

Supplemental data

Schwann cells expressing nociceptive channel TRPA1 orchestrate ethanol-evoked neuropathic pain in mice

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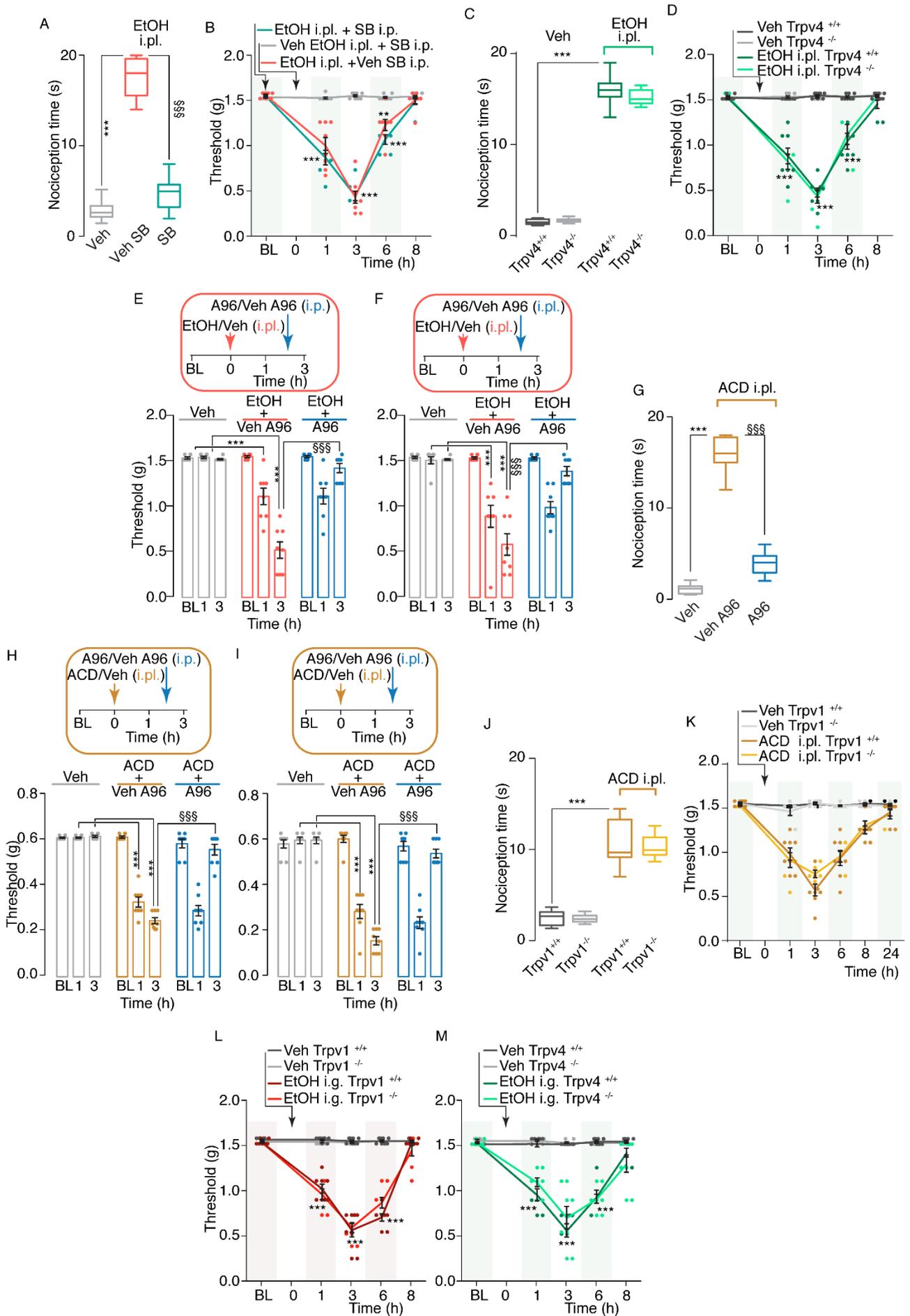
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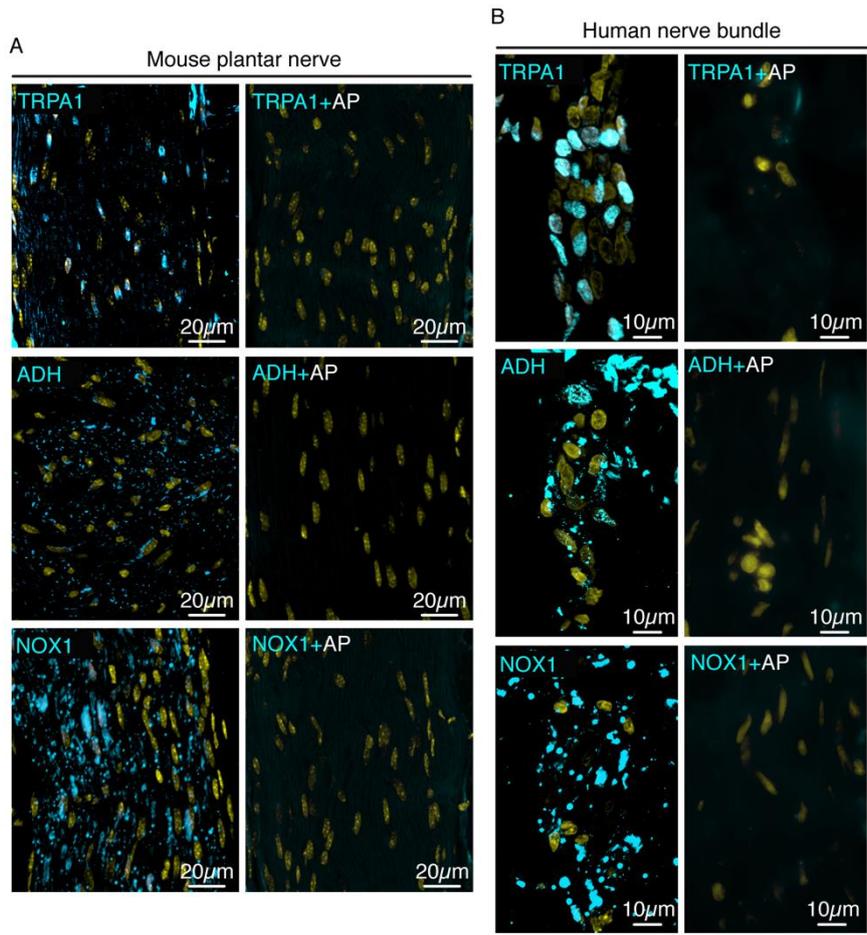
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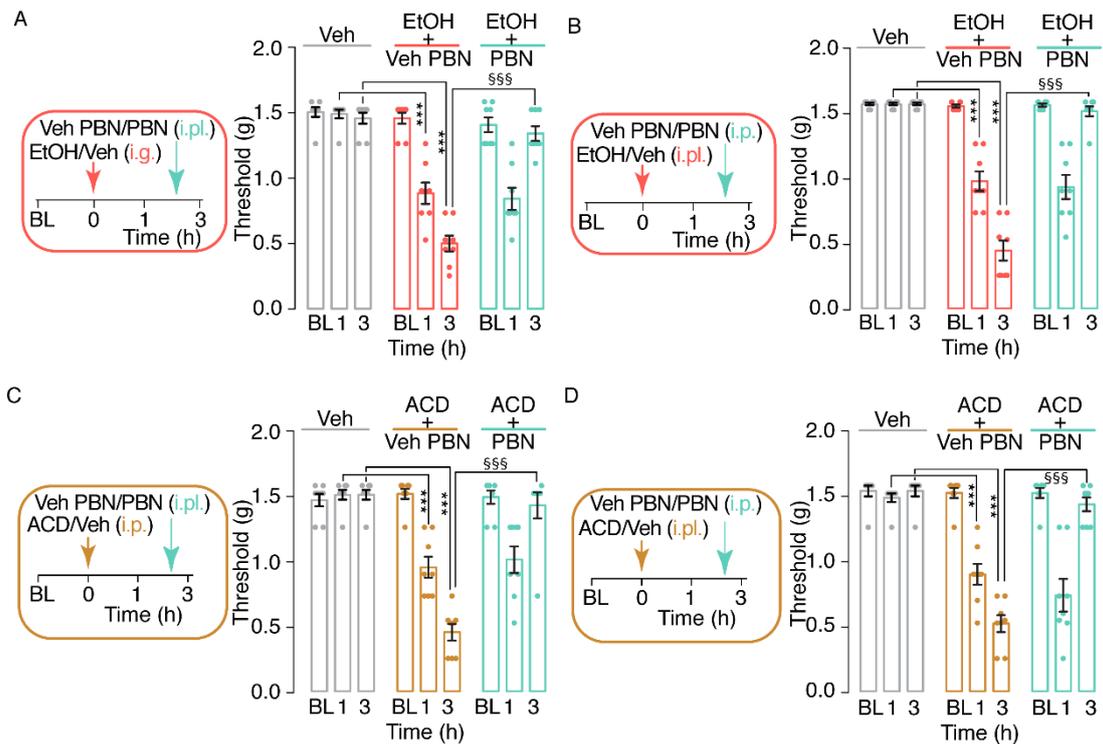
Supplemental figures



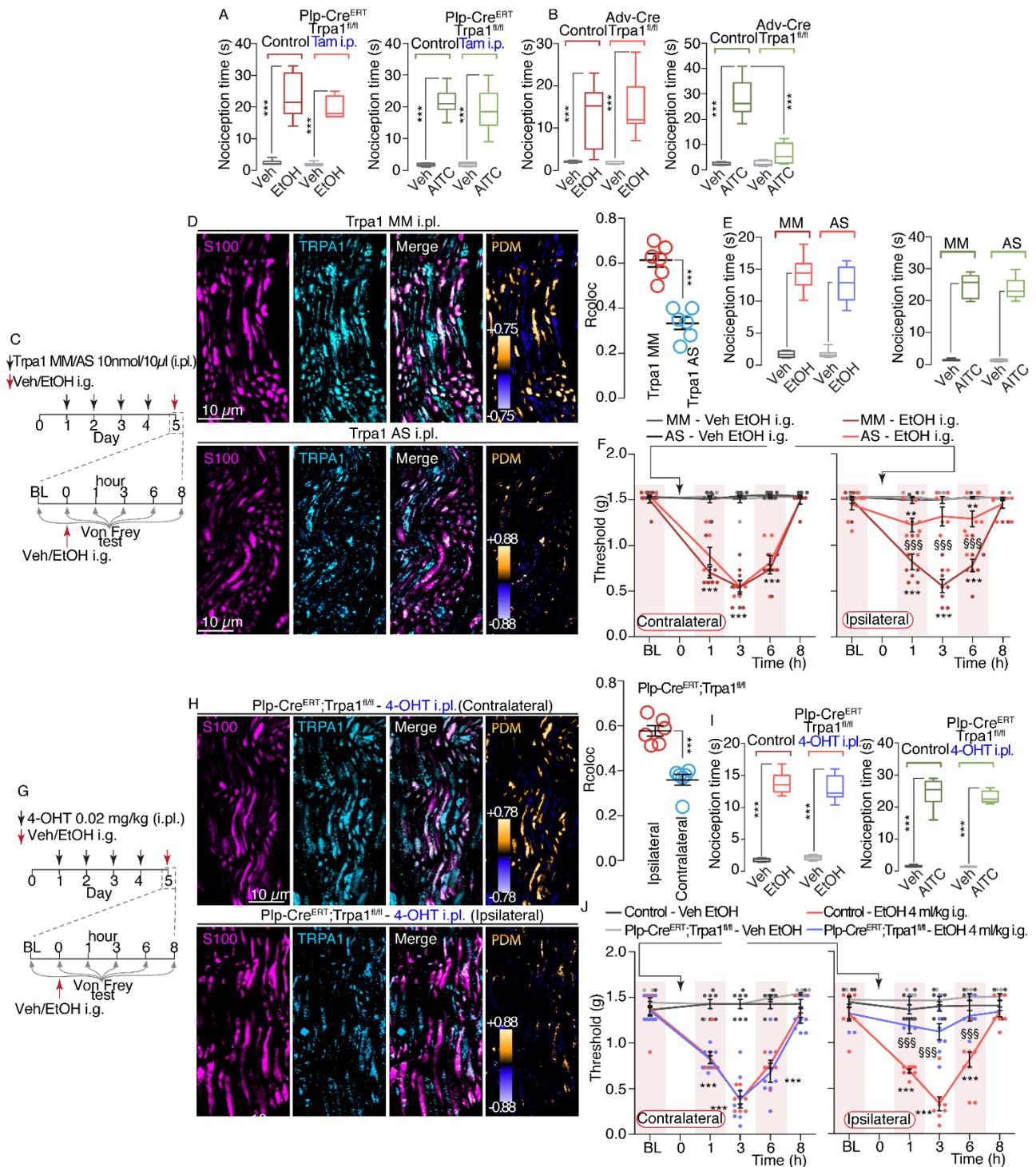
Supplemental Figure 1. (A) Acute nociceptive response and (B) time-dependent hind paw mechanical allodynia evoked by intraplantar (i.pl., 20 μ l) ethanol (EtOH, 30%) or vehicle (Veh) after pretreatment with intraperitoneal (i.p.) SB366791 (SB, 1 mg/kg) or Veh SB in C57BL/6J mice. Acute nociception response (C) and time-dependent mechanical allodynia (D) after EtOH (30%, i.pl.) or Veh in *Trpv4*^{+/+} and *Trpv4*^{-/-} mice. (E and F) Mechanical allodynia evoked by EtOH (30%, i.pl.) or Veh after A967079 (A96, 100 mg/kg, i.p. or 100 μ g, i.pl.) or Veh A96 in C57BL/6J mice. (G) Acute nociceptive response evoked by acetaldehyde (ACD, 20 nmol, i.pl.) after A96 (100 mg/kg, i.p.) or Veh A96 in C57BL/6J mice. (H and I) Mechanical allodynia evoked by ACD (20 nmol, i.pl.) or Veh, after A96 (100 mg/kg i.p. or 100 μ g, i.pl.) or Veh A96 in C57BL/6J mice. Acute nociception response (J) and time-dependent mechanical allodynia (K) after ACD (20 nmol, i.pl.) or Veh in *Trpv1*^{+/+} and *Trpv1*^{-/-} mice. Time-dependent mechanical allodynia after intragastric (i.g.) EtOH (15%, 4 ml/kg) or Veh in *Trpv1*^{+/+} and *Trpv1*^{-/-} (L) and *Trpv4*^{+/+} and *Trpv4*^{-/-} (M) mice. BL, baseline. Veh is the vehicle of EtOH and ACD. (A, C, G and J) box plots with horizontal lines at the 25th percentile, the median, and the 75th percentile and the vertical lines which extend to the minimum and maximum values; all other data are mean \pm SEM with individual data points overlaid; n=8 mice for each experimental condition. **P<0.01, ***P<0.001 vs. Veh; §§§P<0.001 vs. EtOH-A96, ACD-A96, one-way (A, C, G and J) or two-way (B, D-F, H, I and K-M) ANOVA with Bonferroni post-hoc correction.



Supplemental Figure 2. Representative images of TRPA1, NOX1 and ADH staining with primary antibodies pre-incubated with or without their respective antigen peptides (AP) in mouse plantar nerve (A) and nerve bundles in biopsies of human skin (B).

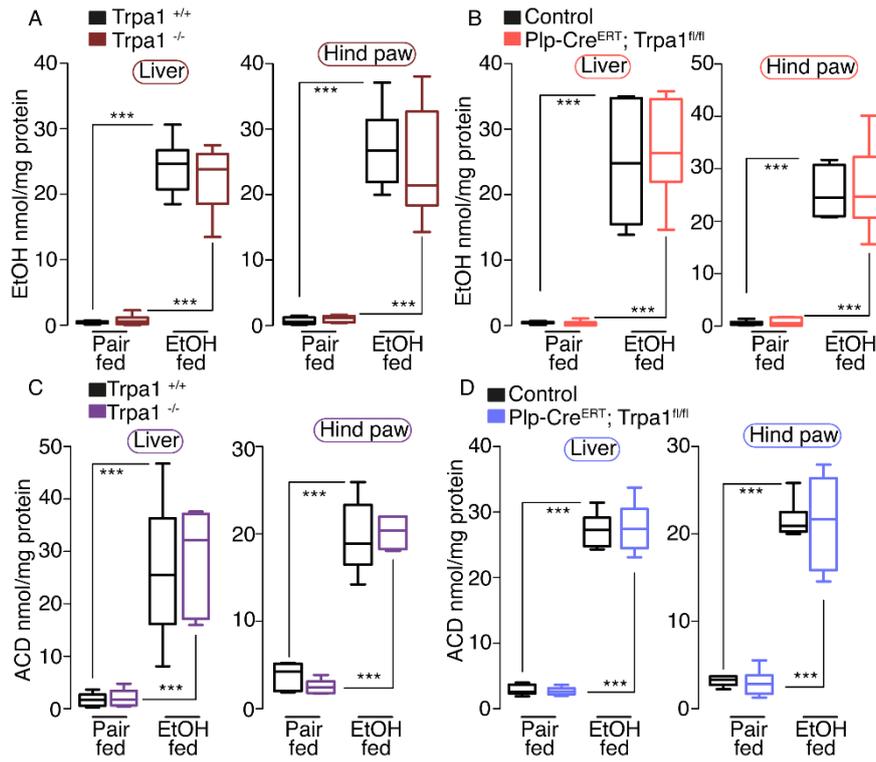


Supplemental Figure 3. (A) Hind paw mechanical allodynia after intragastric (i.g.) ethanol (EtOH, 15% 4 ml/kg) or vehicle (Veh) and intraplantar (i.pl., 20 μ l) phenyl- α -tert-butyl nitron (PBN, 100 μ g) or Veh PBN, in C5BL/6 mice. (B) Mechanical allodynia after EtOH (30%, i.p.) or Veh and intraperitoneal (i.p.) PBN (50 mg/kg, i.p.) or Veh PBN in C5BL/6 mice. (C) Mechanical allodynia after acetaldehyde (ACD, 0.1 mg/kg, i.p.) or Veh and PBN (100 μ g, i.pl.) or Veh PBN in C5BL/6 mice. (D) Mechanical allodynia after ACD (20 nmol, i.pl.) or veh and PBN (50 mg/kg, i.p.) or vehicle PBN in C5BL/6 mice. BL, baseline. Veh is the vehicle of EtOH and ACD. Data are mean \pm SEM with individual data points overlaid; n=8 mice for each experimental condition. ****P<0.001 vs. Veh; sssP<0.001 vs. EtOH and ACD; (A-D) two-way ANOVA with Bonferroni post-hoc correction.

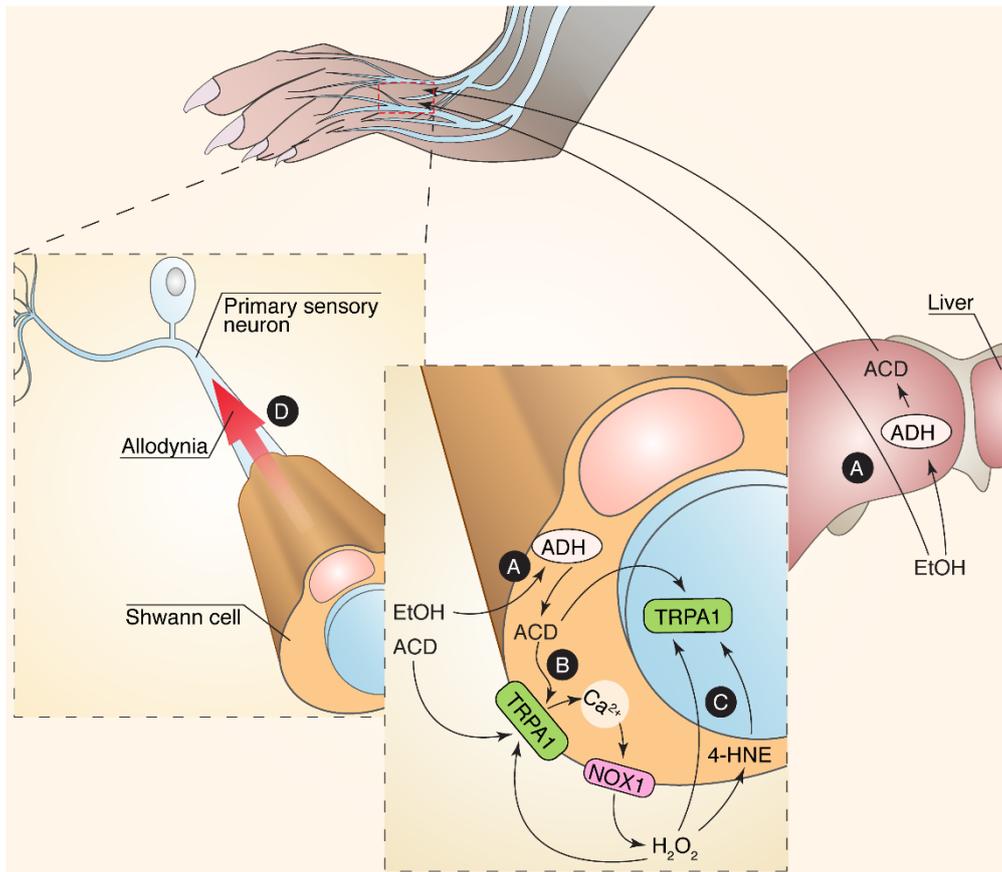


Supplemental Figure 4. (A) Acute nociceptive response induced by intraplantar (i.pl., 20 μ l) ethanol (EtOH, 30%) or vehicle (Veh) and allyl isothiocyanate (AITC, 10 nmol, i.pl.) or Veh in *Plp1-Cre^{ERT};Trpa1^{fl/fl}* and *Control* mice treated with tamoxifen (Tam, 1 mg/100 μ l, intraperitoneal, i.p.). (B) Acute nociception response induced by EtOH (30%, i.pl.) or Veh and AITC (10 nmol, i.pl.) or Veh in *Adv-Cre;Trpa1^{fl/fl}* and *Control* mice.

Veh is the vehicle of EtOH and AITC. Schematic representation of treatment protocols of (C) C57BL/6J mice received TRPA1 AS/MM-ODN (1 nmol/ μ l, i.pl.) and (G) *Plp1-CreERT;Trpa1^{fl/fl}* and *Control* mice received 4-hydroxytamoxifen (4-OHT) (0.02 mg/10 μ l, i.pl.) for 4 consecutive days. Ipsilateral indicates the treated hind paw and the untreated contralateral hind paw was used as control. (D and H) Representative images and qualitative analysis of colocalization by PDM image and colocalization value (Rcoloc) of S100 and TRPA1 expression in plantar nerve. The orange color indicates colocalized pixels and the blue color means segregation. Acute nociceptive response induced by ethanol (EtOH, 30%, i.pl.) or Veh and AITC (10 nmol, i.pl.) or Veh in C57BL/6J mice (E) and in *Plp1-CreERT;Trpa1^{fl/fl}* and *Control* mice (I). Mechanical allodynia after intragastric (i.g.) ethanol (EtOH, 15% 4 ml/kg) or Veh in C5BL/6J mice (F) and in *Plp1-CreERT;Trpa1^{fl/fl}* and *Control* mice (J). (A, B, E, I) Box plots with horizontal lines at the 25th percentile, the median, and the 75th percentile and the vertical lines which extend to the minimum and maximum values, all other data are mean \pm SEM with individual data points overlaid; n=8 mice for each experimental condition. ***P<0.001 vs. Veh; §§§P<0.001 vs. AITC (Control); unpaired two-tailed Student's t-test (D and H), one-way (A, B, E and I) or two-way (F and J) ANOVA with Bonferroni post-hoc correction.



Supplemental Figure 5. Ethanol (EtOH) and acetaldehyde (ACD) levels in liver and hind paw tissue after 28 days of an EtOH 5% (vol/vol)- or control-diet in (A and C) *Trpa1*^{+/+} and *Trpa1*^{-/-} mice and, (B and D) *Plp1-CreERT;Trpa1*^{fl/fl} and *Control* mice. Box plots with horizontal lines at the 25th percentile, the median, and the 75th percentile and the vertical lines which extend to the minimum and maximum values; n=8 mice for each experimental condition. ***P<0.001 vs. pair-fed mice; (A-D) one-way ANOVA with Bonferroni post-hoc correction.



Supplemental Figure 6. (A) Intra-gastric ethanol is converted into acetaldehyde (ACD) by alcohol dehydrogenase (ADH) in the liver and in Schwann cells. (B) Hepatic or locally produced ACD targets the Ca²⁺ dependent TRPA1/NOX1 pathway in Schwann cells to release H₂O₂. (C) H₂O₂ and its carbonylic byproduct, 4-hydroxynonenal (4-HNE), gating the neuronal TRPA1 (D) signal allodynia.