Supplementary Information

A subpopulation of nociceptors specifically linked to itch

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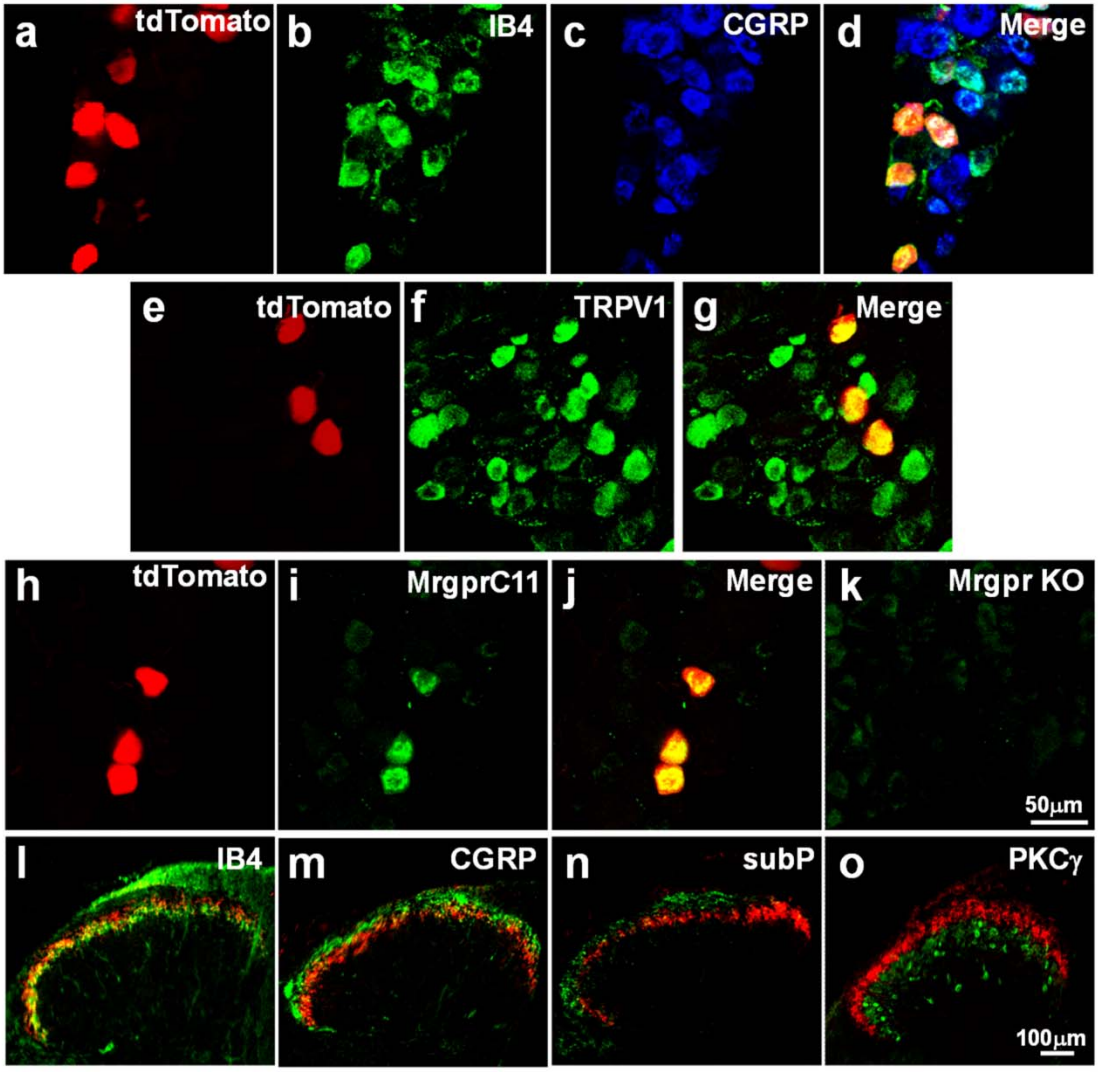

Supplementary Figure 1. Further characterization of MrgprA3^{+} neurons. (a-d) Triple labeling of tdTomato (a), IB4 (b) and CGRP (c) of L4-L6 DRG sections from *MrgprA3*$^{GFP\text{-}Cre}$; *ROSA26*^{tdTomato} mice. 61.32% of MrgprA3^{+} neurons co-express both IB4

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and CGRP (d). (e-k) L4-L6 DRG sections from *MrgprA3*$^{GFP\text{-}Cre}$; *ROSA26*^{tdTomato} mice stained with TRPV1 (e-g) and MrgprC11 (h-k) antibodies. 88.3% of MrgprA3^{+} neurons were labeled with TRPV1 and 93% of MrgprA3^{+} neurons co-express MrgprC11. Rabbit polyclonal MrgprC11 antibody was custom-made from Proteintech Group, Inc. It did not show any positive signal in Mrgpr-cluster knockout mice, demonstrating that this antibody is specific for MrgprC11 (k). (l-o) MrgprA3 axons terminate in lamina II_{middle} of the dorsal spinal cord. Confocal images of thoracic regions of adult spinal cord sections from *MrgprA3*$^{GFP\text{-}Cre}$; *ROSA26*^{tdTomato} mice stained with IB4 (l), CGRP (m), substance P (n) and PKCγ (o) MrgprA3^{+} axons were visualized by tdTomato fluorescence. Axonal terminals expressing other markers were stained green. Axons of MrgprA3^{+} neurons terminated in lamina II_{middle}, ventral to the lamina with terminals expressing substance P^{+} and CGRP^{+} and dorsal to the lamina with terminals expressing PKCγ.

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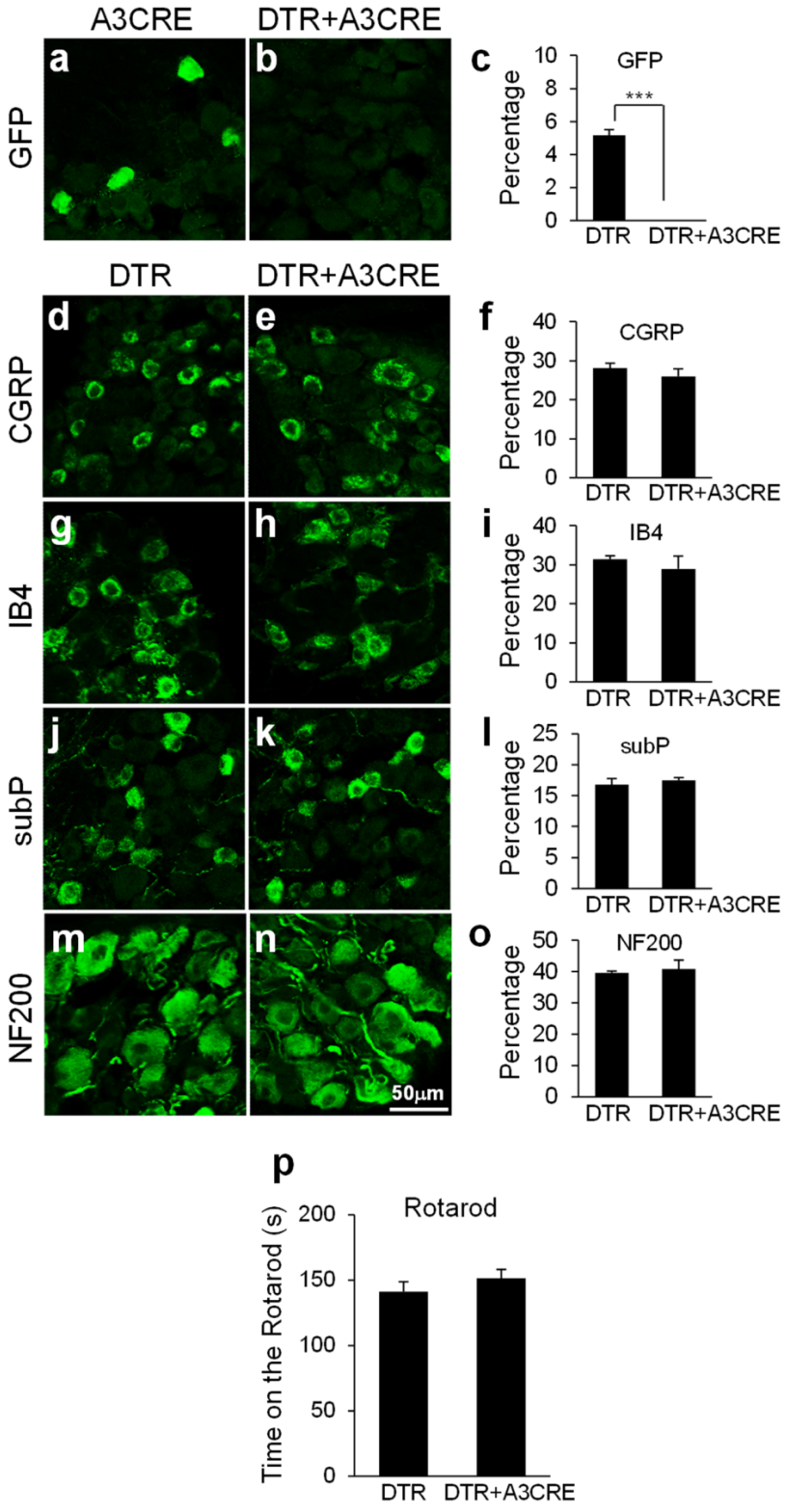

Supplementary Figure 2. DTX treatment did not produce neurotoxic effects. (a-b) L4-L6 DRG sections from DTX-treated *MrgprA3*$^{GFP\text{-}Cre}$ mice or DTX-treated *MrgprA3*$^{GFP\text{-}Cre}$;

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ROSA26^{DTR} mice stained with GFP antibodies. (c) The percentage of the total number of DRG neurons expressing GFP. 100% of the GFP^{+} neurons were lost in DTX treated *MrgprA3*$^{GFP\text{-}Cre}$; *ROSA26*^{DTR} mice (n=3, p=0.002). (d-e, g-h, j-k, m-n) L4-L6 DRG sections from DTX-treated *ROSA26*^{DTR} mice or DTX-treated *MrgprA3*$^{GFP\text{-}Cre}$; *ROSA26*^{DTR} littermates stained with various molecular markers. (f, i, l, o) Of the total number of DRG neurons, the percentages of IB4^{+}, CGRP^{+}, substance P^{+} and NF200^{+} neurons were unaffected by the ablation of the MrgprA3^{+} neurons (n=3, IB4, p=0.33, CGRP, p=0.42, substance P, p=0.46, NF200, p=0.63). (p) DTX-treated *MrgprA3*$^{GFP\text{-}Cre}$; *ROSA26*^{DTR} mice did not differ from littermate control mice in motor function as measured with the rotarod test (n=7, p=0.32). All data are presented as the mean ± SEM. ***p < 0.005; two-tailed unpaired Student's *t*-test.

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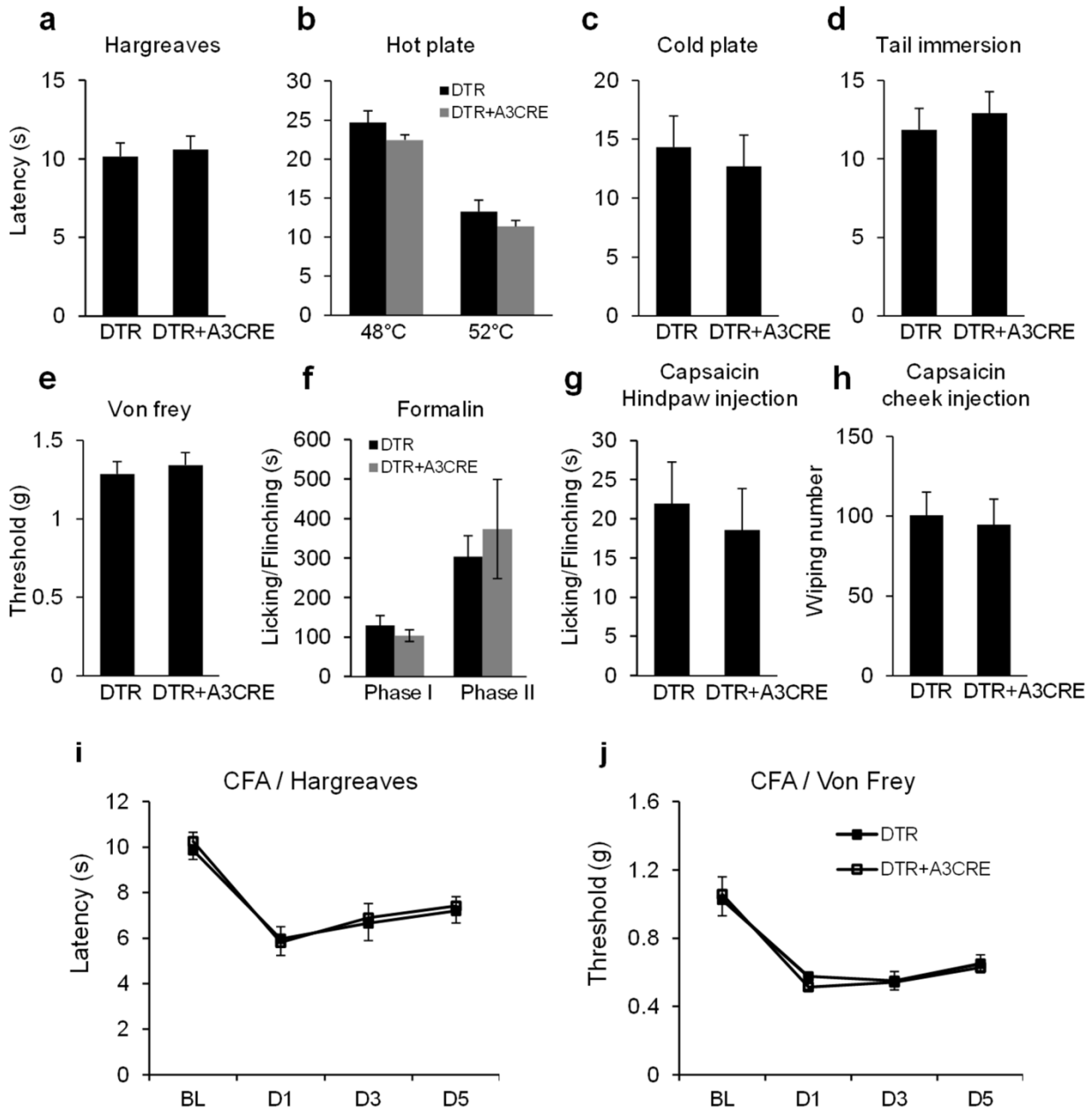

Supplementary Figure 3. The ablation of MrgprA3^{+} neurons does not affect pain behavior. (a-d) DTX-treated *MrgprA3*$^{GFP\text{-}Cre}$; *ROSA26*^{DTR} and *ROSA26*^{DTR} mice showed normal responses to noxious acute thermal stimuli. Response latencies in the Hargreaves, hot plate (48°C and 52°C), cold plate (0°C), and tail immersion tests (48°C) did not differ between groups (n ≥ 6 for each group, p > 0.5 for each test). (e) Paw withdrawal threshold to punctate mechanical stimuli (von Frey) was comparable

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between groups (n = 7, p = 0.55). (f,g) Licking and flinching behaviors induced by formalin (2%, 6 µl) (f) (n = 7, p = 0.35) and intraplantar injection of capsaicin (0.3mM) (g) (n = 7, p = 0.63) did not differ between groups. (h) Facial wiping behavior induced by the s.c. cheek injection of capsaicin (3.3 mM, 10 µl) was comparable in DTX-treated *MrgprA3*$^{GFP\text{-}Cre}$; *ROSA26*^{DTR} mice and *ROSA26*^{DTR} littermates (ablated, n = 8, control, n = 7, p = 0.91). (i,j) DTX-treated *MrgprA3*$^{GFP\text{-}Cre}$; *ROSA26*^{DTR} mice and *ROSA26*^{DTR} mice displayed a similar degree of hyperalgesia to radiant heat (Hargreaves test) and mechanical stimuli (von Frey test) after intraplantar injection of complete Freund's adjuvant (CFA, 50%, 6 µl) (n=7, p>0.45 for every timepoint). All data are presented as the mean ± SEM. Two-tailed unpaired Student's *t*-test.

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Supplementary Table 1: Axonal projections of *MrgprA3*$^{GFP\text{-}Cre}$; *ROSA26*^{tdTomato} neurons in the mouse.

Tissue	MrgprA3 projection	Tissue	MrgprA3 projection
Nervous system		Digestive System	
Dorsal root ganglion	+	Tongue	–
Trigeminal ganglion	+	Esophagus	–
Spinal cord	+	Liver	–
Brain		Stomach	–
Spinal Trigeminal Nucleus	+	Pancreas	–
Other Brain Areas	–	Small intestine	–
Skin		Colon	–
Glabrous skin	+	Circulatory system	–
Hairy skin	+	Heart	–
Respiratory system		Blood vessel	–
Trachea	–	Other Tissues	–
Lung	–	Eye-cornea	–
Urinary system		Eye-retina	–
Kidney	–	Skeletal muscle	–
Bladder	–	Spleen	–

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