

Supporting Information

Imamachi et al. 10.1073/pnas.0905605106

SI Text

Behavioral Studies. Paw edema was measured with a spring-loaded digital caliper (Mitutoyo). Mechanical sensitivity was measured with a series of von Frey filaments with increasing stiffness (0.16, 0.4, 0.6, 1.0, 1.4, and 2.0 g). Each filament was applied 10 times to the same hindpaw, with at least a 1-min interval between 2 consecutive stimuli. Results are expressed as the average percentage of withdrawal responses for a given stimulus force. Thermal sensitivity was tested using a Hargreaves radiant heat source (IITC). The scratching assay was performed as described in ref. 9. α -Me-5-HT, clobenpropit dihydrobromide, and histamine-trifluoromethyl-toluidine (HTMT) were purchased from Tocris; endothelin-1 was from Calbiochem; SLIGRL-NH₂ from Bachem. All other pruritic compounds were purchased from Sigma-Aldrich. Briefly, the itch-evoking compounds were s.c. injected into the nape of the neck, via a 26 1/2-G needle in a volume of 100 μ L after acclimatization to the test chamber for >30 min. The resultant bouts of scratching were counted at 5-min intervals over a 30-min observation period. An intraplantar (i.pl.) injection of pruritic compounds in a volume of 20 μ L was made through a 30-G needle. Time spent licking the injected hindpaw for the next 30 min after injection was measured. All experiments were carried out blind to the genotype of the animals.

Histology. To document pruritogen-induced Fos expression ($n = 4-8$ mice per genotype), 2 h after the pruritogen injection, the mice were anesthetized with pentobarbital and perfused intracardially with buffered 10% formalin. Twenty micrometer transverse frozen sections of the cervical spinal cord were processed for Fos. Digitized images of 10 randomly selected sections per animal were captured using both brightfield and darkfield illumination. Using the darkfield image as a guide, a line was drawn at the border between the relatively dark substantia gelatinosa (lamina II) and the more lucent lamina III. From the brightfield image, we counted the number of Fos-immunoreactive neurons in laminae I/II and V in segments of the cervical enlargement and plotted them on the darkfield-derived sketch.

To determine the distribution of PLC β 3 in TRPV1⁺ neurons, the following primary antibodies were used: 1:300 rabbit anti-PLC β 3 (a gift from the Sternweis Lab at University of Texas Southwestern, Dallas) and 1:400 guinea pig anti-TRPV1 (Neuromics). Secondary antibodies were conjugated to Alexa Fluor 488 or 568 fluorochromes (Molecular Probes). Using a Leica TCS-NT confocal microscope system, we captured 14 confocal images from adult wild-type mice and counted the number of PLC β 3⁺ neurons that coexpressed TRPV1.

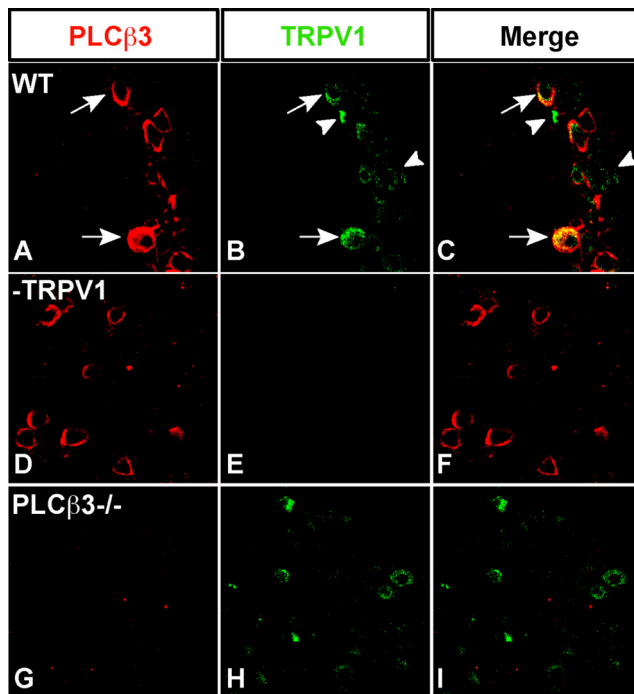


Fig. S1. TRPV1-expressing DRG neurons are composed of at least 2 different subsets; PLC β 3⁺TRPV1⁺ and PLC β 3-TRPV1⁺. (A–C) Double immunostaining for PLC β 3 (red) and TRPV1 (green) shows that PLC β 3 is partially expressed in TRPV1⁺ neurons. (D–F) Double immunostaining in the absence of TRPV1 primary antibody. (G–I) Double immunostaining of the DRG section derived from PLC β 3^{-/-} mice. Shown are 1.0- μ m confocal optical sections of adult mouse DRG neurons. Arrows indicate double-labeled cells (PLC β 3⁺TRPV1⁺). Arrowheads indicate PLC β 3-TRPV1⁺ neurons.