Science Advances
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Supplementary Materials for

Sortilin gates neurotensin and BDNF signaling to control peripheral neuropathic pain

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Published 19 June 2019, *Sci. Adv.* **5**, eaav9946 (2019)
DOI: 10.1126/sciadv.aav9946

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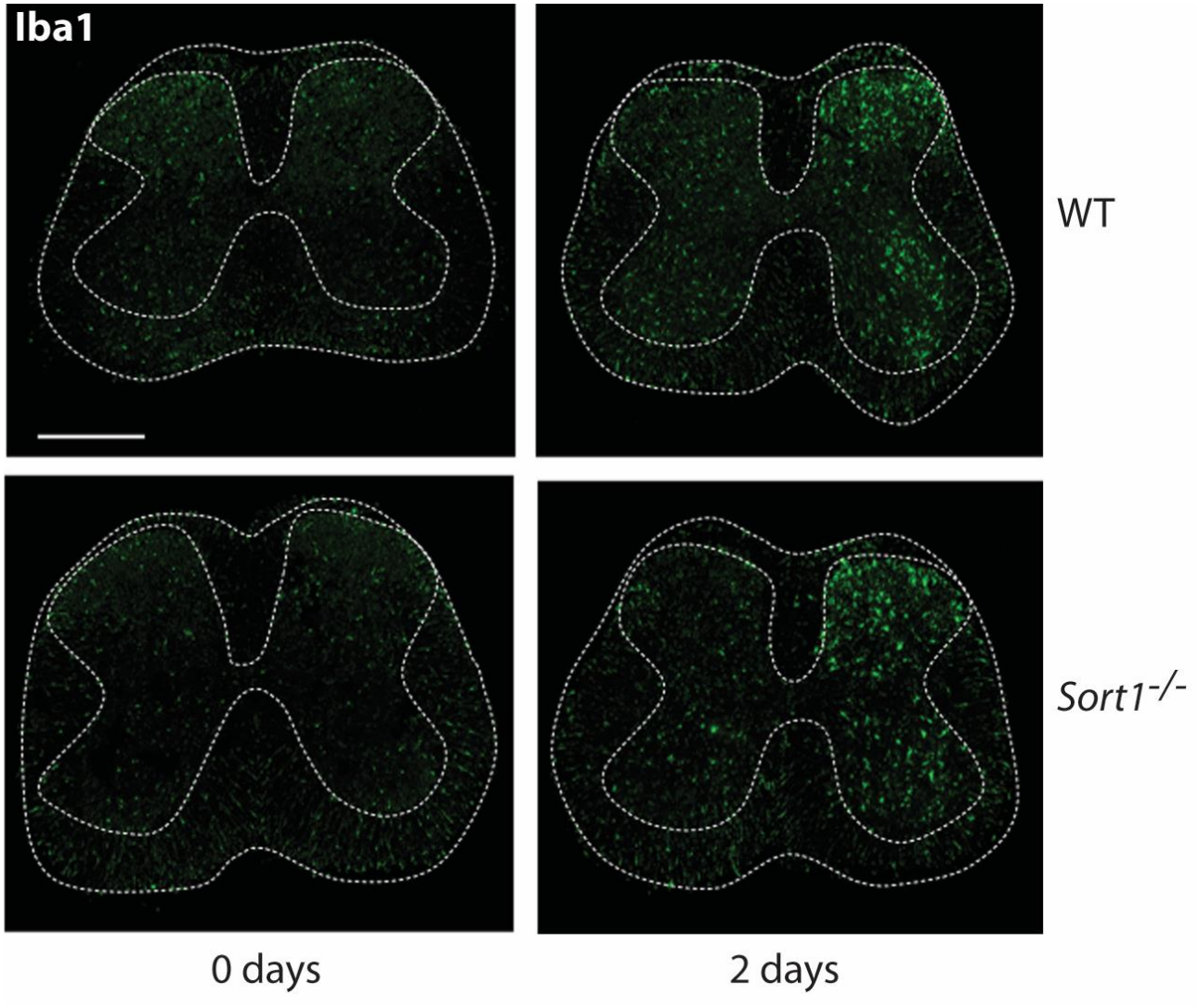


Fig. S1. Injury-induced microglia reactivity in WT and *Sort1*$^{-/-}$ lumbar spinal cord.Representative images of immunofluorescence labelling of Iba1 (green, microglia marker) in spinal L3-L5 sections of WT and *Sort1* $^{-/-}$ mice 0 and 2 days post SNI (injury on right side); scale bar = 500 µm, n = 3.

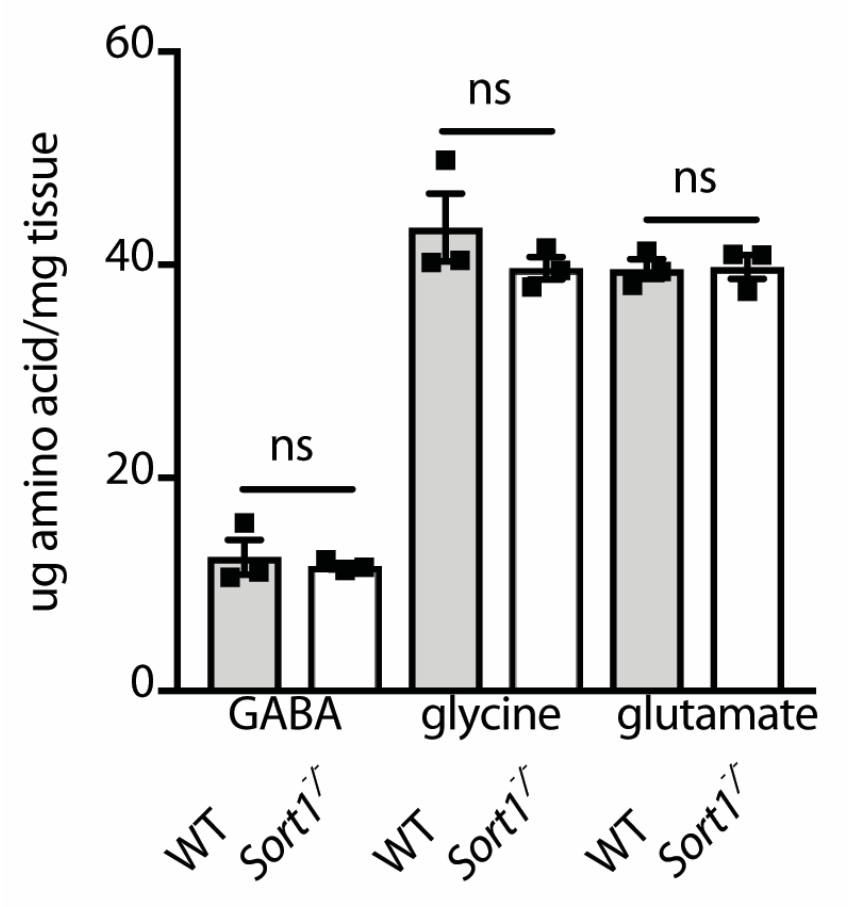


Fig. S2. Neurochemical markers remain intact in sortilin-deficient mice. GABA, glycine and glutamate in spinal L3-L5 sections of naïve WT and *Sort1* $^{-/-}$ mice (n = 3, unpaired t-test, ns = not significant, means ± SEM).

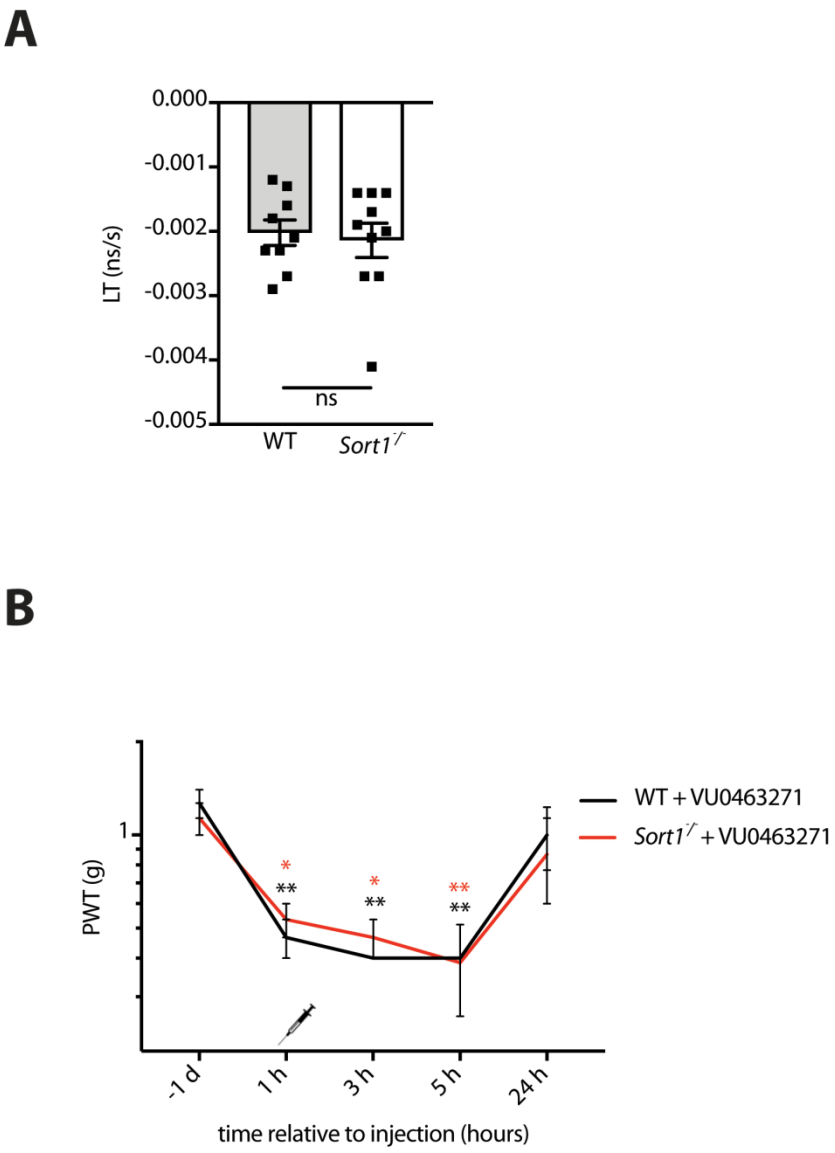


Fig. S3. Direct KCC2 inhibition induces pain hypersensitivity. (A) Measurements of KCC2 activity in reverse mode by K^+-driven uptake of Cl^- in superficial dorsal horn neurons of lumbar spinal cord, as lifetime (LT) change in nanoseconds per second (ns/s). 56-60 cells in 9-10 lumbar spinal cord slices from 4 animals were analyzed for each genotype (n = 9-10, t-test, ns = not significant, means ± SEM). (B) PWT to tactile stimuli of naïve WT and *Sort1* $^{-/-}$ mice with i.t. injection of VU0463271 (* $P < 0.03$, ** $P < 0.007$ versus -1 d of the same mouse, n = 3, RM two-way ANOVA with post hocSidak's test ($F(1,4) = 0.1266$, $P = 0.7400$), means ± SEM).

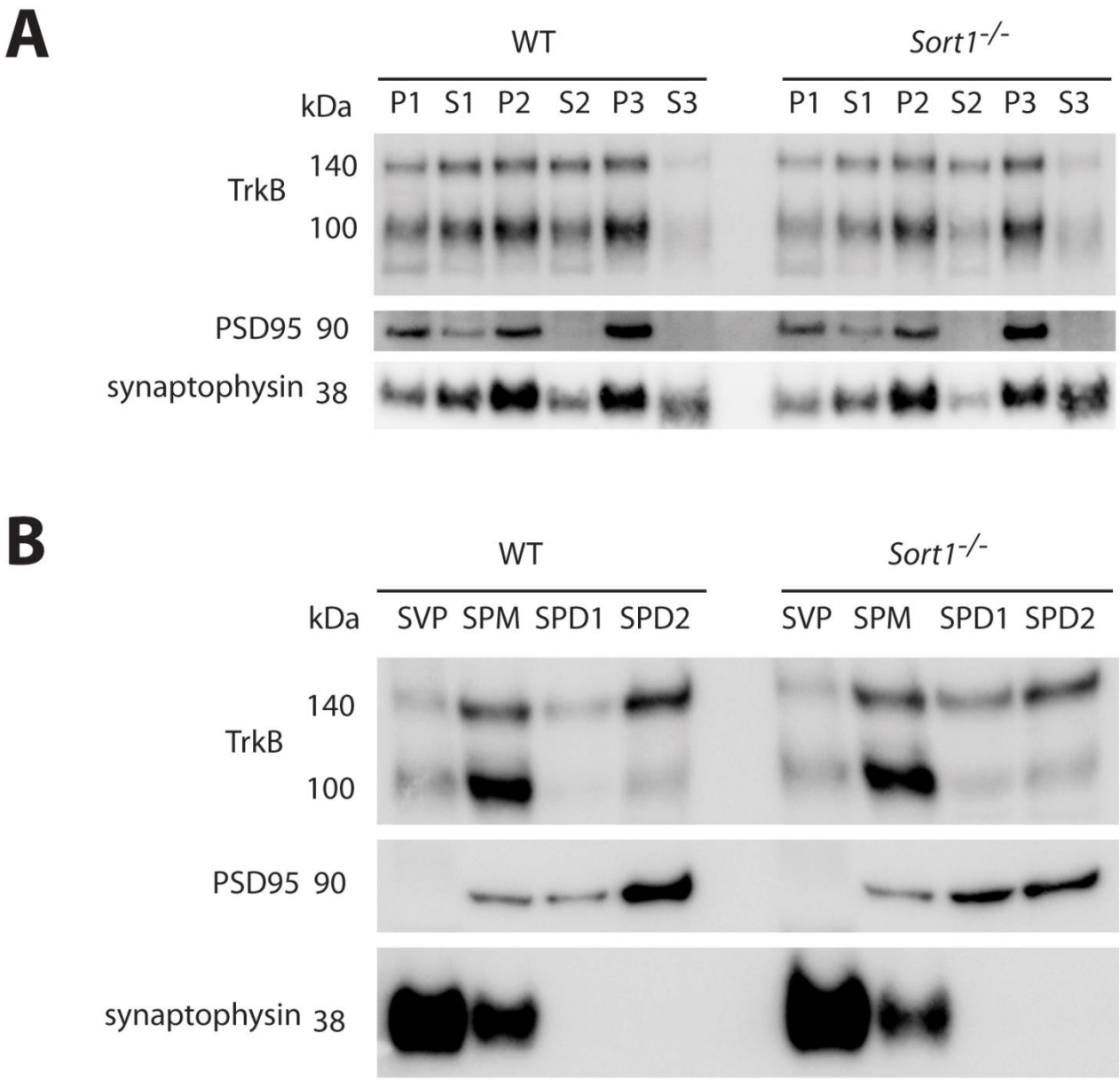


Fig. S4. Subcellular localization of TrkB is unchanged in SDH of sortilin-deficient mice. WB of subcellular fractionation of 8 pooled SDH of spinal L3-L5 from naïve WT and *Sort1* $^{-/-}$ mice (A) TrkB, PSD95 and synaptophysin in fractions P1 to S3, n = 1. (B) TrkB, PSD95 and synaptophysin in fractions SVP to SPD2.

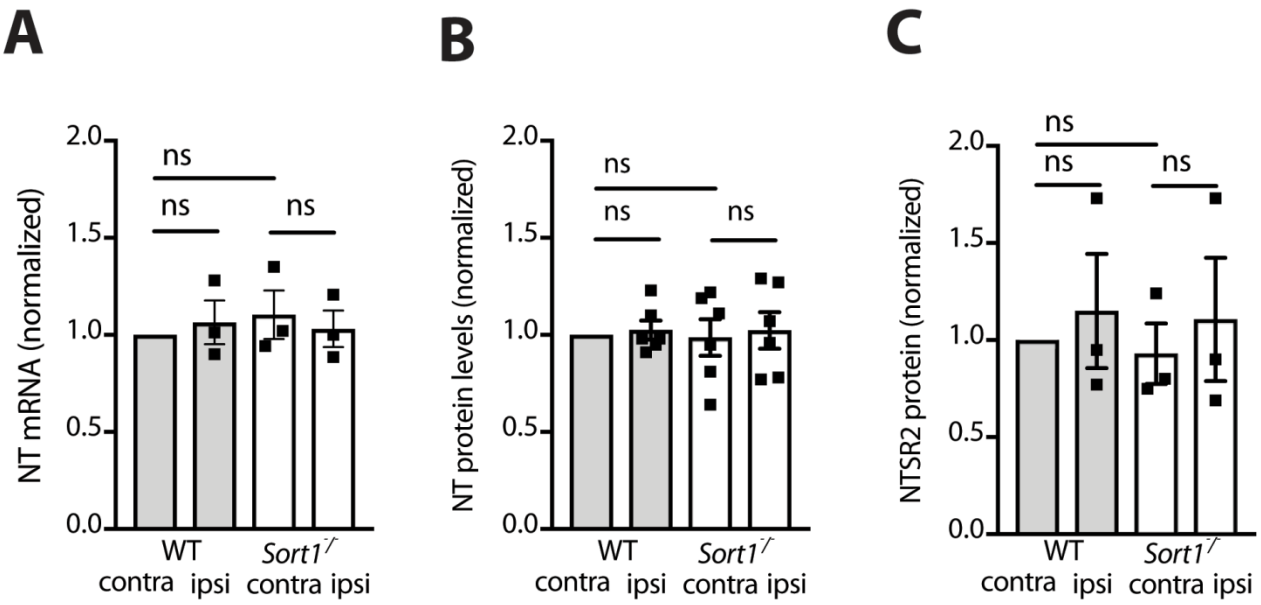


Fig. S5. mRNA and protein levels of neurotensin and NTSR2 are unchanged in sortilin-deficient mice. (A) qPCR of neurotensin mRNA levels (delta-CT) in SDH L3-L5 of WT and *Sort1* $^{-/-}$mice 6 d post SNI (n = 3, one-way ANOVA with *post hoc* Tukey's test, ns = not significant, means ± SEM). (B) MS of neurotensin levels in SDH L3-L5 of WT and *Sort1* $^{-/-}$mice 6 d post SNI (n = 6, one-way ANOVA with *post hoc* Tukey's test, means ± SEM). (C) NTSR2 protein levels (by WB) in SDH L3-L5 of WT and *Sort1* $^{-/-}$mice (n = 3, one-way ANOVA with *post hoc* Tukey's test, means ± SEM).

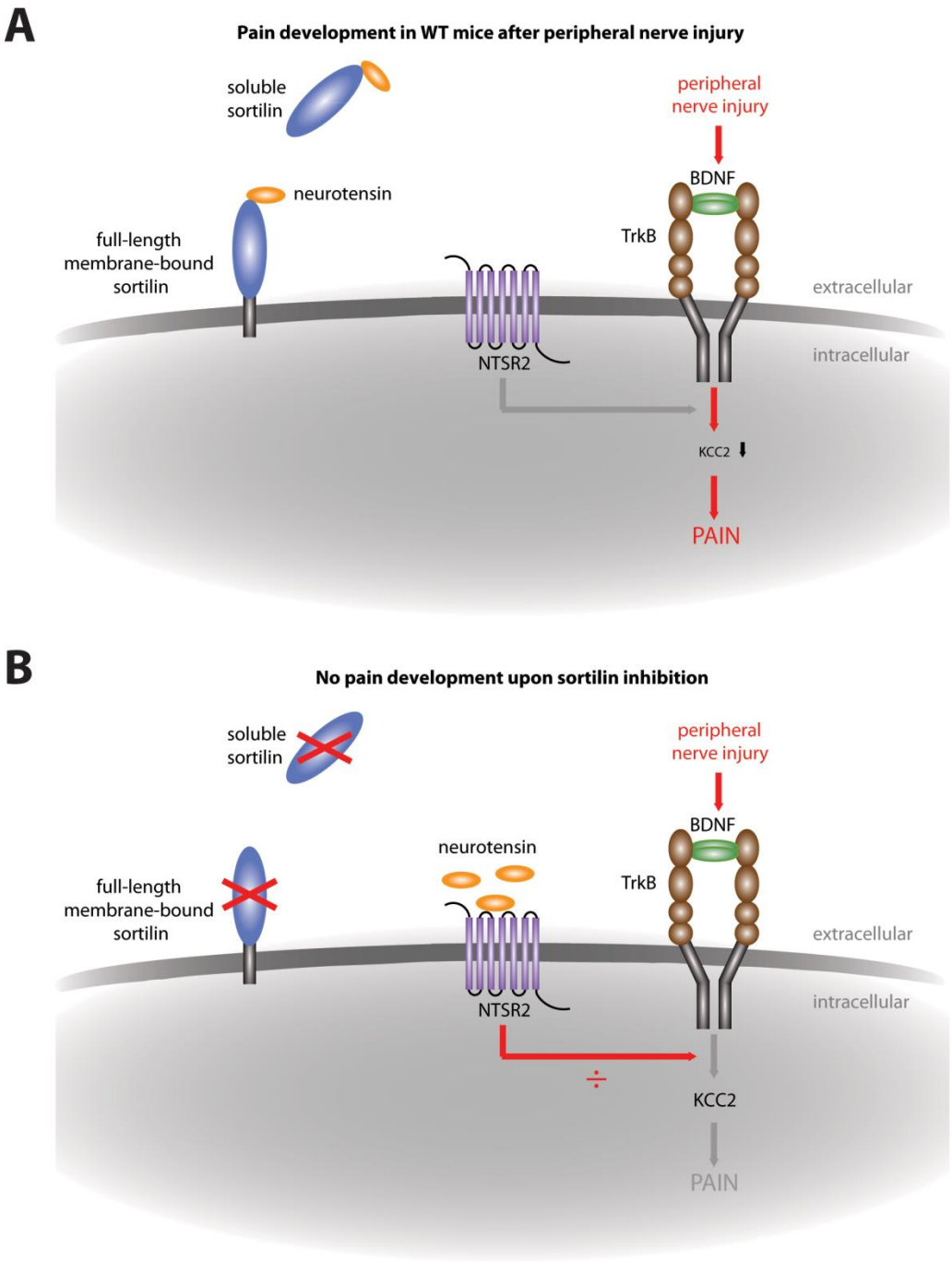
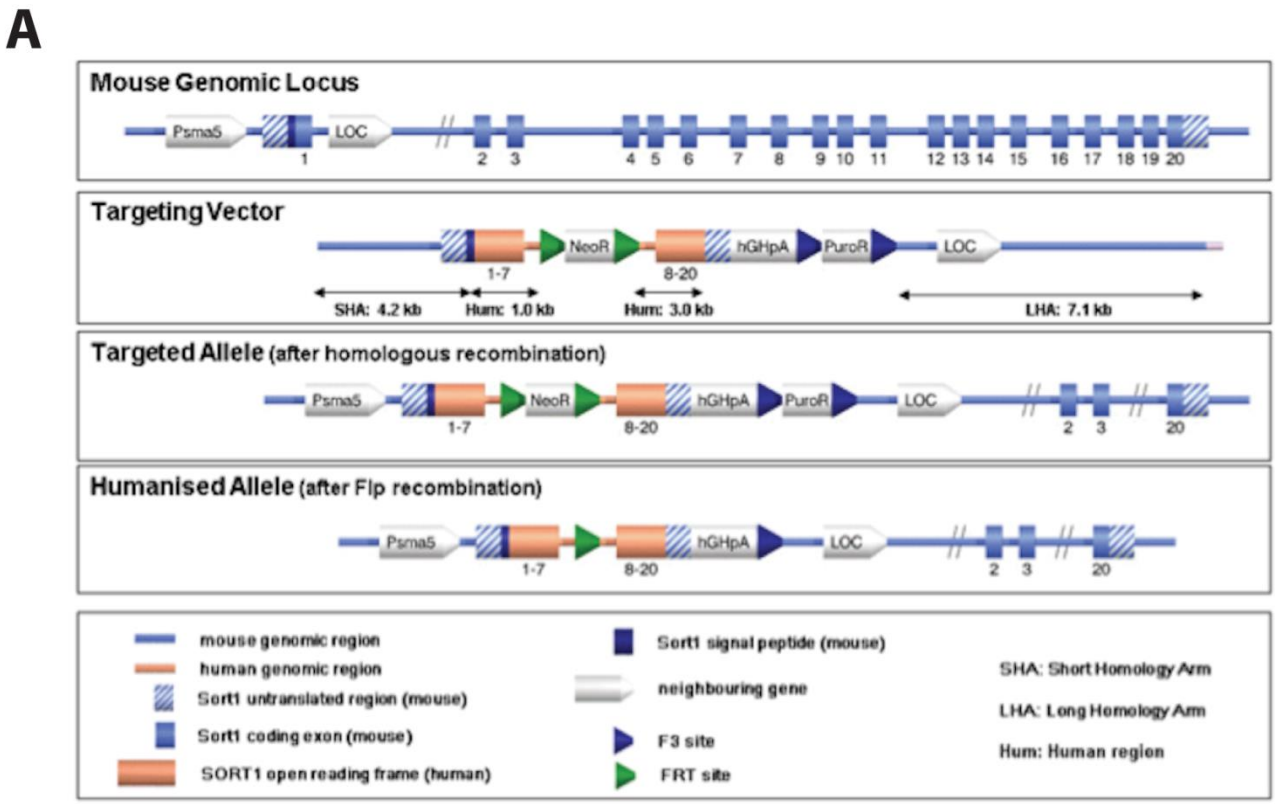


Fig. S6. Sortilin allows direct manipulation of the pain response to neuronal injury. (A) Upon neuronal injury, released BDNF activates the central BDNF-TrkB pain inducing pathway leading to KCC2 downregulation. In WT mice, sortilin (possibly both membrane-bound and soluble forms) scavenges neurotensin thereby preventing neurotensin from activating NTSR2, permitting the BDNF-TrkB pain pathway to induce KCC2 downregulation and neuropathic pain (red arrows). (B) In absence of or upon inhibition of sortilin (by antibodies or the small-molecule AF38469), neurotensin activates NTSR2, which inhibits BDNF-TrkB pathway induced KCC2 downregulation and pain development (red arrow). I.t. injection of soluble sortilin to injured *Sort1* $^{-/-}$ mice (not shown) restores the neurotensin scavenging capacity allowing pain development.



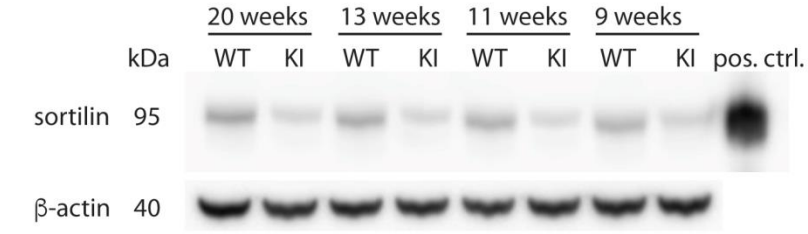


Fig. S7. *Hum-sortilin-KI* mouse model. C57BL/6J mice expressing the human *SORT1* gene under the control of the endogenous mouse *Sort1* promoter (*hum-sortilin-KI*). (A) The coding region in mouse exon 1 was replaced with a human *SORT1* cDNA, see Methods section for details. (B) Protein expression was confirmed by Western blotting of nerve tissue