SUPPLEMENTAL FIGURE LEGENDS:

Impaired glutamate recycling and GluN2B-mediated neuronal calcium overload in mice lacking TGF-β1 in the CNS

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Supplemental Figure 1 (S1): Schematic of the TGF-β1-transgene introduced in T-lymphocytes. In [IL2-TGFβ1Tg] mice, activation of the T-cell receptor (TCR) drives the expression of TGF-β1 in T-cells through the T-cell specific Il-2 promoter.

Supplemental Figure 2 (S2): Pathological examination of CNS-TGFβ1$^{-/-}$ mice (A) CNS-TGFβ1$^{-/-}$ mice do not show any gross pathological abnormities as compared to CNS-TGFβ1$^{+/+}$ or wild-type mice at age of 4 (as shown here) and 8 wks. **(B)** CNS-TGFβ1$^{-/-}$ mice (third row) do not show inflammatory infiltrates as seen in TGF-β1$^{-/-}$ animals (second row) and they appear similar to CNS-TGFβ1$^{+/+}$ mice (first row). Photomicrographs show representative H & E stained tissue slices from heart, lung, liver, pancreas and stomach from all genotypes at the age of 4 weeks. Note strong cell infiltrates and tissue damage in TGF-β1$^{-/-}$ mice.

Supplemental Table 1: Blood Chemistry results from CNS-TGFβ1$^{-/-}$ and CNS-TGFβ1$^{+/+}$ mice. Representative blood results from CNS-TGFβ1$^{-/-}$ and CNS-TGFβ1$^{+/+}$ mice. Venous blood was taken from the tail vain of both genotypes and analyzed. Results display mean values from n = 3/genotype.

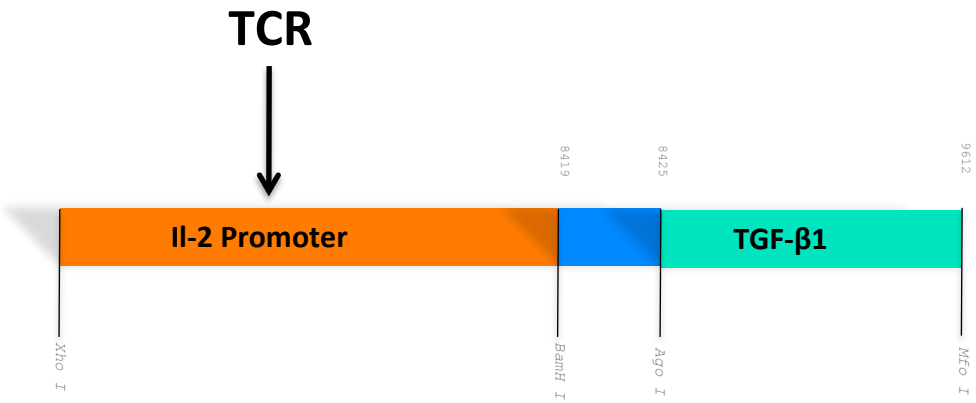
Supplemental Figure 3 (S3): The CNS of CNS-TGFβ1$^{-/-}$ mice is deprived of TGF-β1. (A) TGF-β1 mRNA (expression normalized to GAPDH) is undetectable in the hippocampus of CNS-TGFβ1$^{-/-}$ mice (n=3) whereas the expression of TGF-β2 and TGF-β3 mRNA remains unaffected. (**B**) Transgenic T-cells do not secrete TGF-β1 into the serum of CNS-TGFβ1$^{-/-}$ mice since TGF-β1 was absent from the serum of those mice ($p < 0.001$). (**C-D**) Representative results from fluorescent-activated cell sorting (FACS) analysis show the quantification of CD4^{+} and CD8^{+} T-lymphocytes isolated from the brain of 3-month old CNS-TGFβ1$^{-/-}$ mice (**C**) or wild-type mice that have been induced with EAE (**D**). Note high amounts of T-lymphocytes in the CNS of EAE mice (upper left and lower right quadrant in the dot plot) as compared to low T-lymphocyte counts in the brain of CNS-TGFβ1$^{-/-}$ mice. (**E-F**) The protein phosphorylation level of Smad2 and Smad3 is equivalent in CNS-TGFβ1$^{+/+}$ and CNS-TGFβ1$^{-/-}$ mice (n = 3). Bar graphs represent relative P-Smad/Smad ratios, normalized to the value of CNS-TGFβ1$^{+/+}$ mice.

Supplemental Figure 4 (S4): CNS-TGFβ1$^{-/-}$ mice do not show signs of enhanced neuronal cell death (A-F) Representative microscopic images from CNS-TGFβ1$^{-/-}$ mice and CNS-TGFβ1$^{+/+}$ mice (Scale bar: 100 µm). Brain tissue and spinal cord sections were tripple-stained for NeuN/Cleaved Caspase 3 and TUNEL and analysed by confocal microscopy. White inserted box shows a high magnification photomicrograph (Scale bar: 10 µm). (**G-I**) The number of TUNEL- and cleaved caspase-3 double-positive cells was not significantly different between the two genotypes in neither of the analysed regions. (CNS-TGFβ1$^{+/+}$: 5.08±0.78/section, CNS-TGFβ1$^{-/-}$: 4.97±0.47/section, n = 40/3 (Sections/Animals), $p > 0.05$; Hippocampus CNS-TGFβ1$^{+/+}$: 19.64±1.58/section, CNS-TGFβ1$^{-/-}$: 22.09±1.24/section, n = 33/3 (Sections/Animals), $p > 0.05$; Spinal Cord CNS-TGFβ1$^{+/+}$: 2.25±0.41/section, CNS-TGFβ1$^{-/-}$: 2.56±0.72/section, n = 16/3 (Sections/Animals), $p > 0.05$). [*$p < 0.05$; **$p<0.01$; ***$p<0.001$]

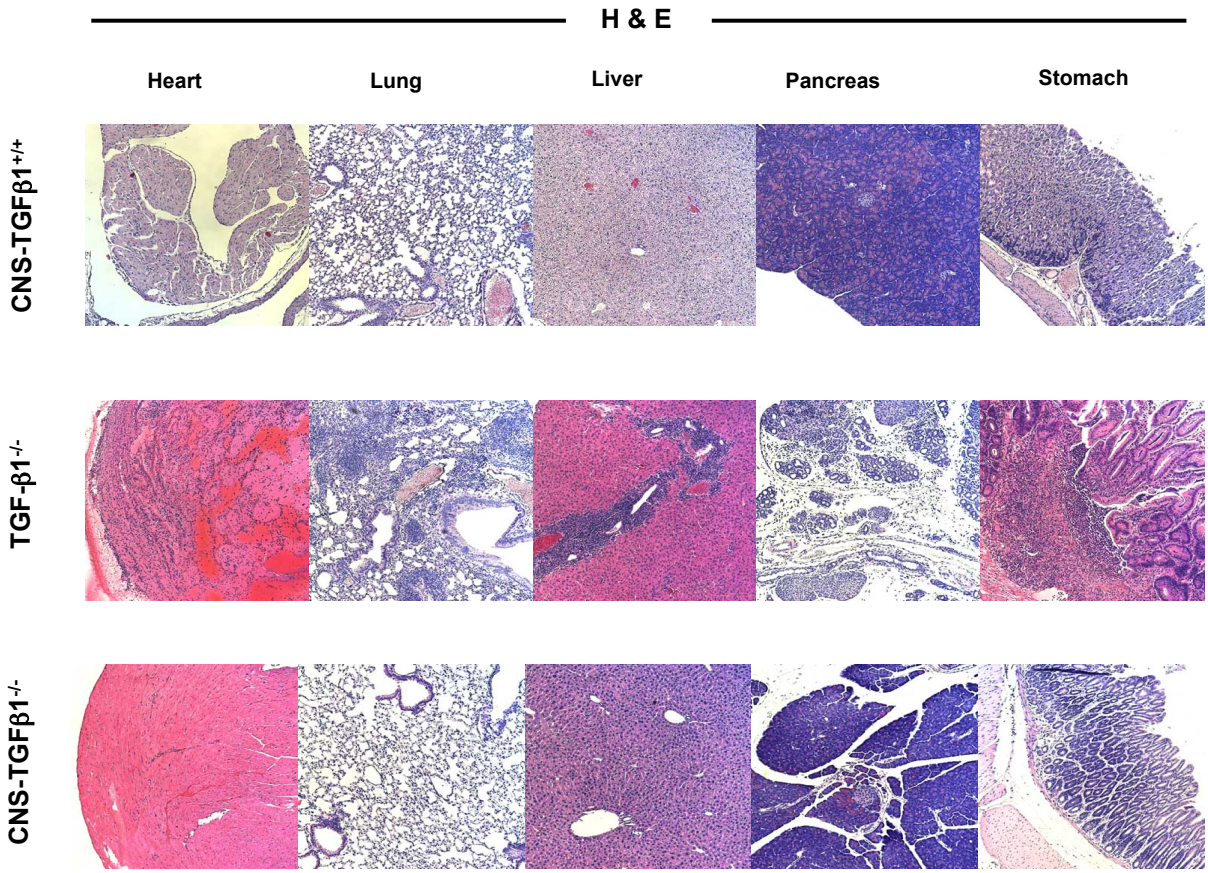
Supplemental Figure 5 (S5): CNS-TGF-β1-deficient mice display increased numbers of astrocytes in the hippocampus. (A) Photomicrographs showing GFAP- and S100B-stained hippocampal sections from CNS-TGFβ1$^{-/-}$ and CNS-TGFβ1$^{+/+}$ mice (Scale bar: 50 μm). **(B)** Bar graph illustrates the number of S100B-positive astrocytes/mm^2 in CNS-TGFβ1$^{-/-}$ and CNS-TGFβ1$^{+/+}$ mice. CNS-TGFβ1$^{-/-}$ animals display increased numbers of S100B-positive astrocytes in the hippocampus. CNS-TGFb1$^{+/+}$: 120.8±10.1/section, CNS-TGFb1$^{-/-}$:177.6±8.2/section, n = 6/3 (sections/animals), p<0.001). [*p < 0.05; **p<0.01; ***p<0.001].

Supplemental Figure 6 (S6): Protein abundance of glutamate receptors and PSD-95 in CNS-TGFβ1$^{-/-}$ and CNS-TGFβ1$^{+/+}$ mice. (**A**) Representative Western blots illustrating the protein amount of glutamate receptors in CNS-TGFβ1$^{-/-}$ mice and CNS-TGFβ1$^{+/+}$ mice. (**B**) TGF-β1-deficient mice display equal amounts of PSD95, GluR2, GluN1, GluN2A and GluN2B protein in the hippocampus.

Supplementary Figure 7 (S7): CNS-TGFβ1$^{-/-}$ mice display increased sensitivity to excitotoxic injury. (A) Representative photomicrographs (Scale bar: 50 μm) from hippocampus tissue sections 5 days after mice had been subjected to kainic acid-induced seizures (dotted white line indicates the location of the CA3 hippocampal pyramidal cell layer). Sections from either genotype were stained with IBA1 (labels microglia and macrophages), NeuN (labels neuronal nuclei) and TUNEL (labels apoptotic nuclei). (**B**) Statistical analysis demonstrates that CNS-TGFβ1$^{-/-}$ mice had increased numbers of TUNEL-positive cells in the hippocampus as compared to CNS-TGFβ1$^{+/+}$ animals. (# TUNEL$^+$/Caspase$^+$ cells/ section CNS-TGFβ1$^{+/+}$: 55.56±2.71, n = 25/3 (Sections/Animals), CNS-TGFβ1$^{-/-}$: 116.7±7.42, n = 25/3 (Sections/Animals), P < 0.001). [*p < 0.05; **p<0.01; ***p<0.001]



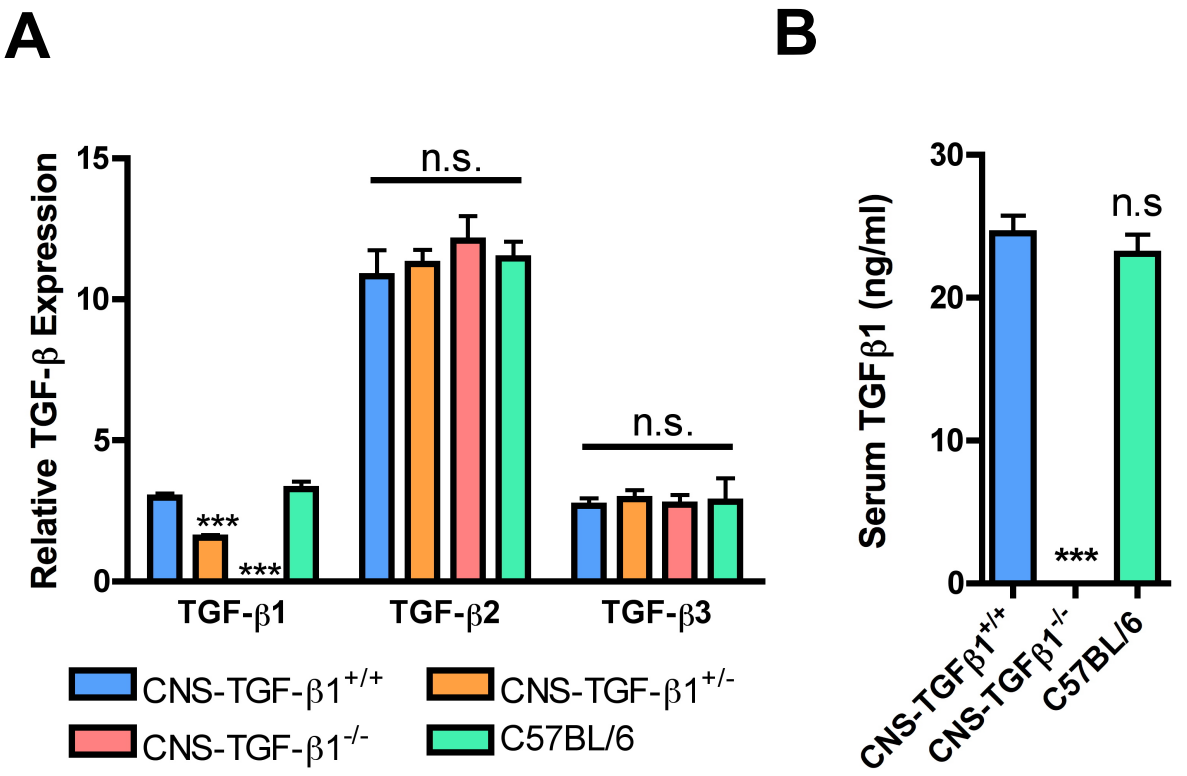
– Supplemental Figure 1 (S1) –

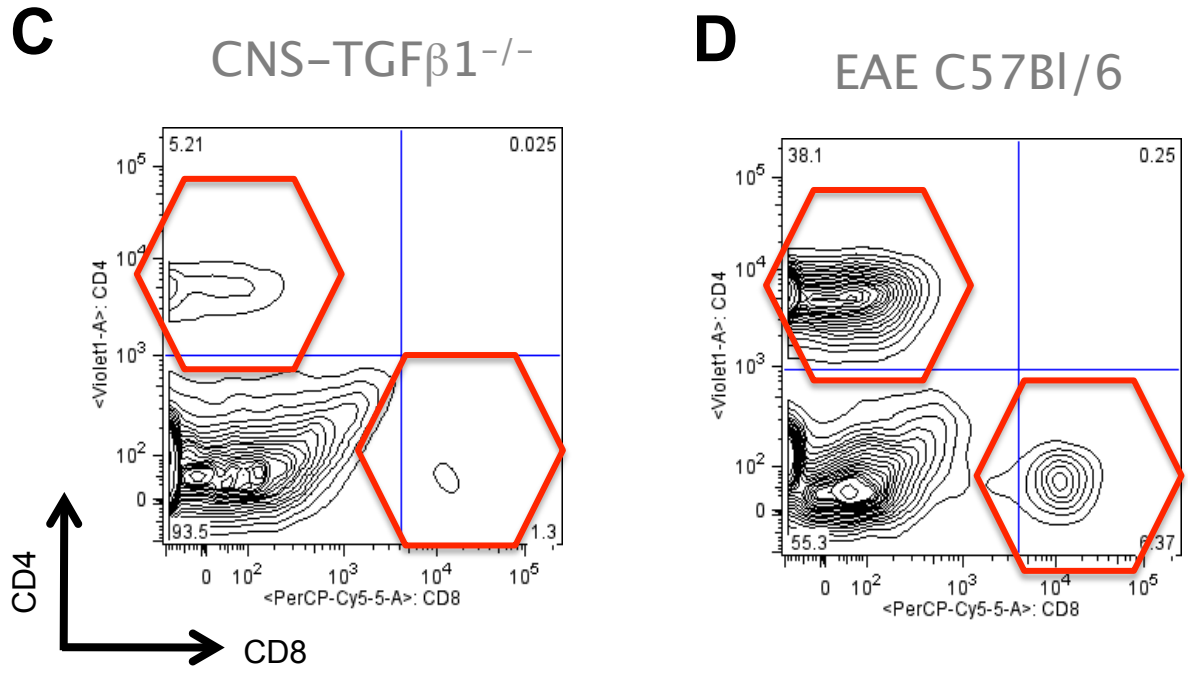


– Supplemental Figure 2 (S2) –

Test	CNS-TGFβ1$^{+/+}$	CNS-TGFβ1$^{-/-}$	Reference Range	Units
Renal & Glucose				
Glucose	186	208	50-118	mg/dl
Urea	36	22	4-25	mg/dl
Creatinine	0.26	0.22	0.1-1.3	mg/dl
Sodium	151	152	135-145	mmol/l
Potassium	7.6	6.9	3.5-7.0	mmol/l
Chloride	116	108	96-108	mmol/l
Total CO_2	24	27	23-32	mmol/l
Anion Gap	11	17	4-12	mmol/l
Enzymes & Billirubin				
ALT/GPT	29	24	0-54	U/l
AST/GOT	159	48	9-74	U/l
ALK Phos	18	46	36-300	U/l
Total Billirubin	0.2	0.1	0.1-1.2	mg/dl
Direct Billirubin	0	0	0-0.8	mg/dl
Chemistry				
Total Protein	4.9	5.5	4.4-8.0	g/dl
Albumin	2.3	2.8	2.9-5.4	g/dl
Globulin	2.6	2.9	2.0-4.0	g/dl
Calcium	9.8	10	7.0-12.0	mg/dl
Phosphate	6.7	6.4	2.4-8.9	mg/dl

- Supplemental Table 1 -





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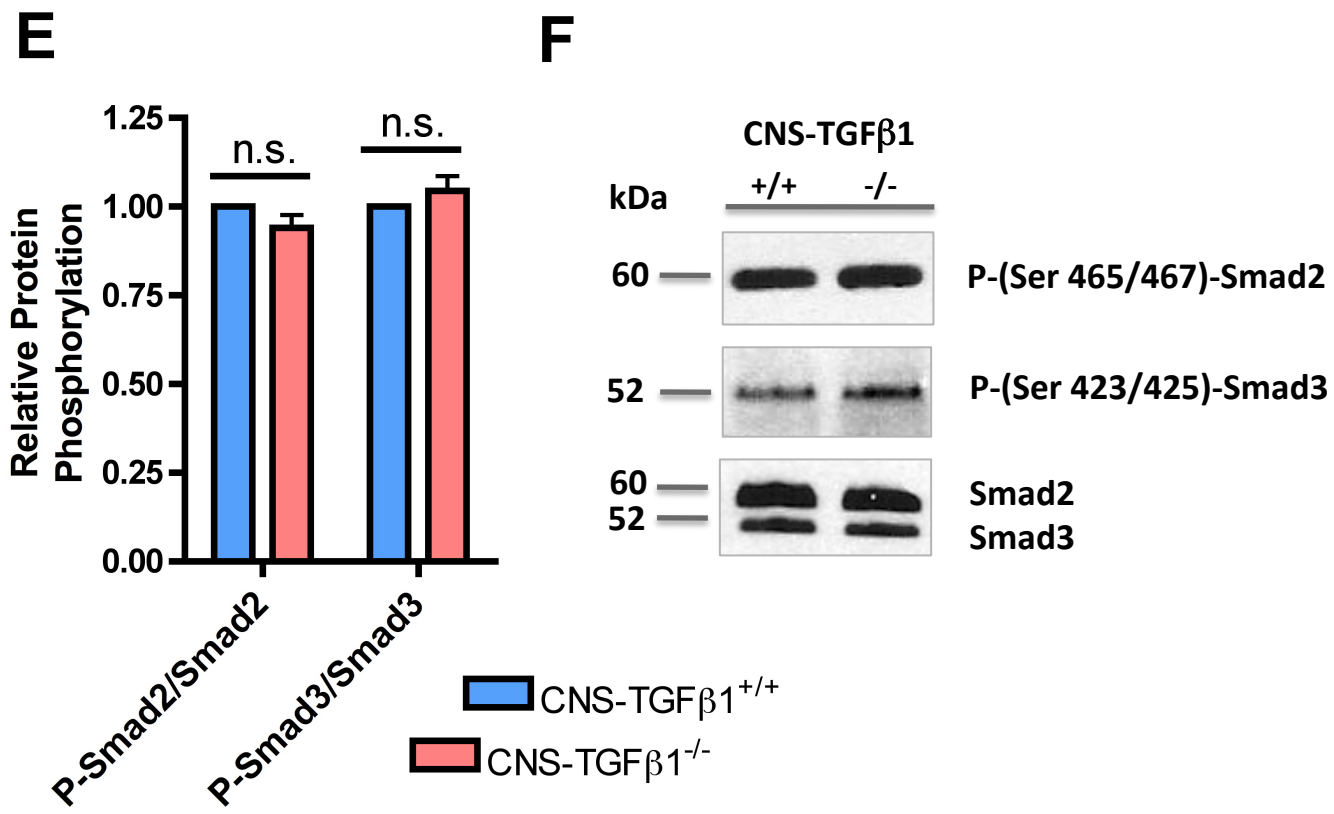

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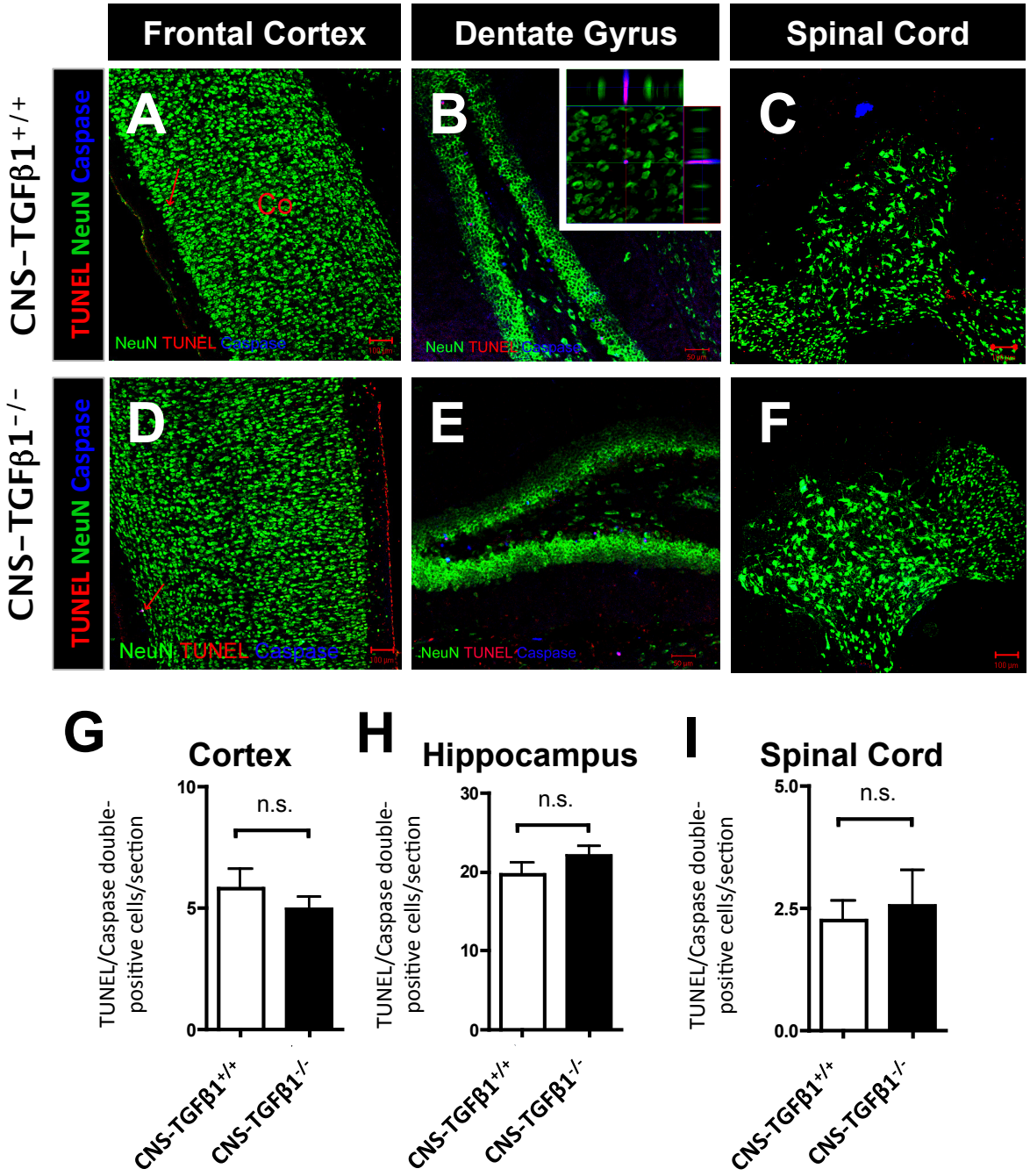

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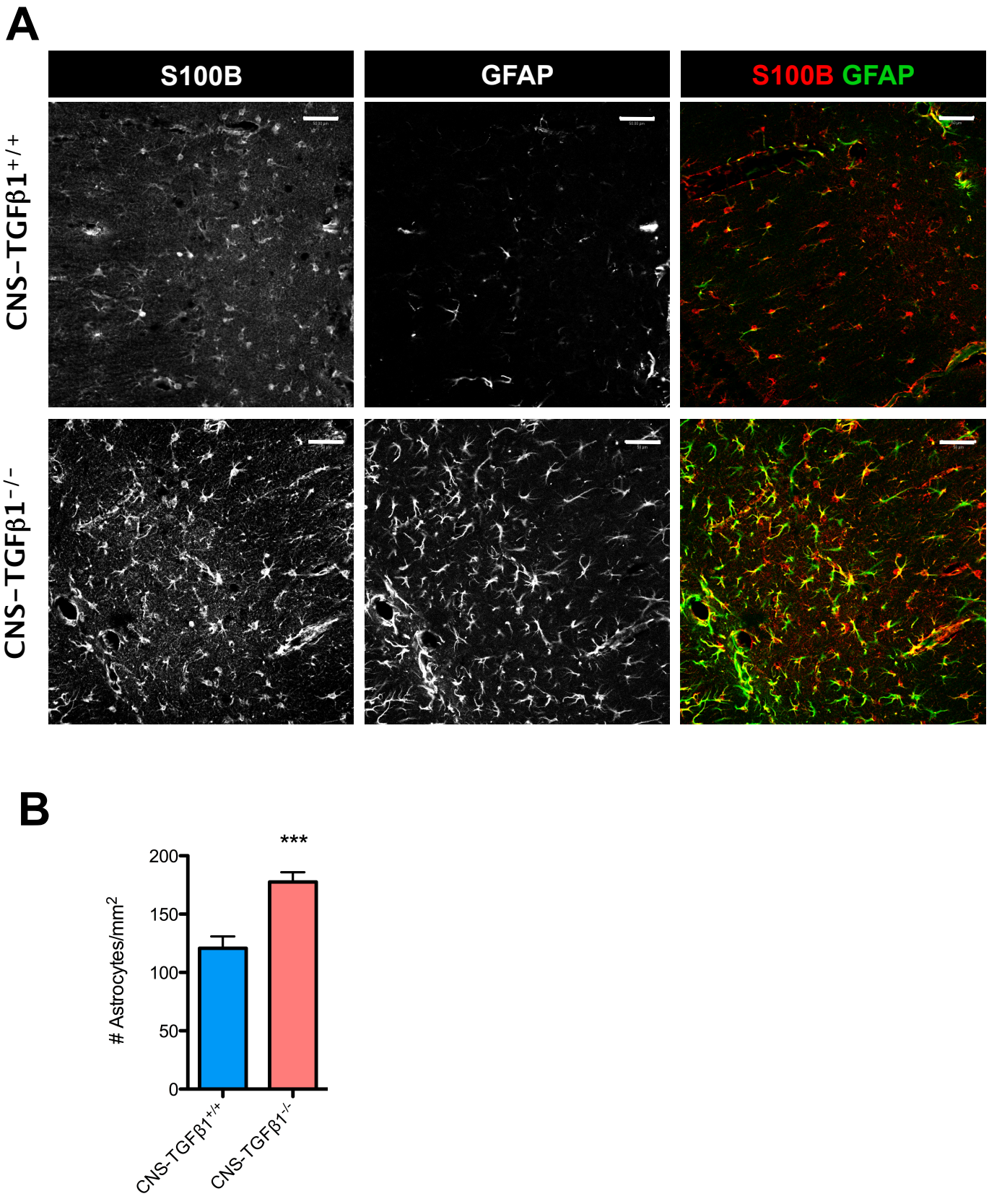

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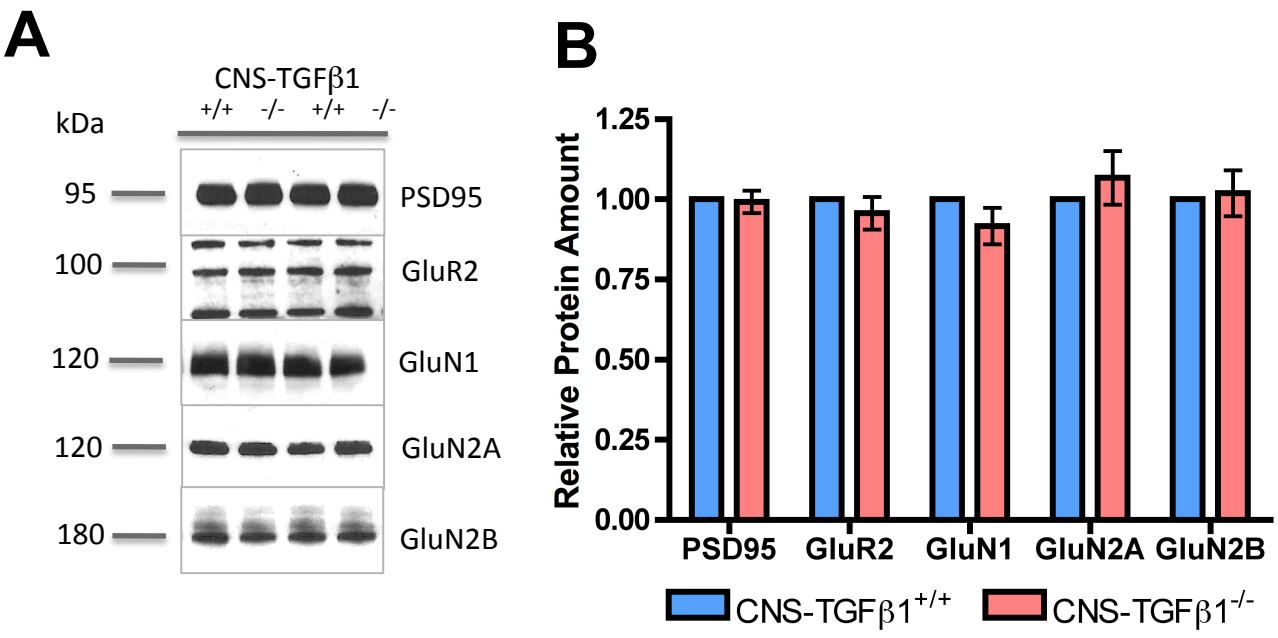
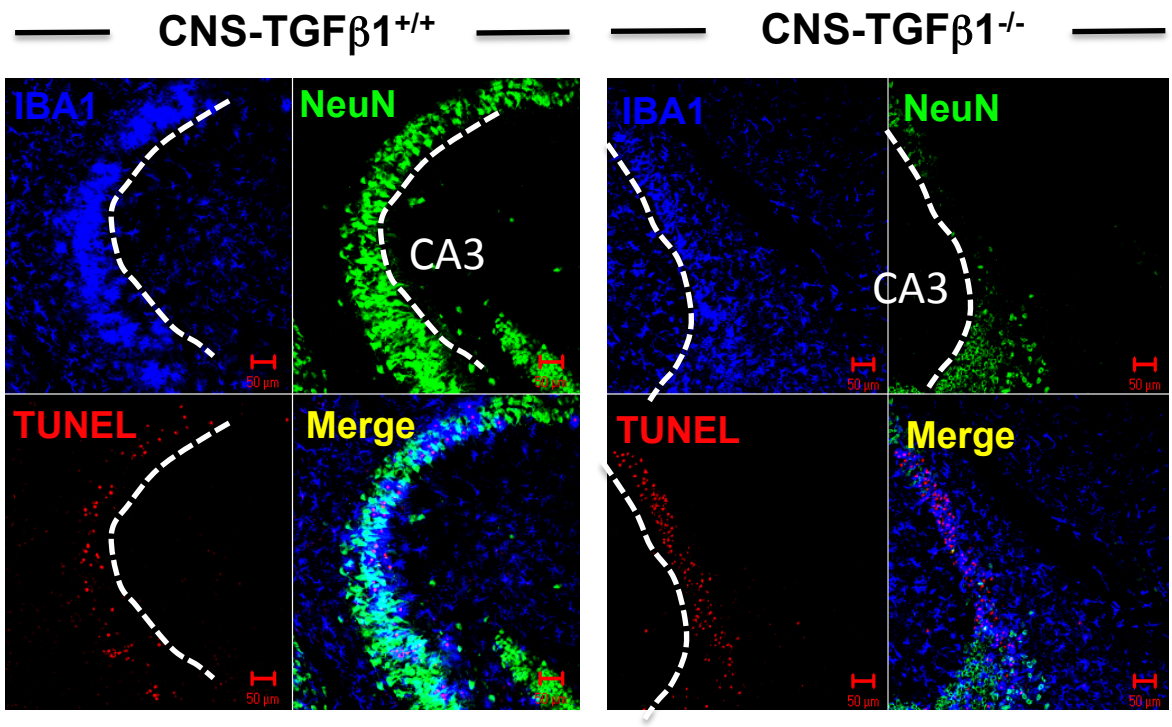


– Supplemental Figure 6 (S6) –

5 d.p.i. kainic acid

A



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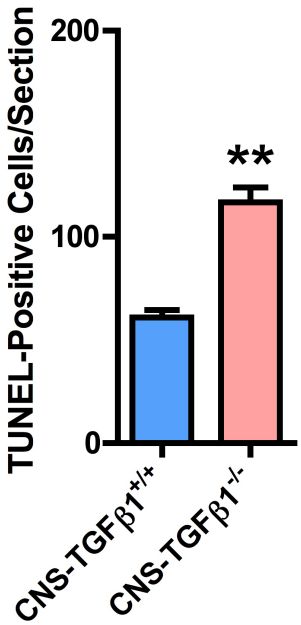

– Supplemental Figure 7 (S7) –