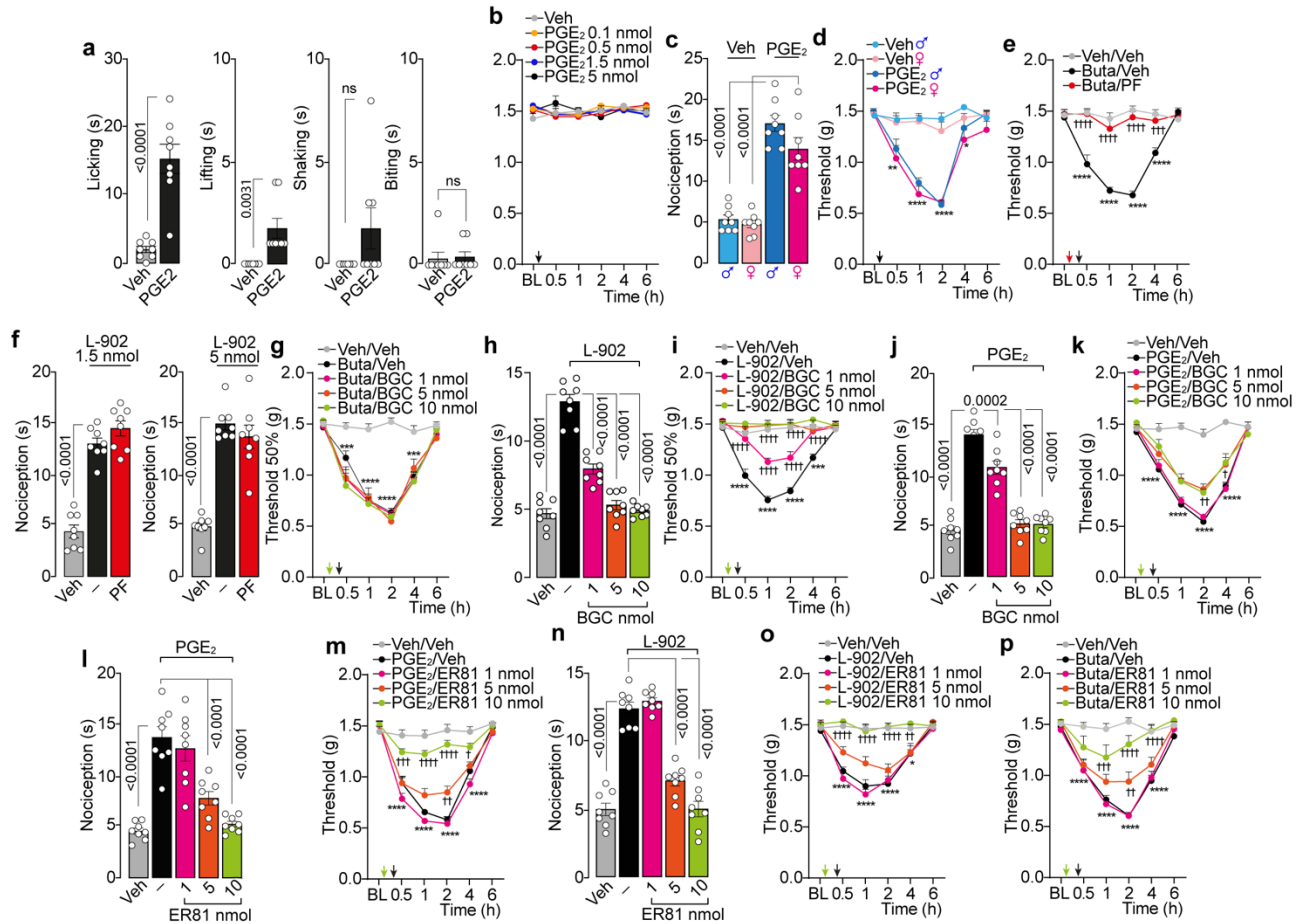


Supplementary information

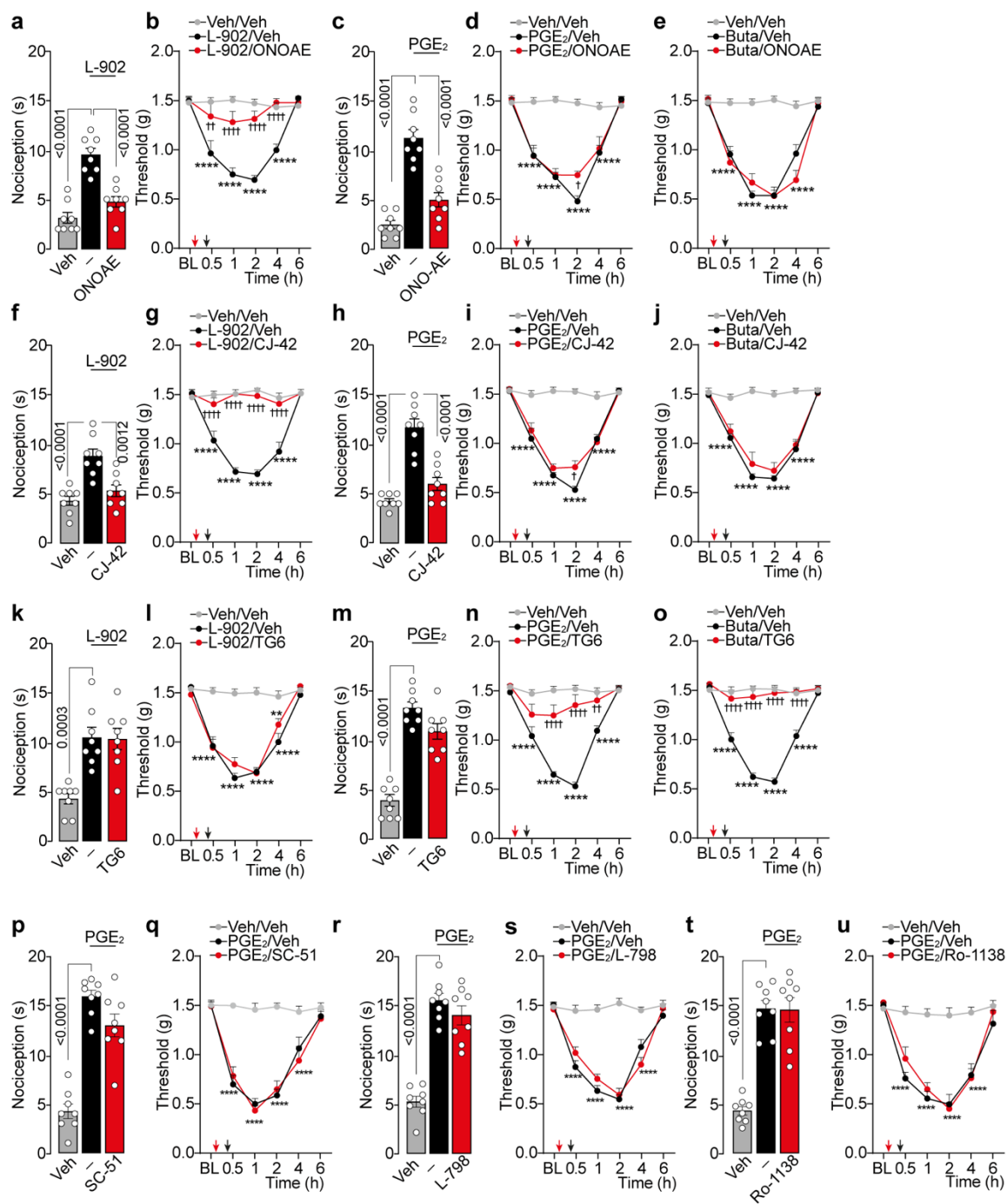
Supplementary Figures



Supplementary Fig. 1. EP2 mediates hind paw mechanical allodynia (allodynia)

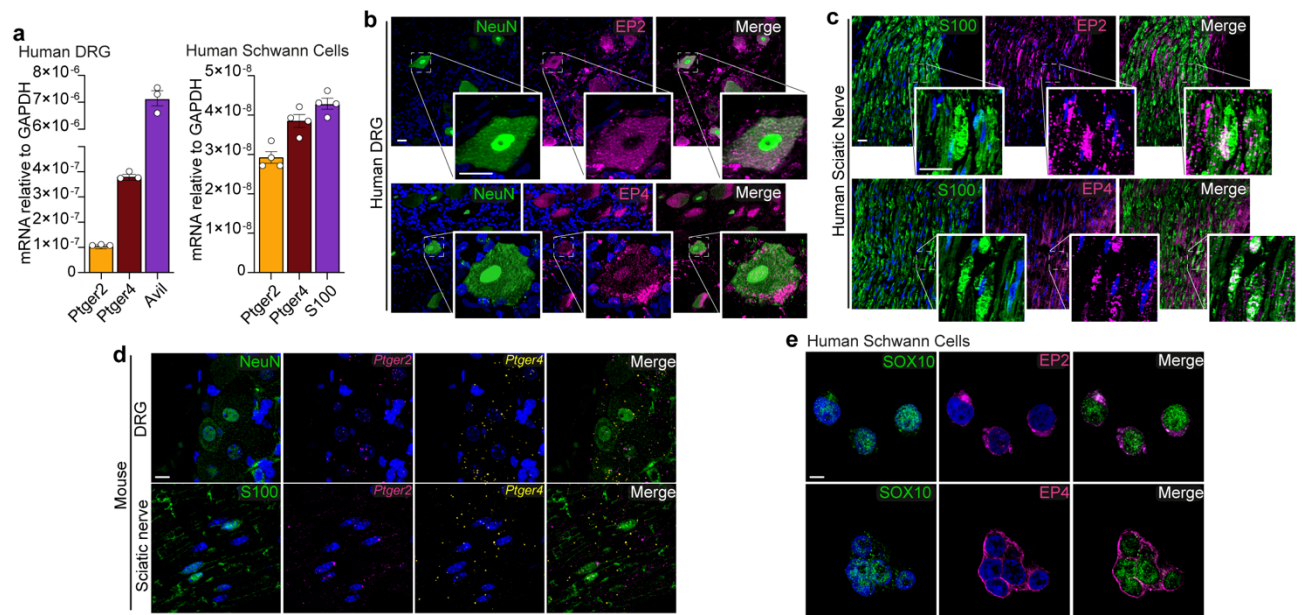
by PGE₂. **a**, Licking, lifting, shaking, and biting behaviour after intraplantar (i.pl.) injection of PGE₂ (1.5 nmol) or vehicle (Veh) in C57BL/6J mice (B6). **b**, Allodynia in the contralateral paw after i.pl. PGE₂ (1.5 nmol) or Veh in B6 mice. **c,d**, Non-evoked nociception, and allodynia in male and female B6 after i.pl. PGE₂ (1.5 nmol) or Veh. **e**, Allodynia after i.pl. (R)-Butaprost (Buta, 1.5 nmol) or Veh in B6 pretreated with PF-04448948 (PF, 5 nmol) or Veh. **f**, Non-evoked nociception after i.pl. L-902,688 (L-902, 1.5 and 5 nmol) or Veh in B6 pretreated with PF (5 nmol) or Veh. **g**, Allodynia after i.pl. Buta (1.5 nmol) or Veh in B6 pretreated with BGC-20-1531 (BGC). **h,i**, Non-evoked nociception and allodynia after i.pl. L-902 (5 nmol) or **j,k**, PGE₂ (1.5 nmol) or Veh in B6 pretreated with BGC or Veh. **l,m**, Non-evoked nociception and allodynia after i.pl. PGE₂ (1.5 nmol) or **n,o**, L-902 (5 nmol) or

p, Buta (1.5 nmol) or Veh in B6 mice pretreated with ER-819762 (ER81) or Veh. All panels, n=8 mice/group. Data are mean $\pm$ s.e.m. **a**, Student's t test, **c,f,h,j,l,n**, 1-way or **b,d,e,g,i,k,m,o,p**, 2-way ANOVA, Bonferroni correction. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 vs. Veh †P<0.05, ††P<0.01, †††P<0.001, ††††P<0.0001 vs. Buta, L-902, PGE₂/Veh. Source data are provided as a Source Data file.

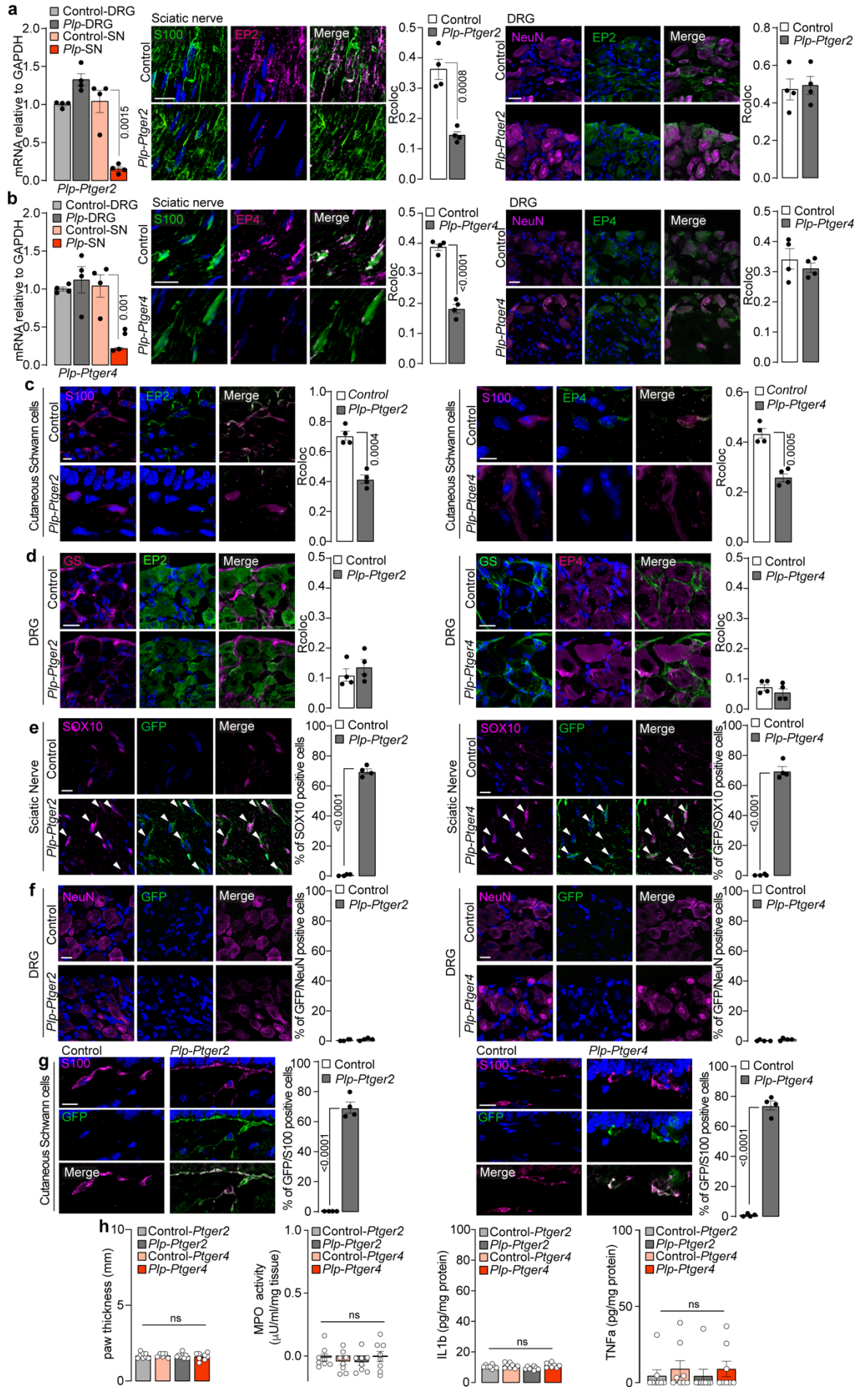


Supplementary Fig. 2. EP4 and EP2 mediate non-evoked nociception (nociception) and mechanical allodynia (allodynia), respectively, in response to PGE₂ in mouse hind paw. Nociception and allodynia after intraplantar (i.p.) injection of the selective EP4 agonist, L-902,688 (L-902, 5 nmol), PGE₂ (1.5 nmol) or the selective EP2 agonist, butaprost (Buta, 1.5 nmol) or vehicle (Veh) in C57BL/6J mice (B6) pretreated with i.p. injection of the selective EP4 antagonists, (a-e) ONOAE3-208 (ONOAE, 5 nmol) (a-e)

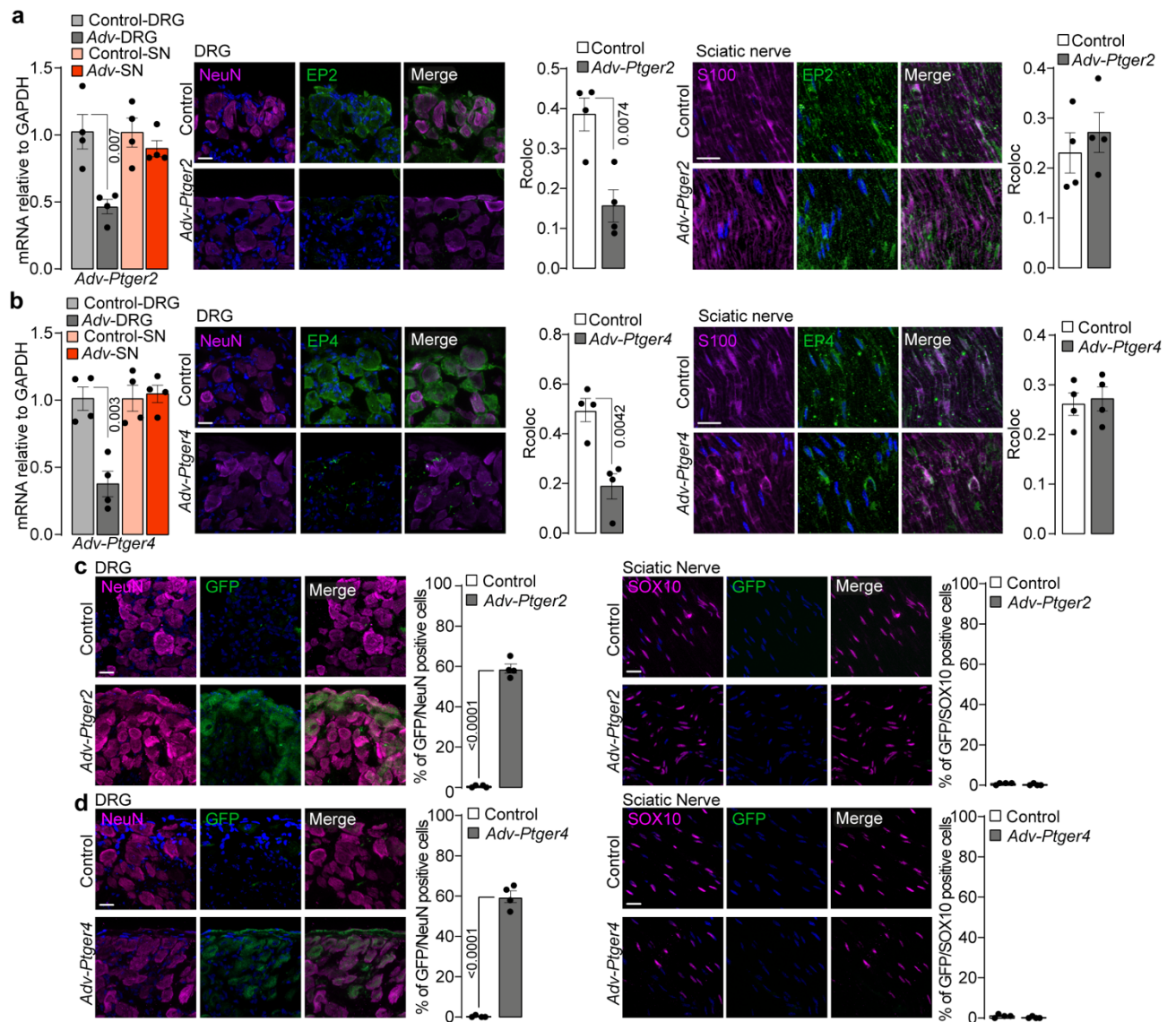
and CJ-42794 (CJ-42, 5 nmol) (**f-j**) or the selective EP2 antagonist TG6-10-1 (TG6, 5 nmol) or Veh (**k-o**). Non-evoked nociception and allodynia after i.pl PGE₂ (1.5 nmol) or Veh in B6 mice pretreated with the selective EP1 antagonist, SC-51322 (SC-51, 5 nmol), the selective EP3 antagonist, L-798,106 (L-798, 5 nmol), the selective IP antagonist, Ro-1138452 (Ro-1138, 5 nmol) or Veh (**p-u**). All panels, 8 mice/group. Data are mean $\pm$ s.e.m. **a,c,f,h,k,h,m, p,r,t** 1-way or **b,d,e,g,i,j,l n,o,q,s,u** 2-way ANOVA, Bonferroni correction. **P<0.01, ****P<0.0001 vs. Veh †P<0.05, ††P<0.01, †††P<0.001, ††††P<0.0001 vs. Buta, L-902, PGE₂/Veh. Source data are provided as a Source Data file.



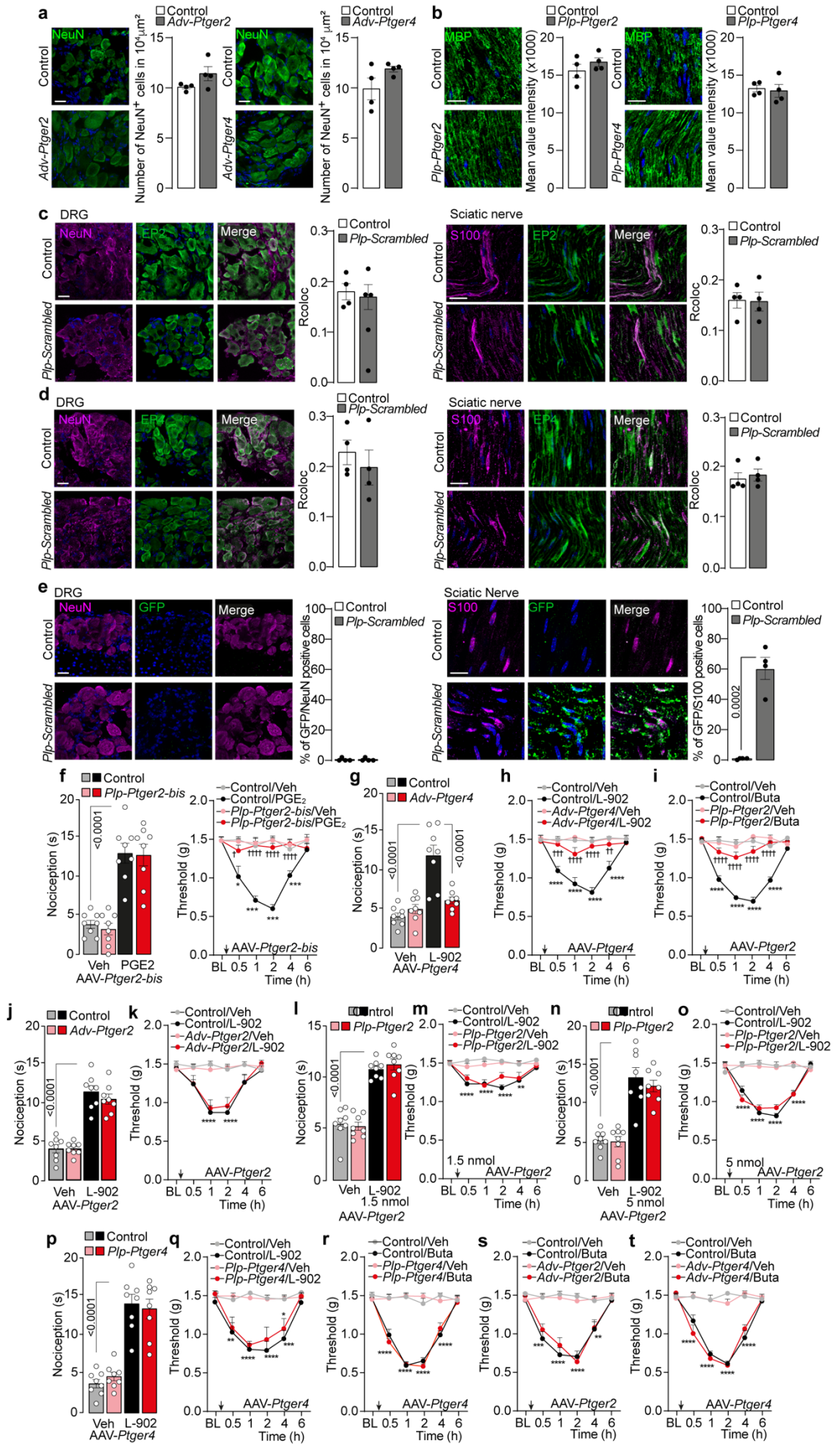
Supplementary Fig. 3. EP2 and EP4 expression in human dorsal root ganglia and Schwann cells. **a**, RT-qPCR for *Ptger2*, *Ptger4*, *Avil*, and *S100* mRNA in human dorsal root ganglia (DRG) and Schwann cells (SCs) (n=3 and 4 independent experiments, respectively). **b**, Representative images of EP2, EP4, NeuN and S100B expression in human DRG and **c**, sciatic nerve tissue (scale bar: 20 μm, inset 20 μm) (n=3 subjects). **d**, Representative RNAscope images of *Ptger2* and *Ptger4* mRNA and S100 and NeuN protein expression in mouse DRG and sciatic nerve tissues (scale bar: 20 μm) (n=3 independent experiments). **e**, Representative images of EP2, EP4 and SOX10 expression in human SCs (n=3 independent experiments). Data are mean ± s.e.m. Source data are provided as a Source Data file.



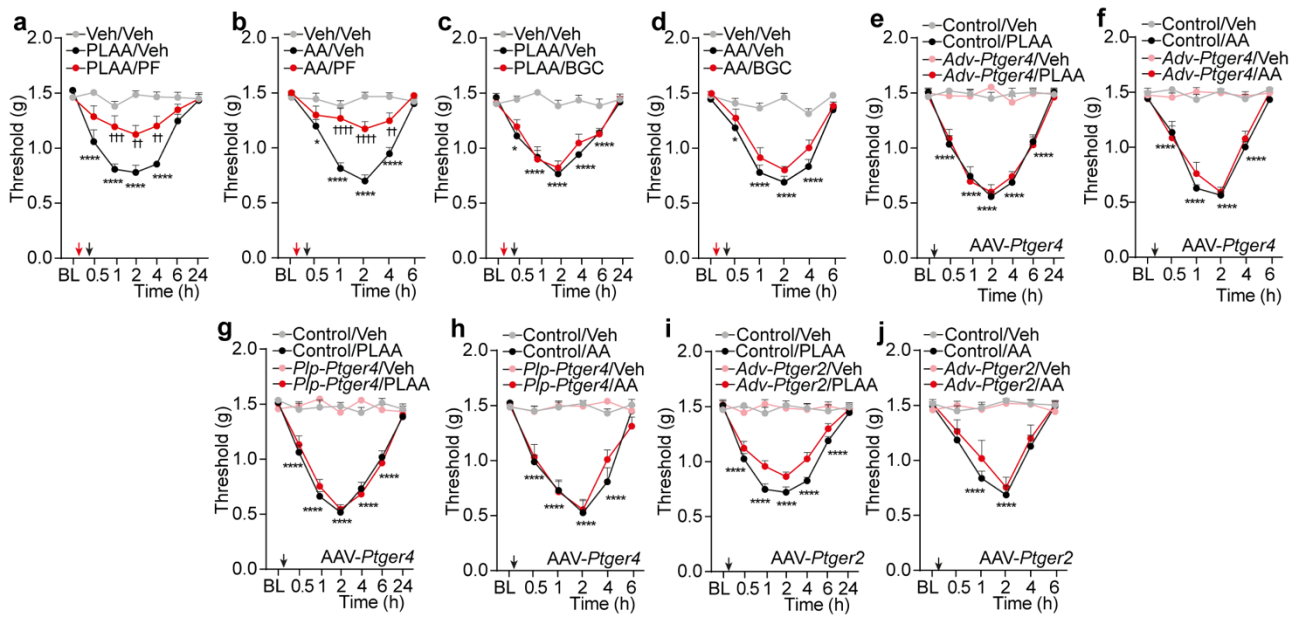
Supplementary Fig. 4. AAV mediated EP2 and EP4 selective silencing in Schwann cells. **a, b** RT-qPCR for *Ptger2* and *Ptger4* mRNA and representative images of EP2, EP4, NeuN and S100B protein expression and colocalization data (Rcoloc) in mouse sciatic nerve and dorsal root ganglia (DRG) in *Plp-Cre* and Control mice infected with AAV for selective silencing of EP2 (*Plp-Ptger2*) or EP4 (*Plp-Ptger4*). **c**, Representative images of EP2, EP4 and S100B protein expression and Rcoloc in mouse paw skin tissue of *Plp-Ptger2*, *Plp-Ptger4* and Control mice. **d**, Representative images of EP2, EP4 and satellite glial cell marker (GS) protein expression and Rcoloc in DRG of *Plp-Ptger2*, *Plp-Ptger4* and Control mice. **e,f,g**, Representative images of SOX10, NeuN, S100B and GFP protein expression and percentage of GFP positive cells in mouse sciatic nerve, DRG and paw skin tissue of *Plp-Ptger2*, *Plp-Ptger4* and Control mice (n=4 subjects). (scale bar: 20 μ m) **h**, Paw thickness, myeloperoxidase (MPO) activity, IL-1 β and TNF- α levels in paw tissue homogenates of *Plp-Ptger2*, *Plp-Ptger4* and Control mice (n=8 mice/group). (ns, not significant). Data are mean $\pm$ s.e.m. **a,b,h**, 1-way or **a-g**, 2-Tailed Student's t test. Source data are provided as a Source Data file.



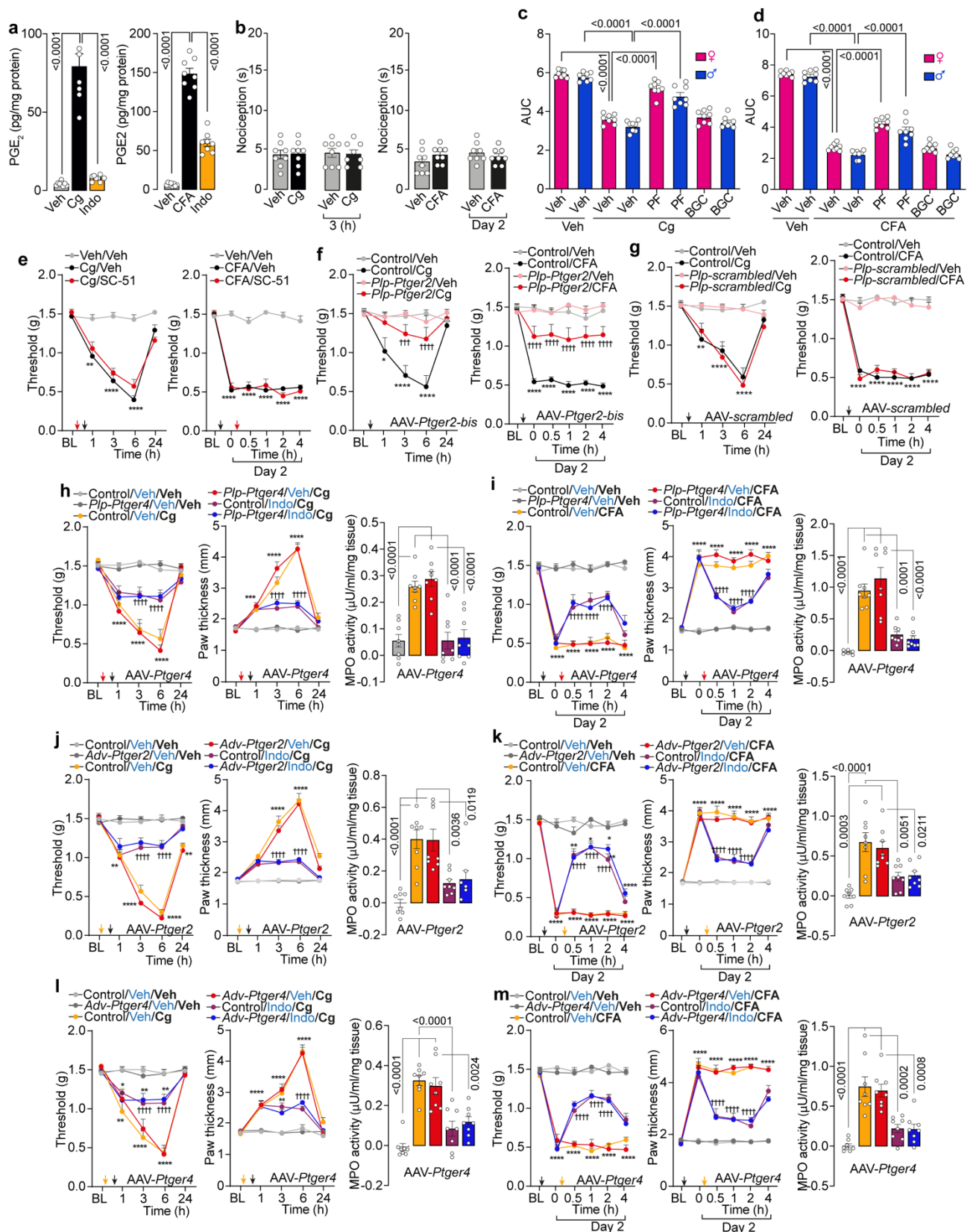
Supplementary Fig. 5. AAV mediated EP2 and EP4 selective silencing in primary sensory neurons. a, b, RT-qPCR for *Ptger2* and *Ptger4* mRNA and EP2, EP4, NeuN and S100B protein expression and colocalization data (Rcoloc) in mouse dorsal root ganglia (DRG) and sciatic nerve tissue in *Adv-Cre* and Control mice infected with AAV for selective silencing of EP2 (*Adv-Ptger2*) or EP4 (*Adv-Ptger4*). **c,d,** Representative images of NeuN, SOX10 and GFP protein expression and percentage of GFP positive cells in mouse DRG and sciatic nerve tissue of *Adv-Ptger2*, *Adv-Ptger4* and Control mice. (scale bar: 20 μ m) (n=4 subjects). Data are mean $\pm$ s.e.m. **a,b,** 1-way or **a-d,** Student's t test. Source data are provided as a Source Data file.



Supplementary Fig. 6. Non-evoked nociception and mechanical allodynia (allodynia) mediated by EP2 and EP4 selective agonist in mice with EP2 and EP4 selective deletion. **a**, Representative images and cumulative data of NeuN expression in dorsal root ganglia (DRG) in *Adv-Cre* and Control mice infected with AAV for selective silencing of EP2 (*Adv-Ptger2*) or EP4 (*Adv-Ptger4*). (scale bar: 20 μ m) **b**, Representative images and fluorescence intensity of myelin basic protein (MBP) in mouse sciatic nerve of *Plp-Cre* and Control mice infected with AAV for selective silencing of EP2 (*Plp-Ptger2*) or EP4 (*PLp-Ptger4*). (scale bar: 20 μ m) **c,d**, Representative images of EP2, EP4, NeuN and S100B protein expression and colocalization data (Rcoloc) in DRG and mouse sciatic nerve of *Plp-scrambled* and Control mice. (scale bar: 20 μ m) **e**, Representative images of NeuN, S100B and GFP protein expression and percentage of GFP positive cells in mouse DRG and sciatic nerve tissue of *Plp-scrambled* and Control mice. (scale bar: 20 μ m) **f**, Non-evoked nociception and allodynia after i.pl. PGE₂ (1.5 nmol) or Veh in *Plp-Cre* and Control mice infected with AAV for a selective silencing of EP2 (*Plp-Ptger2-bis*). **f,g,j,l,n,p**, Non-evoked nociception, and **h,k,m,o,q** allodynia after i.pl. L-902,688 (L-902, 5 nmol) or Veh in **g,h**, *Adv-Ptger4* **j,k** *Adv-Ptger2*, **l-o** *Plp-Ptger2*, **p,q**, *Plp-Ptger4* and Control mice (n=8 mice/group) Allodynia after i.pl. Butaprost (Buta, 1.5 nmol) or Veh in **i**, *Plp-Ptger2*, **r**, *Plp-Ptger4*, **s**, *Adv-Ptger2*, **t**, *Adv-Ptger4* and Control mice (n=8 mice/group). Data are mean $\pm$ s.e.m. **a-e** Student's t test, **f,g,j,l,n,p**, 1-way or **f,h,l,k,m,o,q-t** 2-way ANOVA, Bonferroni correction, *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 vs. Veh †P<0.05, ††P<0.01, †††P<0.001, ††††P<0.0001 vs. Buta, L-902, PGE₂/Veh. Source data are provided as a Source Data file.

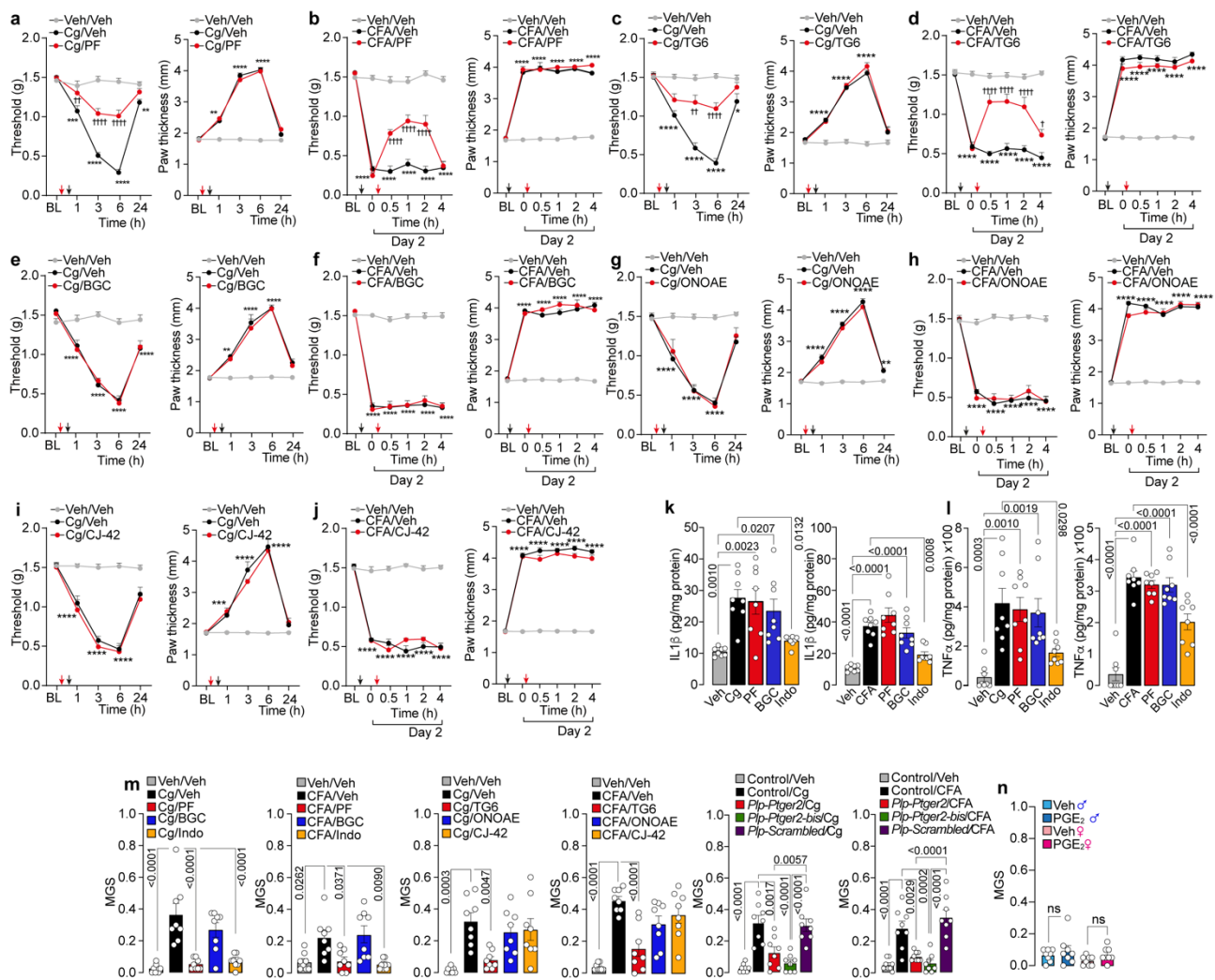


Supplementary Fig. 7. EP2 in Schwann cells mediates hind paw mechanical allodynia (allodynia) by Phospholipase A2 Activating Protein (PLAA) and Arachidonic Acid (AA). **a-d**, Allodynia after intraplantar (i.p.) PLAA (2 nmol), AA (10 nmol) or vehicle (Veh) in C57BL/6J (B6) mice pretreated with PF-04448948 (PF, 5 nmol), BGC-20-1531 (BGC, 5 nmol) or Veh. **e-j**, Allodynia after i.p. PLAA, AA or Veh in *Plp-Cre*, *Adv-Cre* or Control mice infected with AAV for selective silencing of EP2 (-Ptger2) or EP4 (-Ptger4) (*Plp-Ptger2* or *Adv-Ptger2* and *Plp-Ptger4* or *Adv-Ptger4*). All panels, n=8 mice/group. Data are mean $\pm$ s.e.m. 2-way ANOVA, Bonferroni correction. *P<0.05, ****P<0.0001 vs. Veh, Control/Veh, ††P<0.01, †††P<0.001, ††††P<0.0001 vs. PLAA, AA/Veh. Source data are provided as a Source Data file.



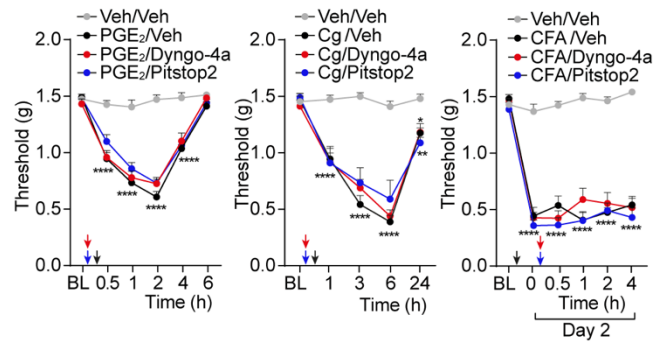
Supplementary Fig. 8. EP2 in Schwann cells mediates carrageenan and CFA hind paw mechanical allodynia (allodynia) but not inflammation. **a**, PGE₂ assay in paw tissue homogenates of C57BL/6J (B6) mice after intraplantar (i.pl.) carrageenan (Cg, 300 µg) or Complete Freund Adjuvant (CFA) or vehicle (Veh) pretreated with indomethacin

(Indo, 280 nmol) or Veh. **b**, Non-evoked acute or delayed nociception after i.pl. Cg, CFA or Veh in B6 mice. **c,d** Allodynia expressed as area under the curve (AUC) after i.pl. Cg (300 µg), CFA or Veh in male and female B6 mice pretreated with PF-04448948 (PF, 5 nmol), BGC-20-1531 (BGC, 5 nmol) or Veh. **e**, Allodynia after i.pl. Cg, CFA or Veh in B6 mice pretreated with SC-51322 (SC-51, 5 nmol) or Veh. **f,g**, Allodynia after i.pl. Cg, CFA or Veh in *Plp-Cre* or Control mice infected with AAV for selective silencing of EP2 (*Ptger2 bis*) or scrambled (*Plp-Ptger2-bis* or *Plp-scrambled*). **h-m**, Allodynia, paw thickness and MPO activity assay after i.pl. Cg, CFA or Veh in *Plp-Cre*, *Adv-Cre* or Control mice infected with AAV for selective silencing of EP2 (-*Ptger2*) or EP4 (-*Ptger4*) (*Plp-Ptger2* or *Adv-Ptger2* and *Plp-Ptger4* or *Adv-Ptger4*) and pretreated with indomethacin (Indo, 280 nmol) or Veh. All panels, n=8 mice/group. Data are mean ± s.e.m. **a,c,d,h-m** MPO activity 1-way or **e-m** allodynia and paw thickness 2-way ANOVA, Bonferroni correction. *P<0.05, **P<0.01 ***P<0.0001, ****P<0.0001 vs. Control/Veh/Veh or Veh/Veh; ††††P<0.0001 vs. Control/Veh/Cg, CFA or Cg, CFA/Veh. Source data are provided as a Source Data file.

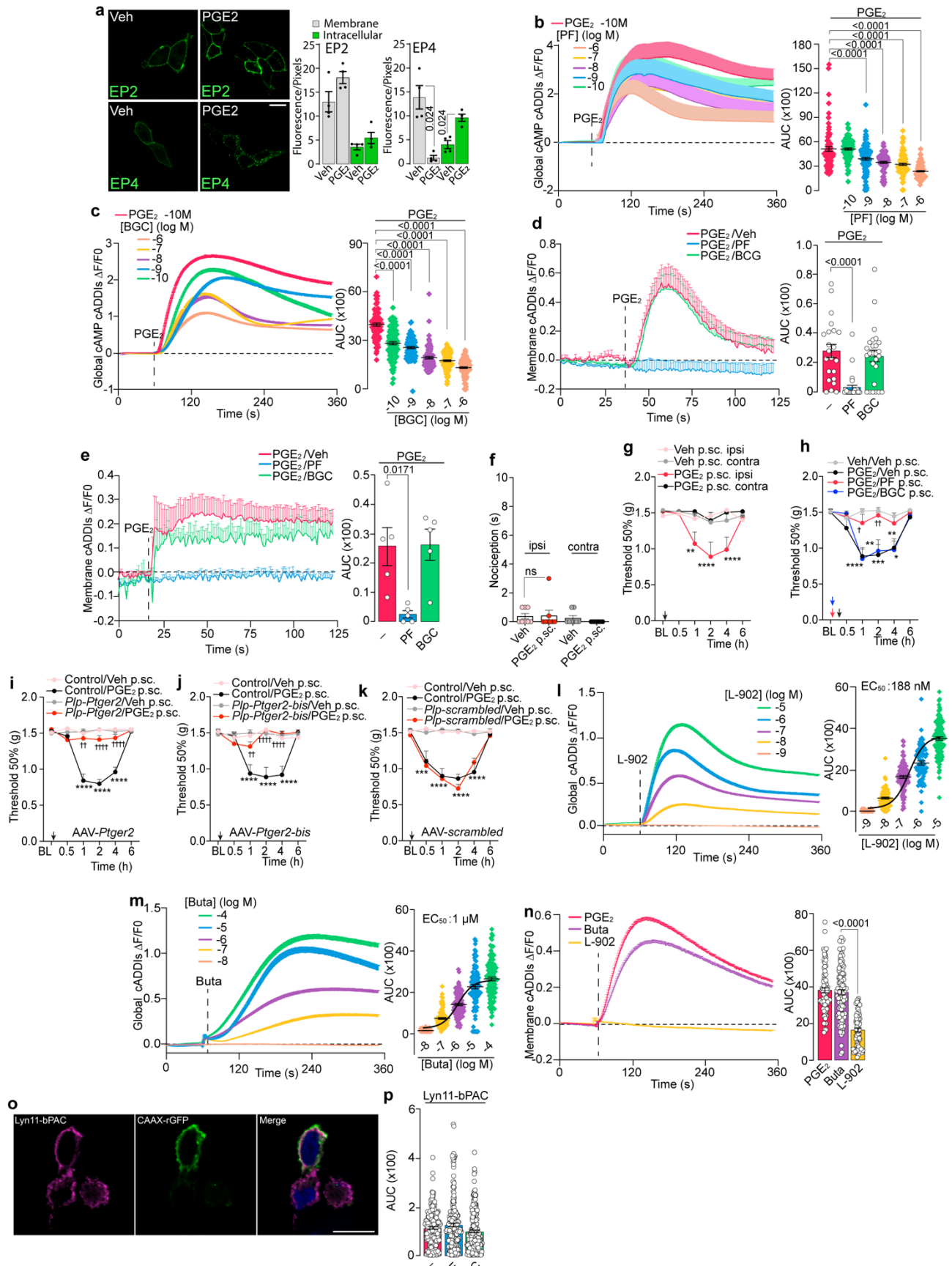


Supplementary Fig. 9. EP2 in Schwann cells mediates carrageenan and CFA hind paw mechanical allodynia (allodynia) and grimace behavior (grimace) but not inflammation. **a-j** Allodynia, paw thickness after intraplantar (i.pl.) carrageenan (Cg, 300 μ g) or Complete Freund Adjuvant (CFA) or vehicle (Veh) in C57BL/6J (B6) mice pretreated with PF-04448948 (PF, 5 nmol), TG6-10-1 (TG6, 5 nmol), BGC-20-1531 (BGC, 5 nmol), ONOA3-208 (ONOA, 5 nmol), CJ-42794 (CJ-42, 5 nmol) or Veh. **k, l** IL1 β and TNF α assay in paw tissue homogenates after i.pl. Cg, CFA or Veh in B6 mice pretreated with PF (5 nmol), BGC (5 nmol) indomethacin (Indo, 280 nmol) or Veh. **m**, Mouse grimace score (MGS) after i.pl. Cg, CFA or Veh in B6 mice pretreated with PF (5 nmol), BGC (5 nmol), Indo (280 nmol), TG6 (5 nmol), ONOA (5 nmol), CJ-42 (5 nmol) or Veh or in *Plp-Cre* or Control mice infected with AAV for selective silencing of EP2 (*Ptger2* and *Ptger2-bis*) or scrambled (*Plp-Ptger2*, *Plp-Ptger2-bis* or *Plp-scrambled*). **n**, MGS after i.pl. PGE₂ in B6 male and female mice. All panels, n=8 mice/group. Data are mean $\pm$ s.e.m. **a-j** 2-way or **k-m** 1-way ANOVA, Bonferroni correction **n** Student's t test. **P<0.01 ***P<0.0001,

****P<0.0001 vs. Veh/Veh; ††P<0.01, ††††P<0.0001 vs. Cg/Veh, CFA/Veh. Source data are provided as a Source Data file.

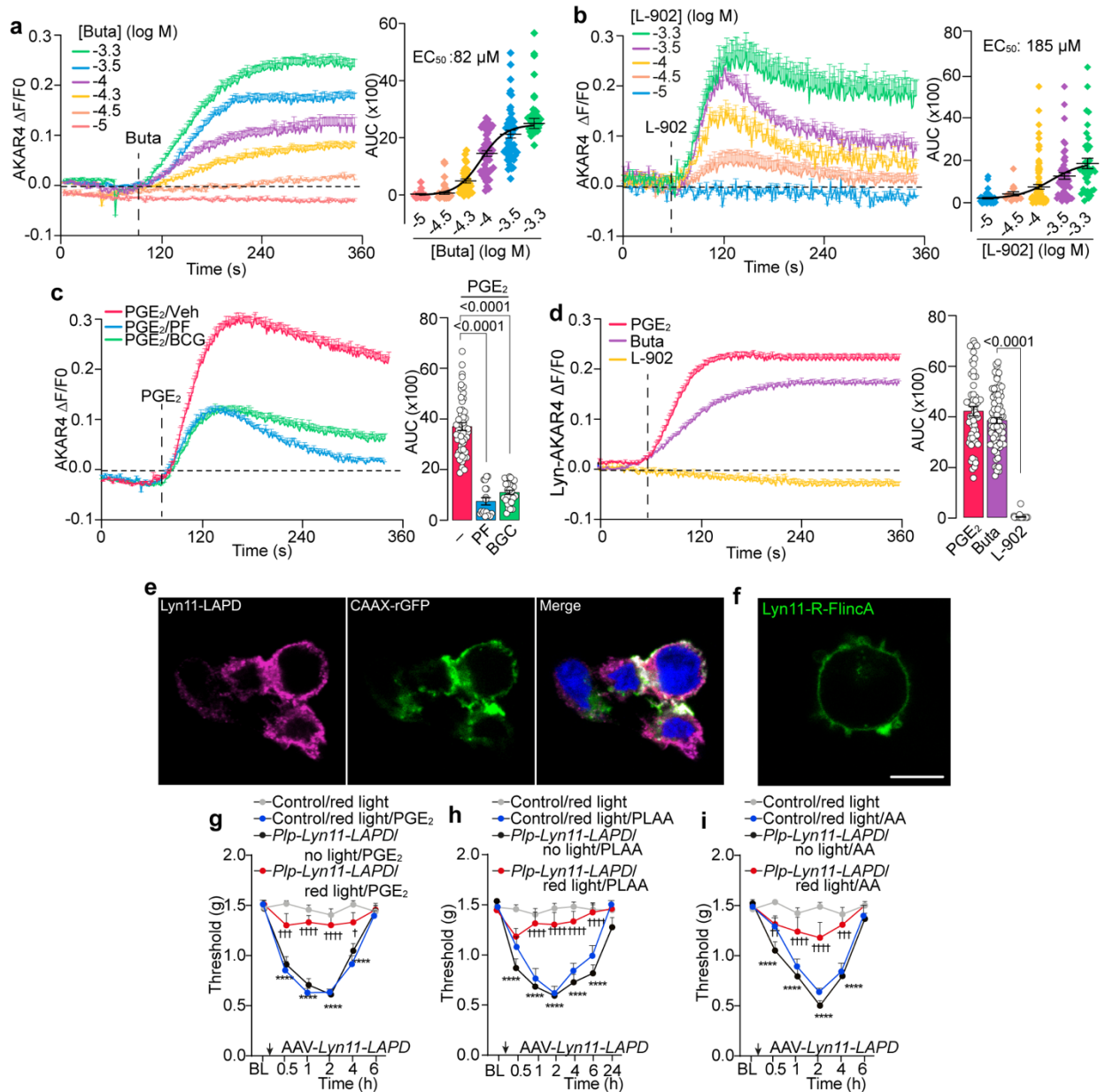


Supplementary Fig. 10. Clathrin and dynamin inhibitors do not mediate PGE₂, carrageenan and CFA hind paw mechanical allodynia (allodynia). Allodynia after intraplantar (i.p.l.) injection of PGE₂ (1.5 nmol), carrageenan (Cg, 300 µg), Complete Freund Adjuvant (CFA) or vehicle (Veh) in C57BL/6J (B6) mice pretreated with Dyngo-4a (500 pmol), Pitstop2 (500 pmol) or Veh (n=8 mice/group). Data are mean ± s.e.m. 2-way ANOVA, Bonferroni correction. *P<0.05, **P<0.01, ****P<0.0001 vs. Veh/Veh. Source data are provided as a Source Data file.



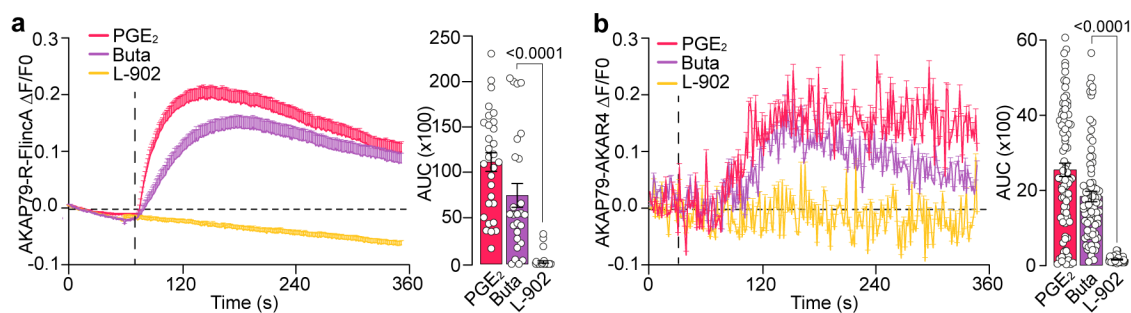
Supplementary Fig. 11. Schwann cells cAMP nanodomains promote EP2 hind paw mechanical allodynia (allodynia). a, Representative images and cumulative data of membrane

and intracellular localization of EP2 and EP4 in HEK293T cells, with or without pre-incubation with unlabeled PGE₂ (10 μ M, 30 min) (n=4 independent experiments). (Scale bar, 20 μ m). **b,c**, Global cAMP measurement after PGE₂ (100 pM) stimulation of human Schwann cells (hSCs) in the presence of PF-04448948 (PF) (cells number: -6 M=109, -7M=89, -8 M=75, -9M=99, -10M=99), BGC-20-1531 (BGC) (cells number: -6 M=96, -7M=102, -8 M=87, -9M=84, -10M=95) or Veh (cells number: n=78 or n=91) (n=3 independent experiments). **d,e**, Membrane cAMP measurement after PGE₂ (10 nM) stimulation of mouse sciatic nerve and cutaneous SCs in the presence of PF (1 μ M) (cells number: sciatic nerve SCs n=27, cutaneous SCs n=5), BGC (1 μ M) (cells number: sciatic nerve SCs n=26, cutaneous SCs n=5) or Veh (cells number: sciatic nerve SCs n=22, cutaneous SCs n=5) (n=3 independent experiments). **f-h** Non-evoked nociception and allodynia after peri-sciatic (p.sc.) injection of PGE₂ (1.5 nmol) or vehicle (Veh) in C57BL/6J mice (B6) pretreated with PF-04448948 (PF, 5 nmol), BGC-20-1531 (BGC, 5 nmol) or Veh. **i,j,k**, Allodynia after p.sc. PGE₂ (1.5 nmol) or vehicle (Veh) in *Plp-Cre* or Control mice infected with AAV for selective silencing of EP2 (*Ptger2* and *Ptger bis*) or scrambled (*Plp-Ptger2*, *Plp-Ptger2-bis* or *Plp-scrambled*). **g-k**, n=8 mice/group. **l,m**, Global cAMP measurement after L-902,688 (L-902) (cells number: -5M=112,-6M=73,-7M=94,-8M=82,-9M=94, n=3 independent experiments) and (R)-Butaprost (Buta) (cells number: -4M=109,-5M=96,-6M=99,-7M=94,-8M=98, n=3 independent experiments) stimulation of hSCs. **n**, Membrane cAMP measurement after PGE₂ (10 nM), Buta (100 μ M) or L-902 (100 μ M) stimulation of hSCs (cells number: PGE₂=86, Buta=103, L-902=79, n=3 independent experiments). **o**, Colocalization of Lyn11-bPAC and the plasma membrane marker CAAX in HEK293 cells (scale bar: 10 μ m). **p**, Membrane cAMP formation in hSCs after blue light (450 nm, 10 min) activation of membrane-tagged bPAC (Lyn11-bPAC) in the presence of PF (1 μ M) (cells number: n=214), BGC (1 μ M) (cells number: n=214) or Veh (cells number: n=214). Data are mean $\pm$ s.e.m. **a** Student's t test, **b-f, n,p** 1-way, **j-k** 2-way ANOVA Bonferroni correction. AUC, area under the curve. *P<0.05, **P<0.01 ***P<0.0001, ****P<0.0001 vs. Veh, Control/Veh, †P<0.05, ††P<0.01, †††P<0.0001 vs. Control/PGE₂. Source data are provided as a Source Data file.

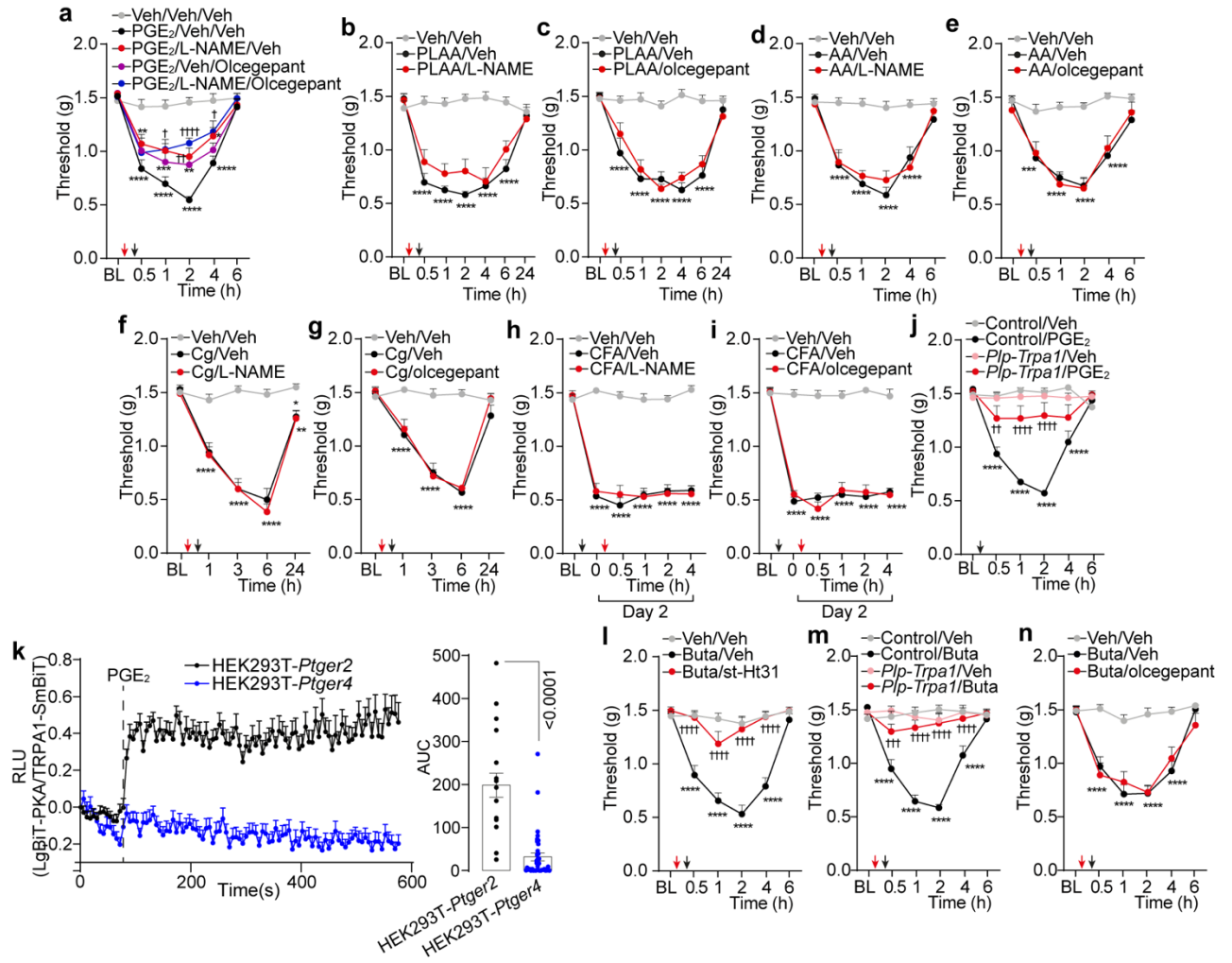


Supplementary Fig. 12. Schwann cells membrane-bound PKA/AKAP drives EP2 signaling. **a,b** Global PKA activation induced by Buta (cells number:-3.3M=37,-3.5M=48,-4M=49,-4.3M=35,4.5M=44,-5M=37, n=3 independent experiments) and L-902 (cells number:-3.3M=37,-3.5M=52,-4M=95,-4.5M=16,-5M=51, n=3 independent experiments) stimulation of human Schwann cells (hSCs). **c**, global PKA activation induced by PGE₂ (10 nM) in the presence of PF (1 μM), BGC (1 μM) or Veh (cells number: PF=18, BGC=29, PGE₂=52, n=3 independent experiments) in hSCs. **d**, Membrane PKA activation induced by PGE₂, (10 nM) Buta (100 μM) or L-902 (100 μM) stimulation of hSCs (cells number: PGE₂=57, Buta=81, L-902=45, n=3 independent experiments). **e**, Colocalization of Lyn11-LAPD and the plasma membrane marker CAAX in HEK293 cells. (scale bar: 10 μm). **f**, Membrane localization of

Lyn11-R-Flnca in HEK293 cells (scale bar: 10 μ m). **g,h,i**, Allodynia after i.pl. PGE₂, Phospholipase A2 Activating Protein (PLAA, 2 nmol), Arachidonic Acid (AA, 10 nmol) or Veh in Plp-Cre or Control mice after i.pl. infection with AAV-Lyn11-LAPD (Plp- Lyn11-LAPD) and stimulated with red light (n=8 mice/group). Data are mean $\pm$ s.e.m. **c,d**, 1-way or, **g-i**, 2-way ANOVA, Bonferroni correction. AUC, area under the curve. ***P<0.0001 vs. Veh, Control/red light. †P<0.05, ††P<0.01, †††P<0.001, ††††P<0.0001 vs. Plp-Lyn11-LAPD/no light/PGE₂. Source data are provided as a Source Data file.

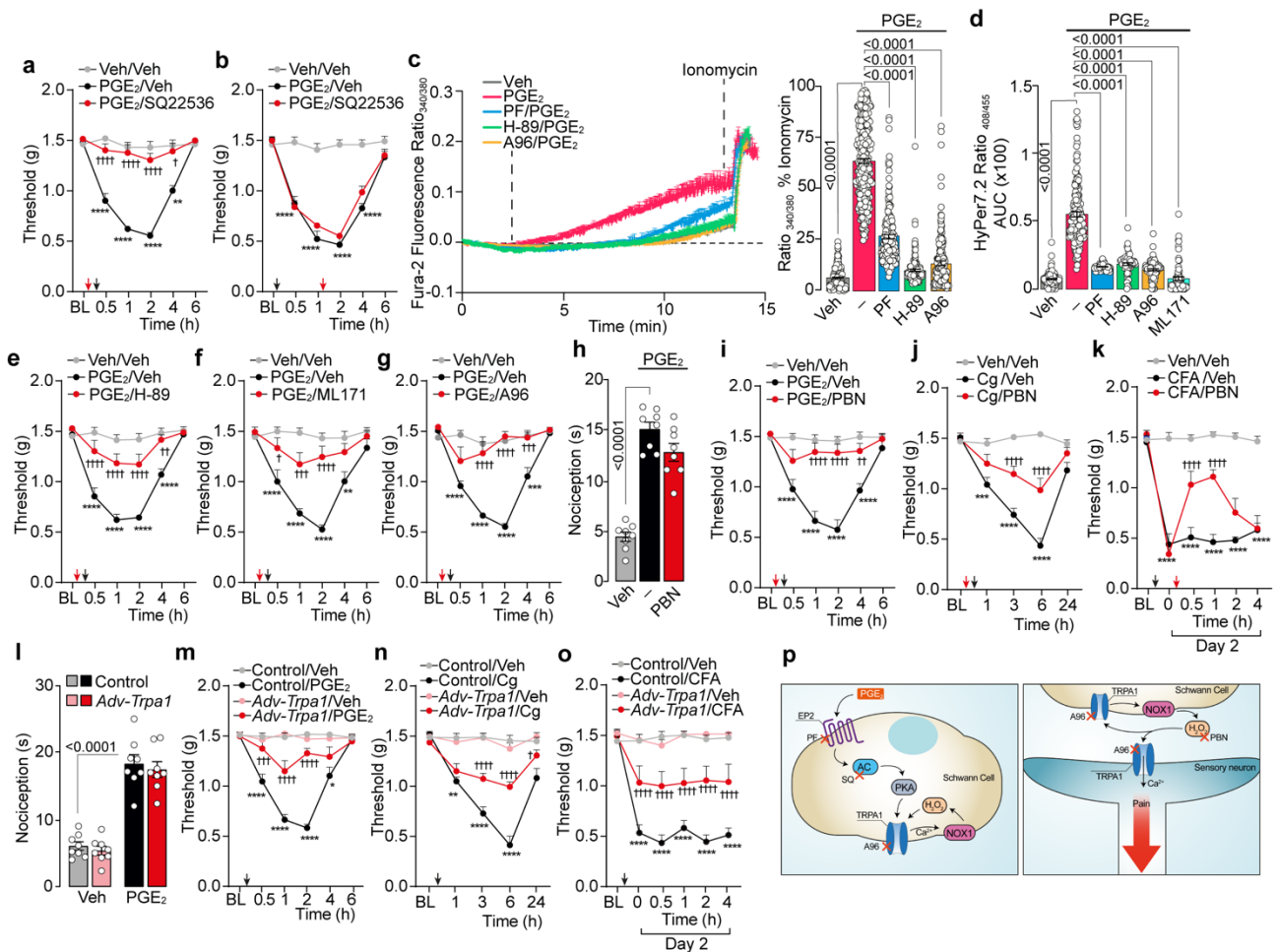


Supplementary Fig. 13. Schwann cells membrane-bound PKA/AKAP drives EP2 signaling. **a**, AKAP79 associated cAMP formation after PGE₂ (10 nM), (R)-Butaprost (Buta, 100μM) or L-902,688 (L-902, 100 μM) stimulation of human Schwann cells (hSCs) (cells number: PGE₂=30, Buta=26, L-902=37, n=3 independent experiments). **b**, AKAP79 associated PKA activation induce by PGE₂ (10 nM), Buta (100 μM) or L-902 (100 μM) stimulation of hSCs (cells number: PGE₂=86, Buta=82, L-902=69, n=3 independent experiments). AUC, area under the curve. Data are mean ± s.e.m. 1-way ANOVA, Bonferroni correction. Source data are provided as a Source Data file.



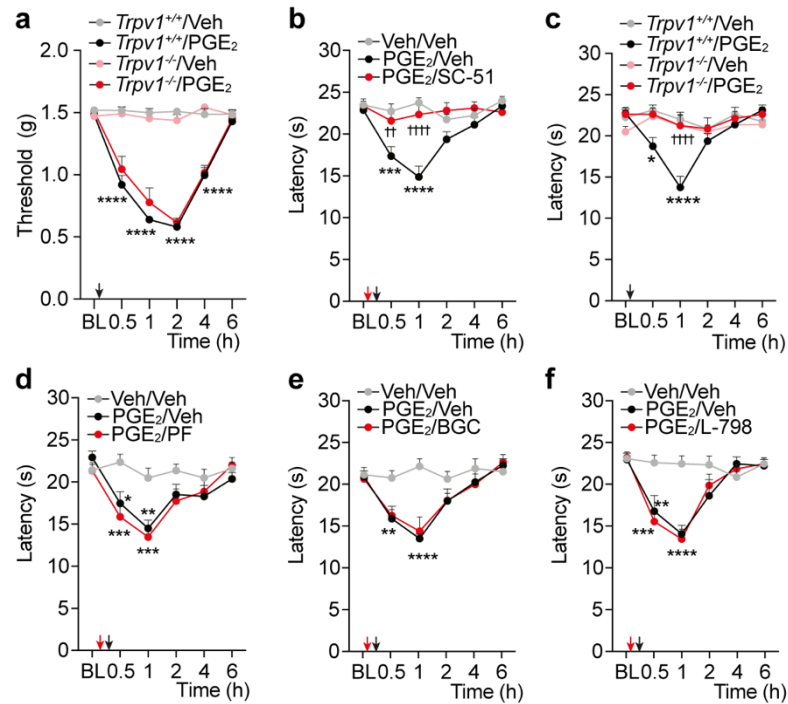
Supplementary Fig. 14. EP2 associates with TRPA1 in Schwann cells signals hind paw mechanical allodynia (allodynia). **a**, Allodynia after intraplantar (i.p.) injection of PGE₂ (1.5 nmol) or vehicle (Veh) in C57BL/6J (B6) mice pretreated with L-NAME (1 μ mol), olcegepant (1 nmol), combination of L-NAME + olcegepant or Veh. **b-i**, Allodynia after i.p. Phospholipase A2 Activating Protein (PLAA, 2 nmol), Arachidonic Acid (AA, 10 nmol), carrageenan (Cg, 300 μ g), Complete Freund Adjuvant (CFA) or Veh in B6 mice pretreated with L-NAME (1 μ mol), olcegepant (1 nmol) or Veh. **j**, Allodynia after i.p. of PGE₂ or Veh in *Plp-Trpa1* or Control mice. **k**, Effect of PGE₂ (100 nM) stimulation in catalytic subunit of PKA and TRPA1 interaction in HEK293T cells transfected with the EP2 (-Ptger2) or EP4 (-Ptger4). **l**, Allodynia after i.p. butaprost (Buta, 1.5 nmol) or Veh in B6 mice pretreated with st-Ht31 (17 nmol) or Veh. **m**, Allodynia after i.p. Buta in *Plp-Trpa1* or Control mice. **n**, Allodynia after i.p. Buta in B6 mice pretreated with olcegepant (1 nmol). **a-j, l-n**, $n=8$ mice/group. Data are mean $\pm$ s.e.m. **k**, 1-way or **a-j, l-n**, 2-way ANOVA, Bonferroni correction. AUC, area under the curve. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$ vs. Veh, Control/Veh † $P < 0.05$,

††P<0.01, †††P<0.001, ††††P<0.0001 vs. PGE₂, PLAA, AA, Cg, CFA, Buta/Veh, or Control/PGE₂, Buta. Source data are provided as a Source Data file.



Supplementary Fig. 15. TRPA1 in Schwann cells sustains PGE₂ hind paw mechanical allodynia (allodynia). **a, b**, Allodynia after intraplantar (i.pl.) injection of PGE₂ (1.5 nmol) or vehicle (Veh) in C57BL/6J (B6) mice pre or post with SQ25536 (25 nmol). (n=8 mice/group). **c**, Typical traces and cumulative data of calcium response after PGE₂ stimulation of human Schwann cells (hSCs) and in the presence of PF-04448948 (PF), H-89, A967079 (A96) or vehicle (Veh) (cells number: PGE₂=262, PF=156, H-89= 170, A96=224, Veh=195, n=3 independent experiments). **d**, Cumulative data of H₂O₂-HyPer7.2 (HyPer7.2), after PGE₂ stimulation of hSCs, PF, A96, H-89, ML171 or Veh (cells number: PGE₂=158, PF=63, H-89=62, A96=99, ML171=68, Veh=111, n=3 independent experiments). **e-g** allodynia after i.pl. PGE₂ or Veh in mice pretreated with H-89 (45 nmol) ML171 (250 nmol) A96 (300 nmol). **h,i**, Non-evoked nociception and allodynia after i.pl. PGE₂ or Veh in B6 mice pretreated with PBN (1 μmol). **j,k**, Allodynia after i.pl. Cg, CFA or Veh in B6 mice treated with PBN (1 μmol). **l,m**, Non-evoked nociception and allodynia after intraplantar (i.pl.) injection of PGE₂ (1.5 nmol) or Veh in *Adv-Trpa1* or Control mice. **n,o** allodynia after carrageenan (Cg, 300 μg), Complete Freund Adjuvant (CFA) or vehicle (Veh) in *Adv-Trpa1* or Control mice. (**a,b,e-o**, n=8 mice/group). **p**, Left panel, intracellular pathways following EP2 activation in Schwann cells.

Upon binding to the EP2 receptor on Schwann cells, PGE₂ activates adenylyl cyclase (AC), leading to increased cAMP levels and subsequent activation of protein kinase A (PKA) and transient receptor potential ankyrin 1 (TRPA1) phosphorylation. Activated TRPA1 elicits a calcium- and NADPH oxidase 1 (NOX1)-dependent release of reactive oxygen species (H₂O₂). Right panel, the released H₂O₂ targets TRPA1 on adjacent nociceptors, leading to sustained pain signaling. Data are mean ± s.e.m. **c,d,h,l** 1-way or **a,b,e-g,i-k,m-o** 2-way ANOVA, Bonferroni correction. AUC, area under the curve. *P<0.05, **P<0.01, ****P<0.0001 vs. Veh, Control/Veh †P<0.05, ††P<0.01, †††P<0.001, ††††P<0.0001 vs. Control PGE₂, Control Cg, Control CFA, PGE₂, Cg, CFA. Source data are provided as a Source Data file.



Supplementary Fig. 16. EP1/TRPV1 signaling mediates PGE₂-induced heat hypersensitivity. **a, b,** Mechanical allodynia after intraplantar (i.pl.) injection of PGE₂ (1.5 nmol) or vehicle (Veh) in *Trpv1*^{+/+} and *Trpv1*^{-/-} mice. Heat hypersensitivity after i.pl. PGE₂ or Veh in **b,** mice pretreated with SC-51322 (SC-51, 5 nmol) in **c,** *Trpv1*^{+/+} and *Trpv1*^{-/-} mice, in **d,** mice pretreated with the EP2 antagonist, PF-04448948 (PF, 5 nmol), in **e,** mice pretreated with the EP4 antagonist, BGC20-1531 (BGC, 5 nmol) and in **f,** mice pretreated with the EP3 antagonist, L-798,106 (L-798, 5 nmol). All panels, n=8 mice/group). Data are mean ± s.e.m. 2-way ANOVA, Bonferroni correction *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 vs. Veh ††P<0.01, ††††P<0.0001 vs. PGE₂. Source data are provided as a Source Data file.

Supplementary Table 1. Pharmacological agents injected subcutaneously in the hind paw (10 µl/site), systemic (intragastric, i.g., intraperitoneal, i.p., 10 ml/kg) or perisciatic (p.sc. 6 µl/site).

Agent	Target	Dose	Supplier and Catalog #
PGE ₂	EP1-EP4 agonist	0.1, 0.5, 1.5, 5 nmol, i.pl. 1.5 nmol p.sc.	Merck Life Science #538904
(R)-Butaprost	EP2 agonist	0.1, 0.5, 1.5, 5 nmol, i.pl.	DBA srl #HY-100448A
L-902,688	EP4 agonist	0.5, 1.5, 5 nmol, i.pl.	DBA srl #HY-119163
AA	arachidonic Acid	1, 10, 100 nmol, i.pl.	Merck Life Science #SML1395
PLAA	Phospholipase A ₂ activating protein	0.5, 2, 10 nmol, i.pl.	Enzo Life Sciences #ALX-165-007
Cg	λ-carrageenan	300 mg, i.pl.	Merck Life Science #22049
CFA	Complete Freund's adjuvant	1 mg/ml, i.pl.	Merck Life Science #F5881
PF-04418948	EP2 receptor antagonist	5 nmol, i.pl., p.sc. 10 mg/kg, i.g.	Tocris Bioscience #4818
TG6-10-1	EP2 receptor antagonist	5 nmol, i.pl.	DBA srl #HY-16978
BGC 20-1531	EP4 receptor antagonist	1, 5, 10 nmol, i.pl. 5 nmol, p.sc.	Tocris Bioscience #5327
ONOA3-208	EP4 receptor antagonist	5 nmol, i.pl.	Merck Life Science #SML2076
CJ-42794	EP4 receptor antagonist	5 nmol, i.pl.	Merck Life Science #TA9H94533261
Indomethacin	Cox inhibitor	280 nmol, i.pl.	Merck Life Science #I7378
SC-51322	EP1 antagonist	5 nmol, i.pl.	Merck Life Science #PZ0132
Ro-1138452	IP antagonist	5 nmol, i.pl.	DBA srl #HY-108912
L-798,106	EP3 receptor antagonist	5 nmol, i.pl.	DBA srl #HY-15274
L-NAME	NOS inhibitor	1 mmole, i.pl.	Merck Life Science #N5751
ER-819762	EP4 antagonist	1, 5, 10 nmol, i.pl.	DBA srl #HY-116099
Dyngo4a	Dynamin inhibitor	500 pmol, i.pl.	Merck Life Science #SML0340
PitStop2	Clathrin inhibitor	500 pmol, i.pl.	Merck Life Science #SML1169
SQ25536	Adenylate cyclase inhibitor	25 nmol, i.pl.	Merck Life Science #S153
Olgecepan	CLR/RAMP1 antagonist	1 nmol, i.pl.	Tocris Bioscience #4561
st-Ht31	Protein kinase anchoring inhibitor	17 nmol, i.pl.	Tocris Bioscience #6286
PBN	Free-radical spin trap	1 mmol, i.pl.	Merck Life Science #203995
Diclofenac	Nonsteroidal anti-inflammatory drug	25 mg/kg, i.p.	Merck Life Science #287840

Supplementary Table 2. AAV vector injected intrasciatic (i.sc. 5µl, 1x10¹² v/g), intrathecal (i.th, 5 µL, 1x10¹² v/g) or intraplantar (i.pl., 10 µl, 1x10¹² v/g).

Vector	Target	Route administration
AAV2/8 shRNA(<i>Ptger2</i>)	Silencing <i>Ptger2</i> in Schwann Cell	i.sc.
AAV2/9n shRNA(<i>Ptger2</i>)	Silencing <i>Ptger2</i> in DRG	i.th.
AAV2/8 shRNA2 (<i>Ptger2</i>) bis	Silencing <i>Ptger2</i> in Schwann Cell	i.sc.
AAV2/8 shRNA(<i>Ptger4</i>)	Silencing <i>Ptger4</i> in Schwann Cell	i.sc.
AAV2/9n shRNA(<i>Ptger4</i>)	Silencing <i>Ptger4</i> in DRG	i.th.
AAV2/8 shRNA(<i>Akap150</i>)	Silencing <i>Akap150</i> in Schwann Cell	i.sc.
AAV2/9n shRNA(<i>Akap150</i>)	Silencing <i>Akap150</i> in DRG	i.th.
AAV 2/8 scrambled-shRNA (<i>Ptger2</i>)	Negative control	i.sc.
AAV2/8 <i>bPac</i>	Knock-in of <i>bPac</i> in Schwann Cell	i.pl
AAV2/8 <i>Akap150-bPac</i>	Knock-in of <i>Akap150-bPac</i> in Schwann Cell	i.pl.
AAV2/8 <i>Lyn11-bPac</i>	Knock-in of <i>Lyn11-bPac</i> in Schwann Cell	i.pl
AAV2/8 <i>Lapd</i>	Knock-in of <i>Lapd</i> in Schwann Cell	i.pl
AAV2/8 <i>Akap150-Lapd</i>	Knock-in of <i>Akap150-Lapd</i> in Schwann Cell	i.pl
AAV2/8 <i>Lyn11-Lapd</i>	Knock-in of <i>Lyn11-Lapd</i> in Schwann Cell	i.pl

Supplementary Table 3. Pharmacological agents used in cultured cells.

Agent	Target	Concentration	Catalog #
PGE ₂	EP1-EP4 agonist	100 fM-10 nM	Merck Life Science #538904
(R)-Butaprost	EP2 agonist	10 nM-500 µM	DBA #HY-100448A
L-902,688	EP4 agonist	1 nM-500 µM	DBA #HY-119163
PF-04448948	EP2 receptor antagonist	100 pM-1 µM	Tocris Bioscience #4818
BGC 20-1531	EP4 receptor antagonist	100 pM-1 µM	Tocris Bioscience #5327
H-89	PKA inhibitor	1 µM	Merck Life Science #B1427
A967079	TRPA1 antagonist	50 µM	Tocris Bioscience #4716
ML171	NOX1 inhibitor	1 µM	Merck Life Science # 492002
CGRP	CGRP receptor agonist	10 µM	Merck Life Science # C0167
st-Ht31	Protein kinase anchoring inhibitor	10 µM	Tocris Bioscience # 6286
Forskolin	Adenylyl cyclase activator	100 µM	Merck Life Science # F6886