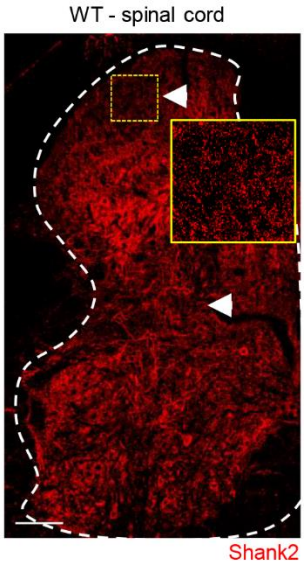
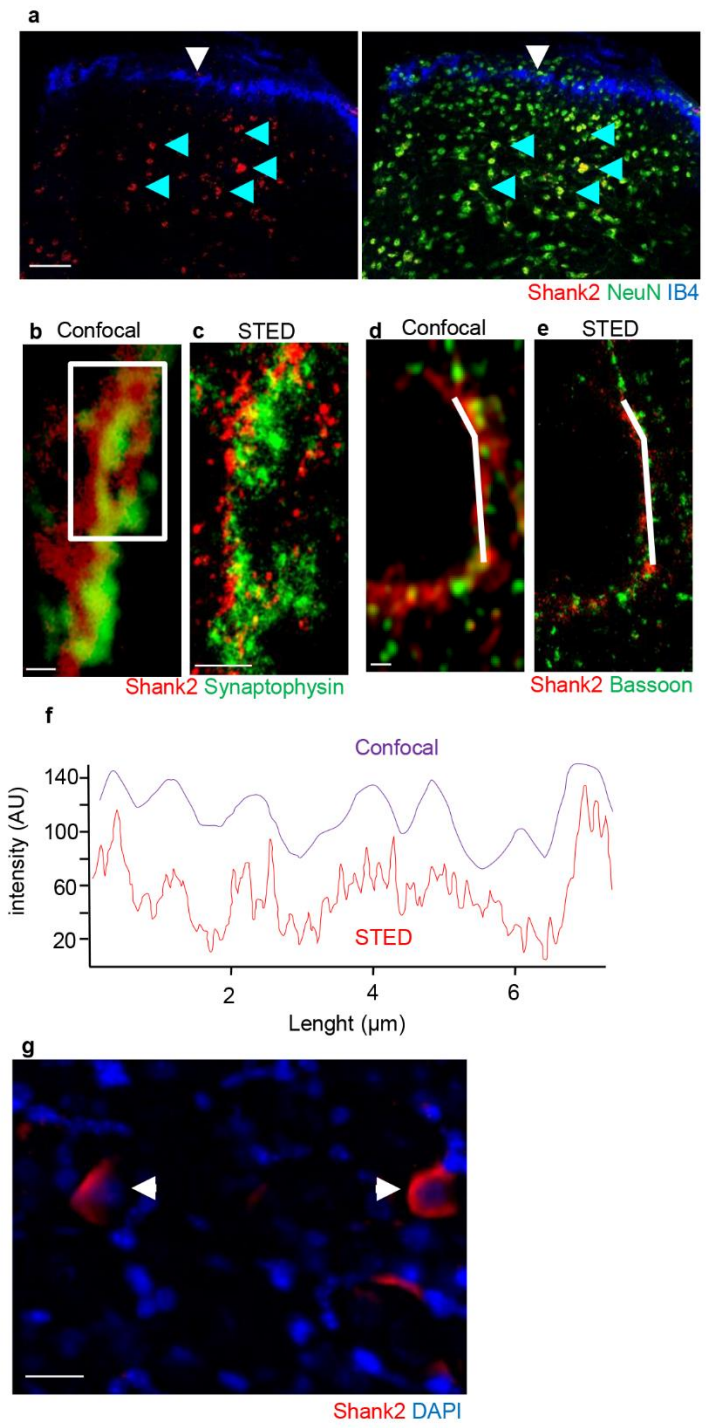
Additional File 1

- Supplementary Figure 1
- Supplementary Figure 2
- Supplementary Figure 3
- Supplementary Figure 4
- Supplementary Figure 5
- Supplementary Figure 6
- Supplementary Figure 7
- Supplementary Table 1
- Supplementary Table 2
- Source data file 1



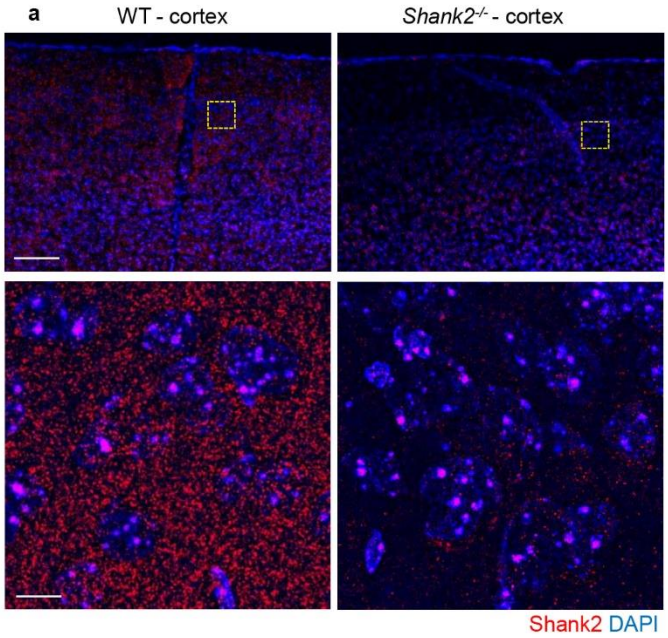
Supplementary Figure 1: Shank2 spinal cord localisation.

Immunofluorescence staining of WT spinal cord sections (unfiltered image from figure 2a) show differential expression level of Shank2 throughout the spinal cord, with a baseline level of Shank2 in laminae I, II and laminae VII (white arrows), a higher expression of Shank2 was observed in laminae III-V (Shank2^{high} cells) and in the ventral horn. N = 3. Scale bar 100µm.



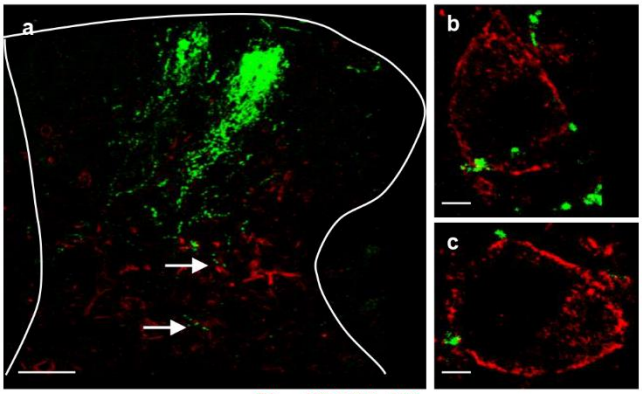
Supplementary Figure 2: Shank2 architecture and localisation.

(a) Confocal images of Shank2 together with IB4 identify Shank2^{high} neurons are located below laminae II (approx. 90%), with a small number of Shank2^{high} neurons in the IB4 ribbon. **(b)** Confocal images of Shank2 and Synaptophysin show a band of Shank2 colocalized with Synaptophysin; (N = 3) scale bar: 500nm. **(c)** STED images of Shank2 and Synaptophysin reveal the punctuated pattern of Shank2 and Synaptophysin; (N = 3) scale bar: 500nm. **(d)** Confocal images of Shank2 and Bassoon show a band of Shank2 colocalized with Bassoon puncta; (N = 3) scale bar: 500nm. **(e)** STED images of Shank2 and Bassoon reveal the punctuated pattern of Shank2 with Bassoon; (N = 3) scale bar: 500nm. **(f)** Graphical view of the fluorescence intensity of STED imaging in comparison to confocal microscopy. **(g)** Dorsal root ganglion staining with Shank2 revealed low shank2 intensity in only a few cells; scale bar: 10µm.



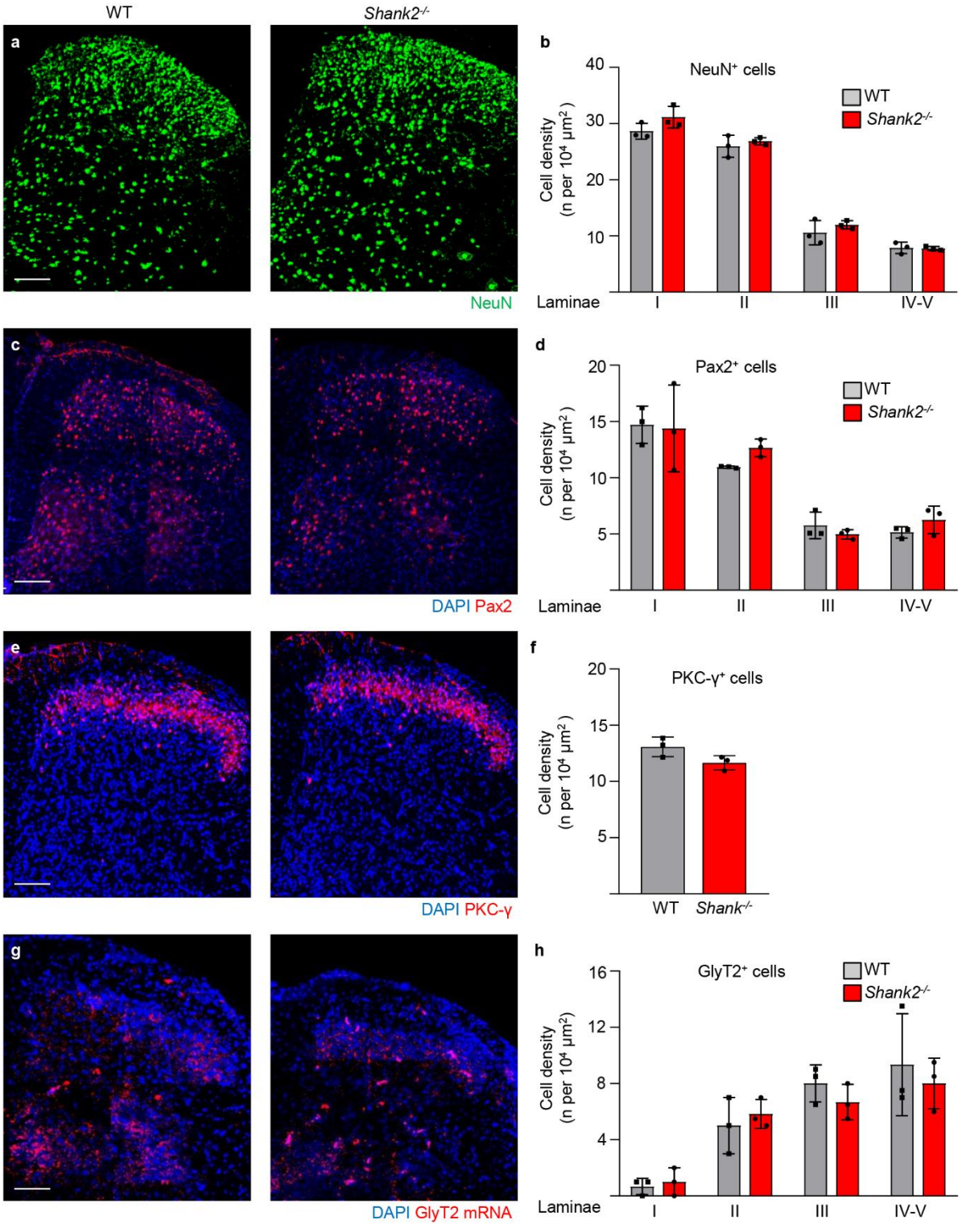
Supplementary Figure 3: Loss of Shank2 immunoreactivity in cerebral cortex from Shank2$^{-/-}$ mice.

(a) Shank2 immunofluorescence staining of WT and Shank2$^{-/-}$ cortical sections shows a homogeneous expression of Shank2 in WT and little to no expression in Shank2$^{-/-}$ sections. N = 3. Scale bar overview: 100µm; insert: 10µm.



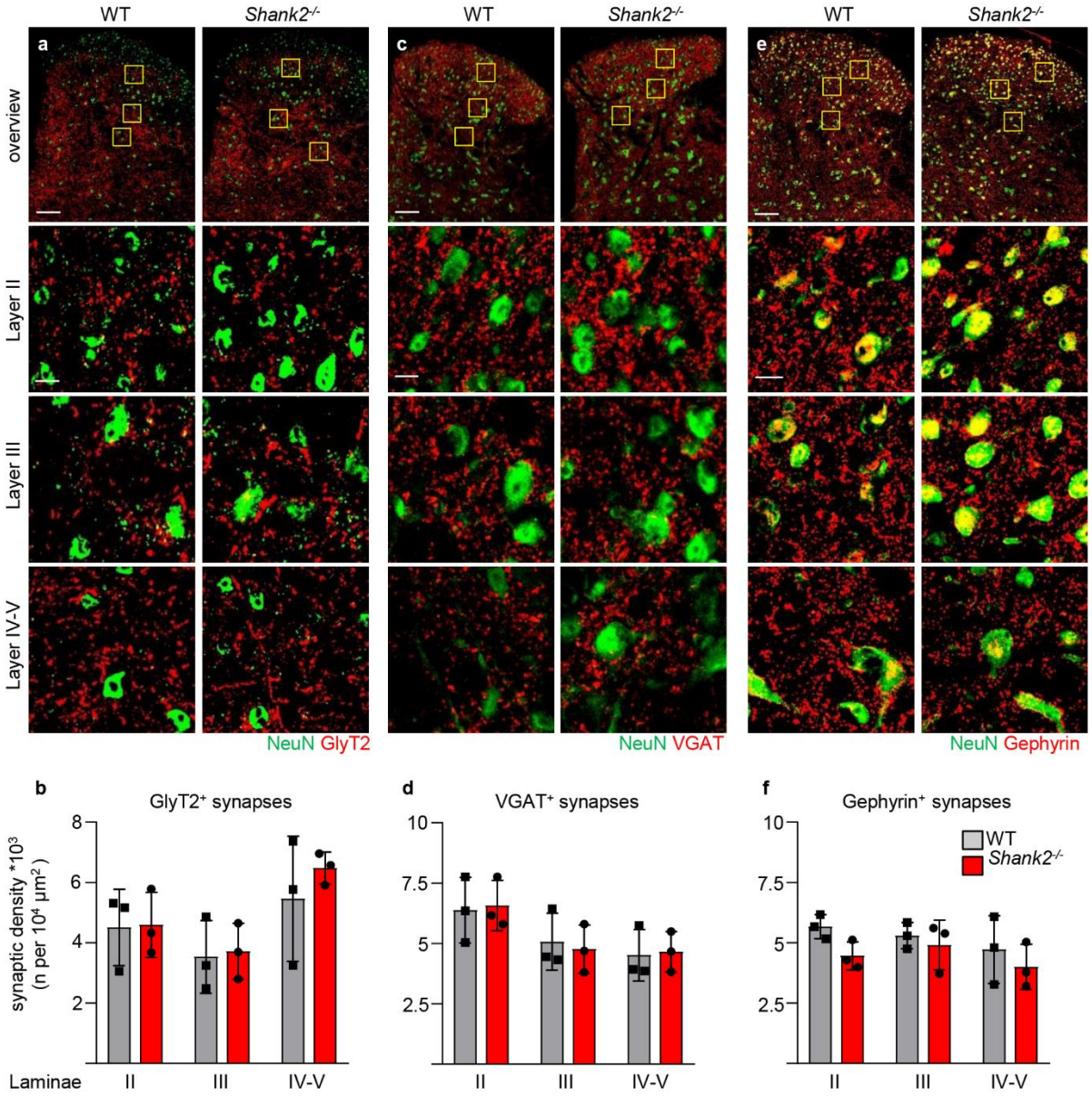
Supplementary Figure 4: CTB-488 injections reveal cutaneous afferents on Shank2^{high} expressing neurons.

(a-c) Detection of GFP positive buttons on Shank2 positive terminals after Cholera Toxin B subunit injection in the hairy skin to show cutaneous afferents on Shank2^{high} expressing neurons; (N = 3) scale bar; A: 50µm, scale bar B and C: 5µm.


WT
Shank2-/-
a
NeuN
b
NeuN+ cells
Cell density (n per 10⁴ µm²)
WT
Shank2-/-
Laminae
I
II
III
IV-V
c
DAPI Pax2
d
Pax2+ cells
e
DAPI PKC-γ
f
PKC-γ+ cells
WT
Shank-/-
g
DAPI GlyT2 mRNA
h
GlyT2+ cells

Supplementary Figure 5: No difference in neuronal architecture in the spinal cord of Shank2$^{-/-}$ mice.

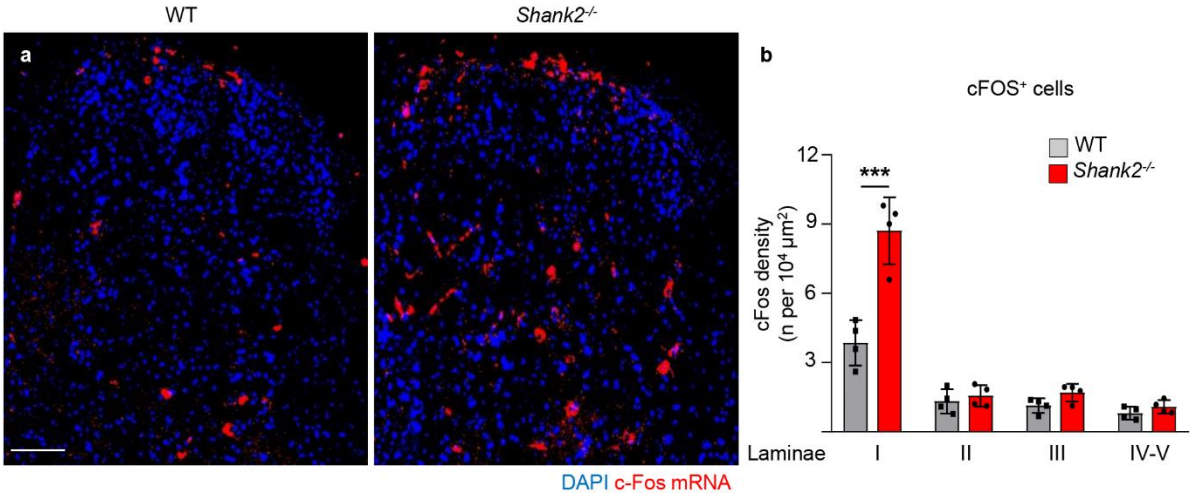
(a, b) Neu-N staining in the spinal cord reveals no differences in Neuronal counts between the WT and Shank2$^{-/-}$ across Laminae (29.4±1.9 vs 30.3±2.3; 26.1±1.6 vs 25.5±2.7; 11.1±2.1 vs 12.0±0.6; 7.8±0.8 vs 7.9±0.4; per 10^4 µm^2; Laminae I-IV/V respectively; WT vs Shank2$^{-/-}$; p>0.05); (N = 3) scale bar: 100nm. **(c, d)** PAX2 staining in the spinal cord shows no differences in the density of inhibitory neurons between the WT and Shank2$^{-/-}$ (15.2±1.4 vs 15.0±3.2; 11.2±0.5 vs 12.2±0.7; 5.9±1.1 vs 4.7±0.7; 5.3±0.4 vs 5.7±1.0; per 10^4 µm^2; Laminae I-IV/V respectively; WT vs Shank2$^{-/-}$; p>0.05); (N = 3) scale bar: 100nm. **(e, f)** PKC-y staining in the spinal cord results in no difference in the number of excitatory cells between WT and Shank2$^{-/-}$ (13.2±1.5 vs 11.7±1.3; per 10^4 µm^2; WT vs Shank2$^{-/-}$; p>0.05); (N = 3) scale bar: 100nm. **(g, h)** single mRNA in situ hybridisation with GlyT2 in the spinal cord reveals no differences in the density of Glycinergic interneurons between WT and Shank2$^{-/-}$ (0.1±0.1 vs 0.1±0.2; 1.4±0.5 vs 1.5±0.4; 1.6±0.5 vs 1.7±0.4; 2.2±0.7 vs 2.1±0.5; per 10^4 µm^2; Laminae I-IV/V respectively; WT vs Shank2$^{-/-}$; p>0.05); (N = 3) scale bar: 100nm. Data shown as average±SD.



Supplementary Figure 6: No difference in inhibitory synapses in the spinal cord of Shank2$^{-/-}$ mice.

(a, b) GlyT2 staining in the spinal cord reveals no differences in the GlyT2 synaptic density between WT and Shank2$^{-/-}$ across all laminae (4.5±1.6 vs 4.6±1.4; 3.5±1.7 vs 3.7±1.1; 5.5±2.1 vs 6.5±8.8 *10^3; per 10^4 µm^2; Laminae II, III and IV respectively; WT vs Shank2$^{-/-}$; p>0.05); (N = 3). **(c, d)** VGAT staining in the spinal cord reveals no differences in the VGAT synaptic density between WT and Shank2$^{-/-}$ across all laminae

(6.3±1.9 vs 6.5±1.5; 4.9±1.6 vs 4.7±1.4; 4.4±1.4 vs 4.6±1.0 *10^3; per 10^4 µm^2; Laminae II, III and IV respectively; WT vs Shank2$^{-/-}$; p>0.05); (N = 3). **(e, f)** Gephyrin staining in the spinal cord reveals no differences in the Gephyrin synaptic density between WT and Shank2$^{-/-}$ across all laminae (5.7±1.0 vs 4.5±1.6; 5.3±1.1 vs 4.9±1.8; 4.7±1.8 vs 4.0±1.5 *10^3; per 10^4 µm^2; Laminae II, III and IV respectively; WT vs Shank2$^{-/-}$; p>0.05); (N = 3). Scale bar overview: 100µm, scale bar insert: 10µm. Data shown as average±SD.



Supplementary Figure 7: Increased number of cFos mRNA+ interneurons in Laminae I upon Formalin injection

(a, b) Single mRNA detection show a strong increase in c-fos expressing cells in laminae I ($p<0.001$), however this effect disappears in Laminae II-IV/V (1.5±0.6 vs 1.6±0.5; 1.2±0.3 vs 1.7±0.4; 0.8±0.2 vs 1.1±0,3; per 10^4 µm^2; Laminae II-IV/V respectively; WT vs Shank2$^{-/-}$; $p>0.05$) upon formalin injection; (N = 4) scale bar A: 100µm. Data shown as average±SD. *** $p < 0.001$.

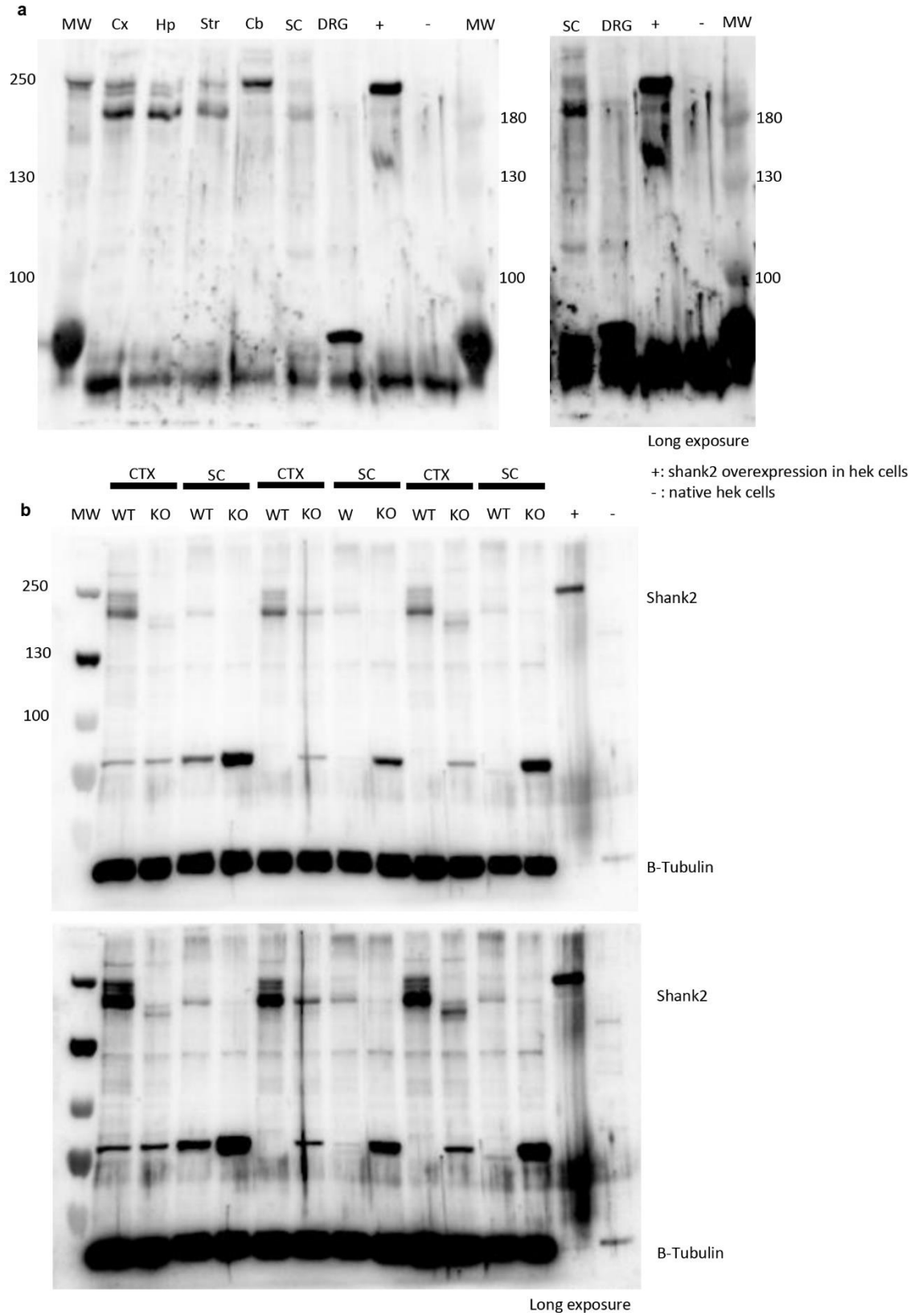
Antigen (primary antibody)	Catalogue number	Company	Dilution
Chicken anti GFP	Ab13970	Abcam	1:1000 (IF)
Rabbit anti RFP	600-401-379	Rockland	1:500 (IF)
Goat anti Parvalbumin	PVG-214	Swant	1:2000 (IF)
Mouse anti Gephyrin	147011	Synaptic Systems	1:500 (IF)
Goat anti Glycine Transporter 2	SC-16705	Santa Cruz	1:200 (IF)
Rabbit anti Glycine Transporter 2	272003	Synaptic Systems	1:200 (IF)
Mouse anti NeuN	MAB377	Millipore	1:100 (IF)
Guinea pig anti vGlut1	135304	Synaptic Systems	1:500 (IF), 1:200 (RNAscope, clearing)
Guinea pig anti vGlut2	135404	Synaptic Systems	1:500 (IF), 1:200 (RNAscope)
Guinea pig anti VGAT	131004	Synaptic Systems	1:500 (IF)
Rabbit anti c-fos	SC-52	Santa Cruz	1:500 (IF)
Mouse anti Bassoon	ADI-VAM-PS003-D	Enzo Life Sciences	1:500 (IF, STED)
Mouse anti Synaptophysin	MAB5258	Millipore	1:1000 (IF, STED)
Rabbit anti Pax-2	PRB-2768	Covance	1:500 (IF)
Rabbit anti PKC-gamma	Ab71558	Abcam	1:200 (IF)
Rabbit anti Shank2	Homemade	SA5192	1:500 (IF, STED, WB), 1:200 (Clearing)
Rabbit anti Homer 1b/c	160022	Synaptic Systems	1:200 (RNAscope)
Mouse anti GluN1	114011	Synaptic Systems	1:200 (RNAscope)
Mouse anti Neurofilament Marker	SMI-311R	Biolegend	1:300 (IF)
Anti Gs-IB4 Alexa Fluor 488 conjugated	121411	Invitrogen	1:1000 (IF)
Chicken anti MAP2	CPCA-MAP2	Encor	1:200

Antigen (secondary antibody)	Catalogue number	Company	Dilution
Donkey anti mouse 488	A21202	Invitrogen	1:500
Donkey anti mouse 568	A10037	Invitrogen	1:500
Donkey anti rabbit 488	A21206	Invitrogen	1:500
Donkey anti rabbit 568	A10042	Invitrogen	1:500
Donkey anti rabbit 647	A31573	Invitrogen	1:500
Donkey anti chicken 488	SAB4600031	Sigma	1:500
Donkey anti goat 568	A11057	Invitrogen	1:500
Donkey anti goat 633	A21082	Invitrogen	1:500
Donkey anti guinea pig 568	20377	Biotium	1:500
Goat anti mouse 594 (Atto)	76085-1ML-F	Sigma-Aldrich	1:1000 (STED)
Goat anti rabbit 647 (Atto)	40839-1ML-F	Sigma-Aldrich	1:1000 (STED)
Goat anti guinea pig 633	A21105	Invitrogen	1:500

Supplementary Table 1: Primary and secondary antibodies used for immunostaining (IF) Fluorescent in Situ Hybridisation (RNAscope) or for western blot (WB).

Lab ID Nr.	Gender	Age
34	Female	69
35	Male	75
39	Female	84
41	Female	85
44	Male	94

Supplementary Table 2: Full details regarding human samples.


a
MW Cx Hp Str Cb SC DRG + - MW
250
130
100
180
130
100
SC DRG + - MW
180
130
100
Long exposure
+: shank2 overexpression in hek cells
- : native hek cells
CTX SC CTX SC CTX SC
b
MW WT KO WT KO WT KO W KO WT KO WT KO + -
250
130
100
Shank2
B-Tubulin
Shank2
B-Tubulin
Long exposure

Source data file 1: uncropped Western Blot of Shank2 in mouse tissue

(a) Shank2 western blot (WB) on mouse tissue samples of various central nervous system (CNS) regions; Cortex (Cx), Hippocampus (Hp), Striatum (Str); Cerebellum (Cb), Spinal Cord (SC), Dorsal Root Ganglia (DRG), positive control (+) and negative control (-). **(b)** Shank2 WB on cortex and spinal cord tissue of WT and Shank2$^{-/-}$ (KO) mice.