

## Supplementary Information

### **A spinal neural circuitry for converting touch to itch sensation**

**Chen et al.**

This pdf file includes:

Supplementary Figure 1

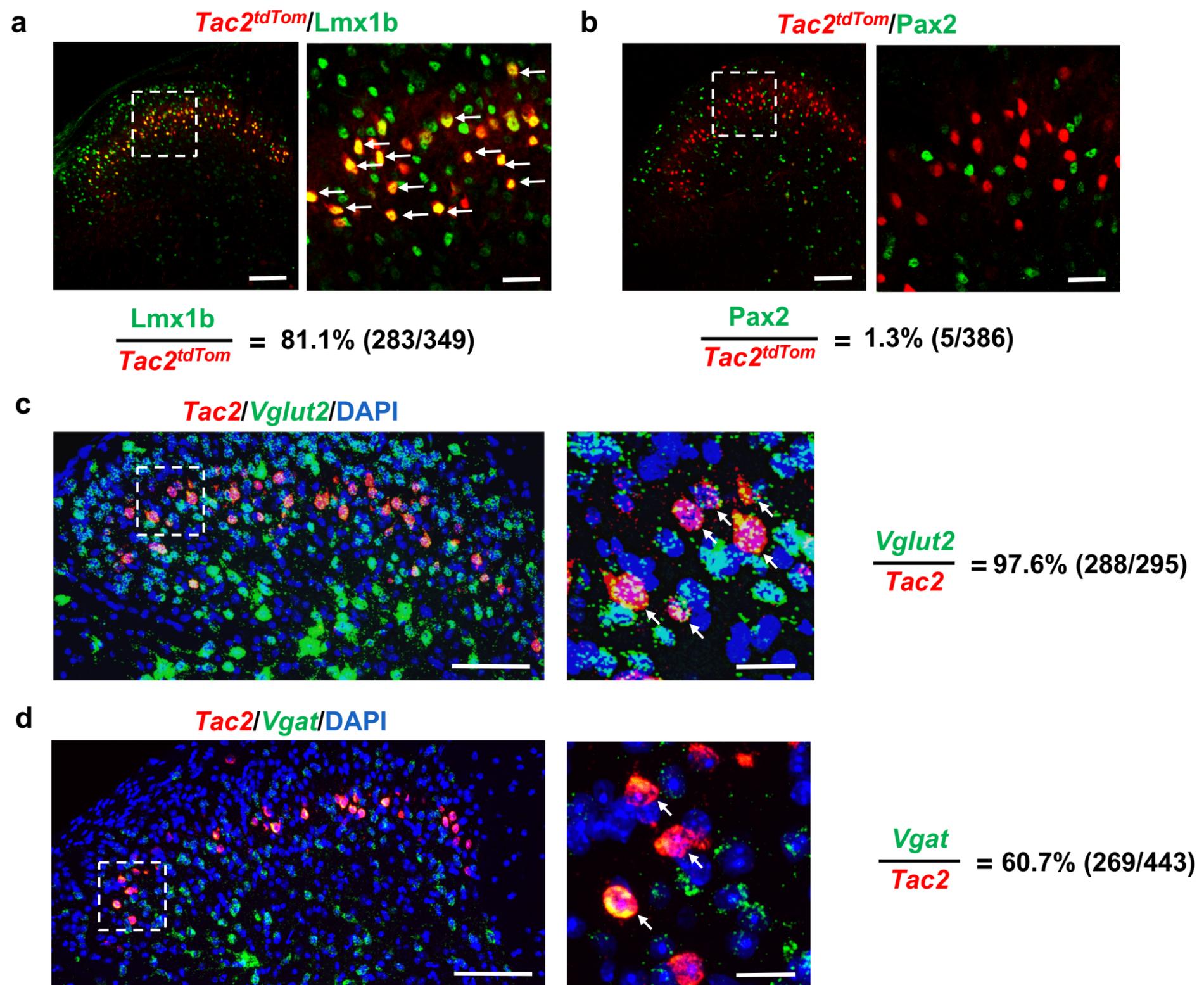
Supplementary Figure 2

Supplementary Figure 3

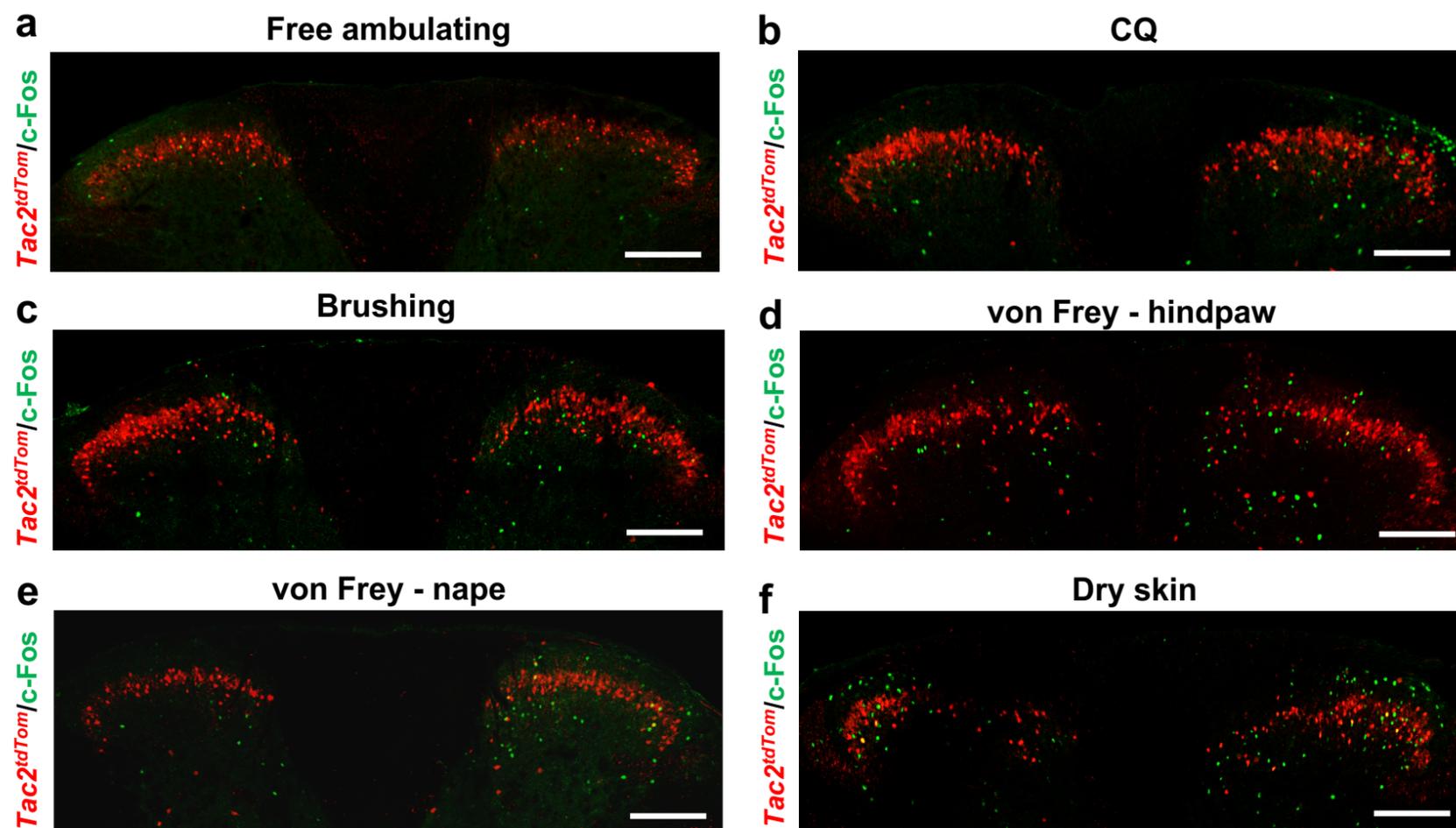
Supplementary Figure 4

Supplementary Table 1

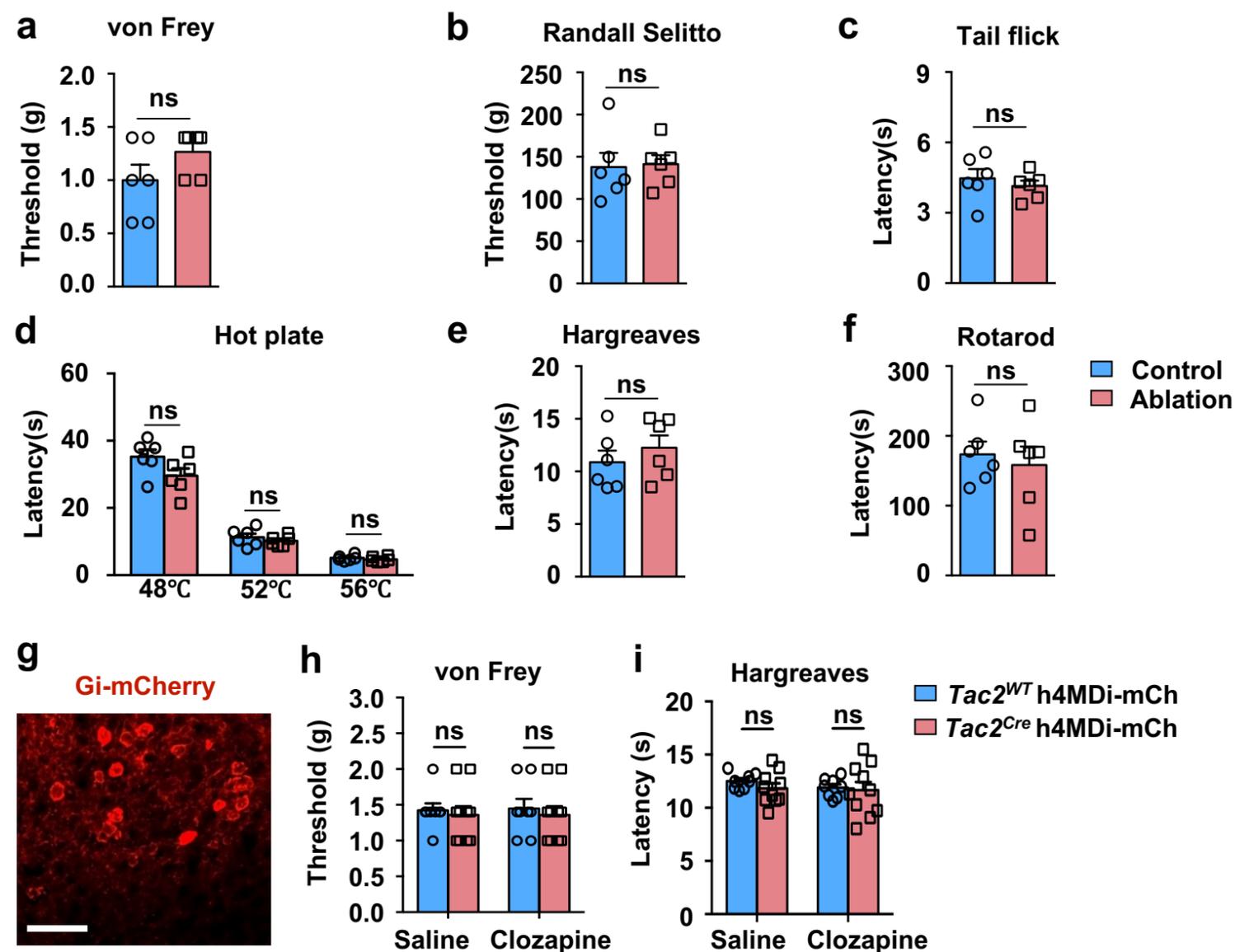
Supplementary Table 2



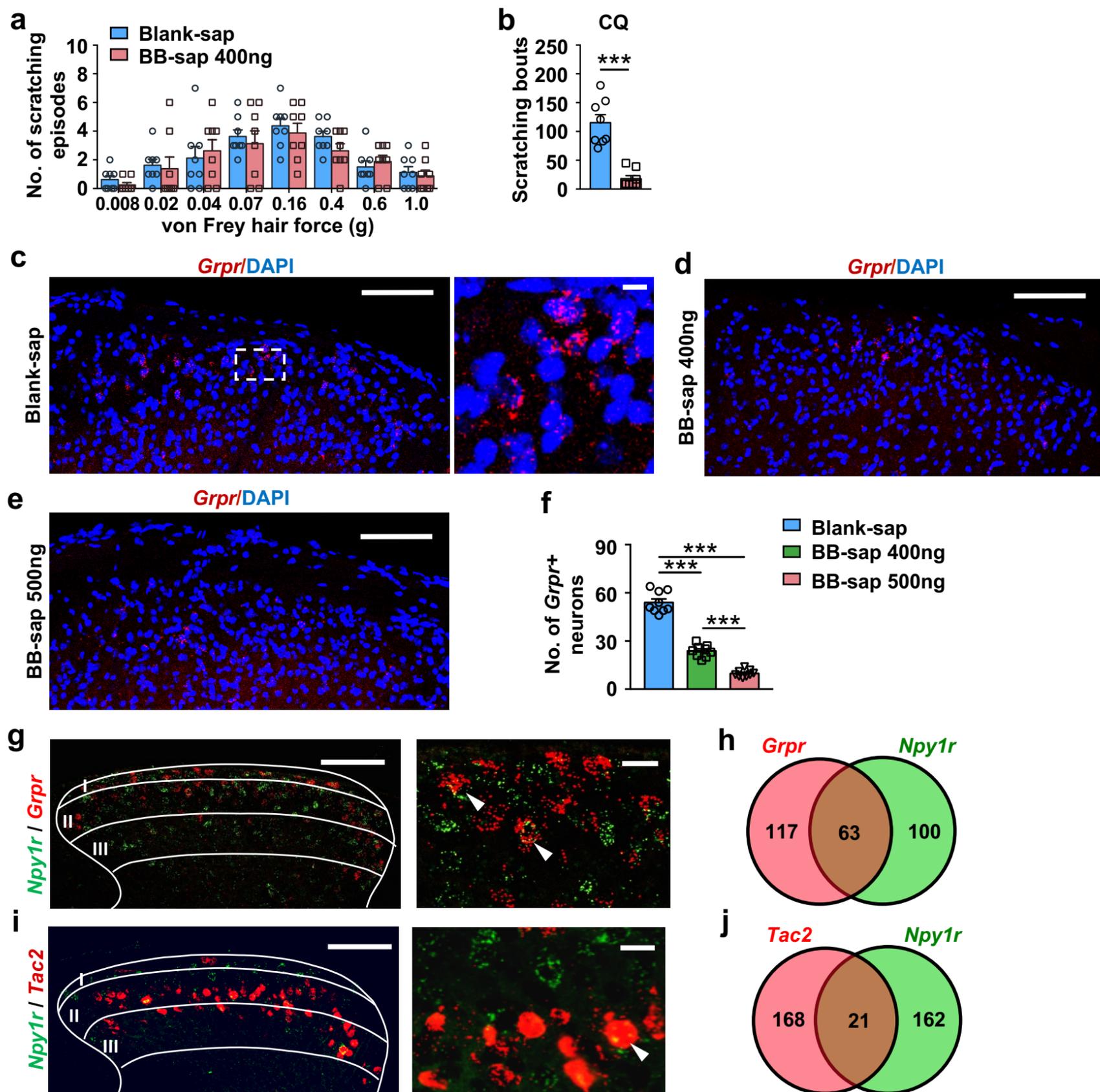
**Supplementary Figure 1. Neurotransmitter phenotype of *Tac2<sup>tdTom</sup>* neurons in the spinal cord. a, b, Representative images of Lmx1b (green) (a) and Pax2 (green) (b) immunofluorescence in the cervical spinal cord of *Tac2<sup>tdTom</sup>* (red) mice. The white box indicates the area of higher magnification (right). The arrows denote Lmx1b<sup>+</sup>/*Tac2<sup>tdTom</sup>*<sup>+</sup> or Pax2<sup>+</sup>/*Tac2<sup>tdTom</sup>*<sup>+</sup> neurons. c, d, Representative images of *Vglut2* (green)(c) and *Vgat* (green)(d) with *Tac2* (red) by RNAscope in the cervical spinal cord of C57BL/6J mice. Nuclei is stained with DAPI (blue). The white box indicates the area of higher magnification (right). The arrows denote *Vglut2*<sup>+</sup>/*Tac2*<sup>+</sup> and *Vgat*<sup>+</sup>/*Tac2*<sup>+</sup> neurons. The percentage is calculated as double-positive neurons over total number of *Tac2<sup>tdTom</sup>*<sup>+</sup> or *Tac2*<sup>+</sup> neurons: Lmx1b (283/349), Pax2 (5/386), *Vglut2* (288/295), and *Vgat* (269/443). n = 9 sections from 3 mice. Scale bars, 100 μm in a-d (left); 25 μm in a-d (right).**



**Supplementary Figure 2. *Tac2<sup>Cre</sup>* neurons are activated by mechanical itch stimulation and dry skin itch. a-f,** Representative images of c-Fos expression (green) in the cervical cord of *Tac2<sup>tdTom</sup>* (red) of free ambulating mice (a), in response to i.d. CQ injection (b), brushing (c), von Frey hair stimulation in hindpaw (d), von Frey hair stimulation in nape (e) and AEW (f). n = 3 mice per group. Scale bars, 200  $\mu$ m in a-f.

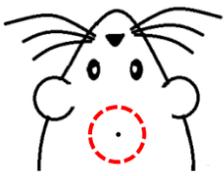


**Supplementary Figure 3. Conditional ablation of spinal *Tac2<sup>Cre</sup>* neurons did not affect pain or motor behaviors.** **a-f**, Pain or motor behavior test in conditional ablation of *Tac2* neurons in spinal cord, including von Frey test (**a**), Randall Selitto test (**b**), Tail flick test (**c**), Hot plate test (**d**), Hargreaves test (**e**) and Rotarod test (**f**). (**a-c**, **e**, **f**; two-tailed Student's unpaired *t*-test, ns : not significant,  $n = 6$  mice per group; **d**; two-way ANOVA with Bonferroni *post hoc*, ns : not significant,  $n = 6$  mice per group). **g**, Representative IHC image of mCherry<sup>+</sup> (red) neurons in the lumbar spinal cord of *Tac2<sup>Cre</sup>* mice infected with AAV8-hSyn-DIO-hM4Di (Gi)-mCherry virus. All 18 *Tac2<sup>Cre</sup>* mice with virus injections were subjected for IHC verifications. Scale bar, 50  $\mu$ m. **h**, **i**, Threshold of von Frey (**h**) and latency of Hargreaves (**i**) after chemogenetic inhibition of *Tac2<sup>Cre</sup>* neurons in the lumbar spinal cord. Two-way ANOVA with Bonferroni *post hoc*, ns: not significant,  $n = 8$  mice for *Tac2<sup>WT</sup>* group and  $n = 10$  mice for *Tac2<sup>Cre</sup>* group. All data are presented as means  $\pm$  s.e.m. and error bars represent s.e.m. Source data are provided as a Source Data file.



**Supplementary Figure 4. Transmission of mechanical itch depends on GRPR neurons.** **a,b**, Mechanical itch test (**a**) and CQ itch test (**b**) after BB-sap 400 ng treatment. (**a**; two-way ANOVA with Bonferroni *post hoc*,  $n = 10$  mice per group; **b**; two-tailed Student's unpaired *t*-test,  $***p = 0.00001$ ,  $n = 10$  mice per group). **c-e**, Representative RNAscope images of *Grpr* mRNA (red) in the cervical spinal cord of mice treated with blank-sap (**c**), BB-sap 400 ng (**d**), and BB-sap 500 ng (**e**). Scale bars, 100  $\mu\text{m}$ . **f**, Comparison of number of *Grpr*<sup>+</sup> neurons among control, BB-sap 400 ng and 500 ng groups. One-way ANOVA with Tukey *post hoc*,  $***p = 0.00001$ ,  $n = 9$  sections from 3 mice per group. **g-j**, RNAscope images in the superficial dorsal horn of the spinal cord (**g**, **i**) and Venn diagrams (**h**, **j**) showing co-expression of *Grpr* (red) (**g,h**) and *Tac2* (red) (**i,j**) with *Npy1r* (green).  $n = 9$  sections from 3 mice per group. Arrow heads indicate co-expression neurons. Scale bars, 100  $\mu\text{m}$  in left images and 20  $\mu\text{m}$  in right images. All data are presented as means  $\pm$  s.e.m. and error bars represent s.e.m. Source data are provided as a Source Data file.

**Supplementary Table 1. Comparison of the methods for mechanical itch tests**

Reference	Stimulation site		Stimulation times	Response percentage	Validation(BB-sap)		
					Dose	CQ 200 $\mu$ g	Mechanical itch
Akiyama et al, 2012	Nape 7 mm away from injection site		3	0(0.07 g)	--	--	--
Bourane et al, 2015	Nape Randomly		5	~10%(0.07 g) ~10%(0.16 g)	400 ng in NPY Abl mice	~50	Normal
Feng et al, 2018	Nape		3 per site 5 sites	~16%(0.07 g) ~5% (0.16 g)	--	--	--
Pan et al, 2019	Ear		5	~80%(0.07 g) ~75%(0.16 g)	400 ng in naïve mice	~50	Normal
Acton et al, 2019	Nape Randomly		10	20%~40% (0.16 g)	400 ng in naïve mice	~100	Normal
Chen et al, 2020*	Nape		10	~40%(0.07 g) ~30%(0.16 g)	400 ng in naïve mice	~20	Normal
					500 ng in naïve mice	~5	Lost

\* Present manuscript

**Supplementary Table 2. Comparison of *Ucn3<sup>tdTom</sup>* and *Tac2<sup>tdTom</sup>* neurons.**

	<b>Ucn 3</b>	<b>Tac 2</b>
Distribution	Lamina II-III	Exclusively Lamina Ili-IIIo
A $\beta$ input	Yes	Yes
Feed-forward inhibition	Yes	Yes
Sensitization of A $\beta$ evoked AP in dry skin itch model	Yes	Yes
Firing pattern in dry skin itch model	Unchanged	Unchanged