

Appendix S1

SUPPLEMENTARY MATERIALS AND METHODS

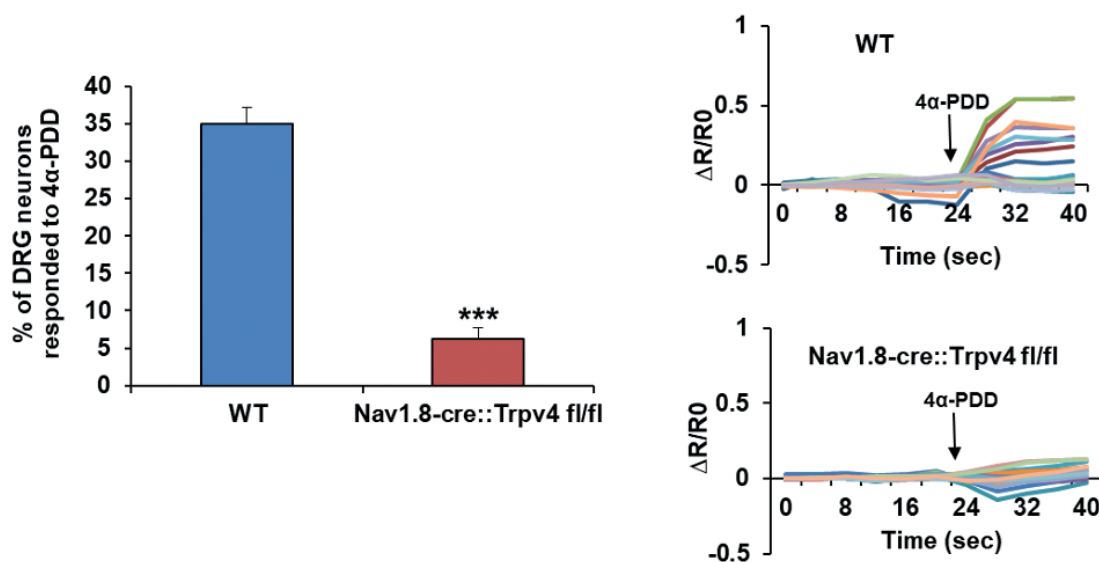
Mice with conditional deletion for *Trpv4* in primary sensory neurons were generated by mating *Trpv4^{fl/fl}* with *Nav1.8-Cre* mice, as described previously (4). Efficiency of targeting *Trpv4* was verified in both DRGs and TGs at gene and protein levels (4), and by functional imaging (SFig. 1). All animal protocols were approved by the Duke University Institutional Animal Care and Use Committee (IACUC). Male and female (2–3 months old) *Trpv4* cKO (*Nav1.8-cre::Trpv4^{fl/fl}*, C57bl/6j background) and wild-type (WT, C57bl/6j) mice were used.

Routine procedures were followed for Ca^{2+} imaging and immunohistochemistry (4), Ca^{2+} imaging in response to 4 α -Phorbol 12,13-didecanoate (Sigma, St. Louis, MO, USA) stimulation was performed in cultured DRG neurones after loading with 5 μM Fura2-AM (Invitrogen, Waltham, MA, USA) for 45 min. Immunostaining was conducted in cervical DRG sections at 12

μm with TRPV4 antibody (Novus Biologicals, Littleton, CO, USA, 1:4000), followed by secondary antibody Alexa Fluor 594 (ThermoFisher, Waltham, MA, USA, 1:600).

All pruritogens were purchased from Sigma-Aldrich. For acute itch, mice received intradermal (i.d.) injection of 50 μl pruritogens in normal saline: histamine (500 μg), 48/80 (100 μg), 5-hydroxytryptamine (5-HT) (25 μg), Ser-Leu-Ile-Gly-Arg-Leu-NH₂ (SLIGRL) (100 μg), and chloroquine (200 μg), into the nape of the neck. For chronic itch, the dry skin model was induced by painting the neck-back skin with acetone and diethyl-ether (1:1) following water (AEW) twice daily (10). An allergic contact dermatitis model was established by applying 1-fluoro-2, 4-dinitrobenzene (DNFB) onto the neck-back skin, as described previously (11). Scratch bouts were analysed as described previously (4), with a blinded method.

Data were expressed as mean \pm standard error of the mean (SEM). Group differences were analysed by 2-tailed *t*-test, 1-way analysis of variance (ANOVA), followed by Tukey *post-hoc* test, or 2-way ANOVA, followed by Bonferroni *post-hoc* test. $p < 0.05$ was considered significant.



SFig. 1. Ca^{2+} imaging shows percentage reduction in cervical dorsal root ganglion (DRG) neurones in response to stimulation by transient receptor potential cation channel subfamily V member 4 (TRPV4) selective agonist 4 α -Phorbol 12,13-didecanoate (4 α -PDD) in *Trpv4* conditional knockout (cKO) male mice ($n = 4$ mice/group, ~ 30 neurones were recorded from each mouse). Right-hand panels show examples of Ca^{2+} signal of cultured neurones in response to 4 α -PDD (10 μM). *** $p < 0.001$ vs wild-type (WT), 2-tailed *t*-test. Note: $\Delta R/R_0$ was determined as the fraction of the increase of a given ratio over baseline ratio divided by baseline ratio (ratiometric 340/380 nm blue light for dual excitation of Fura-2).