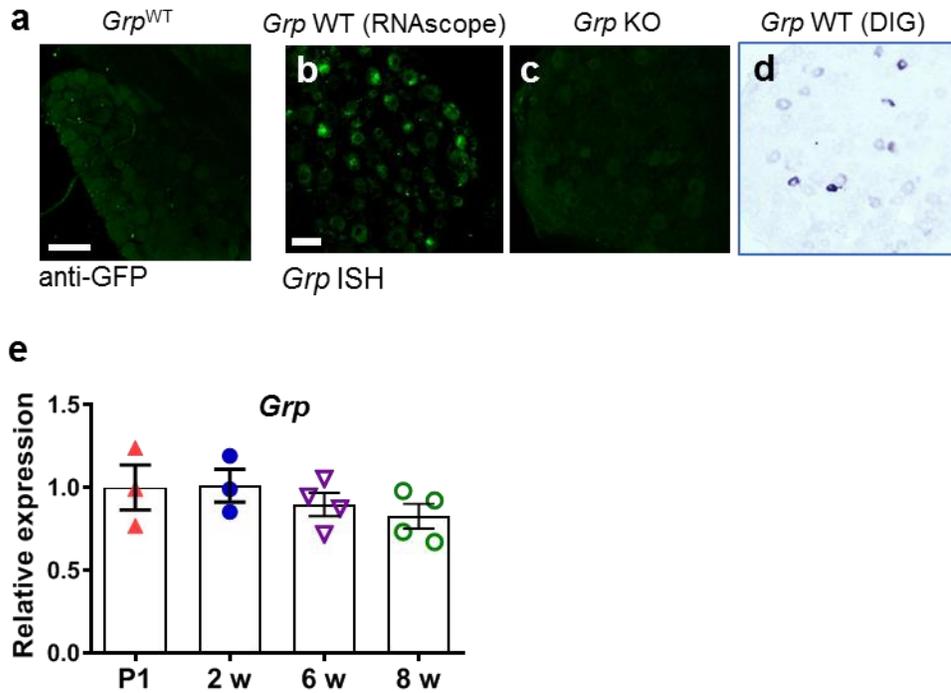


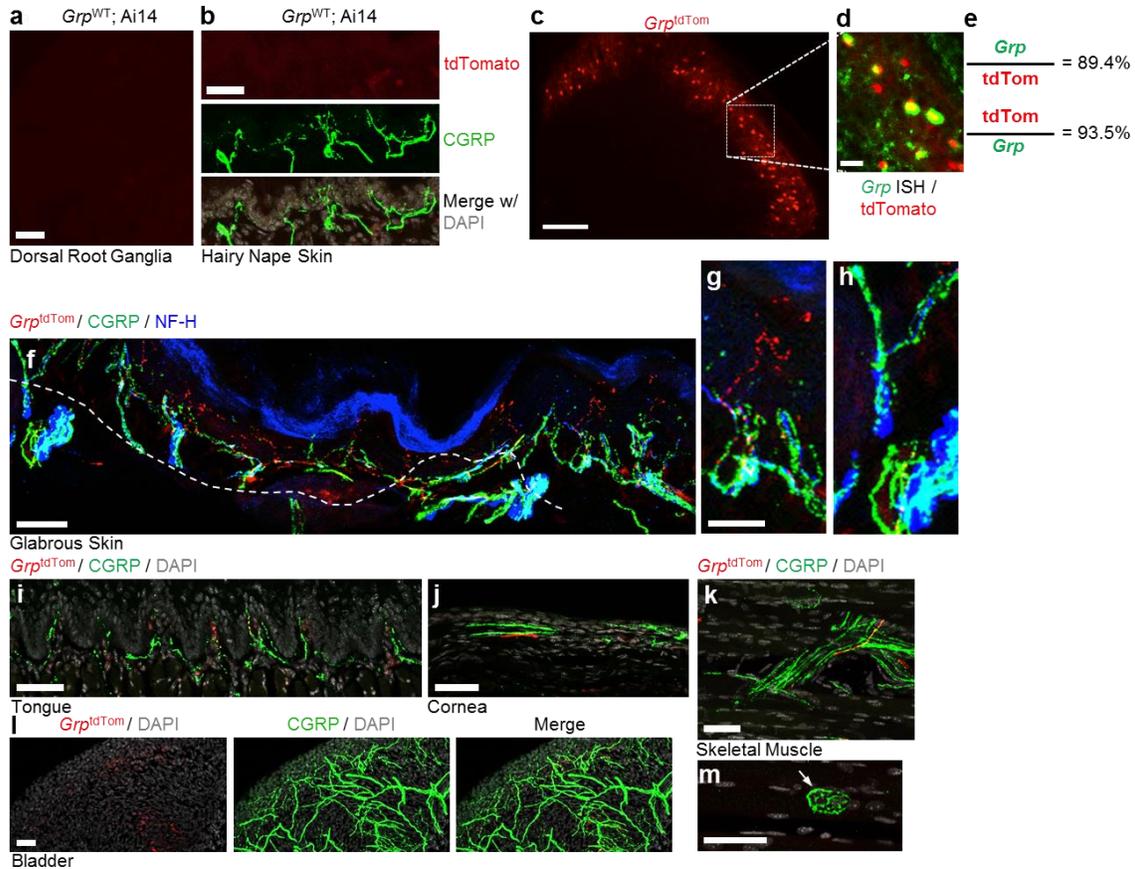
Supplementary Information

Exploration of sensory and spinal neurons expressing gastrin-releasing peptide in itch and pain related behaviors

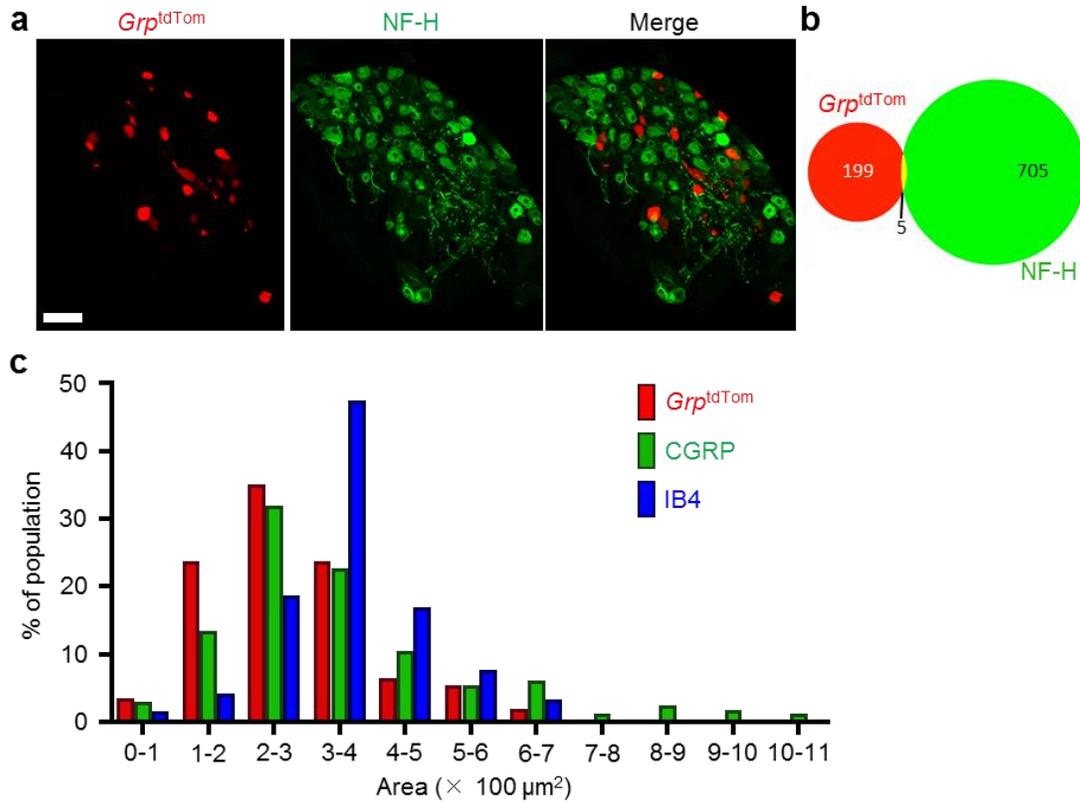
Barry et al.



Supplementary Figure 1. Detection of *Grp* transcript in DRG. **a** IHC with eGFP antibody in *Grp*^{WT} DRG. **b** and **c** RNAscope method of *Grp* ISH in WT DRG (**b**) and *Grp* KO DRG (**c**). **d** *Grp* ISH using conventional DIG method in WT DRG. **e** The levels of *Grp* transcript in DRG are comparable at different age. P1, postnatal day 1. Data represent mean ± s.e.m., *n* = 3-4 mice per age group. Scale bars, 50 μm. Source data are provided as a Source Data file.

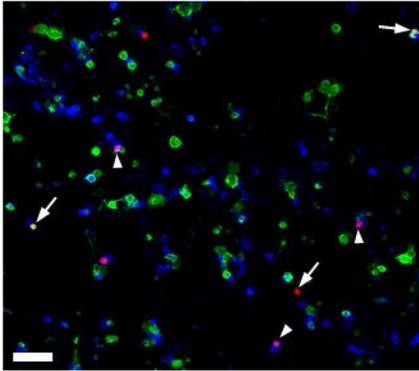


Supplementary Figure 2. Validation of *Grp*^{tdTom} neurons in DRG, spinal cord and skin. **a** Epifluorescent image from *Grp*^{WT}; Ai14 DRG. **b** IHC images of tdTom, CGRP and DAPI in *Grp*^{WT}; Ai14 hairy nape skin. Scale bars, 50 μ m. **c** Epifluorescent images of tdTomato neurons in cervical spinal cord from *Grp*^{tdTom} mice. Scale bar, 100 μ m. **d** ISH of *Grp* with tdTomato neurons. Scale bar, 20 μ m. **e** Percentage of tdTom- or *Grp*-expressing neurons that express *Grp* or tdTom, respectively. n = 3 mice and 9 sections. **f-h** IHC images of tdTom, CGRP and NF-H in *Grp*^{WT}; Ai14 glabrous skin. Image in **g** shows *Grp*^{tdTom} fiber with no NF-H (blue) expression and **h** shows NF-H fibers that do not express tdTom. Scale bar in **f** 50 μ m and in **g**, 20 μ m. **i-m** IHC images of tdTom, CGRP and DAPI in *Grp*^{tdTom} tongue (**i**), cornea (**j**), bladder (**k**) and skeletal muscle (**l**, **m**). Arrow indicates neuromuscular junction in **m**. Scale bars in **l-m**, 50 μ m.

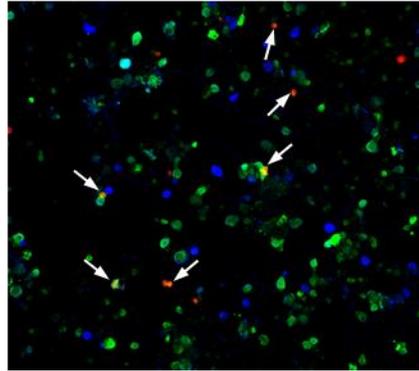


Supplementary Figure 3. Sensory *Grp^{tdTom}* neurons are mostly small sized neurons.
a IHC of tdTomato and NF-H in *Grp^{tdTom}* DRG. Scale bar, 50 μm. **b** Diagram of DRG neurons with tdTomato and NF-H expression. **c** Frequency Distribution (%) of Perikarya Area (μm²) for *Grp^{tdTom}*, CGRP or IB4 neuron populations in DRG. Neurons from 6 DRG sections were measured.

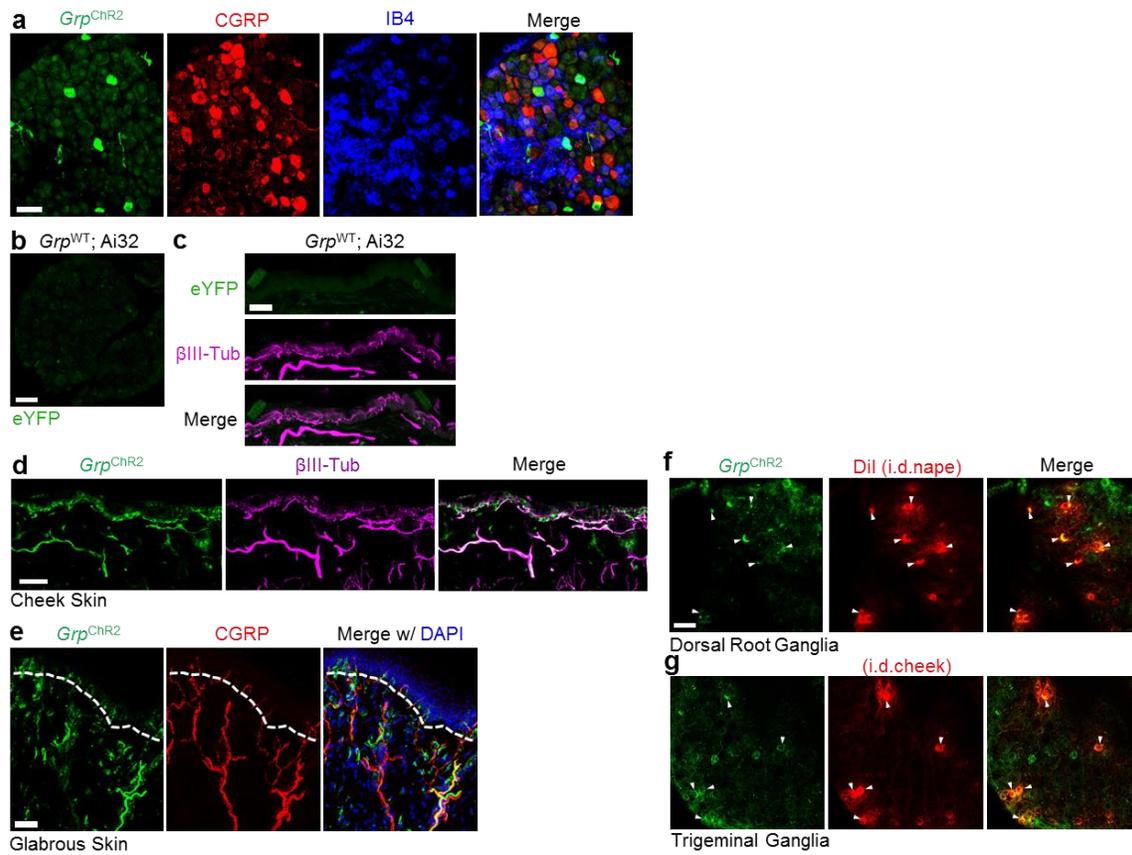
a *Grp*^{tdTom} / CGRP / IB4



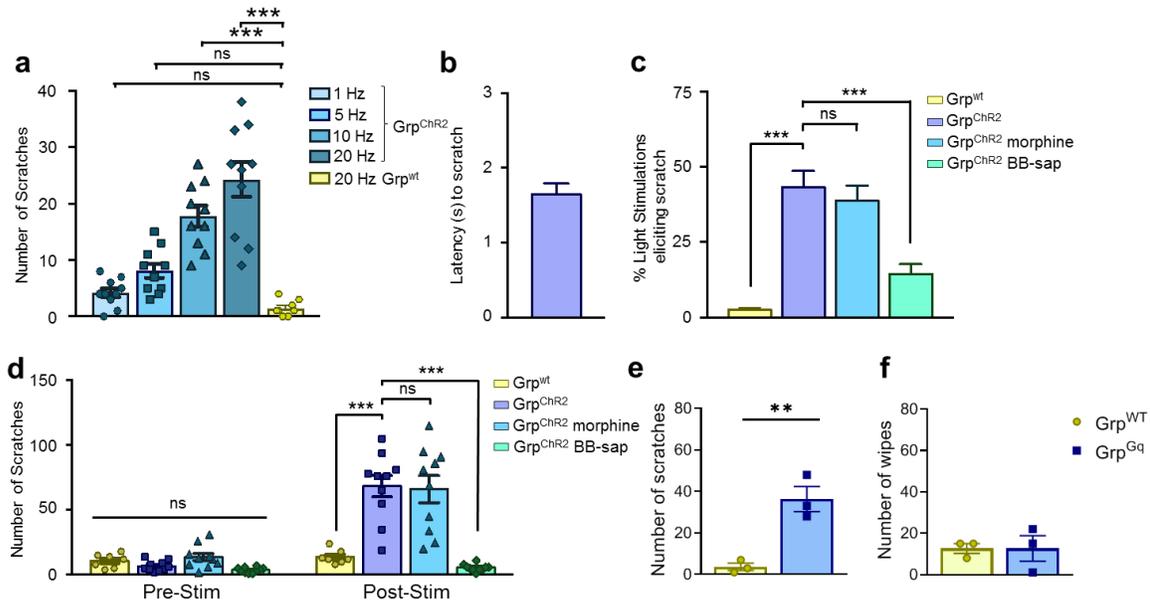
b *Grp*^{tdTom} / TRPV1 / NF-H



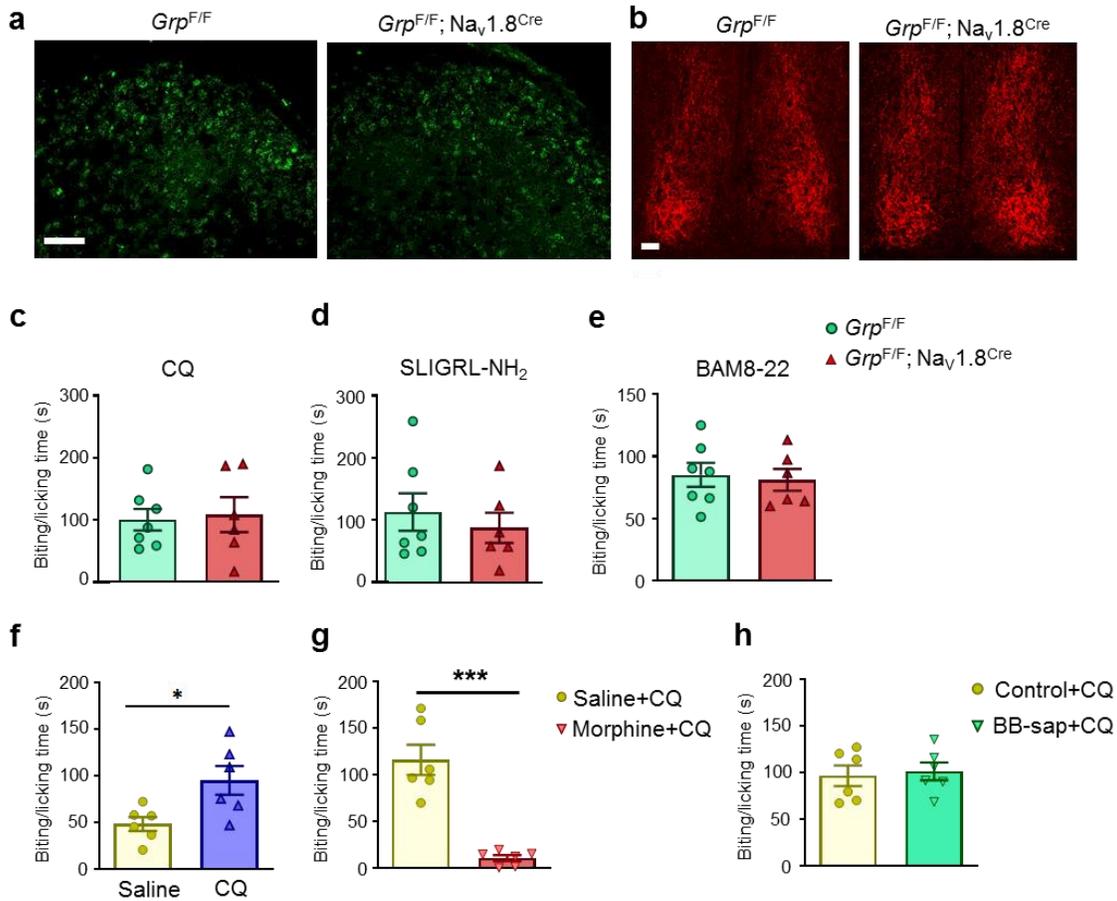
Supplementary Figure 4. Sensory *Grp*^{tdTom} neurons co-express CGRP and TRPV1. a IHC image of tdTomato, CGRP and IB4 from *Grp*^{tdTom} DRG cultures. Arrows indicate *Grp*^{tdTom} neurons co-expressing CGRP and arrowheads indicate IB4-binding. **b** IHC image of tdTomato, TRPV1 and NF-H from *Grp*^{tdTom} DRG cultures. Arrows indicate *Grp*^{tdTom} neurons co-expressing TRPV1. Scale bar, 100 μ m.



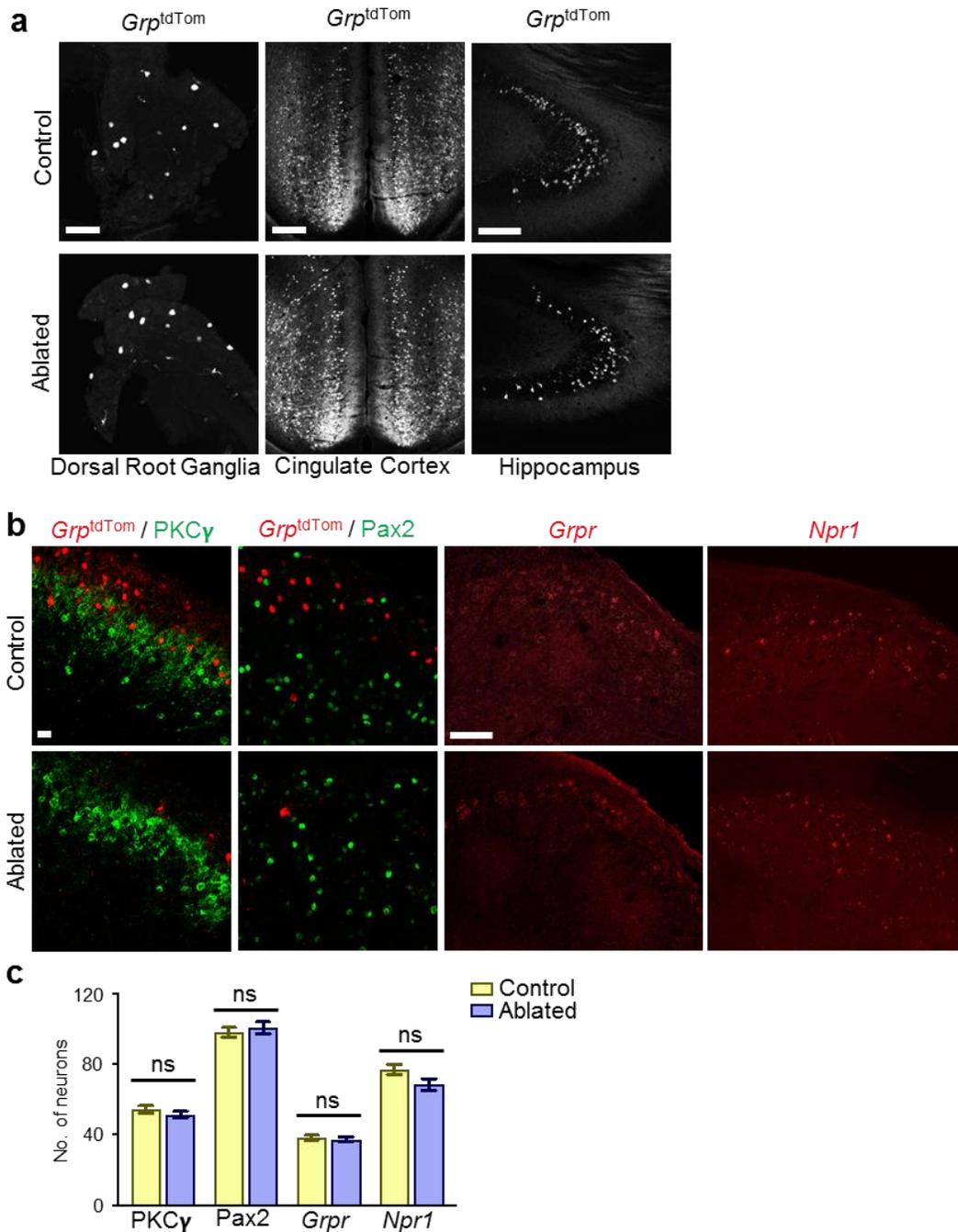
Supplementary Figure 5. Characterization of *Grp*^{ChR2} neurons in DRG and fibers in skin. **a** IHC images of eYFP, CGRP and IB4 in *Grp*^{ChR2} DRG. Scale bar, 50 μ m. **b** eYFP image from *Grp*^{WT}; Ai32 DRG. Scale bar, 50 μ m. **c** IHC image of eYFP, β III-Tubulin and DAPI in *Grp*^{WT}; Ai32 nape skin. Scale bar, 100 μ m. **d** IHC image of eYFP and β III-Tubulin in *Grp*^{ChR2} cheek skin. Scale bar, 100 μ m. **e** IHC images of eYFP, CGRP and DAPI merge in *Grp*^{ChR2} glabrous skin. Scale bar, 100 μ m. **f** and **g** IHC image of eYFP and DiI in *Grp*^{ChR2} DRG (**f**) or TG (**g**) 10 days after i.d. nape or cheek injection of DiI tracer. Scale bar, 50 μ m.



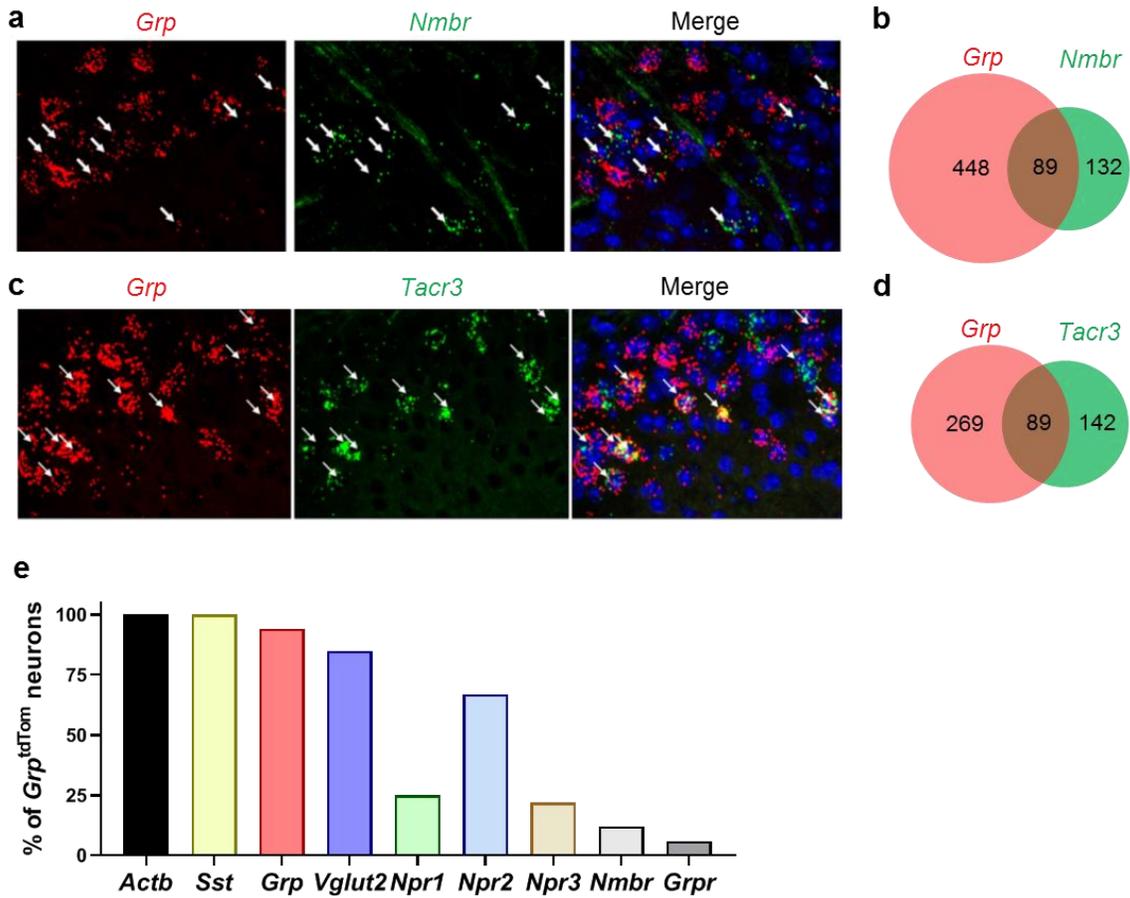
Supplementary Figure 6. Opto-stimulation of *Grp* fibers induces itch-specific behavior. **a** Number of scratches in 5 min induced by 3s – 1, 5, 10 or 20 Hz light stimulation of nape skin in *Grp*^{ChR2} and *Grp*^{WT} mice **(b)** Latency to scratch during 3s – 20 Hz light stimulation of skin in *Grp*^{ChR2} mice. **c** Percentage of 20 Hz light stimulations eliciting scratching behavior in *Grp*^{WT}, *Grp*^{ChR2}, *Grp*^{ChR2} morphine-treated and *Grp*^{ChR2} BB-sap-treated mice. **d** Number of spontaneous scratches in 30 min before and after the 5-min light stimulation experiment in *Grp*^{WT}, *Grp*^{ChR2}, *Grp*^{ChR2} morphine-treated and *Grp*^{ChR2} BB-sap treated mice. **e** and **f** Number of scratches **(e)** and wipes **(f)** in 30 min induced by CNO (1 mM) injections into the cheek skin in *Grp*^{Gq} and *Grp*^{WT} mice. Data are presented as mean ± s.e.m., $n = 8 - 10$ mice in **a-d**, $n = 3$ mice in **e** and **f**, one-way ANOVA with Tukey *post hoc* in **a** and **c**, two-way RM ANOVA with Tukey *post hoc* in **c**, unpaired *t* test in **e** and **f**, ** $p < 0.01$, *** $p < 0.001$, ns – not significant. Source data are provided as a Source Data file.



Supplementary Figure 7. Conditional deletion of sensory *Grp* is specific and does not affect pain responses. **a** ISH images of *Grp* expression in spinal cord sections from *Grp*^{F/F} and *Grp*^{F/F}; *Nav1.8*^{Cre} mice. Right panels are high power images of the boxed areas in left panels. Scale bar, 100 μ m for left panels, 20 μ m for right panels. **b** IHC of GRP in the suprachiasmatic nucleus from *Grp*^{F/F} and *Grp*^{F/F}; *Nav1.8*^{Cre} mice. Scale bar, 50 μ m. **c-e** Mean biting/licking time induced by i.d. calf injection of **(c)** CQ (100 μ g), **(d)** SLIGRL-NH₂ (100 μ g), and **(e)** BAM8-22 (100 μ g) in *Grp*^{F/F} and *Grp*^{F/F}; *Nav1.8*^{Cre} littermates. **f** Mean biting/licking time induced by i.d. calf injection of saline (10 μ l) and CQ (100 μ g). **g** Mean biting/licking time induced by i.d. calf injection of CQ (100 μ g) following i.t. injection of saline (10 μ l) or morphine (0.3 nmol). **h** Mean biting/licking time induced by i.d. calf injection of CQ (100 μ g) on control mice and BB-sap treated mice. Data are presented as mean \pm s.e.m., $n = 6-7$ mice. * $p < 0.05$, *** $p < 0.001$, unpaired t test. Source data are provided as a Source Data file.



Supplementary Figure 8. Ablation of *Grp^{tdTom}* neurons is restricted to the spinal cord.
a Epifluorescent images of tdTomato neurons in DRG, cingulate cortex and hippocampus from *Grp^{tdTom}* control and *Grp^{tdTom}* ablated mice. Scale bars, 50 μ m for DRG, 100 μ m for cingulate cortex and 100 μ m for hippocampus, respectively. **b** IHC images of tdTom with PKC γ or Pax2 and ISH images of *Grpr* or *Npr1* expression in cervical spinal cord sections from *Grp^{tdTom}* control and ablated mice. Scale bars, 20 μ m for tdTom and 100 μ m for ISH. **c** Mean number of PKC γ , Pax2, *Grpr* or *Npr1* neurons from control and ablated mice. Data are presented as mean \pm s.e.m., $n = 3$ mice and 10 sections, unpaired t test in c. Source data are provided as a Source Data file.



Supplementary Figure 9. Spinal GRP neurons are heterogeneous. **a** ISH images of *Grp* and *Nubr* in the superficial dorsal horn. **b** Diagram of overlapping expression of *Grp* and *Nubr* in the superficial dorsal horn. **c** ISH images of *Grp* and *Tacr3* in the superficial dorsal horn. **d** Diagram of overlapping expression of *Grp* and *Tacr3* in the superficial dorsal horn. **e** Single-cell qRT-PCR results from *Grp*^{tdTom} dorsal horn neurons. $n = 10-15$ neurons.

Supplementary Table 1. Fiber projections in *Grp^{tdTom}* mice.

Tissue	Fibers	Tissue	Fibers
Skin (Hairy, Glabrous, Mystacial) Unmyelinated: Epidermal free nerve endings Circular/penetrating follicle neck endings Bush/cluster endings Myelinated: Circumferential nerve endings Lanceolate/club endings Meissner corpuscles Merkel cells and endings Sebaceous gland endings Vibrissa follicle-sinus complex (Vib)		Retina	+(rare)
		Cornea	+(rare)
	+	Tongue	+(rare)
	+	Bladder	+(rare)
	+	Skeletal muscle	-*
		Esophagus	-
	-	Heart	-
	-	Intestine	-
	-	Kidney	-
	-	Liver Lung	-
-	Testis	-	
-	Smooth Muscle	-	

* *Grp^{tdTom}* fibers were observed only in nerve bundles going through muscle but did not innervate any muscle fibers.