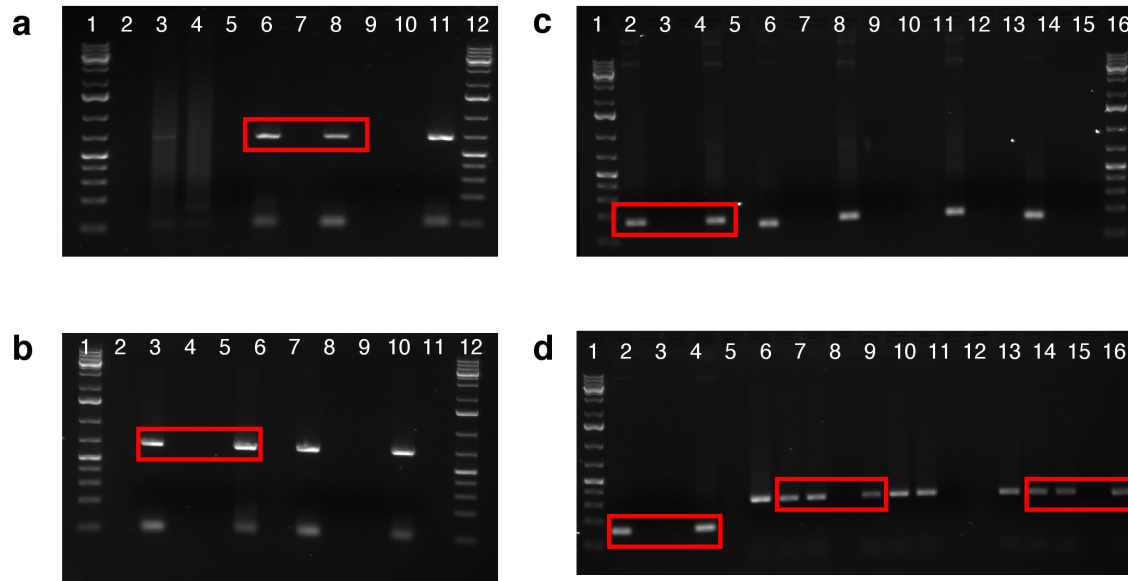


Supplementary Material

TRPA1 is required for histamine-independent, Mas-related G protein coupled receptor-mediated itch.

Sarah R. Wilson¹, Kristin Gerhold¹, Amber Bifolck-Fisher¹, Qin Liu², Kush N. Patel², Xinzhong Dong² and Diana M. Bautista¹



Supplementary Figure 1: PCR analysis of MrgprA3, MrgprC11 and TRPA1 expression in CQ-positive, BAM-positive and CQ/BAM/MO-negative, large diameter sensory neurons. Full-length agarose gels used in Figure 1. BAM- (BM) and CQ- (CQ) responding cells, and large-diameter, non-responsive cells (NR), were selected using calcium imaging. Two to three cell samples were combined for RT-PCR using primers against MrgprA3 (A3), MrgprC11 (C11), TRPA1 (A1) and GAPDH (G). Samples were loaded as described below and are listed as sample name, followed by primer set. Fermentas 1kb Plus DNA Ladder was used in ladder lanes (L). No RT control samples were used as negative controls (NEG). Whole trigeminal ganglia were used as positive controls (POS). Blank, unlabeled lanes were not loaded (B). Regions used in **Figure 1d** are indicated by red boxes.

Gel a.

Lane 1: L, **2:** B, **3:** BM1-A1, **4:** NR1-A1, **5:** NEG-A1, **6:** POS-A1, **7:** B, **8:** BM2-A1, **9:** NR2-A1, **10:** NEG-A1, **11:** POS-A1, **12:** L

Gel b.

Lane 1: L, **2:** B, **3:** CQ3-A1, **4:** NR3-A1, **5:** NEG-A1, **6:** POS-A1, **7:** B, **8:** CQ 4-A1, **9:** NR4-A1, **10:** NEG-A1, **11:** POS-A1, **12:** L

Gel c.

Lane 1: L, **2:** BAM2-C11, **3:** NR2-C11, **4:** NEG-C11, **5:** POS-C11, **6:** B, **7:** CQ2-A3, **8:** NR3-A3, **9:** NEG-A3, **10:** BM3-C11, **11:** NR3-C11, **12:** NEG-C11, **13:** B **14:** BM4-C11, **15:** NR4-C11, **16:** NEG-C11, **17:** CQ4-A3, **18:** NR4-A3, **19:** NEG-A3, **20:** L

Gel d

Lane 1: L, **2:** CQ3-A3, **3:** NR3-A3, **4:** NEG-A3, **5:** POS-A3, **6:** B, **7:** BM2-G, **8:** CQ2-G, **9:** NR2-G, **10:** NEG-G, **11:** POS-G, **12:** BM4-G, **13:** CQ4-G, **14:** NR4-G, **15:** NEG-G, **16:** CQ3-G, **17:** BM3-G, **18:** NR3-G, **19:** NEG-G, **20:** POS-G

Supplementary Movie 1. Itch–evoked scratching in response to chloroquine injection. A wild type mouse displays robust scratching following subcutaneous injection of chloroquine (200 ug/10 μ L, 40 mM) into the cheek. Movie displays a representative 15 second period of scratching, 5 minutes post–injection.