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Supplemental Information

Nppb Neurons Are Sensors of Mast Cell-Induced Itch

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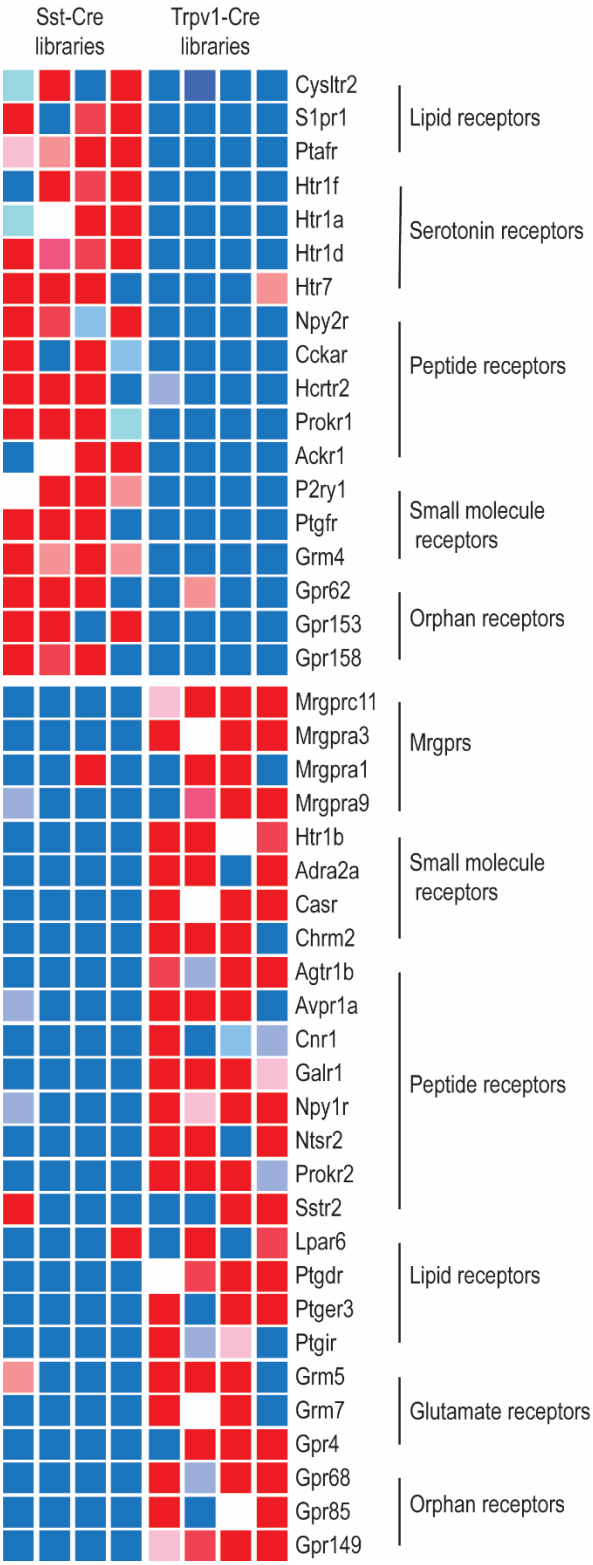

Figure S1, Identification of GPCRs differentially expressed in Nppb- and Trpv1-neurons, related to Fig. 2. GPCRs enriched approximately 2-fold in either Nppb- or in Trpv1-neurons plotted in a heat-map displaying the relative gene expression in each cDNA library (see methods for details). Receptors have been generally divided into groups based on the agonists that stimulate them.

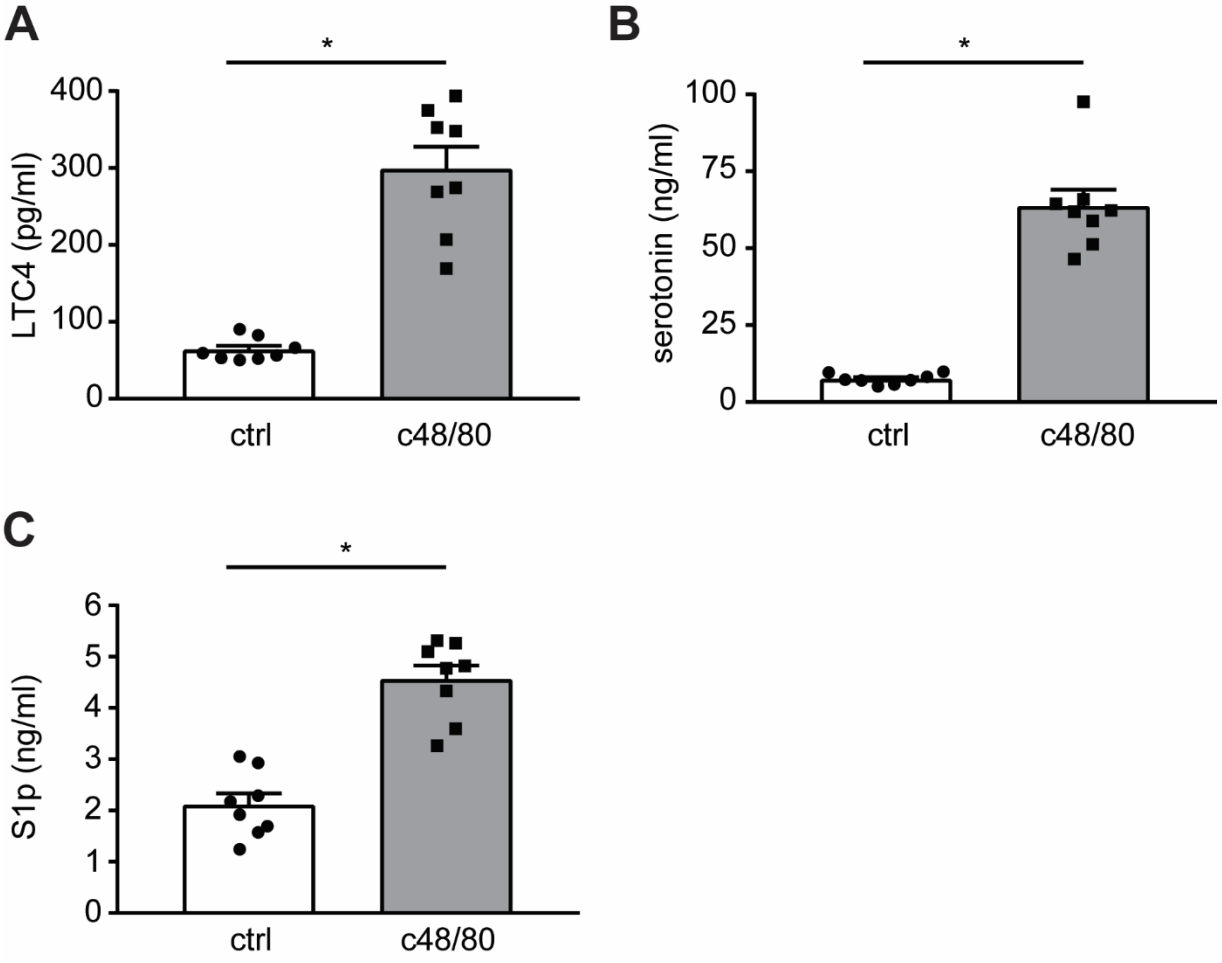

Figure S2, Mast cells produce LTC4, serotonin and S1p upon stimulation, related to Fig. 3. A-C, PDMCs were cultured for 2 weeks in the presence of IL3 (20 ng/ml) and SCF (30 ng/ml). After washing, cells were stimulated in cytokine-free medium without (ctrl) or with compound 48/80 (c48/80, 50 µg/ml) and LTC4 (A), serotonin (B) and S1p (C) release into the supernatant was quantified with commercially available ELISA kits. Note that cells were stimulated for 30 minutes (A+B) or 60 minutes (C), respectively. Significant differences between treatments were assessed by unpaired, two-tailed Student's t-test (*$P < 0.0001$, A-C). Data represent means ± s.e.m. (A-C; cultured mast cells were isolated from n = 8 WT animals and each assayed in duplicate).

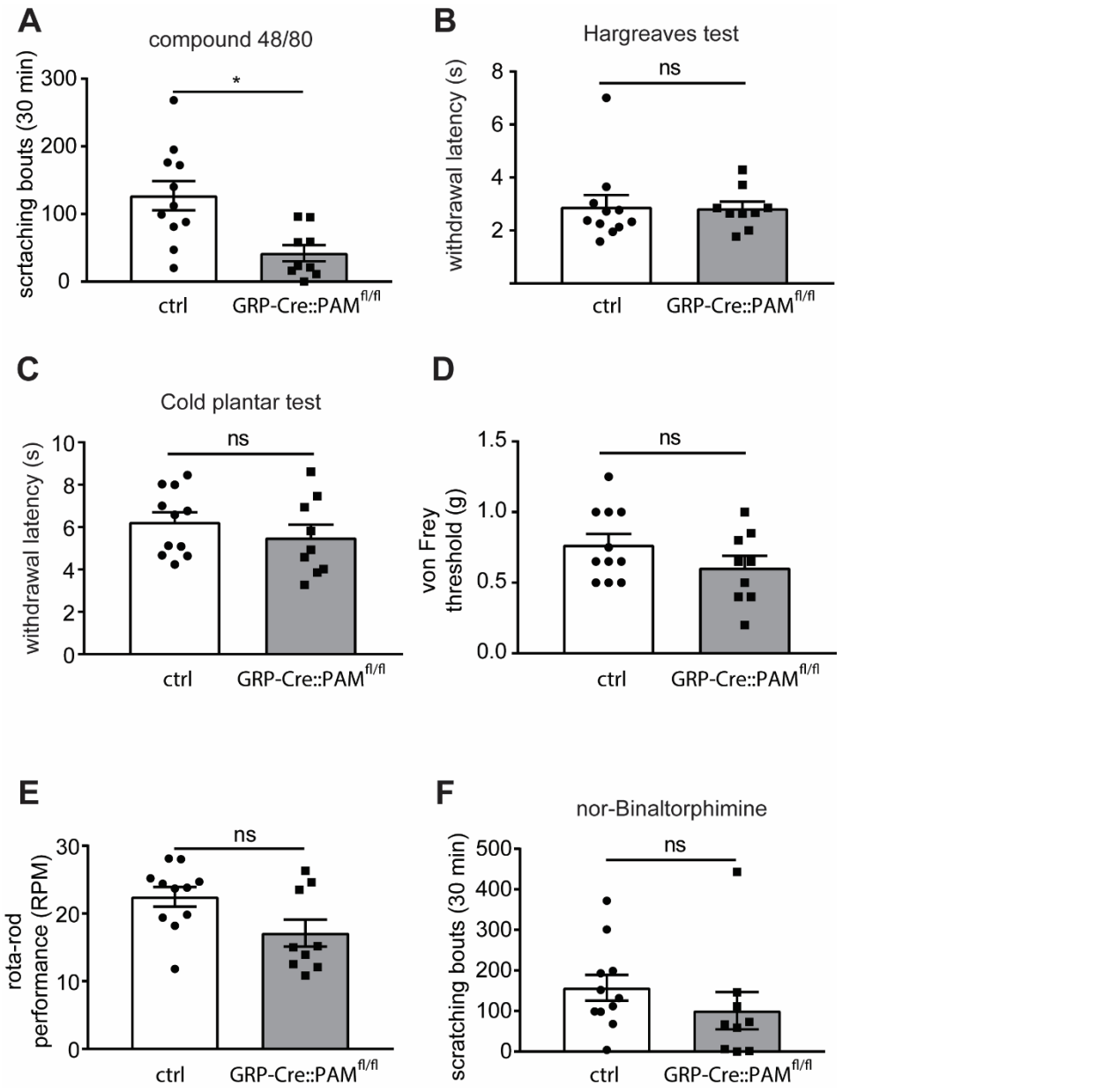

Figure S3, GRP-Cre::PAM$^{fl/fl}$ mice exhibit normal responses to thermal and mechanical stimuli, related to Fig. 3. A, GRP-Cre::PAM$^{fl/fl}$ mice display significantly reduced itch-behavior to compound 48/80 (2 µg) compared to littermate control mice showing that peripherally induced itch is defective in these mutant mice. Significant differences between genotypes were assessed by unpaired, two-tailed Student's t-test (*P = 0.0048). Data represent means ± s.e.m. (n = 11 control and n = 9 GRP-Cre::PAM$^{fl/fl}$ mice). GRP-Cre::PAM$^{fl/fl}$ mice and control littermate animals do not exhibit significantly different behavioral responses in tests for reaction to noxious heat (Hargreaves test; B), to cold (Cold plantar test; C), and to mechanical stimulation (von Frey test; D), for motor coordination (rota-rod test; E), and for itch-responses to treatment with kappa-opioid receptor antagonist (100 µg of nor-Binaltorphimine; F). These results show that GRP-Cre::PAM$^{fl/fl}$ mice have normal responses to most somatosensory modalities and that the itch deficit these animals exhibit occurs upstream of spinal cord GRPR-neurons. Significant differences between genotypes were assessed by unpaired, two-tailed Student's t-test (ns P = 0.9026, B; ns P = 0.3518, C; ns P = 0.1733, D; ns P = 0.0903, E; ns P = 0.3121, F). Data represent means ± s.e.m. (B; n = 11 control and n = 9 GRP-Cre::PAM$^{fl/fl}$ mice, C; n = 11 control and n = 9 GRP-Cre::PAM$^{fl/fl}$ mice, D; n = 11 control and n = 9 GRP-Cre::PAM$^{fl/fl}$ mice, E; n = 11 control and n = 9 GRP-Cre::PAM$^{fl/fl}$ mice, F; n = 11 control and n = 9 GRP-Cre::PAM$^{fl/fl}$ mice).

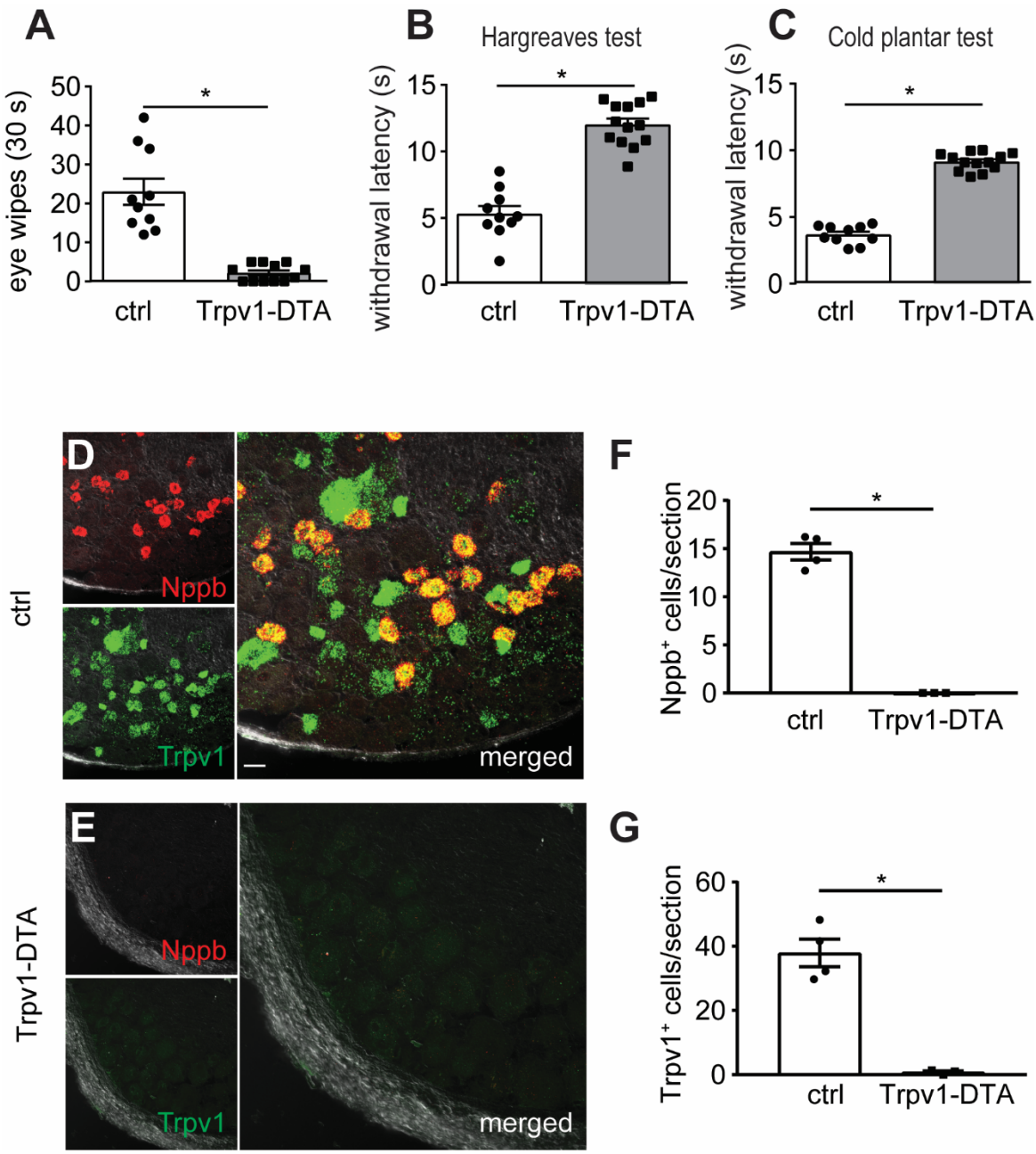

Figure S4, Trpv1-DTA mice have major deficits in responses in thermosensation, related to Fig. 4. A, Trpv1-DTA mice lose responses to capsaicin. Instillation of capsaicin (762.5 ng capsaicin in 50 µl saline) into the eye evokes robust wiping in control littermate mice and this reaction is significantly reduced in Trpv1-DTA animals. B+C, Trpv1-DTA mice exhibit severely compromised reactions to noxious heat (B) and cold (C). Withdrawal responses to heat were assessed with the Hargreaves test, and cold using the cold plantar test. Significant differences between genotypes were assessed by unpaired, two-tailed Student's t-test (*P = 0.0001, A; *P < 0.0001, B; *P < 0.0001, C). Data represent means ± s.e.m. (A; n = 10 control and n = 12 Trpv1-DTA mice, B; n = 10 control and n = 12 Trpv1-DTA mice, and C; n = 10 control and n = 12 Trpv1-DTA mice). D-G, Trpv1-DTA mice have lost expression of Trpv1 and Nppb. In *situ* hybridization of sections of littermate control mice (D) and Trpv1-DTA animals (E) reveal that neurons expressing Trpv1 (green) and Nppb (red) are absent in Trpv1-DTA mice which is also revealed by counting numbers of positively stained Nppb (F) and Trpv1 cells (G). Significant differences between genotypes were assessed by unpaired, two-tailed Student's t-test (*P = 0.0004, F; *P = 0.0030, G). Data represent means ± s.e.m. (n = 4 control and n = 3 Trpv1-DTA mice). Scale bar, 20 µm.

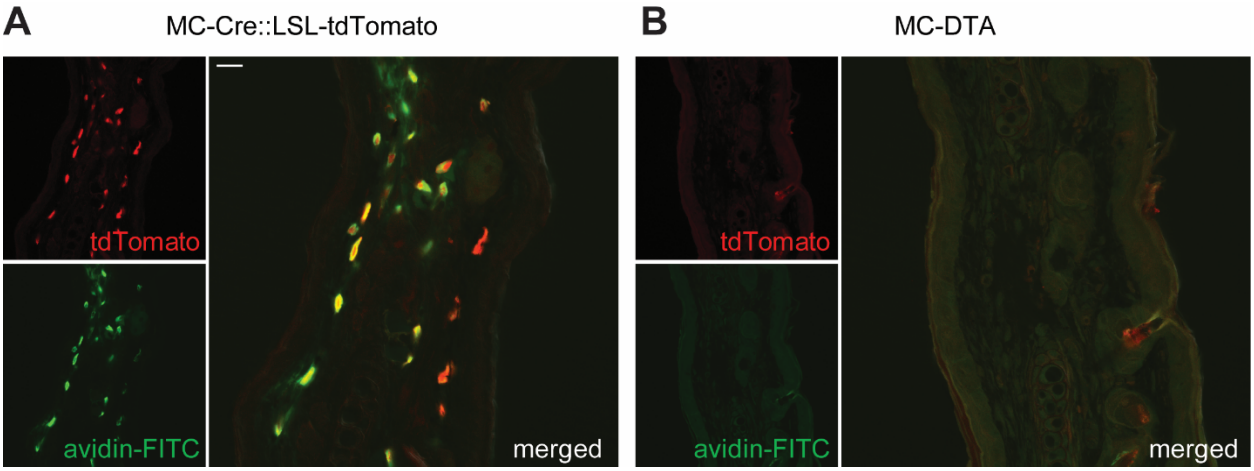

Figure S5, MC-DTA mice have lost dermal mast cells, related to Fig. 4. A, sections from the ears of MC-Cre::LSL-tdTomato mice (A) reveal that the dermal skin layer contains many mast cells marked by avidin-FITC stain (green) and by tdTomato reporter (red). B, in contrast, reporter- and avidin-FITC-stained mast cells are absent from the ears of MC-DTA mice. Scale bar, 20 μm.

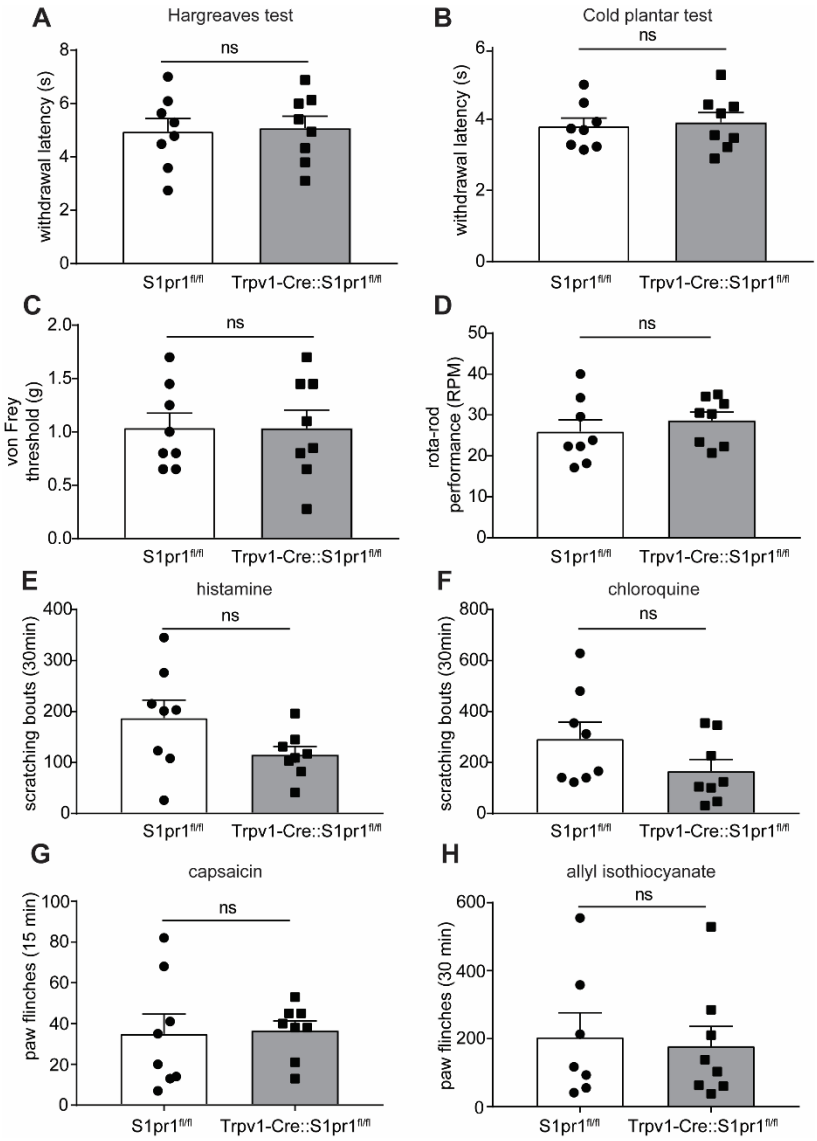

Figure S6, Mice with sensory neuron specific ablation of S1pr1 exhibit normal responses to most somatosensory stimuli, related to Fig. 7. A, noxious heat responses of Trpv1-Cre::S1pr1$^{fl/fl}$ mice are not significantly different from S1pr1$^{fl/fl}$ control littermates. Responses to heat were assessed using the Hargreaves test. B, responses to cold of Trpv1-Cre::S1pr1$^{fl/fl}$ mice are not significantly different from S1pr1$^{fl/fl}$ control littermates. Responses to cold were assessed using the cold plantar test. C, mechanical evoked withdrawal responses were not significantly different between Trpv1-Cre::S1pr1$^{fl/fl}$ mice and S1pr1$^{fl/fl}$ control littermates. Responses to mechanical stimuli were assessed using von Frey microfilaments. D, motor coordination was not significantly different between Trpv1-Cre::S1pr1$^{fl/fl}$ mice and S1pr1$^{fl/fl}$ control littermates using a rota-rod test. E+F, scratching elicited by histamine (100 µg; E) and chloroquine (100 µg; F) in Trpv1-Cre::S1pr1$^{fl/fl}$ mice was not significantly different from S1pr1$^{fl/fl}$ control littermates. G+H, ongoing pain behavior elicited by intraplantar injection of capsaicin (3.1 µg; G) and allyl isothiocyanate (50.6 µg; H) in Trpv1-Cre::S1pr1$^{fl/fl}$ mice was not significantly different from S1pr1$^{fl/fl}$ control littermates. Significant differences between genotypes were assessed by unpaired, two-tailed Student's t-test (ns P = 0.8544, A; ns P = 0.7639, B; ns P = 0.9899, C; ns P = 0.4456, D; ns P = 0.0870, E; ns P = 0.1364, F; ns P = 0.8822, G; ns P = 0.7779, H). Data represent means ± s.e.m. (A; n = 8 control and n = 8 Trpv1-Cre:: S1pr1$^{fl/fl}$ mice, B; n = 8 control and n = 8 Trpv1-Cre:: S1pr1$^{fl/fl}$ mice, C; n = 8 control and n = 8 Trpv1-Cre:: S1pr1$^{fl/fl}$ mice, D; n = 8 control and n = 8 Trpv1-Cre:: S1pr1$^{fl/fl}$ mice, E; n = 8 control and n = 8 Trpv1-Cre:: S1pr1$^{fl/fl}$ mice, F; n = 8 control and n = 8 Trpv1-Cre:: S1pr1$^{fl/fl}$ mice; G, n = 8 control and n = 8 Trpv1-Cre::S1pr1$^{fl/fl}$ mice; H n = 7 control and n = 8 Trpv1-Cre::S1pr1$^{fl/fl}$ mice).

Table S2, Primer list for genotyping, related to STAR methods.

strain	forward primer	reverse primer	amplicon
Trpv1-IRES-Cre	GGTGCAAGTTGAATAACCGG	CAGAGACGGAAATCCATCGC	522bp
Sst-IRES-Cre			
Grp-Cre			
Mcpt5-Cre	ACAGTGGTATTCCCGGGGAGTG T	GTCAGTGCGTTCAAAGGCCA	554bp
R26-LSL-tdTomato (Ai9)	AAGGGAGCTGCAGTGGAGTA	CCGAAAATCTGTGGGAAGTC	WT: 297bp
	CTGTTCCTGTACGGCATGG	GGCATTAAAGCAGCGTATCC	mutant: 196bp
R26-LSL-GCaMP6s (Ai96)	AAGGGAGCTGCAGTGGAGTA	CCGAAAATCTGTGGGAAGTC	WT: 297bp
	ACG AGT CGG ATCTCCCTTTG		mutant: 450bp
LSL-hM3Dq-mCitrine	ATGTCTGGATCCCCATCAAG	GATGTTGCCGATGATGGTCAC	442bp
R26-LSL-DTA	ACCGACAATAAATACGACGCT	CTCAGCGAAGGGAAGGCTGAG	300bp
S1pr1$^{fl/fl}$	GGAGCGGAGGAAGTTAAAAGTG C	CCCCTCCTAAGAGATTGCAGC AA	WT: 221bp
			mutant: 292bp
PAM$^{fl/fl}$	ACTTTATCCTCCTGAGGGCACAC	CACCCGAACAGGGAGGAACA	WT: 268bp
	CGCTCCAGCCTTACTTCGGA	TCATTAATTGCGTTGCGCCATC T	mutant: 461bp