Critical role of the disintegrin metalloprotease ADAM17 for intestinal inflammation and regeneration in mice

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Abbreviations used: ADAM, a disintegrin and metalloprotease; AR, amphiregulin; DSS, dextran sulfate sodium; EGF-R, epidermal growth factor receptor; EXITS, exon-induced translational stop.

Many membrane proteins are cleaved at the plasma membrane to release soluble ectodomains, which may exert different biological activities (Murphy, 2008). Membrane-bound growth factors and cytokines become systemically available upon shedding. Soluble receptors for growth factors and cytokines become antagonists of the cognate cytokines, as is the case for IL-1 and TNF (Müllberg et al., 2000). Alternatively, soluble receptors can be agonistic, i.e., together with their respective ligands they stimulate cells, which are otherwise unresponsive to the cytokine, as demonstrated for IL-6 trans-signaling (Rose-John et al., 2006). A disintegrin and metalloprotease (ADAM) 17 is an important sheddase involved in the proteolysis of membrane proteins such as TNF, IL-6R, L-selectin, and ligands of the epidermal growth factor receptor (EGF-R; Peschon et al., 1998). ADAM17-deficient mice are not viable. Interestingly, these mice were reminiscent of mice lacking TGF-α (Peschon et al., 1998). Recently, conditional ADAM17 knockout animals have been generated (Horiiuchi et al., 2007, 2009). Although these studies confirmed that the protease a disintegrin and metalloprotease (ADAM) 17 cleaves tumor necrosis factor (TNF), L-selectin, and epidermal growth factor receptor (EGF-R) ligands from the plasma membrane. ADAM17 is expressed in most tissues and is up-regulated during inflammation and cancer. ADAM17-deficient mice are not viable. Conditional ADAM17 knockout models demonstrated proinflammatory activities of ADAM17 in septic shock via shedding of TNF.

We used a novel gene targeting strategy to generate mice with dramatically reduced ADAM17 levels in all tissues. The resulting mice called ADAM17ex/ex were viable, showed compromised shedding of ADAM17 substrates from the cell surface, and developed eye, heart, and skin defects as a consequence of impaired EGF-R signaling caused by failure of shedding of EGF-R ligands. Unexpectedly, although the intestine of unchallenged homozygous ADAM17ex/ex mice was normal, ADAM17ex/ex mice showed substantially increased susceptibility to inflammation in dextran sulfate sodium colitis. This was a result of impaired shedding of EGF-R ligands resulting in failure to phosphorylate STAT3 via the EGF-R and, consequently, in defective regeneration of epithelial cells and breakdown of the intestinal barrier. Besides regulating the systemic availability of the proinflammatory cytokine TNF, our results demonstrate that ADAM17 is needed for vital regenerative activities during the immune response. Thus, our mouse model will help investigate ADAM17 as a potential drug target.

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ADAM17 is the major endotoxin-stimulated TNF sheddase in myeloid cells in vivo and that ADAM17 is involved in the control of physiological bone remodeling, they did not clarify the role of ADAM17 in complex settings such as inflammation and cancer. Studies of ADAM17 have been complicated by the fact that it is not clear whether the protease and its substrates need to be expressed on the same cell or whether shedding in trans (i.e., the protease is expressed on one cell, the substrate on a different cell) is possible (Janes et al., 2005). Therefore, the choice of cre-transgenic mice for tissue-specific deletion of the ADAM17 gene in conditional mice is ambiguous.

In this paper, we developed a novel strategy to generate mice with barely detectable levels of ADAM17 in all tissues. The strategy is based on the generation of a new exon within the ADAM17 gene, which starts with an in-frame translational stop codon and which was flanked by splice donor/acceptor sites, which slightly deviated from the canonical consensus sequence. This strategy has been named exon-induced translational stop (EXITS). Homozygous mice used the new exon for ~95% of the ADAM17 mRNAs, resulting in a dramatic loss of ADAM17 protein in all cell types. Nevertheless, homozygous ADAM17<sup>ex/ex</sup> mice were viable and developed eye, hair, and skin defects reminiscent of mice lacking TGF-α. Although the intestine of the homozygous ADAM17<sup>ex/ex</sup> mice showed no overt abnormalities, the animals displayed dramatically increased susceptibility to intestinal inflammation induced by dextran sulfate sodium (DSS) as a consequence of impaired EGF-R–dependent regeneration caused by failure of shedding of EGF-R ligands. Results show that during inflammation, ADAM17 is not only involved in shedding the proinflammatory cytokine TNF but also in the regulation of regenerative responses. Thus, our mouse model will help investigate ADAM17 as a potential drug target in TNF- and/or EGF-R–dependent pathologies in inflammation and cancer.

**RESULTS AND DISCUSSION**

To construct a targeting vector for the ADAM17 gene, we inserted a loxP sequence followed by a noncanonical donor splice site downstream of a cryptic acceptor splice site within intron11. This manipulation generated a new exon between exons 11 and 12, which started with an in-frame translational stop codon (Fig. 1 A). Importantly, mice homozygous for the ADAM17 ex allele (Fig. 1 A; Fig. S1 A) were viable. The usage of the new exon between exons 11 and 12 of the mouse ADAM17 gene was tested by RT-PCR, as outlined in Fig. 1 B. Whereas in WT animals only a band of 380 bp was detected, heterozygous ADAM17<sup>WT/ex</sup> mice showed an additional band of 550 bp corresponding to the insertion of the new exon.
sequence. In homozygous ADAM17<sup>ex/ex</sup> mice, ~95% of the ADAM17 mRNAs contained the new exon. Consequently, almost no ADAM17 protein was detected in all tissues analyzed (Fig. 1 C; Fig. S1 B). These results indicated that we had generated viable mice with barely detectable ADAM17 protein levels in all tissues. ADAM17<sup>ex/ex</sup> mice were born at a frequency clearly below the expected Mendelian distribution pattern (Table S1). Homozygous ADAM17<sup>ex/ex</sup> mice showed abnormalities of the eyes, skin, and heart, but not in the brain, body segmentation, or vascular development, indicating that ADAM17 is not crucial for developmental Notch or Notch ligand function in vivo (Fig. S2).

Generation of soluble L-selectin and TNF was completely abrogated in ADAM17<sup>ex/ex</sup> mice and levels of sTNF-RII were significantly reduced, indicating that shedding of ADAM17 substrates was severely impaired (Fig. 1, D–F). ADAM17 has been implicated in the cleavage of membrane-bound ligands of the EGF-R (Sternlicht et al., 2005). Soluble, but not membrane-bound, amphiregulin (AR) has been shown to be sufficient and necessary for the formation of milk ducts in developing female animals (Sternlicht et al., 2005). As shown in Fig. 1 G, in contrast to WT mice, milk duct development was reduced in ADAM17<sup>ex/ex</sup> mice at 12 wk (Fig. 1 G, right), indicating that AR-induced milk duct formation required ADAM17-mediated cleavage of this EGF-R ligand.

Blockade of TNF has been shown to be beneficial for the treatment of patients with Crohn's disease (van Dullemen et al., 1995). Therefore, it was of interest to examine such a condition in ADAM17<sup>ex/ex</sup> animals. When WT and heterozygous ADAM17<sup>WT/ex</sup> mice were treated with a mild regimen (2%) of DSS, they showed mild weight loss (Fig. 2 A) and minimal signs of inflammation upon endoscopy (Fig. 2 B). In contrast, homozygous ADAM17<sup>ex/ex</sup> mice exhibited severe weight loss and five of eight mice died. Colonoscopy revealed severe inflammation and ulcerations in these animals (Fig. 2 B) and an increase in endoscopic index of colitis severity (Fig. S3). ADAM17 protein was expressed in the crypts of the intestine of WT mice and was up-regulated upon DSS challenge but could not be detected in ADAM17<sup>ex/ex</sup> animals (Fig. 2 C). In ADAM17<sup>ex/ex</sup> animals, the crypt structure was completely lost.

**Figure 2.** ADAM17<sup>ex/ex</sup> mice are highly susceptible to DSS–induced colitis. (A) Body weight after the onset of treatment with DSS. Numbers of mice per group used are indicated. The experiment was performed three times. (B) Colonoscopy of untreated (top) and treated (bottom) mice. Representative pictures are shown. (C) DSS–treated (4 d) and untreated WT and ADAM17<sup>WT/ex</sup> mice were immunostained with anti–ADAM17 (magnification, 100×). Bars, 100 µm. Representative microscopic pictures of four mice per group are shown. (D) Hematoxylin and eosin (H&E), BrdU, anti–phospho-STAT3, and anti-cyclin D1 staining of colons from mice challenged for 10 d with DSS. Bars: (H&E) 200 µM; (BrdU, p-STAT3, and cyclin D1) 100 µm. Representative microscopic images of three experiments (H&E and BrdU), two experiments (pSTAT3), and one experiment (cyclin D1) of ADAM17<sup>WT/WT</sup>, ADAM17<sup>WT/ex</sup>, and WT controls (five mice per group) are shown. (E) DSS colitis was induced and plasma FITC–dextran concentrations in ADAM17<sup>WT/WT</sup> (n = 7) and ADAM17<sup>ex/ex</sup> (n = 7) mice 4 h after FITC–dextran administration by oral gavage (60 mg/100 g of body weight) are shown. Data are shown as mean values ± SD. The experiment was performed twice with similar results.
proliferation in colon tissue sections was detected (Fig. 3 B). Gene expression analysis in the intestine of unchallenged mice revealed significant up-regulation of genes involved in immunity and defense as well as of genes playing a role in interferon-mediated immunity (Fig. 3 C and Fig. S4). These findings indicated that loss of ADAM17 activity, although it did not result in an overt inflammatory phenotype, led to a significant inflammatory gene expression signature, possibly resulting in sensitization toward inflammatory stimuli.

In DSS-treated animals we detected a strong increase in myeloperoxidase activity in ADAM17<sup>ex/ex</sup> animals (Fig. S5 A), which is considered a marker for activated neutrophils (Breckwoldt et al., 2008). In colon organ cultures from DSS-treated animals the inflammatory chemokines KC and MCP-1 were elevated in ADAM17<sup>ex/ex</sup> mice. Interestingly, the anti-inflammatory cytokine IL-10 and the IL-6–related cytokine IL-11 were also up-regulated in these animals (Fig. S5, B–E). Such an up-regulation of IL-10 and IL-11 was also seen in DSS-challenged mice with a deletion of STAT3 in intestinal epithelial cells (Bollrath et al., 2009). Consequently, an increase of CD68-positive mononuclear cells and CD3-positive T cells in intestinal tissue sections of DSS-treated ADAM17<sup>ex/ex</sup> animals was detected (Fig. S5, F and G). The cytokines TNF, IL-21, IL-12, and IL-17 were not significantly up-regulated (unpublished data).

DSS is toxic to gut epithelial cells of the basal crypts and affects the integrity of the mucosal barrier (Wirtz et al., 2007). Colon sections of WT and ADAM17<sup>WT/ex</sup> mice exhibited proliferating nuclei throughout the crypts of the gut (Fig. 2 D). No such staining was seen in ADAM17<sup>ex/ex</sup> mice. In WT and ADAM17<sup>WT/ex</sup> mice, but not in ADAM17<sup>ex/ex</sup> mice, phosphorylated STAT3 and cyclin D1 were readily detectable (Fig. 2 D). These results indicated that in ADAM17<sup>ex/ex</sup> mice the proliferative response of intestinal epithelial cells to DSS was blocked, presumably as a result of the inability to activate EGF-R–mediated STAT3 phosphorylation (Itoh et al., 2006). Importantly, upon DSS challenge, the intestinal barrier became highly permeable for FITC–dextran in ADAM17<sup>ex/ex</sup> mice (Fig. 2 E).

Surprisingly, in unchallenged ADAM17<sup>ex/ex</sup> mice the colon mucosa was regularly arranged (Fig. 3 A) and unaltered proliferation in colon tissue sections was detected (Fig. 3 B). Gene expression analysis in the intestine of unchallenged mice revealed significant up-regulation of genes involved in immunity and defense as well as of genes playing a role in interferon–mediated immunity (Fig. 3 C and Fig. S4). These findings indicated that loss of ADAM17 activity, although it did not result in an overt inflammatory phenotype, led to a significant inflammatory gene expression signature, possibly resulting in sensitization toward inflammatory stimuli.

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The inflammatory response seen in homozygous ADAM17ex/ex mice was not the result of an intrinsic activation of the immune system in these animals, as demonstrated in a T cell transfer colitis model where T cells from WT or homozygous ADAM17ex/ex mice were transferred into rat−/− animals (Powrie et al., 1994; Atreya et al., 2000). Importantly, no significant difference in colitis development was detected when comparing animals transplanted with WT or ADAM17ex/ex T cells (unpublished data).

We hypothesized that the failure of intestinal epithelial cells to proliferate was caused by the fact that ADAM17ex/ex mice could not cleave and thereby systemically activate ligands of the EGF-R (Berasain et al., 2009). As shown in Fig. 4 A, after DSS challenge, higher levels of cell surface–expressed TGF-α were detected in ADAM17ex/ex mice as compared with WT animals. We therefore asked whether injection of recombinant TGF-α would restore proliferation of intestinal epithelial cells. As shown in Fig. 4 B, treatment of ADAM17ex/ex mice with this EGF-R ligand induced intestinal epithelial cell proliferation to a degree, which clearly surpassed the proliferation seen in WT animals. Concomitantly, phosphorylation of STAT3 was induced in TGF-α–treated mice. Interestingly, WT and ADAM17ex/ex mice upon daily injection of EGF-R ligand lost less weight than PBS-injected animals (Fig. 4 C). These results demonstrated that in ADAM17ex/ex mice signaling downstream of the EGF-R, was intact and that ectopic stimulation of this pathway compensated for the loss of proliferation caused by the deficiency of ADAM17.

Although the cre/loxP technology (Kühn et al., 1995) has been helpful in the analysis of gene defects in animals when total deletion of the gene of interest was lethal, there are limitations to this method. In cases when the expression profile and the activity of the gene of interest is complex or when it is not known which cell type is responsible for the synthesis and secretion of a soluble protein, it can be difficult to choose the appropriate transgenic cre mouse. In the case of ADAM17, it is not clear whether the protease exclusively cleaves its targets in cis or whether shedding in trans is possible (Janes et al., 2005). Furthermore, many functions of ADAM17 remained unknown as a result of embryonic lethality caused by ADAM17 gene deletion (Peschon et al., 1998). The phenotypes described in this paper are based on the development of a novel knockin targeting strategy, leading to an almost complete absence of functional ADAM17 protein as the result of a premature translational stop codon encoded by the artificially introduced exon. Our approach can be adapted to other genes as we showed in the case of the human CNTF gene (unpublished data) and will therefore be generally applicable.

Our experiments showed that for all substrates tested, shedding in ADAM17ex/ex animals was severely compromised. Physiological proliferation in the intestine of ADAM17ex/ex mice was normal, indicating that ADAM17 activation was not needed. In contrast, formation of milk ducts in 12-wk-old mice was abrogated as a result of the lack of AR processing. During the regenerative response i.e., after DSS treatment, the STAT3-mediated proliferative response of the intestinal epithelial cells was completely absent in ADAM17ex/ex mice. This finding is consistent with a study showing that mice lacking TGF-α have an increased susceptibility to DSS–induced colitis (Egger et al., 1997) and that TGF-α–overexpressing mice were less susceptible to DSS–induced colitis (Egger et al., 1998). The EGF-R can...
be stimulated by its cognate ligands in a paracrine and in a systemic fashion (Rodland et al., 2008). Thus, ADAM17ex/ex mice will be a good model to study the differences between EGF-R signaling via membrane-bound and soluble ligands.

The activation mechanisms of ADAM17 are not fully understood. In contrast to matrix metalloproteases, which are activated by the removal of the prodomain, ADAM17 additionally needs to be activated by intra- or extracellular signaling (Murphy, 2008). Recently, we showed that induction of apoptosis in neutrophils led to selective activation of ADAM17 and subsequent shedding of the IL-6R (Chalaris et al., 2007) and that this mechanism might represent a gauge to measure the extent of damage encountered by neutrophilic cells. In view of the pleiotropic activities governed by ADAM17, it will be interesting to study how the activity of this protease is regulated under stress conditions.

The intestine of unchallenged ADAM17ex/ex mice appeared normal but failed to respond adequately to the tissue damage by DSS, although we detected an up-regulation of inflammatory genes. These results show that ADAM17 plays a role in the coordination of reactions toward stress caused by wounding. The intestinal epithelium acts as an intrinsic barrier against microbial invaders. Our data show that lack of regeneration and failure to maintain the barrier function of the intestine act dominantly over the reduced activity of the immune system as a result of impaired coordination of reactions toward stress caused by wounding. The activation mechanisms of ADAM17 are not fully understood.

In summary, we presented the novel EXITS method for gene targeting, which will be applicable to other genes. Using this technique, viable mice with greatly diminished ADAM17 activity in all tissues were generated. Models of inflammatory bowel disease showed that these mice were highly susceptible to intestinal damage by DSS and point to a function of ADAM17 in the coordination of pro- and antiinflammatory activities in response to body stress and damage.

MATERIALS AND METHODS
Reagents and antibodies. Reagents were purchased from Carl Roth unless otherwise specified. Restriction enzymes, molecular weight markers, Taq-polymerase, and reverse transcription were obtained from Fermentas. Cell culture media, trypsin, FCS, L-glutamine, and penicillin/streptomycin were obtained from PAA Laboratories. All other reagents were obtained from the following sources: TGF-α (PeproTech); LPS, FITC-dextran, and myeloperoxidase (Sigma-Aldrich); PMA (EMD); anti-CD3, anti-CD28, anti-CD4-FITC, anti-CD25-FITC, and anti-L-selectin-PE (BD); antiphospho-STAT3 and anti-γ-cyclin-D1 (Cell Signaling Technology); and horse-radish peroxidase-coupled anti-goat antibody (Thermo Fisher Scientific). The anti-ADAM10 and anti-ADAM7 antisera were a gift from S. Weber (Institute of Biochemistry, Christian-Albrechts-University, Kiel, Germany).

Generation of ADAM17<sup>ex/ex</sup> knockin mice. We created hypomorphic and conditional knockout mouse models of ADAM17. The ADAM17 gene is composed of 18 exons. We designed the targeting vector in a way that exon 11, which encodes the catalytic zinc-binding domain, is flanked by two loxP sites and the FTR-flanked neo cassette. A cryptic acceptor splice site within the intron 11 of ADAM17 was predicted using the NetGene2 program. Hypomorphic expression of ADAM17 was achieved by inserting a loxP site together with a cryptic noncanonical donor splice site (GAG-GTAATT instead of the canonical sequence A/CAG-GTAAGT, where the hyphen denotes the exon-intron border) downstream of the cryptic acceptor splice site into intron 11 and thereby generating an additional, artificial, exon 11a (Fig. 1 A). Alternative splicing/use of the artificial exon 11a led to premature disruption of ADAM17 protein translation as a result of the in-frame stop codon TAG in exon 11a. If needed, deletion of exon 11 could be achieved by tissue-specific cre recombinase-mediated deletion of exon 11 of the ADAM7 gene. The ADAM17 targeting plasmid was assembled in pBR322 using standard cloning techniques. In brief, the 5′ADAM7 small homology was followed by the first loxP site, the FTR-neomycin-FTR cassette, partial intron 10, exon 11, and the first half of intron 12 (including the cryptic but canonical splice acceptor site), the second loxP site, an artificial noncanonical splice donor site, and the 3′ADAM7 large homology. Mouse ADAM17 is located on the minus strand of chromosome 12 from 21,329,378 to 21,379,454 bp (from VEGA annotation of National Center for Biotechnology Information Build 37). To simplify annotation of the ADAM17 sequences subcloned into our targeting plasmid, we defined the adenine base from the start codon ATG of the ADAM7 gene as position 1. The subcloned fragments were: 5′ADAM7 small homology from position 35,626 to 36,574, partial intron 10, exon 11, and the first half of intron 12 from position 36,375 to 37,976 and 3′ADAM7 large homology from position 37,977 to 43,640. The ADAM7 gene fragments were subcloned into pBR322 from a genomic lambda clone. The resulting plasmid was named pBR322-ADAM7-targeting. 129Ev ES cells were transfected with the ADAM17 targeting vector to obtain clones with hypomorphic recombination. Targeted embryonic stem cells were injected into C57BL/6 blastocysts to produce chimeras, and germ-line-transmitted ADAM17<sup>ex/ex</sup> mice were obtained by mating chimeras with C57BL/6 mice (InGenious Targeting Laboratory). Because ADAM17<sup>exneo/exne</sup> mice were not viable, presumably because of the presence of the neomycin gene cassette, we deleted this cassette by crossing with ACTB:FLPe mice expressing FLP recombinase under the control of the β-actin promoter (Rodriguez et al., 2000). These mice were obtained from F. Stewart (BioInnovation Zentrum, Technical University Dresden, Dresden, Germany). Homozygous ADAM17<sup>ex/ex</sup> mice were viable but showed drastically reduced ADAM17 protein levels. To verify whether this strategy may be generally applicable, we introduced a DNA sequence containing the same noncanonical acceptor splice site and donor splice site into the single intron of the human CNTF gene. This approach led to the same result seen for the mouse ADAM7 gene. The introduced exon was only partly used in transfected HepG2 cells, leading to inefficient translation of the CNTF protein. We therefore concluded that the EXITS strategy should be applicable to other genes to generate hypomorphic phenotypes.

Southern blotting. 10 µg of genomic DNA was digested with HindIII, separated on 0.8% agarose gels, and transferred to a nylon membrane, which was hybridized at 65°C overnight with the α-32P–dATP-labeled probe as indicated in Fig. 1 A. The membrane was washed at 65°C and signals were detected by phosphoimaging.

RT-PCR. RNA was isolated from different organs and 2 µg was reverse transcribed into cDNA using Reverse Aid M-MuLV reverse transcription...
Flow cytometric analysis. 10^7 mouse splenocytes were stimulated with 100 nM PMA for 2 h, 1 µg/ml LPS for 24 h, or 5 µg/ml anti-CD3 and 1 µg/ml anti-CD28 for 24 h and subsequently washed twice with FACS buffer (PBS, 1% BSA, and 0.01% NaN₃). To block Fc receptors, the cell suspension was incubated with mouse Fc block CD16/32 mAb (BD) for 10 min and further stained with fluorescence-coupled antibodies against the B cell marker B220, T cell marker CD4, or L-selectin. Cells were washed with 500 µl PBS buffer and analyzed by FACS. The fold change was calculated based on the Student’s t test. Differences were considered to be statistically significant if P ≤ 0.05.

Online supplemental material. Fig. S1 shows Southern blots and Western blots of mice demonstrating homologous recombination of the ADAM17 allele and lack of ADAM17 protein expression in brain, liver, and spleen. Fig. S2 shows the open eye, skin, heart, and brain phenotype of ADAM17 alleles. Fig. S3 demonstrates the endoscopic score and disease activity of mice after DSS treatment. Fig. S4 depicts a gene expression analysis in the intestine of WT and ADAM17 alleles. Fig. S5 demonstrates increased intestinal inflammation in DSS-treated ADAM17 alleles. Table S1 demonstrates segregation of offspring from ADAM17 alleles. Online supplemental material is available at http://www.jem.org/cgi/content/full/jem.20092366/DC1.

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**Figure S1. Generation and analysis of ADAM17<sup>WT/ex</sup> 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<sup>WT/ex</sup>, and homozygous ADAM17<sup>ex/ex</sup> 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<sup>ex/ex</sup> mice. (A) ADAM17<sup>ex/ex</sup> 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<sup>ex/ex</sup> 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) 8×; (bottom) 10×. 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<sup>ex/ex</sup> (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<sup>ex/ex</sup> (bottom right) hearts. Aortic valve (av) and mitral valve (mv) are indicated. Note that the mitral cusps in ADAM17<sup>ex/ex</sup> mice were rather short compared with the ventricle volume. When seen from the atrial side (bottom right, inset), the mitral cusps appeared macroscopic intact and spanned the entire area of the atioventricular 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<sup>ex/ex</sup> 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, infrapyramidal blade (of dentate gyrus); Fl, 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<sup>WT/ex</sup>, and Adam17<sup>ex/ex</sup> mice after DSS treatment. Mean values ± SD from three untreated Adam17<sup>ex/ex</sup> and control mice, as well as five DSS-treated Adam17<sup>ex/ex</sup> and control mice per group, are shown.
Figure S4. Gene expression analysis in WT and ADAM17ex/ex mice. Hierarchical cluster of regulated genes showing transcripts regulated in the intestine between WT (n = 3) and ADAM17ex/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. ADAM17ex/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<sup>ex/ex</sup> mice. (A) Myeloperoxidase (MPO) levels in the colon of ADAM17<sup>ex/ex</sup> mice (n = 5) were increased after DSS-treatment as compared with homozygous ADAM17<sup>WT/WT</sup> 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<sup>WT/ex</sup>, n = 5; ADAM17<sup>ex/ex</sup>, n = 6; B) and MCP-1 (day 10; WT, n = 10; ADAM17<sup>WT/ex</sup>, n = 5; ADAM17<sup>ex/ex</sup>, n = 6; C) and of the cytokines IL-10 (day 10; WT, n = 10; ADAM17<sup>WT/ex</sup>, n = 5; ADAM17<sup>ex/ex</sup>, n = 6; D) and IL-11 (day 5; WT, n = 4; ADAM17<sup>WT/ex</sup>, n = 4; ADAM17<sup>ex/ex</sup>, n = 3; E). Data are shown as mean values ± SD from two independent experiments. (F) Colonic tissue sections obtained from WT and ADAM17<sup>ex/ex</sup> mice were stained with an anti–CD68 mAb (magnification, 400×). 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<sup>ex/ex</sup> mice were stained with an anti–CD3 mAb (magnification, 400×). Representative microscopic pictures of untreated WT and ADAM17<sup>ex/ex</sup> mice (three mice per group), as well as DSS-treated WT and ADAM17<sup>ex/ex</sup> 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<sup>WT/ex</sup> mice

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