SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure S1. *Trpm8*$^{-/-}$ mice are hypersusceptible to acute but not chronic experimental colitis.

(a) *Trpm8*$^{-/-}$ mice showed shorter colons without pelleted contents after DSS treatment compared to WT mice. Typical examples are shown. **(b)** Quantification of colon lengths from WT and *Trpm8*$^{-/-}$ mice after DSS treatment. **(c)** Decreased expression of *Muc2* and, **(d)** *Muc3* transcripts in intestinal epithelial cells harvested from *Trpm8*$^{-/-}$ mice compared to WT, as determined by Q-PCR. Results were normalized to *Gapdh*. **(e)** Mean bodyweights of WT and *Trpm8*$^{-/-}$ mice during treatment with 3 cycles of DSS. Significant differences between groups were only observed during the first cycle *i.e.* the acute stage of colitis. **(f)** No statistically significant difference in DAI between WT and *Trpm8*$^{-/-}$ mice after 3 cycles of DSS. **(g)** The extent of mucosal injury and inflammatory cell infiltration were comparable between WT and *Trpm8*$^{-/-}$ mice after 3 cycles of DSS. **(h)** No difference in pro-inflammatory cytokine production between WT and *Trpm8*$^{-/-}$ mice after 3 cycles of DSS in colon explant cultures. **(i)** Similar or diminished levels of T-cell cytokines produced by MLN cells stimulated with anti-CD3/CD28 Abs in the *Trpm8*$^{-/-}$ cohort compared to WT. **(j)** Mean bodyweights of WT and *Trpm8*$^{-/-}$ mice after topical re-challenge with 2.5% (w/v) TNBS or EtOH (negative control) on day 0 after cutaneous sensitization with 1% (w/v) TNBS on day -7. No statistically significant differences were observed between the two cohorts throughout follow-up. **(k)** Reduced signs of colitogenic activity in colons from *Trpm8*$^{-/-}$ compared to WT mice after TNBS treatment. **(l)** No differences between WT and *Trpm8*$^{-/-}$ mice in innate (TNF-α, IL-1β and IL-6) or T-cell derived (IFNγ, IL-17A) pro-inflammatory cytokines in colon explant cultures after TNBS treatment. **(m)** Significantly reduced TNF-α production but

similar levels of IFNγ and IL-17A by anti-CD3/CD28 restimulated MLN cells isolated from *Trpm8*$^{-/-}$ mice compared to WT controls. Data are mean ± SEM. **P*<0.05 by Student's t-test (c-d, i, m) or ANOVA (e). Scale bars = 500 μm (g) or 250 μm (k).

Supplementary Figure S2. Lack of epithelial traits predisposing for colitis in *Trpm8*$^{-/-}$ mice. **(a)** Similar expression levels of *Muc2* and *Muc3* in colonic epithelial cell lysates from naïve WT and *Trpm8*$^{-/-}$ mice, as determined by Q-PCR. Results were normalized for housekeeping gene *Gapdh*. **(b)** Comparable intestinal barrier functions measured in naïve WT and *Trpm8*$^{-/-}$ mice. Fecal albumin was measured by ELISA to quantify albumin leakage from the circulation to the intestinal lumen. **(c)** No difference in the uptake of dextran polymer FD-4 between WT and *Trpm8*$^{-/-}$ mice *in vivo*. FD-4 levels in the blood serum were quantified by measuring fluorescence, 2 hrs after oral gavage. **(d)** No differences in baseline active ion secretion (short-circuit current, I_{sc}), paracellular permeability (conductance, G) or macromolecular permeability (FITC dextran) as measured by Ussing chamber experiments with distal colon segments from naïve WT and *Trpm8*$^{-/-}$ mice. **(e)** No difference in the expression of proliferation marker *Mki67* in intestinal epithelial cells between WT and *Trpm8*$^{-/-}$ mice. *Mki67* expression was determined in intestinal epithelial cell lysates from the small intestine and colon, respectively, by Q-PCR (normalized for *Gapdh*). **(f)** No difference in the number of Ki67 positive cells in the small intestine or colon between WT and *Trpm8*$^{-/-}$ mice. Ki67 immunoreactivity in paraffin sections was detected with DAB (brown). Counterstaining was performed with hematoxylin (blue). Scale bar = 100 μm. **(g)** No expression of *Trpm8* in intestinal epithelial cell lysates, as determined by Q-PCR. Positive control: spine homogenate. *Neurofilament* (neuronal marker). *Villin* (intestinal epithelial cell marker). Data are presented as mean ± SEM.

Supplementary Figure S3. Optimization of anti-CGRP and anti-TRPM8 Abs for immunofluorescent staining.

(a) Immunofluorescent (IF) staining with anti-CGRP Ab (Abcam) of WT colon sections, detection with AF488 conjugated secondary Abs. Left panel: no primary Ab (control). Arrowheads: positive staining in myenteric plexus. Asterisk: positive staining fibre in the mucosal layer. **(b)** IF staining with anti-TRPM8 Abs (Abcam) of WT colons, detection with AF488 secondary Ab. Left panel: no primary Ab (control). Arrowheads: positive staining in myenteric plexus. Double asterisks: positive staining in serosal layer. **(c)** Staining of PFA-fixed colon sections prepared from WT mice treated with water or DSS 2% (w/v) for 3 days. The mucosal layer was visualized with differential interference contrast (DIC) microscopy and merged with fluorescence images (right panels). Typical results are shown (n=4/group), demonstrating that TRPM8-IR+ fibres are located between colonic crypts. **(d)** Double staining of DSS-treated WT colon section with anti-CGRP (green) and anti-TRPM8 (red) Abs. See additional comments in legends to **Figure 3g**. Imaging was performed at 63X.

Supplementary Figure S4. SP receptor deficiency protects against colitis.

(a) WT and *Nk1*$^{-/-}$ mice received DSS 2% (w/v) in drinking water for 5 days. Mice were euthanized on day 9 for analysis. *Nk1*$^{-/-}$ mice showed significantly less body weight loss during follow-up, less mucosal injury and hallmarks of colonic inflammation compared to WT mice. **(b)** Deficiency of the SP receptor in bone marrow derived cells protects against colitis. WT mice were reconstituted with either WT or *Nk1*$^{-/-}$ bone marrow followed by DSS 2% (w/v) treatment 6 weeks later. Mice were euthanized on day 10. Recipients of *Nk1*$^{-/-}$ bone marrow were protected

against acute colitis as demonstrated by reduced weight loss and less mucosal injury compared to control mice. Data are mean ± SEM. **P*<0.05 (2-way ANOVA). Scale bars = 100 μm.

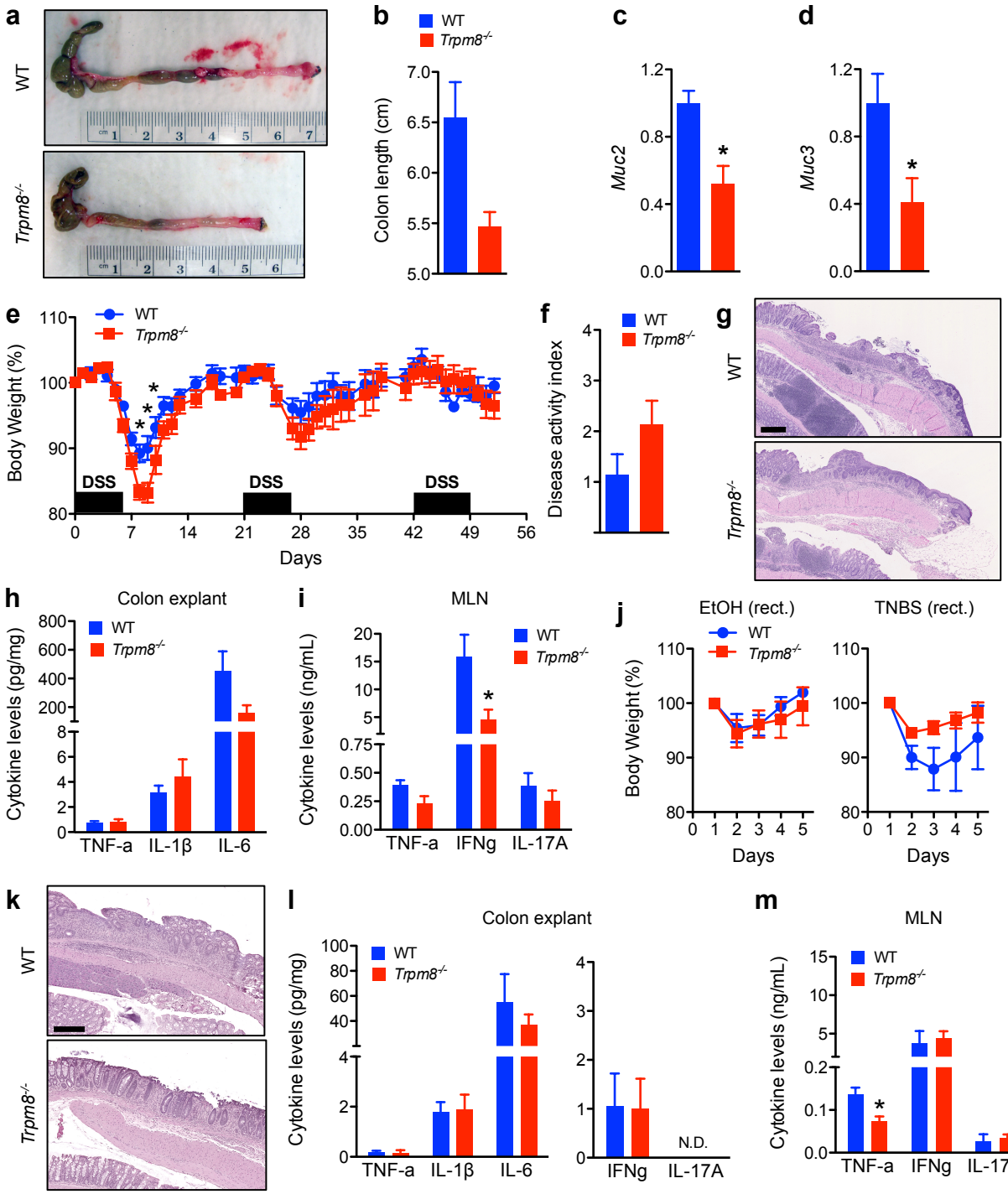
Supplementary Figure S5. CGRP antagonism aggravates DSS colitis in WT mice.

(a) WT mice showed an increased susceptibility to DSS colitis during treatment with the CGRP antagonist, $CGRP_{8-37}$. WT and *Trpm8*$^{-/-}$ mice received DSS 2% (w/v) in drinking water for 5 days and were treated once daily with $CGRP_{8-37}$ (2 μg, i.p.). Whereas WT mice in the PBS group started recovering from the DSS-induced bodyweight loss at day 7, WT mice of the $CGRP_{8-37}$ group did not. No statistically significant differences were observed in mean bodyweights between the treatment groups on the *Trpm8*$^{-/-}$ background. **(b)** $CGRP_{8-37}$ vs. PBS treatment resulted in a significantly increased DAI in WT but not in *Trpm8*$^{-/-}$ mice. **(c)** WT mice showed more extensive mucosal injury and colonic inflammation in the $CGRP_{8-37}$ group compared to PBS-treated mice. A similar degree of colitis was observed in PBS vs. $CGRP_{8-37}$ treated *Trpm8*$^{-/-}$ mice. Scale bar = 500 μm. **(d)** Increased production of TNF-α and IL-6 in colon explants of $CGRP_{8-37}$ treated WT mice, but not *Trpm8*$^{-/-}$ mice. **P*<0.05 $CGRP_{8-37}$ vs. PBS by ANOVA (a), Mann-Whitney (b) or t-test (d).

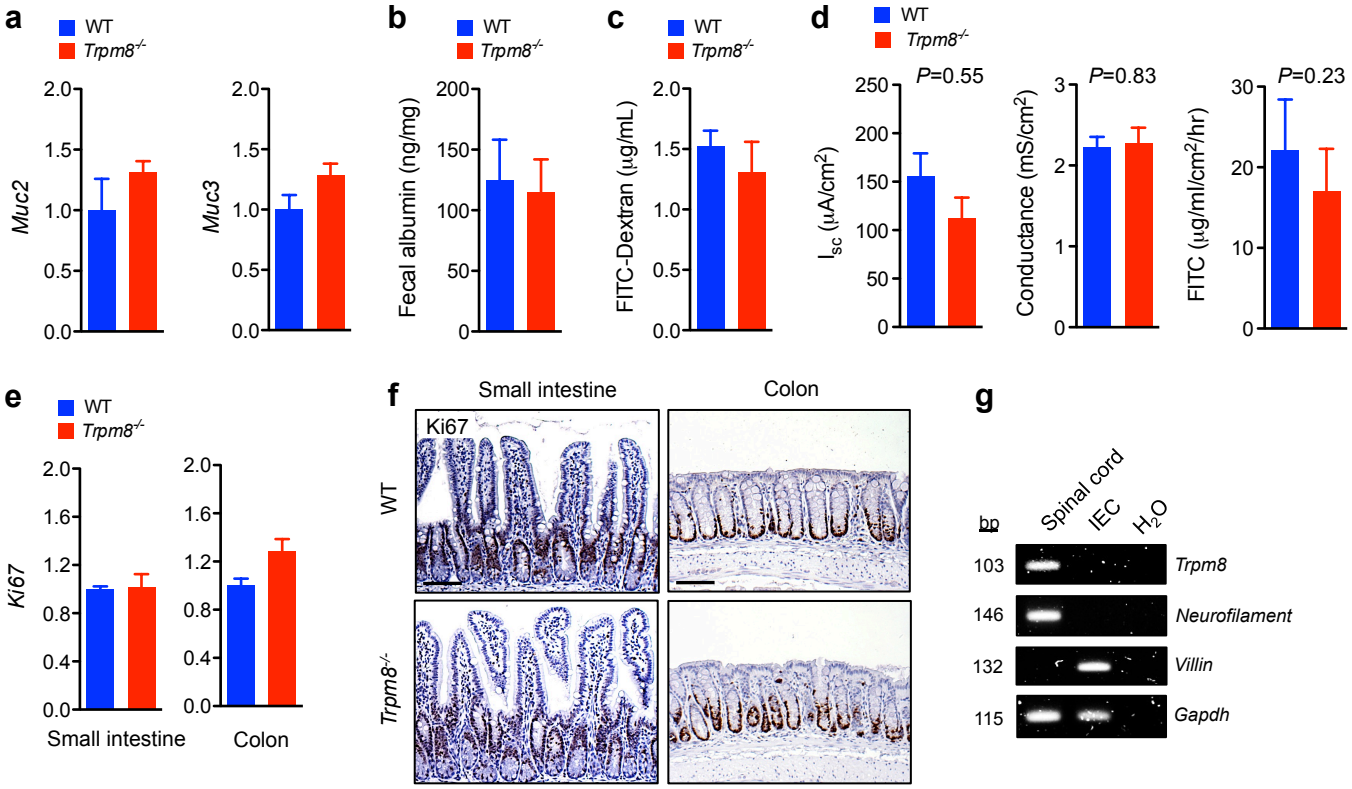
Supplementary Table 1. Primer sequences.

Gene	Accession number	Sequence 5'-3'
Cd11c	NM_021334.2	CTGAGAGCCCAGACGAAGACA TGAGCTGCCCACGATAAGAG
Clr	NM_018782.1	GTTGCCAACGGATCACATTGC ACAAAGCAGCACAAATCGGAC
Gapdh	NM_008084	TCAACAGCAACTCCCACTCTT ACCCTGTTGCTGTAGCCGTAT
Muc2	XM_975612.2	ACATCACCTGTCCCGACTTC GAGCAAGGGACTCTGGTCTG
Muc3	XM_355711.9 XM_001478962.2	GTGATCCTCGTGATCCTCCT GATGCTCTGCCTTCCTCTTC
Mki67	NM_001081117.2	TGCCCGACCCTACAAAATG GAGCCTGTATCACTCATCTGC
Nefl	NM_010910	GGCCTTGGACATCGAGATTG TCTGCAAGCCACTGTAAGC
Ramp1	NM_016894.2	GACGCTATGGTGTGACT GAGTGCAGTCATGAGCAG
Trpm8	NM_134252.3	GCTGTACAAAGCCTTCAGCAC CTCATCACTGGCAAGGTCCA
Vil	NM_009509	TGGAAGAGTTTCAACAGAGGG CTGGTCTCGGATCTCTTTGG

Supplementary Figure S1.
***Trpm8*$^{-/-}$ mice are hypersusceptible to acute but not chronic experimental colitis.**



Supplementary Figure S2.
Lack of epithelial traits predisposing for colitis in *Trpm8*$^{-/-}$ mice.



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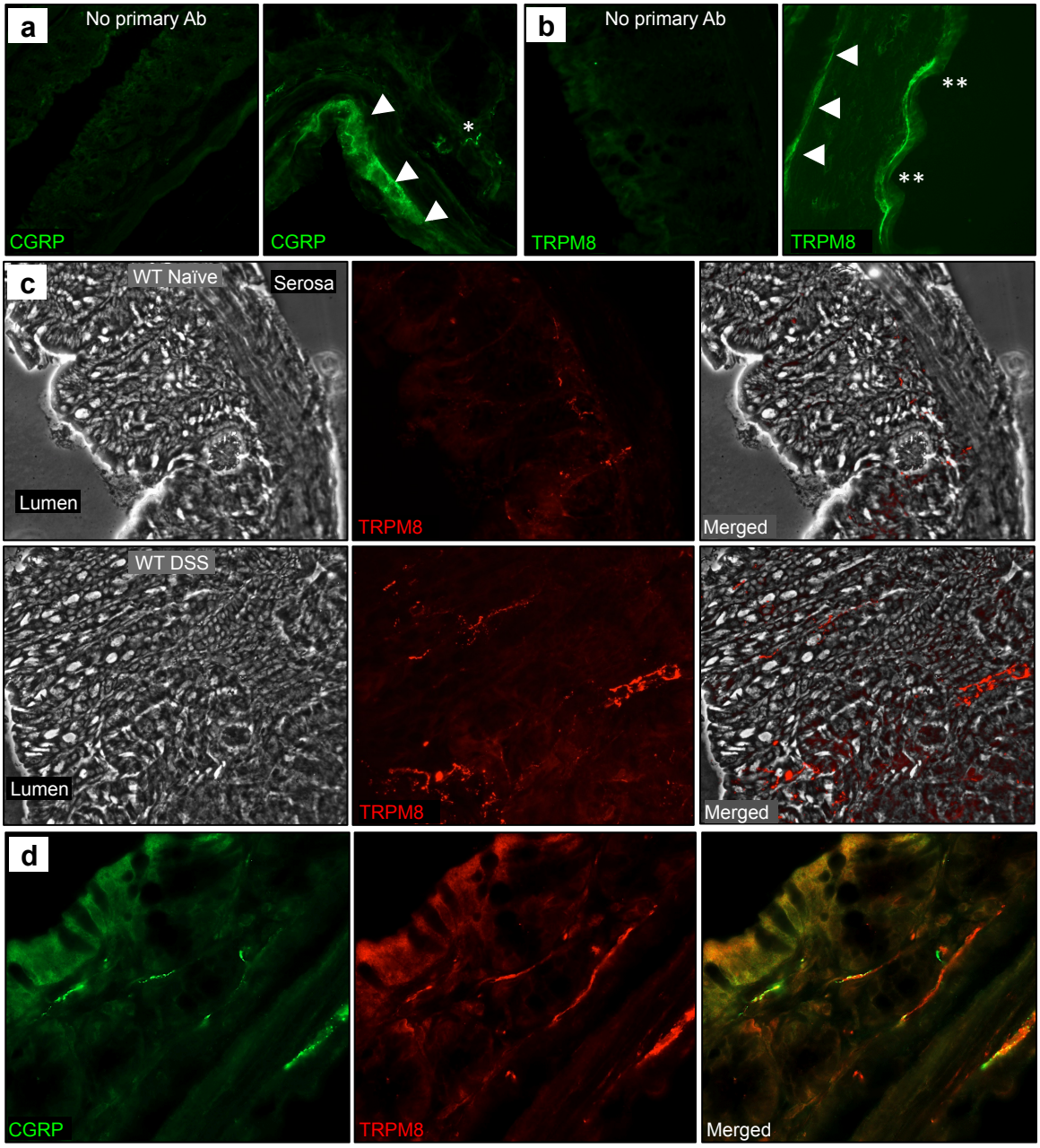
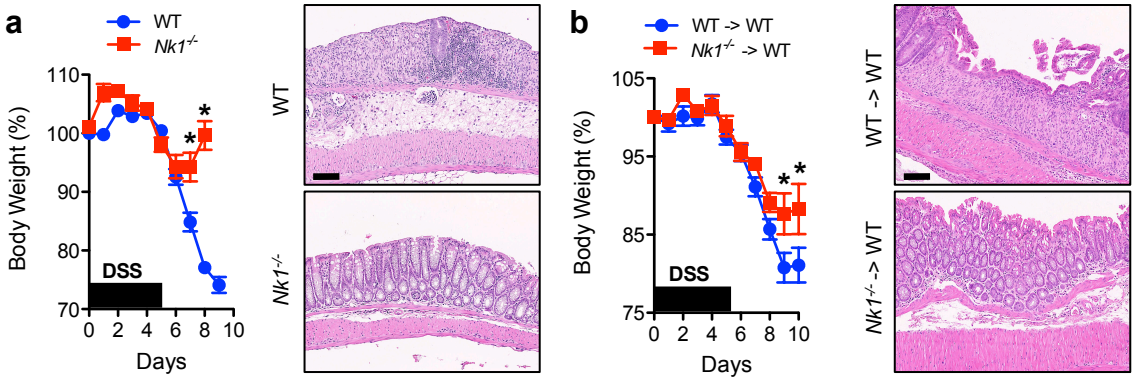


Supplementary Figure S4.
SP receptor defiency protects against colitis.



Supplementary Figure S5.
CGRP antagonism aggravates DSS colitis in WT mice.