

SUPPLEMENTAL MATERIAL

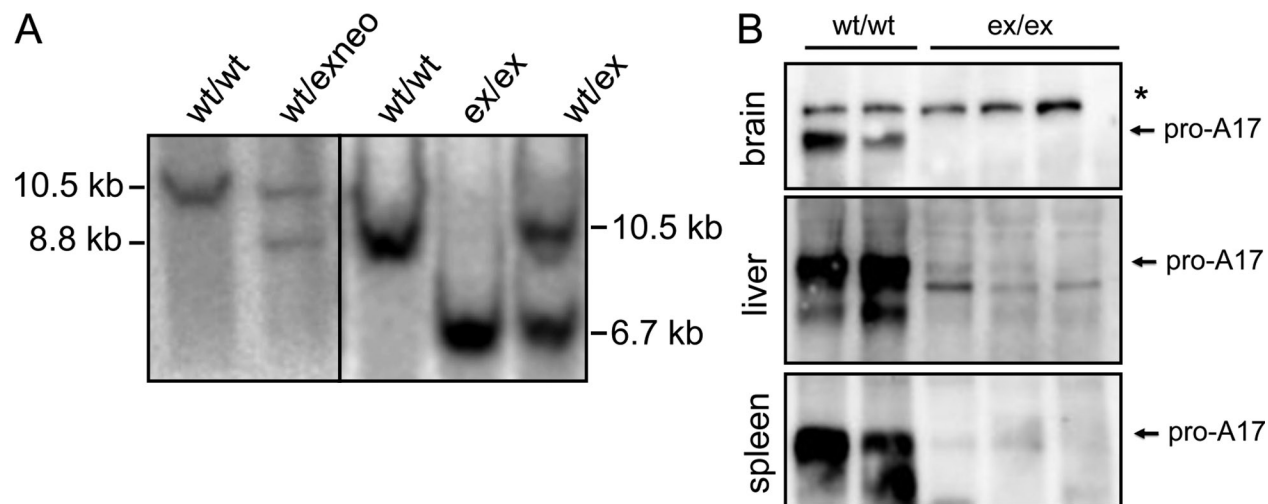
Chalaris et al., <http://www.jem.org/cgi/content/full/jem.20092366/DC1>

Figure S1. Generation and analysis of ADAM17^{WT/ex} mice. (A) Southern blotting of genomic DNA. Left: genomic DNA isolated from embryonic stem cells was digested with HindIII and used for Southern blot analysis. The positive clone, showing a 10.5-kb band corresponding to the WT allele and an 8.8-kb band corresponding to the recombined allele (including FRT-Neo-FRT-cassette), was used for injection into early stage mouse embryos. Right: Southern blots of genomic DNA from WT mice, heterozygous ADAM17^{WT/ex}, and homozygous ADAM17^{ex/ex} mice after breeding the mice with FLP recombinase transgenic mice leading to in vivo excision of the neomycin resistance cassette. The black line indicates that intervening lanes were spliced out. (B) ADAM17 Western blots of membrane fractions of brain, liver, and spleen tissues. The asterisk denotes an unspecific band.

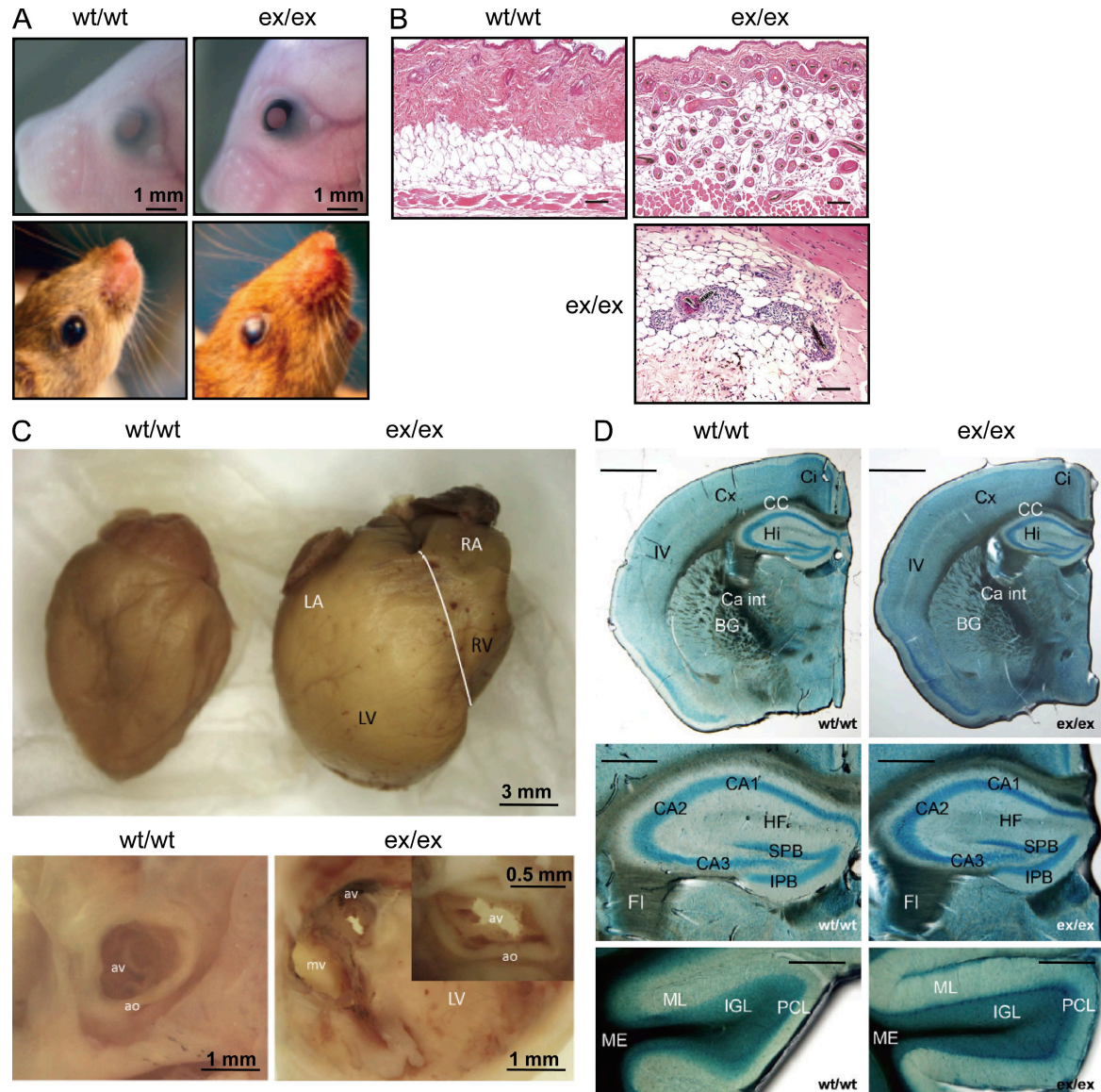


Figure S2. Histological and functional characterization of ADAM17^{ex/ex} mice. (A) ADAM17^{ex/ex} embryos at day 17 showed open eyes as compared with their WT littermates. Adult mice had opaque eyes. Bars, 1 mm. (B) H&E-stained sections of the mid-dorsal skin showing disorganized arrangement of partly waved hair follicles and secondary inflammatory changes in ADAM17^{ex/ex} mice (top right) as compared with WT mice (left). Bottom right: destruction of hair follicles. A (peri)follicular mixed inflammatory infiltrate with variable numbers of multinucleated giant cells. Magnification: (top) 8x; (bottom) 10x. Bars, 100 μm. Representative macroscopic (A) and microscopic (B) pictures of eight mice per group are shown. (C) Hearts of age-matched WT (left) and ADAM17^{ex/ex} (right) mice. Note the massive left-ventricular enlargement, pushing the right ventricle toward the upper right quadrant. The position of the anterior interventricular sulcus is indicated by a white line. Aortic valve of age-matched WT (bottom left) and ADAM17^{ex/ex} (bottom right) hearts. Aortic valve (av) and mitral valve (mv) are indicated. Note that the mitral cusps in ADAM17^{ex/ex} mice were rather short compared with the ventricle volume. When seen from the atrial side (bottom right, inset), the mitral cusps appeared macroscopically intact and spanned the entire area of the atrioventricular opening without obvious valve insufficiency. Representative microscopic pictures of four analyzed mice per group are shown. Bars, 3 mm. LA, left atrium; RA, right atrium; LV, left ventricle; RV, right ventricle. (D) No abnormalities in the brain of ADAM17^{ex/ex} mice. The overall architecture of cerebral hemispheres was indistinguishable between both genotypes. For instance, lamina IV of the parietal isocortex (Cx) was clearly discernible. Likewise, no major abnormality was found in basal ganglia (BG), internal capsule (Ca Int), and hippocampus (Hi). Likewise, the basic cortical layering of both hippocampal and cerebellar cortex are indistinguishable from controls. Representative microscopic pictures of four analyzed mice per group are shown. Bars: (top) 1 mm; (middle and bottom) 500 μm. CC, corpus callosum; IV, lamina IV of neocortex; Ci, cingulum; SPB, suprapyramidal blade (of dentate gyrus); IPB, infra-pyramidal blade (of dentate gyrus); FI, fimbria hippocampi; HF, hippocampal fissure; ME, medulla of cerebellum; ML, molecular layer of cerebellum; IGL, internal granular layer of cerebellum; PCL, Purkinje cell layer.

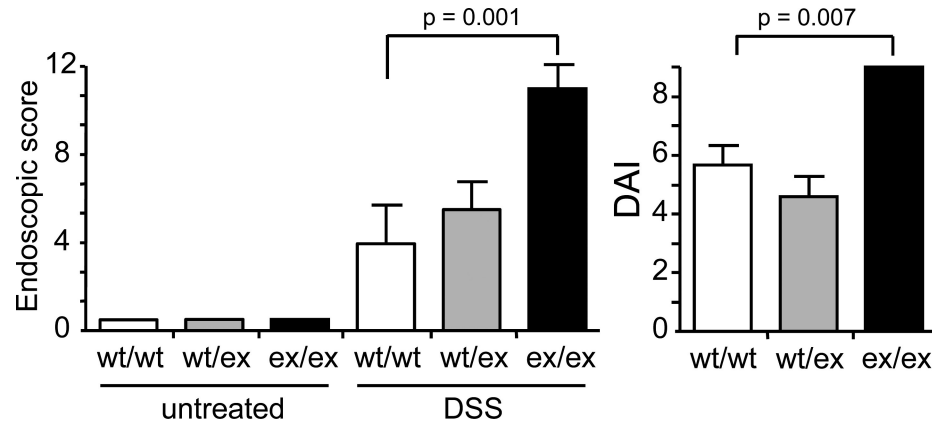


Figure S3. Endoscopic score and disease activity of mice after DSS treatment. Endoscopic score (MEICS; left) and disease activity index (DAI; right) of WT, Adam17^{WT/ex}, and Adam17^{ex/ex} mice after DSS treatment. Mean values \pm SD from three untreated Adam17^{ex/ex} and control mice, as well as five DSS-treated Adam17^{ex/ex} and control mice per group, are shown.

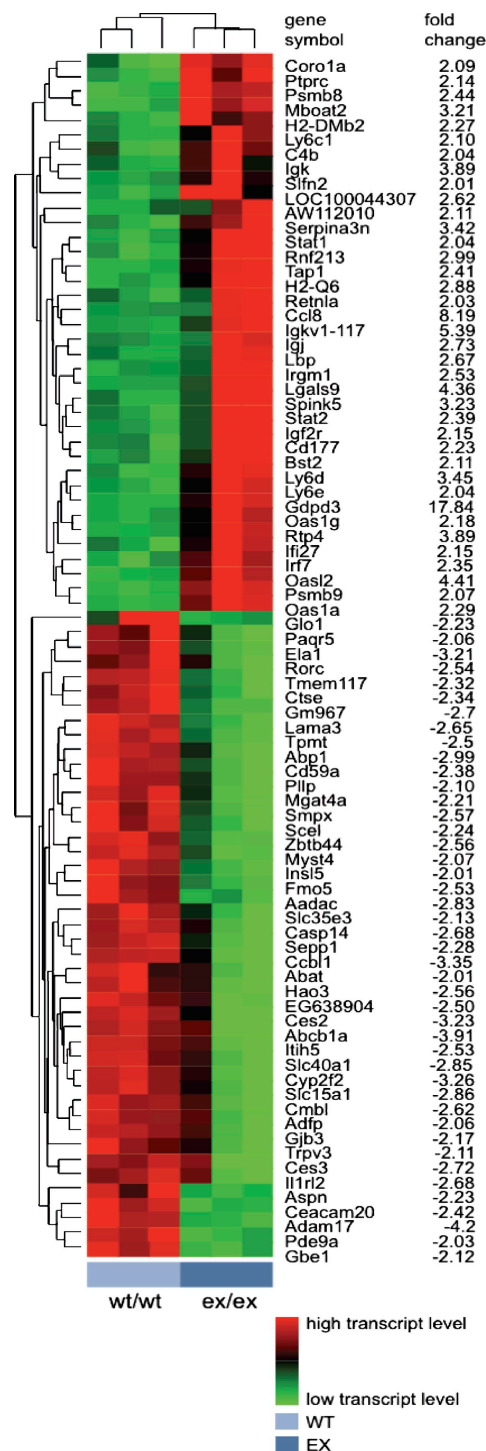


Figure S4. Gene expression analysis in WT and ADAM17^{ex/ex} mice. Hierarchical cluster of regulated genes showing transcripts regulated in the intestine between WT ($n = 3$) and ADAM17^{ex/ex} ($n = 3$) mice. Red represents high transcript level, and green represents low transcript levels. The hierarchical cluster was generated based on regulated genes using the correlation as the similarity measure and UPGMA (unweighted mean) as the clustering method. The fold change was calculated based on the ratios of the two medians (WT vs. ADAM17^{ex/ex} mice). Criteria for inclusion in the cluster for each transcript were: regulation fold change $>+2$ or <-2 , rank sum difference ≥ 2 , transcript is associated to a characterized gene.

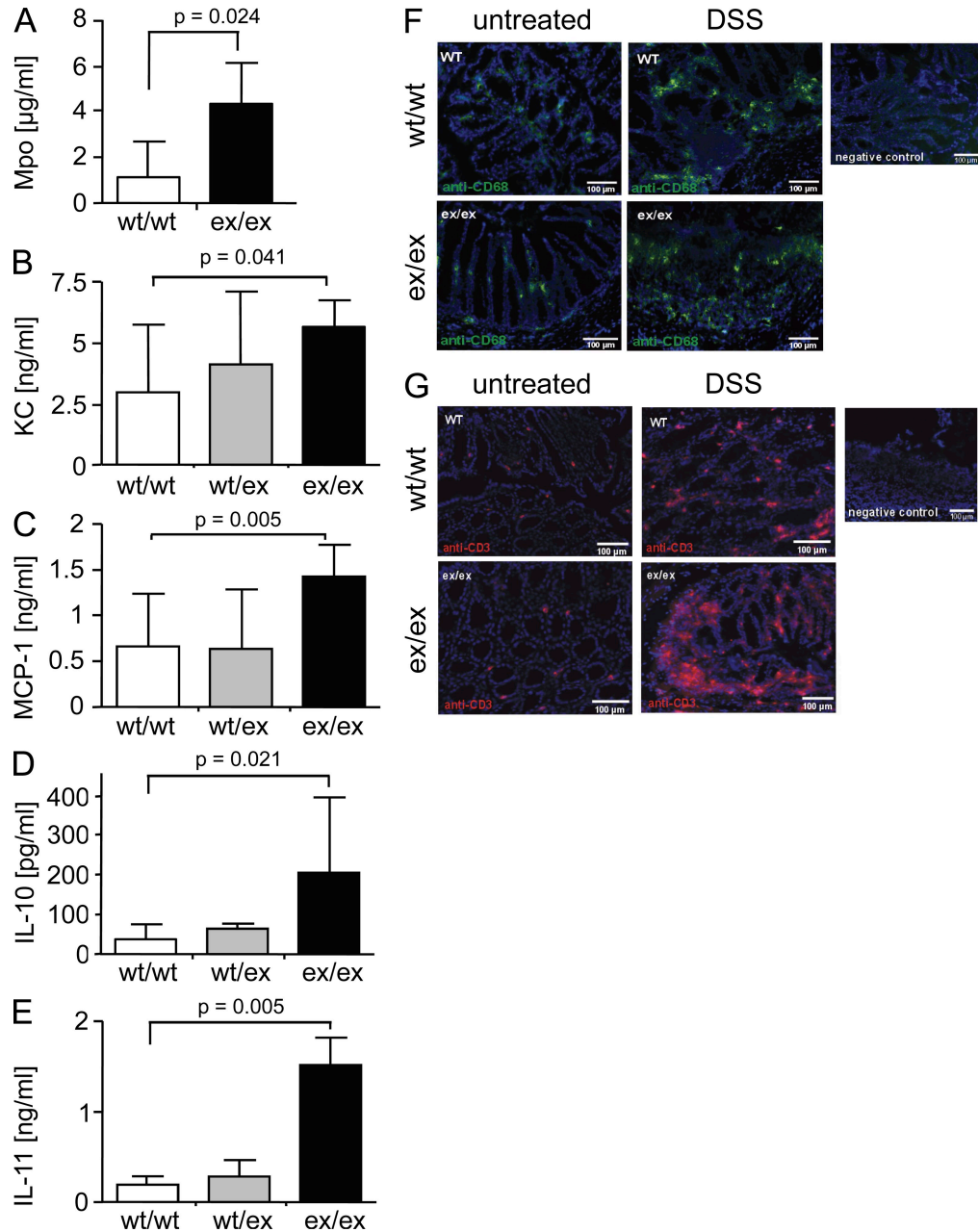


Figure S5. Increased intestinal inflammation in DSS-treated ADAM17^{ex/ex} mice. (A) Myeloperoxidase (MPO) levels in the colon of ADAM17^{ex/ex} mice ($n = 5$) were increased after DSS-treatment as compared with homozygous ADAM17^{WT/WT} mice ($n = 4$). (B–E) Supernatants from organ cultures were assayed by ELISA for levels of the chemokines KC (day 10; WT, $n = 10$; ADAM17^{WT/ex}, $n = 5$; ADAM17^{ex/ex}, $n = 6$; B) and MCP-1 (day 10; WT, $n = 10$; ADAM17^{WT/ex}, $n = 5$; ADAM17^{ex/ex}, $n = 6$; C) and of the cytokines IL-10 (day 10; WT, $n = 10$; ADAM17^{WT/ex}, $n = 5$; ADAM17^{ex/ex}, $n = 6$; D) and IL-11 (day 5; WT, $n = 4$; ADAM17^{WT/ex}, $n = 4$; ADAM17^{ex/ex}, $n = 3$; E). Data are shown as mean values \pm SD from two independent experiments. (F) Colonic tissue sections obtained from WT and ADAM17^{ex/ex} mice were stained with an anti-CD68 mAb (magnification, 400 \times). Bars, 100 μm . The experiment was performed three times with three or more mice per group. (G) Colonic tissue sections obtained from WT and ADAM17^{ex/ex} mice were stained with an anti-CD3 mAb (magnification, 400 \times). Representative microscopic pictures of untreated WT and ADAM17^{ex/ex} mice (three mice per group), as well as DSS-treated WT and ADAM17^{ex/ex} mice (five mice per group), are shown. Bars, 100 μm . The experiment was performed three times with three or more mice per group.

Table S1. Offspring from heterozygous matings of ADAM17^{WT/ex} mice

Breeding	Offspring			
	Total	WT/WT	WT/ex	ex/ex
(WT/ex) × (WT/ex)	107	44 (37.6%)	49 (41.9%)	14 (11.9%)
(WT/ex) × (ex/ex)	104	-	75 (72.1%)	29 (27.9%)