Supporting Information

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SI Methods

In vitro permeability. Caco2-bbe human intestinal adenocarcinoma cells (ATCC) were maintained in DMEM supplemented with 10% FCS, 0.1 mM nonessential amino acids, 1 mM sodium pyruvate, 10 mM Hepes, and 1× penicillin/streptomycin (all Invitrogen) in a humidified incubator (5% CO_2, 37 °C). Cells (5×10^5) were seeded on snapwells (12-mm diameter, 0.4-μm pore; Corning) and cultured for 18–21 days. TER was monitored with an EVOM/Endohm (Costar), only snapwells with TER >250 Ω*cm^2 were used. Baseline TER was measured and recombinant human chymase (0.005–0.5 U/mL; Sigma) added to the basolateral chamber. TER was monitored over 24 h, with some snapwells placed in Ussing chambers after 30 min or 4 h of stimulation for permeability assays using FITC-dextran as described above. Additional snapwells were treated for 12 h with 0.05 U/mL chymase preincubated with the serine protease inhibitor chymostatin (100 μM; Sigma) or vehicle (DMSO), and TER measured.

Epithelial Cell Proliferation, Migration, and Apoptosis. Bromo-2′-deoxyuridine (BRDU) in PBS was injected i.p. (0.2 mg/g body weight) and mice killed 24 h later. BRDU-labeled cells in the jejunum were detected using a BRDU staining kit (Zymed) per manufacturer's instructions. A minimum of 25 well-oriented, villus-crypt units were counted for BRDU$^+$ cells to quantify proliferation. The distance from the crypt base to the farthest BRDU$^+$ epithelial cell was measured for epithelial migration using ImageProPlus. Apoptotic cells were detected by cleaved caspase-3 immunohistochemistry (Cell Signaling).

Immunofluorescence. Jejunum was fixed in 4% paraformaldehyde followed by 30% sucrose. Frozen sections were brought to room temperature for 30 min, permeabilized in ice-cold acetone for 10 min, and blocked in 5% goat serum per 1% BSA per 0.05% Triton-X-100/TBS. Sections were incubated with primary antibodies: rabbit anti-fibronectin (1:80, Millipore), rabbit anti-claudin-3 (1:25, Zymed), rat anti-E-cadherin (1:500, Zymed), rabbit anti-Mcpt-1 or -4 [1:25, 1:250 (25)] or isotype control overnight at 4 °C. After washing, slides were incubated with AlexaFluor 488-conjugated goat anti-rabbit or AlexaFluor 594 goat anti-rat secondary antibodies (1:250, Molecular Probes) for 90 min and coverslipped with DAPI/Supermount G.

1. Miller HR, Pemberton AD (2002) Tissue-specific expression of mast cell granule serine proteinases and their role in inflammation in the lung and gut. *Immunol* 105:375–390.
2. Wattanasirichaigoon S, Menconi MJ, Delude RL, Fink MP (1999) Effect of mesenteric ischemia and reperfusion or hemorrhagic shock on intestinal mucosal permeability and ATP content in rats. *Shock* 12:127–133.

Caco2 Cell Immunofluorescence. Confluent Caco2-bbe cells grown on transwells were stimulated basolaterally with 0.05 U/mL human chymase for 0, 12, or 24 h. Cells were rinsed, fixed in 4% paraformaldehyde for 30 min on ice, permeabilized in 0.5% Triton-X-100/TBS for 10 min and blocked in 5% goat serum per 1% BSA per 0.05% Triton-X-100/TBS for 30 min. Cells were incubated with primary antibodies: rat anti-E-cadherin and rabbit anti-ZO-1 (1:250, Zymed) for 2 h followed by AlexaFluor 488-conjugated goat anti-rabbit Ig or AlexaFluor 594 goat anti-rat Ig (1:250, Molecular Probes). Transwell filters were placed on slides and coverslipped with DAPI/Supermount G.

Protein and RNA Analysis. Jejunum protein (30 μg) was separated under reducing and denaturing conditions on 4–12% Bis-Tris gels and transferred to nitrocellulose membranes. Membranes were blocked in 5% milk per 0.1% Tween-20 per TBS for 2 h. Rabbit primary antibodies against actin (1:2,000, Sigma), claudins-1, -2, and -3 (1 μg/mL, Zymed), and E-cadherin (1:1,000 Zymed), and a peroxidase-conjugated goat anti-rabbit Ig secondary antibody (1:10,000, Calbiochem) were used. Proteins were detected by chemiluminescence (ECL Plus, Amersham). Densitometric analysis was performed using Image J (National Institutes of Health). RNA analysis by quantitative real-time PCR was performed as described in ref. 1.

Everted Gut Sac Method. Jejunal mucosal permeability to FITC-Dextran (FD4) was assessed using the everted gut sac method as described in ref. 2. Permeability was expressed as the mucosal-to-serosal clearance of FD4 and fluorescence was determined by spectrophotofluorometry (Biotek).

IgE-Mediated Passive Anaphylaxis. Mice were injected with 50 μg anti-IgE antibody [EM-95 (21)] or saline. Rectal temperature was monitored for 1 h and serum collected. Mcpt1 serum levels were measured by ELISA according to manufacturer's instructions (Moredun Scientific).

Hydroxyproline Assay. Intestinal tissue was hydrolyzed overnight in 6 N HCl at 100 °C. The supernatant was neutralized with 1% phenolphthalein and titrated against 10 N NaOH, then mixed with isopropanol and chloramine-T/citrate buffer solution (pH 6.0). Erlich's reagent was added and incubated for 25 min at 60 °C then measured at 558 nm. Hydroxyproline levels were calculated using 4-hydroxy-L-proline (Calbiochem) as a standard.

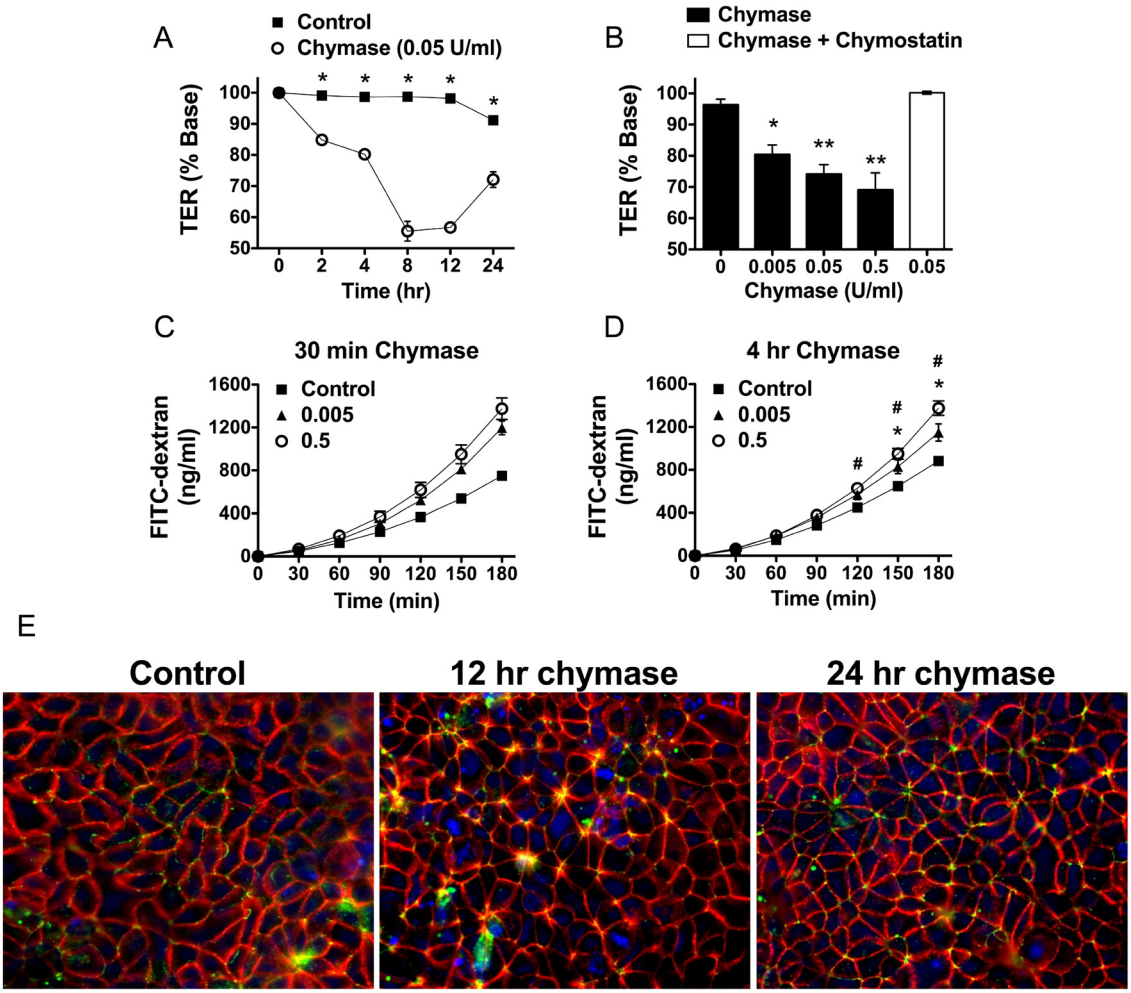

Fig. S1. Decreased jejunal permeability to FD4 in $Kit^{W\text{-}sh/W\text{-}sh}$ (Wsh) and Mcpt4$^{-/-}$ mice assessed by the everted gut sac method. Everted jejunum sacs were incubated in Krebs buffer containing FD4 and the mucosal-to-serosal clearance of FD4 was measured. Values represent mean ± SEM; n = 5–12 per group. Statistical significance is: *, $P < 0.005$ and **, $P < 0.001$ vs. control.

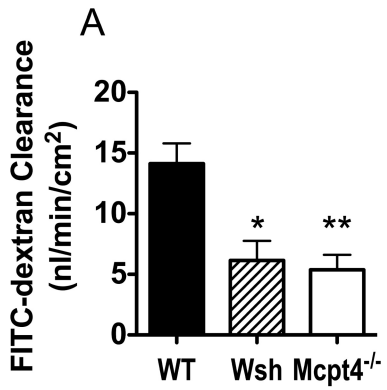

Fig. S2. Mcpt4-deficiency does not alter homeostatic intestinal mast cells. (*A*) Chloroacetate esterase reactive mast cells in the jejunum of WT, Mcpt4$^{-/-}$, and *Kit*$^{W\text{-}sh/W\text{-}sh}$ (Wsh) mice were quantified per villus crypt unit (VCU) and localized to the villus, villus-crypt junction (V/C jxn), crypt, and submucosa. No positive cells were detected in the jejunum of mast cell-deficient *Kit*$^{W\text{-}sh/W\text{-}sh}$ mice. Values represent mean ± SEM of 100 VCUs per mouse; $n = 6–9$ mice per group. (*B*) Immunofluorescent detection of Mcpt1 (green; *Top*) and Mcpt4 (green; *Bottom*) in the jejunum of WT (*Left*) and Mcpt4$^{-/-}$ (*Right*) mice. Note the absence of Mcpt4 detection in Mcpt4$^{-/-}$ jejunum (*Bottom Right*). Nuclei are shown by DAPI (blue). (*C*) Serum Mcpt1 measurement and (*D*) rectal temperature change (Δ Temp °C) at 15 min in WT and Mcpt4$^{-/-}$ mice after iv administration of 20 μg anti-IgE (EM-95) or control antibody. Values represent mean ± SEM; $n = 4–6$ mice per group.

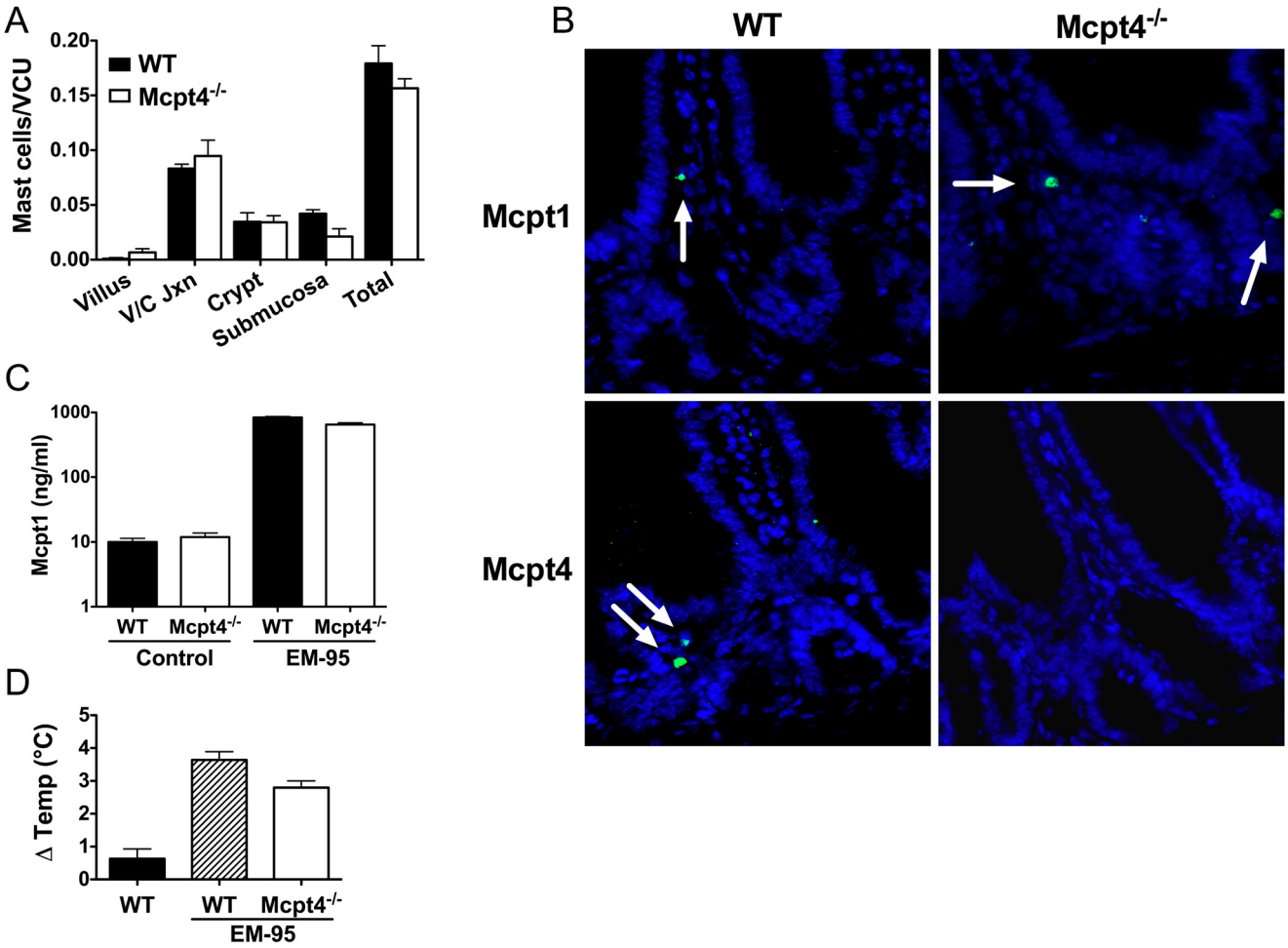

Fig. S3. Mast cell chymase alters intestinal epithelial permeability. Confluent Caco2-bbe cells cultured on snapwells were stimulated basolaterally with recombinant human chymase. (*A*) TER was measured from 0 to 24 h and expressed as a percentage of baseline TER (0 min) in the absence (■) or presence (○) of 0.05 U/mL chymase. (*B*) Dose-response TER changes to chymase were determined at 12 h in the absence (■) or presence (□) of the serine protease inhibitor, chymostatin 100 μM. (*C* and *D*) Apical to basolateral flux of FITC-dextran measured at 30 min (*C*) and 4 h (*D*) after 0–0.5 U/mL chymase stimulation. To confirm Caco2-bbe cellular integrity and viability, (*E*) confluent Caco2-bbe cells cultured on transwells were stimulated with human chymase for 12 (*Middle*) or 24 (*Right*) h or with media alone as a control (*Left*) and E-cadherin (red) and ZO-1 (green) immunofluorescence was performed; nuclei stained by DAPI (blue). Values represent mean ± SEM; $n = 6$–9 per group. Statistical significance is: (*A*) *, $P < 0.001$ chymase vs. control; (*B*) *, $P < 0.05$ and **, $P < 0.001$ chymase vs. control and chymase vs. chymostatin; (*D*) *, $P < 0.05$ low dose chymase (0.005 U/mL) vs. control and #, $P < 0.05$ high dose chymase (0.5 U/mL) vs. control.

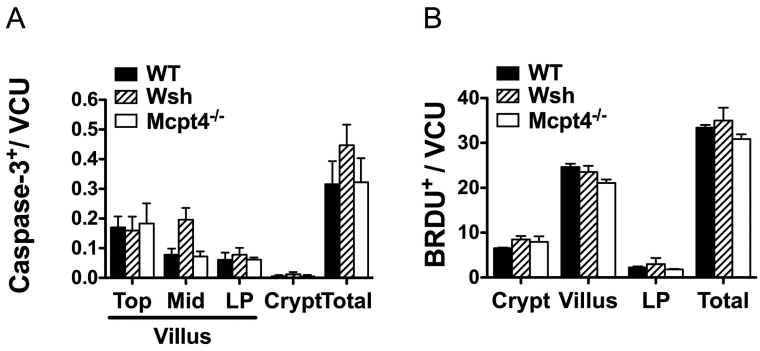

Fig. S4. *Kit*$^{W\text{-}sh/W\text{-}sh}$ and Mcpt4$^{-/-}$ mice do not display altered intestinal apoptosis or proliferation. Apoptotic and proliferating cells in the jejunum of WT, *Kit*$^{W\text{-}sh/W\text{-}sh}$ (Wsh), and Mcpt4$^{-/-}$ mice were quantified per villus crypt unit (VCU) and localized to the villus, crypt, and lamina propria. (*A*) Apoptotic cells were stained for cleaved caspase-3 and positive cells quantified per VCU. (*B*) Epithelial proliferation was measured by the incorporation of BRDU and quantified as BRDU^{+} cells per VCU. Values represent mean ± SEM; n = 5–7 mice per group.

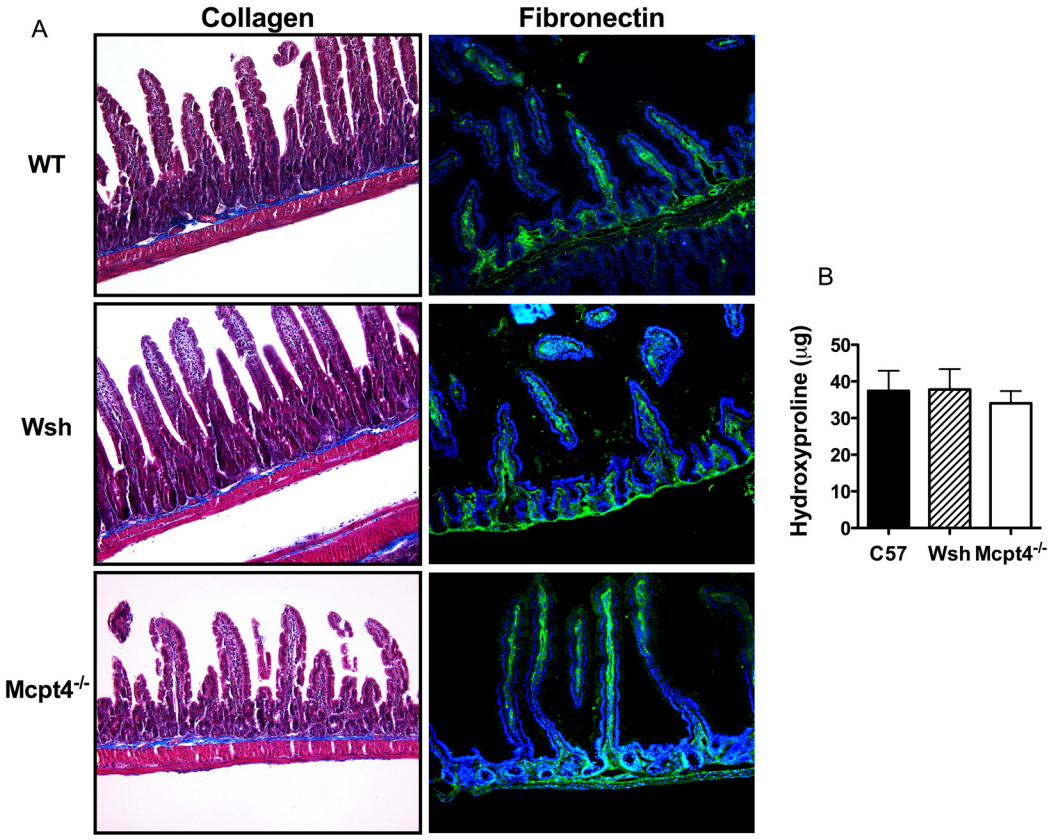

Fig. S5. Mast cell- and Mcpt4-deficiency does not alter the intestinal extracellular matrix. (*A* and *B*) Collagen and fibronectin expression were examined in the jejunum of WT, $Kit^{W\text{-}sh/W\text{-}sh}$ (Wsh), and $Mcpt4^{-/-}$ mice. (*A*) Collagen was examined by Trichrome staining (blue, *Left*) and by quantification of (*B*) hydroxyproline in the small intestine. (*A*) Fibronectin was analyzed by immunofluorescence (green, *Right*) with nuclei shown by DAPI (blue). Values represent mean ± SEM; n = 5–7 mice per group.