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Supplemental Information

Activation of Mast-Cell-Expressed Mas-Related G-Protein-Coupled Receptors Drives Non-histaminergic Itch

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SUPPLEMENTAL FIGURES

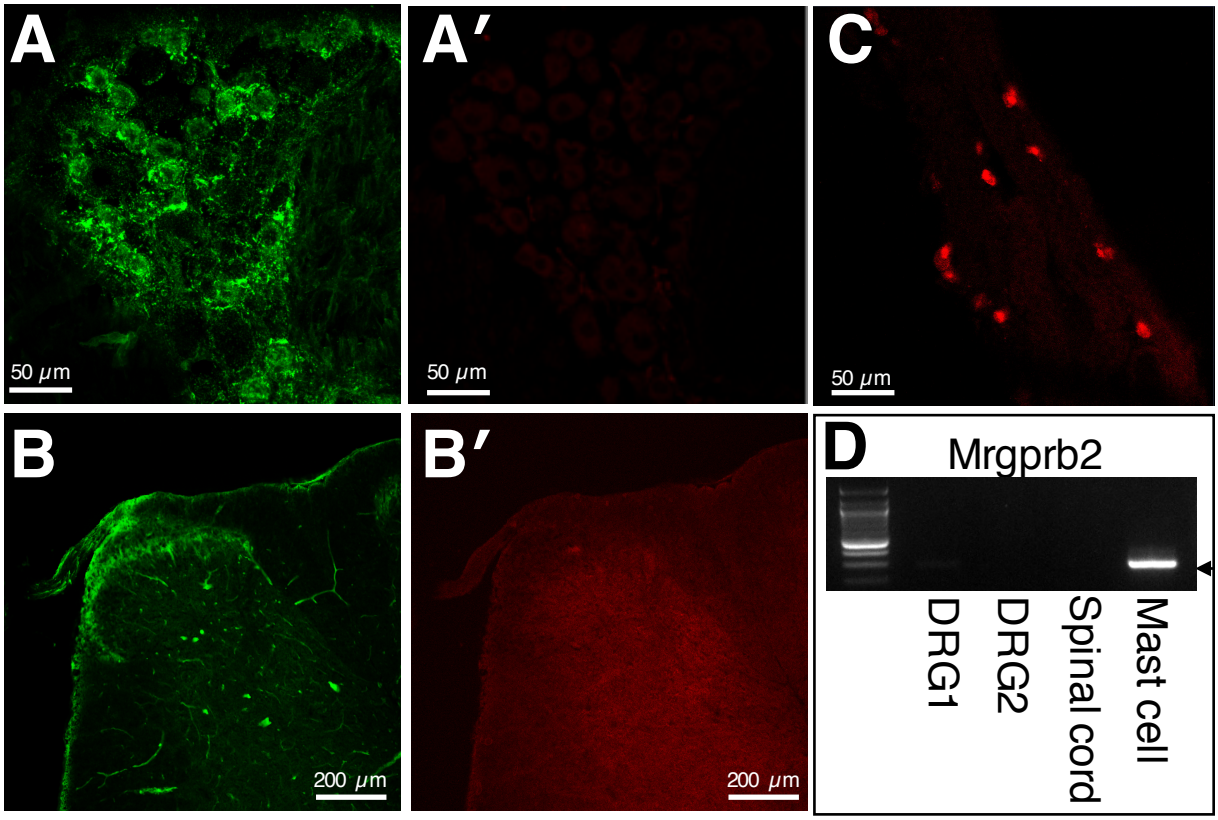

Figure S1 related to Figure 1. Mrgprb2 is not expressed in dorsal root ganglia sensory neurons and the spinal cord. Dorsal root ganglia (DRG) (**A, A′**) and spinal cord (**B, B′**) sections taken from Mrgprb2-Cre; tdTomato animals, mice expressing both Mrgprb2-Cre and lox-stop-lox tdTomato (tdT) fluorescent reporter. Isolectin B4 conjugated to Alexa Fluor 488 depicted in green in DRG (**A**) and the spinal cord (**B**). tdT fluorescent reporter is undetectable in both DRG (**A′**) and the spinal cord (**B′**). (**C**) Skin section taken from MRgprb2-Cre; tdT mouse. Red depicts positive tdT staining of mast cells. (**D**) Stringent RT-PCR for Mrgprb2 from cDNA collected from two dorsal root ganglia, spinal cord, and mast cell preps. Experiment was repeated at least two times. The arrow indicates the expected band size for Mrgprb2.

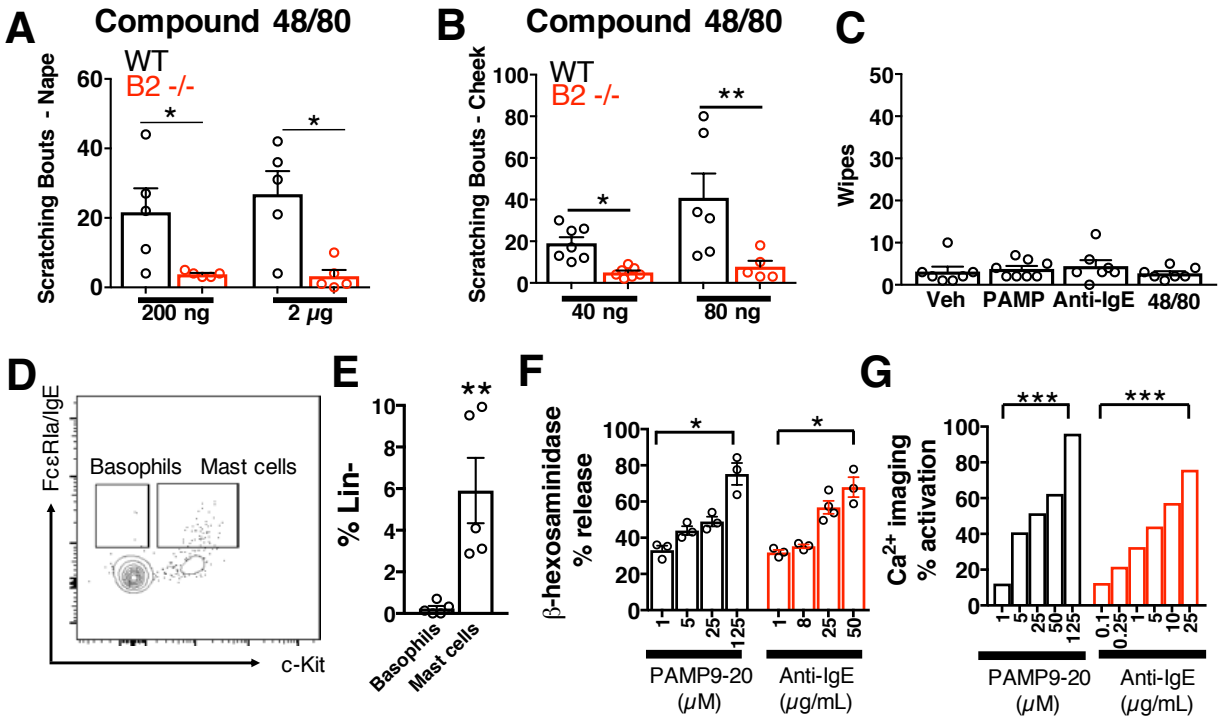

Figure S2 related to Figure 1. Mrgprb2 agonists elicit itch and not pain. (**A-C**) Mean plus s.e.m. depicted. Each open circle represents an individual mouse. (**A**) Scratching bouts from injection (50µL) of compound 48/80 into the nape of the neck of WT and Mrgprb2-/- mice. For 200 ng: WT n= 5, B2 -/- n= 5; 2 µg: WT n=5, B2 -/- n= 5. (**B**) Scratching bouts associated with injection (10µl) of compound 48/80 into cheek of WT or B2 -/- animals. For 40 ng: WT n= 7, B2 -/- n= 7; 80 ng: WT n=6, B2-/- n= 5. (**C**) Wiping associated with injection (10µl) of the indicated substance into the cheek of WT animals. 300µM PAMP9-20, 10µg/mL compound 48/80, and 100µg/mL Anti-IgE were injected. Veh n= 7, PAMP9-20 n=8, Compound 48/80 n= 7, anti-IgE n=5. (**D**) Representative FACS plot of Live CD45+ Lin- CD11b- cells (basophils) from naïve cheek skin. (**E**) Quantification of the frequency of c-Kit+ Mast cells and c-Kit- Basophils (average ± SD) as a percentage of Lin- cells of n = 2 mice. (**F**) β-hexosaminidase release of mouse peritoneal mast cells, displayed as a percentage of total β-hexosaminidase. For all conditions, n=3. Open circles depict independent replicates. (**G**) Percent activation as detected by

Ca^{2+} imaging of mouse peritoneal mast cells. ***, $P < 0.001$ by chi-square test. (**A-G**) Mean plus s.e.m. depicted. Each open circle represents an individual mouse. *, $P < 0.05$; **, $P < 0.01$, n.s. = not significant by two-tailed unpaired Student's *t*-test.

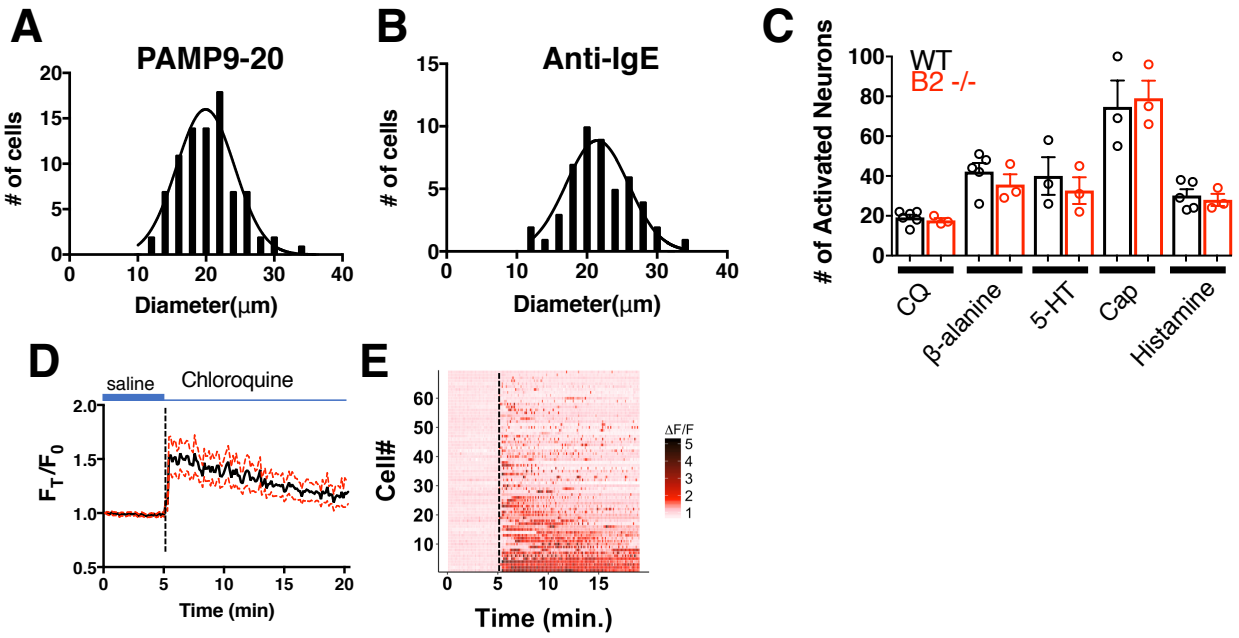

Figure S3 related to Figure 2 and 3. Diameters and total numbers of identified activated neurons associated with mast cell agonists and neuronal activators. (**A**) Histogram of diameters of neurons activated *in vivo* by 300μM PAMP9-20. (**B**) Histogram of diameters of neurons activated *in vivo* by 100μg/mL Anti-IgE. (**C**) The total number of activated neurons identified within each imaging trial period from the labeled test compound. For CQ: WT n= 6, B2 -/- n=3; β-alanine: WT n= 5, B2 -/- n=3; 5-HT: WT n=3, B2 -/- n=3; capsaicin (cap): WT n=3, B2 -/- n=3; histamine: WT n=5, B2 -/- n=3. Mean plus s.e.m. depicted. Open circles represent individual, imaged mouse DRGs. Averaged Ca^{2+} imaging traces (**D**) and heatmaps (**E**) of individual neurons (included in the average) activated by 10mM chloroquine, n= 69. Black dotted lines indicate the start of the test compound imaging period. The solid black line depicts the averaged change in fluorescence of representative, activated sensory neurons, whose individual fluorescent changes are illustrated in the heatmap. The red dotted lines (**D**) are 95% confidence intervals.

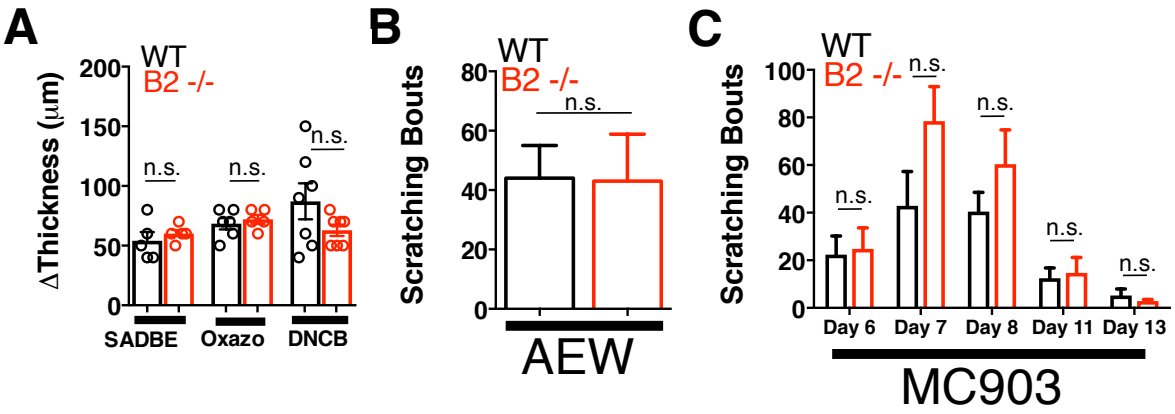

Figure S4 related to Figure 4. Mrgprb2-/- animals had intact itch in AEW and MC903 chronic itch models. (**A**) Changes in thickness of ear skin from WT and B2 -/- animals treated with either SADBE, Oxazolone, or DNCB contact dermatitis models. Mean ± s.e.m. depicted. n.s, not significant by two-tailed student's t-test. For SADBE: WT n= 5, B2 -/- = 5; for Oxazolone: WT n= 6, B2 -/- = 5; for DNCB: WT n= 7, B2 -/- = 7. (**B**) Scratching bouts from WT and B2 -/- animals subjected to the acetone, ether, water (AEW) model of dry skin. WT n= 4, B2 -/- n= 4. (**C**) Scratching bouts from WT and B2 -/- animals treated with calcipotriol (MC903). For Day 6-8: WT n= 9, B2 -/- n=9; For Day 11: WT n= 7, B2 -/- n= 7; For Day 13: WT n= 5, B2 -/- n= 8. Mean plus s.e.m. depicted. n.s, not significant by two-tailed student's t-test (**B**) or two-way ANOVA (**C**).