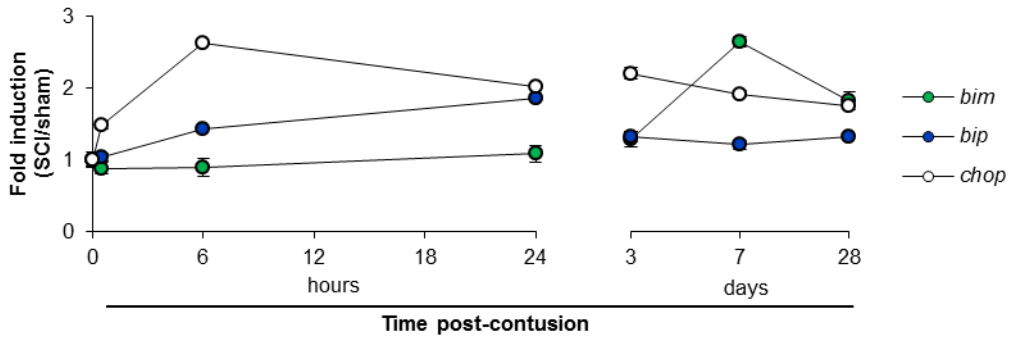


Valenzuela et al., SI Fig. 2



Valenzuela et al., SI Fig. 3

a



b

	Time post-surgery						
	hours				days		
Fold induction (SCI / sham)	0	0.5	6	24	3	7	28
edem	1	0.84	0.88	1.22	1.87	1.62	1.32
sec61	1	0.92	0.99	2.14	1.57	2.13	1.53
grp58	1	0.78	0.92	1.23	2.10	2.81	1.29
xbp-1	1	1.05	1.13	1.50	1.16	1.04	1.34
grp78	1	1.06	1.47	1.90	1.36	1.24	1.36
ho1	1	1.08	2.38	5.68	3.23	1.93	1.39
bim	1	0.89	0.9	1.11	1.29	2.65	1.83
atf4	1	1.16	1.74	2.06	1.24	1.20	0.89
chop	1	1.49	2.64	2.03	2.21	1.95	1.75
calnexin	1	1.03	1.11	1.26	0.86	0.95	1.01
calreticulin	1	1.13	1.09	1.57	1.06	1.18	1.29
actin	1	0.97	0.96	1.16	0.93	0.85	1.18

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