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Supplemental Information

A Mast-Cell-Specific Receptor Mediates Neurogenic Inflammation and Pain

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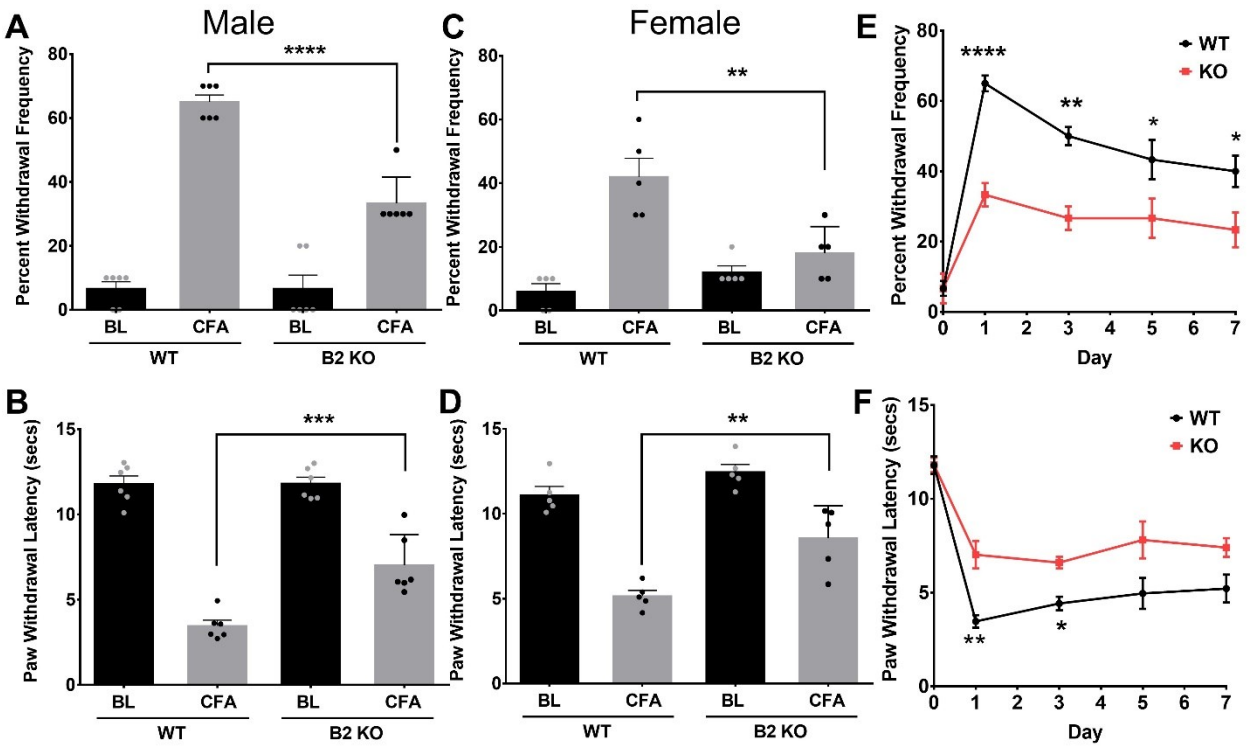

Figure S1. Mrgprb2$^{-/-}$ mice exhibit significantly less inflammation induced hypersensitivity in models of inflammatory pain. Related to Figure 1.

CFA was injected into the hind-paw of male (A and B) female (C and D) WT and Mrgprb2$^{-/-}$ mice. Mechanical (A and C) and thermal hypersensitivity (B and D) peaked at 24 hrs after injection (BL = baseline, no difference was observed in baseline between WT and Mrgprb2$^{-/-}$ mice). Time course of CFA induced mechanical (E) and thermal hypersensitivity (F) was measured starting 24 hours after CFA hind-paw injection for up to 7 days. WT vs Mrgprb2$^{-/-}$ CFA induced mechanical (E) and thermal (F) hypersensitivity was observed for seven days. Data was analyzed using 2-tailed Student's t-test and two-way ANOVA with Bonferroni's post-hoc test (>2 groups), *p<0.05, **p<0.01, ****p<0.001 n = 6-7/group, error bar: S.E.M.

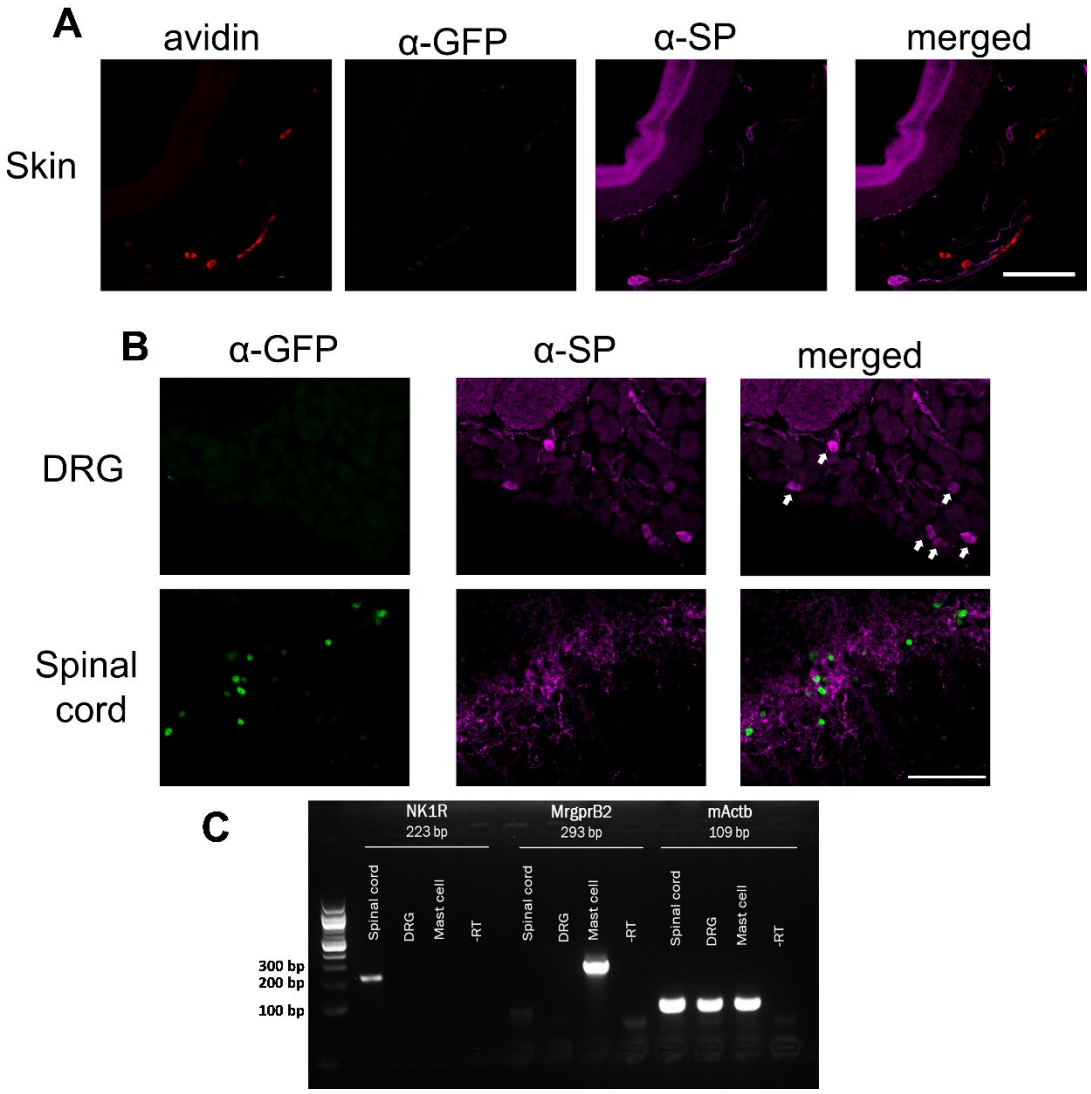

Figure S2. NK-1 receptor is not expressed in mast cells or primary sensory neurons. Related to Figure 2.

Confocal microscopy images of immunofluorescence staining of sections from mice using rhodamine-conjugated avidin (red) to stain mast cells, anti-GFP antibody to stain NK-1 receptor-GFP CRE (green) and anti-substance P antibody (magenta). (A) Skin section with avidin stained mast cells found in the dermis in the vicinity or contact with SP positive nerve fibers. (B) Double immunofluorescence staining of DRG (L5) and spinal cord (lamina I). NK-1 receptor-GFP (green) and anti-substance P antibody (magenta) found no positive NK-1 staining in the DRG. Arrows point to SP positive cell bodies. NK-1 receptor-GFP (green) staining was found in lamina I along with SP (magenta) positive terminals. Scale bars are 100 μm. (C) RT-PCR analysis of NK-1 receptor, Mrgprb2, and actin expression of tissue samples from spinal cord, DRG, and peritoneal mast cells. The size of each PCR product is indicated at top of gel.

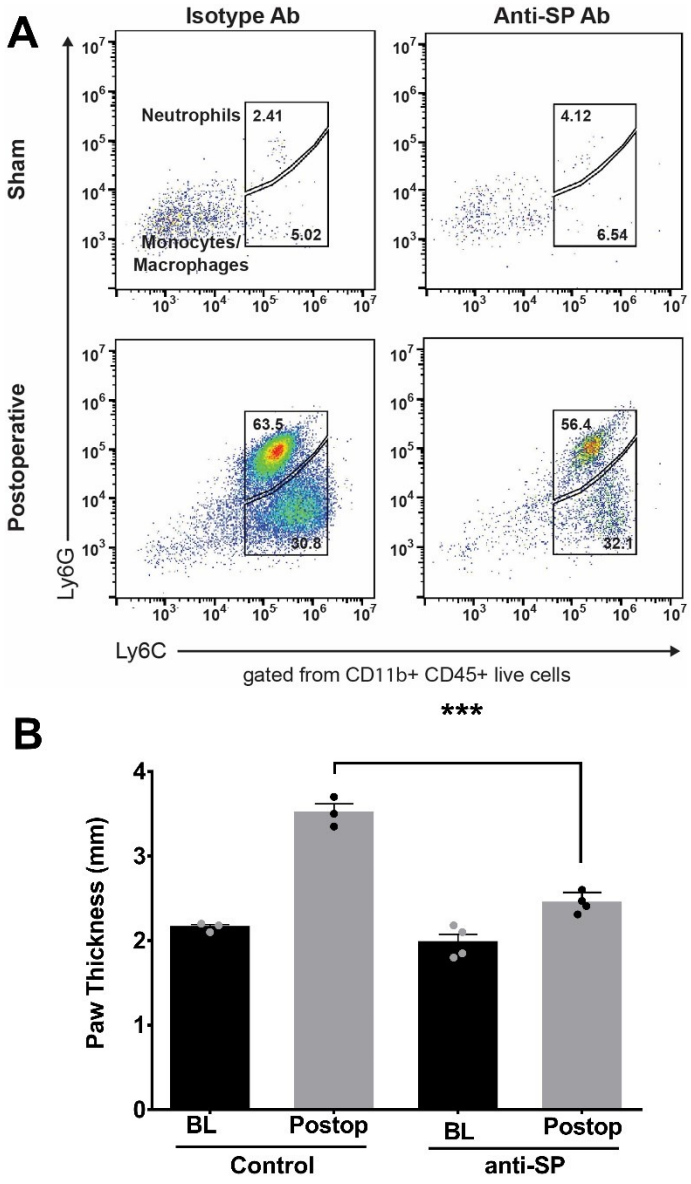

Figure S3. Substance P promotes innate immune cell recruitment and inflammation. Related to Figure 3.

(A) Representative flow cytometric profiles of biopsies taken from WT hind paw skin injected with control (isotype Ab) or anti-substance P antibodies (15 µg). Numbers indicate the percentage of cells pre-gated on viability, CD45+, CD11b+. FcεRI+. (B) Quantification of incision induced inflammation, measured by paw thickness, in WT mice treated with control (isotype Ab) or anti-SP antibodies.

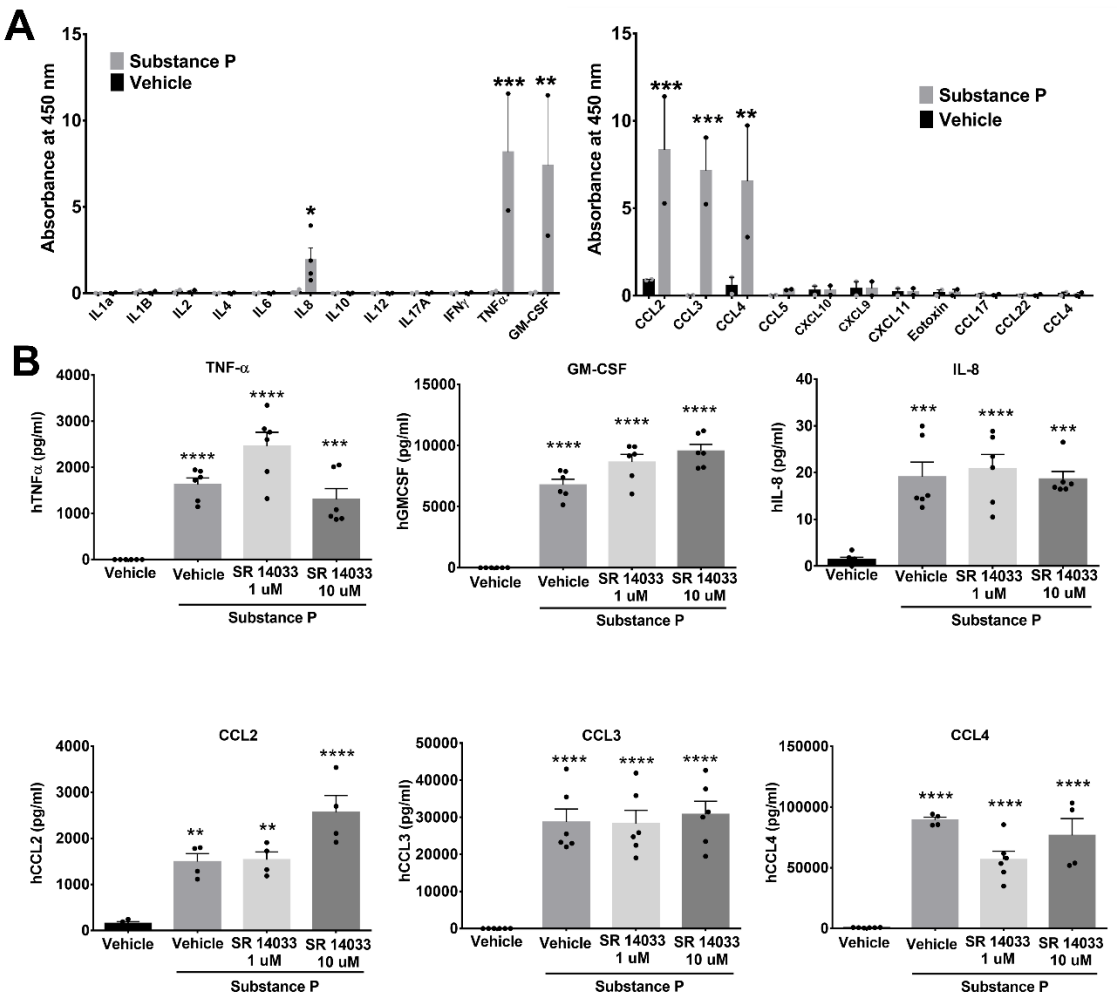

Figure S4. Human mast cells release specific inflammatory cytokines and chemokines that is MRGPRX2 dependent. Related to Figure 4.

(A) Cytokines and chemokines released after exposure to substance P (1 μM) or vehicle were measured using a multi-analyte ELISA array. Data are represented as absorbance values. Data was analyzed using 2-tailed Student's t-test (*p<0.05, **p<0.01 **p<0.01, ****p<0.001 error bar: S.E.M.). (B) LAD2 mast cells were treated with vehicle or the NK-1 receptor antagonist SR 140333 (1 or 10 μM). Concentrations of cytokines TNF-a, GM-CSF, IL-8, and chemokines CCL2, CCL3, and CCL4 in supernatant taken from vehicle or substance-P (1 μM) treated LAD2 mast cells were determined by ELISA. Data was analyzed using a one-way ANOVA (*p<0.05, **p<0.01, ****p<0.001 error bar: S.E.M.).