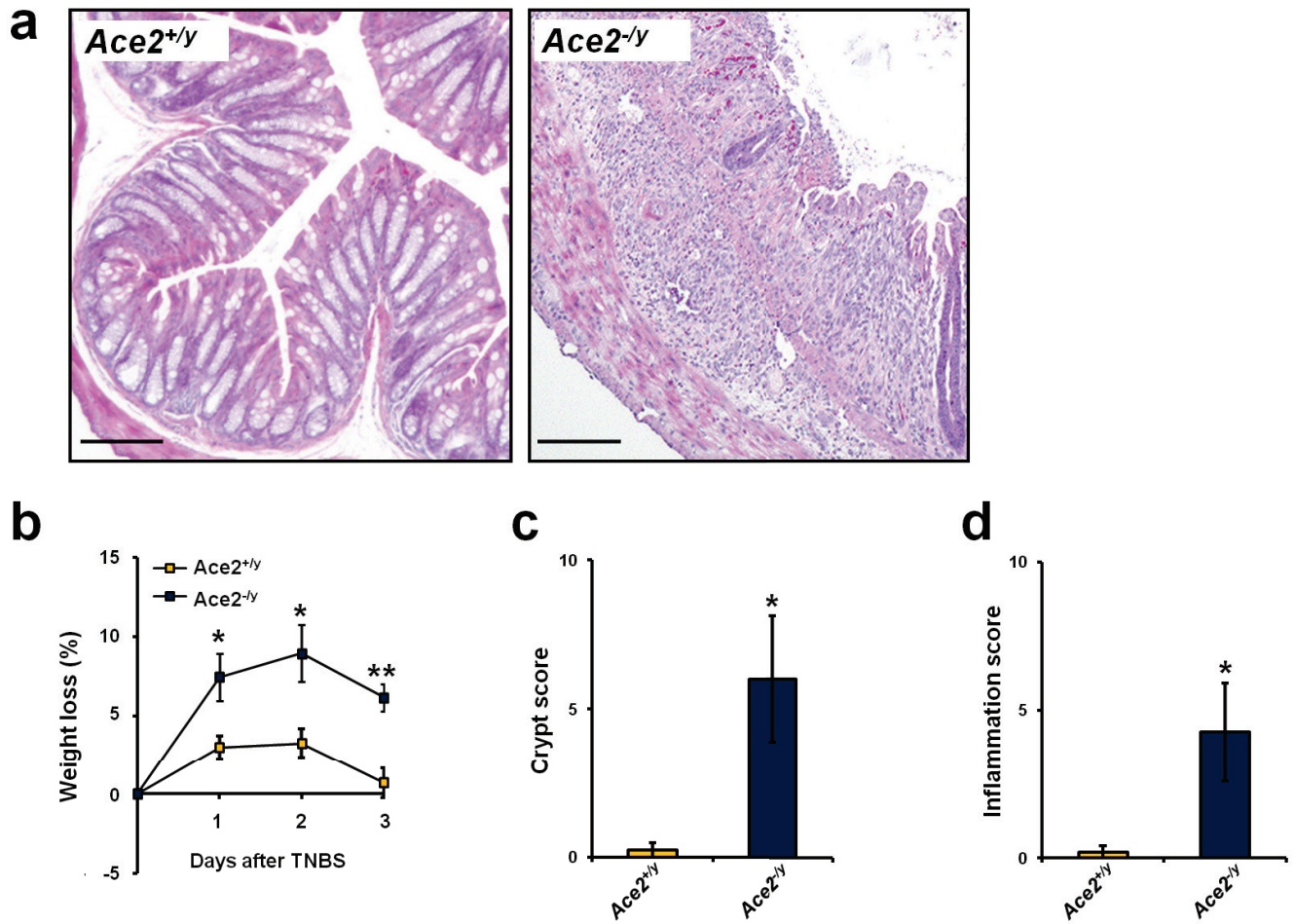


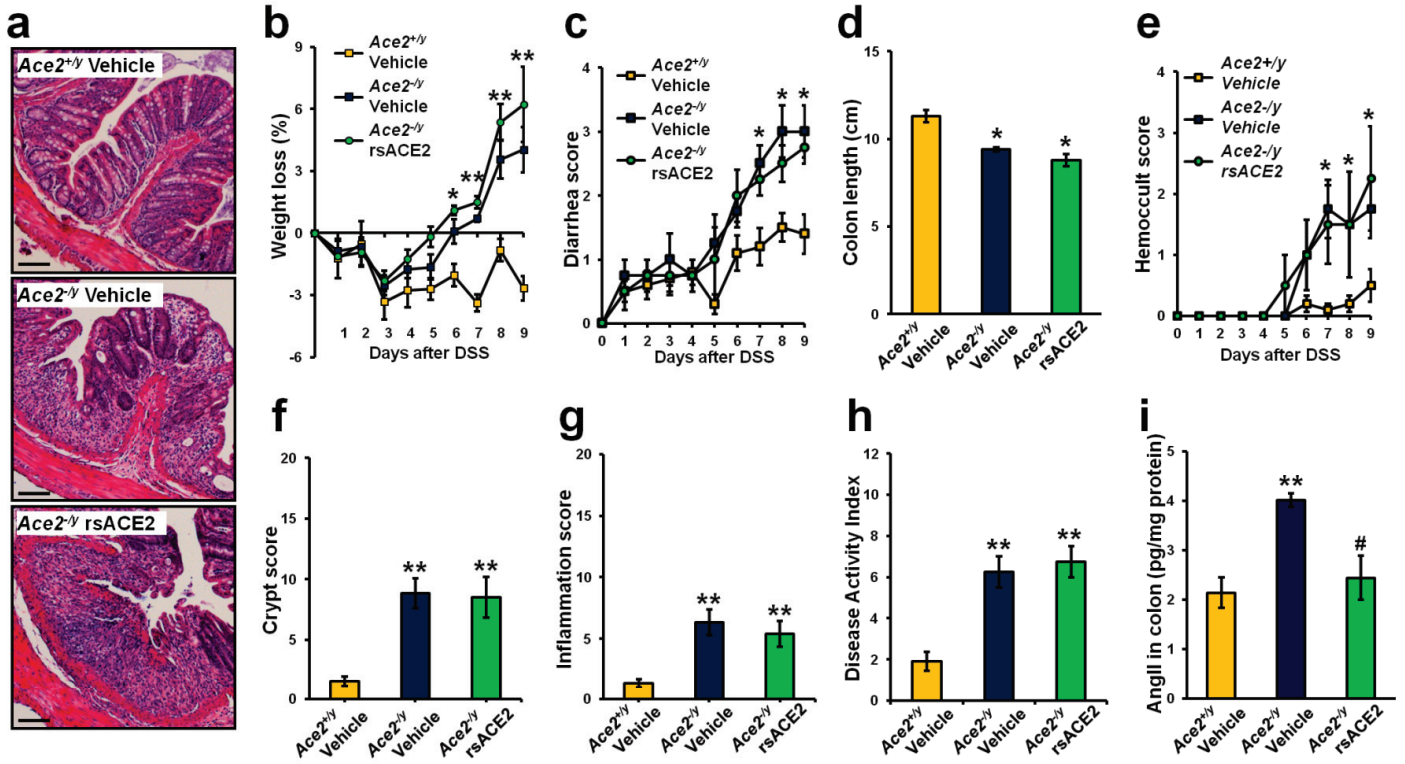
Supplementary Figure 1. ACE2 controls the severity of DSS induced colitis.

a, Colon length, **b**, hemocult scores indicative of intestinal bleeding, **c**, crypt scores, **d**, inflammation scores, and **e**, disease activity index, indicative of severity of colitis, in control and DSS treated littermate *Ace2*^{+y} and *Ace2*^{-y} mice. All values are mean ± SEM of 5-9 mice per group. **P*<0.05, ***P*<0.01 comparing DSS-treated *Ace2*^{+y} vs. *Ace2*^{-y} littermates (paired-t-test). Of note, although we present data from male mice (*Ace2*^{-y}, the *Ace2* gene being located on the X chromosome) throughout the study, we observed a similar phenotype in female *Ace2*^{-/-} mice.



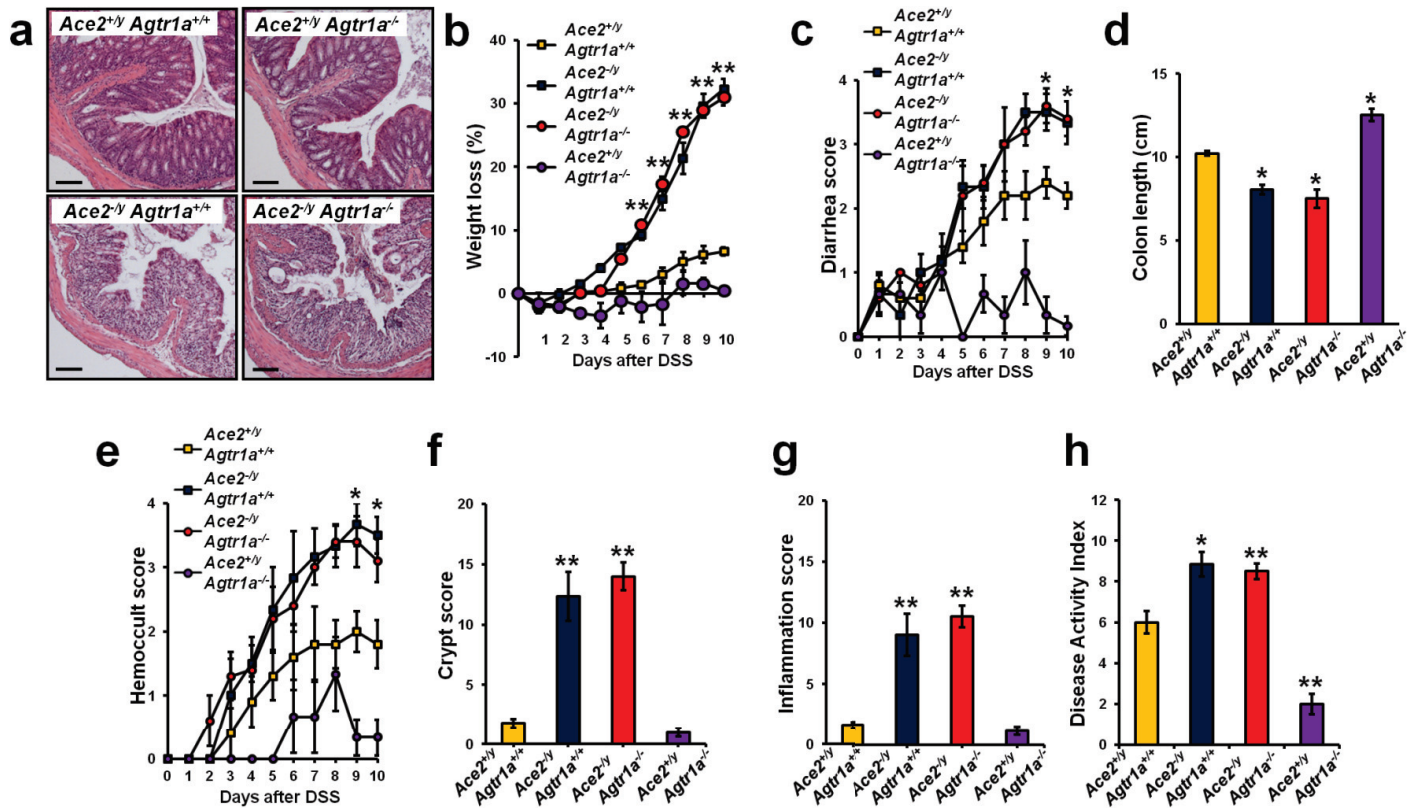
Supplementary Figure 2. ACE2 controls the severity of TNBS induced colitis.

a, Colon histopathology (H&E staining; scale bar, 100µm), **b**, percent weight loss, **c**, crypt scores, and **d**, inflammation scores in TNBS treated littermate *Ace2*^{+/y} and *Ace2*^{-/y} mice. All values are mean ± SEM of 5-9 mice per group. **P*<0.05, ***P*<0.01 comparing TNBS-treated *Ace2*^{+/y} vs. *Ace2*^{-/y} littermates (paired-t-test).



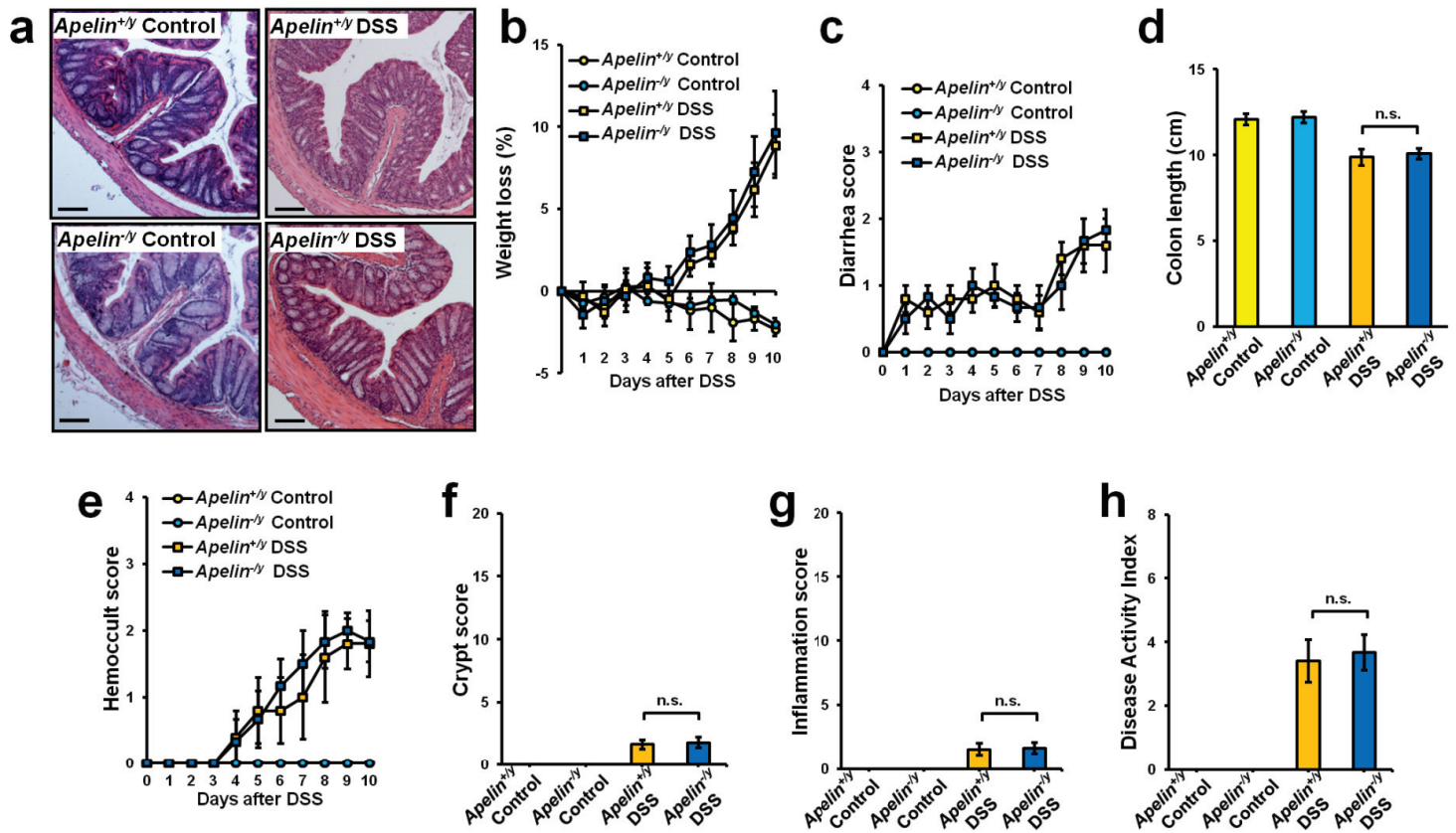
Supplementary Figure 3. Recombinant soluble ACE2 does not rescue DSS-induced colitis.

a, Colon histopathology (H&E staining; scale bar, 100 μm), **b**, percent weight loss, **c**, diarrhea scores, **d**, colon length, **e**, hemocult scores, **f**, crypt scores, **g**, inflammation scores, **h**, disease activity index, and **i**, colon angiotensin II (AngII) levels in DSS-challenged control and *Ace2*^{-y} littermates receiving vehicle or recombinant soluble ACE2 (rsACE2). Values are mean ± SEM of 5-10 mice per group. **P*<0.05, ***P*<0.01 comparing vehicle or rsACE2 treated *Ace2*^{-y} mice vs. *Ace2*^{+/y} mice; # *P*<0.05 comparing vehicle vs. rsACE2 treated *Ace2*^{-y} mice (paired-t-test).



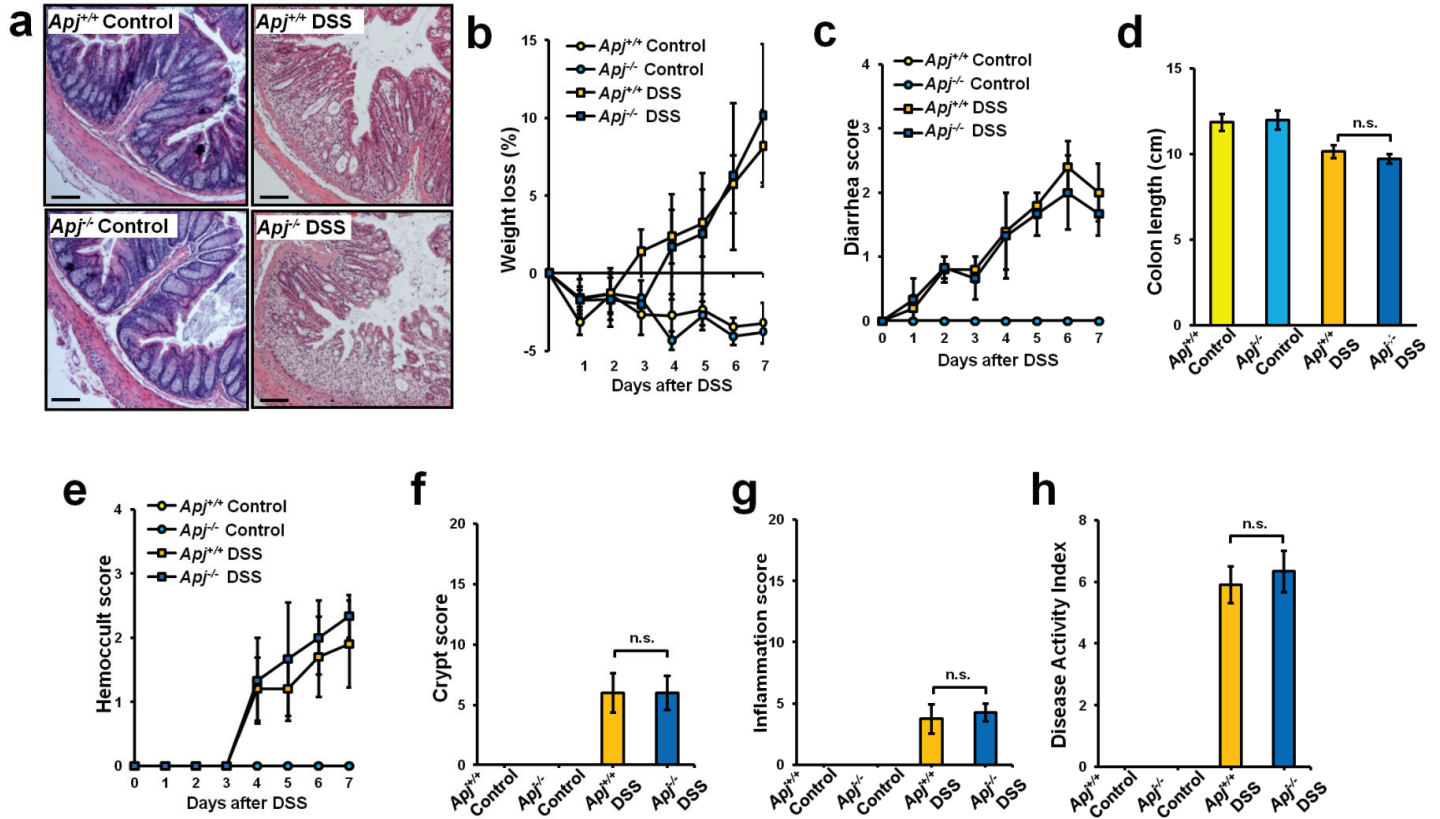
Supplementary Figure 4. AT1a receptor deficiency does not rescue the severe DSS-induced colitis phenotype of *Ace2^{-/-}* mice.

a, Colon histopathology (H&E staining; scale bar, 100 μm), **b**, percent weight loss, **c**, diarrhea scores, **d**, colon length, **e**, hemocult scores, **f**, crypt scores, **g**, inflammation scores, and **h**, disease activity index in DSS-challenged *Ace2^{+/-} Agtr1a^{+/+}*, *Ace2^{+/-} Agtr1a^{-/-}*, *Ace2^{+/-} Agtr1a^{+/+}*, and *Ace2^{+/-} Agtr1a^{-/-}* littermates. Note that *Atgr1a* deficiency improves colitis on an *Ace2* wild type background; the reason for this needs to be further explored. Values are mean ± SEM of 5-6 mice per group. * $P < 0.05$, ** $P < 0.01$ comparing *Ace2^{-/-} Agtr1a^{+/+}*, *Ace2^{-/-} Agtr1a^{-/-}*, or *Ace2^{+/-} Agtr1a^{-/-}* mice vs. *Ace2^{+/-} Agtr1a^{+/+}* mice (paired-t-test).



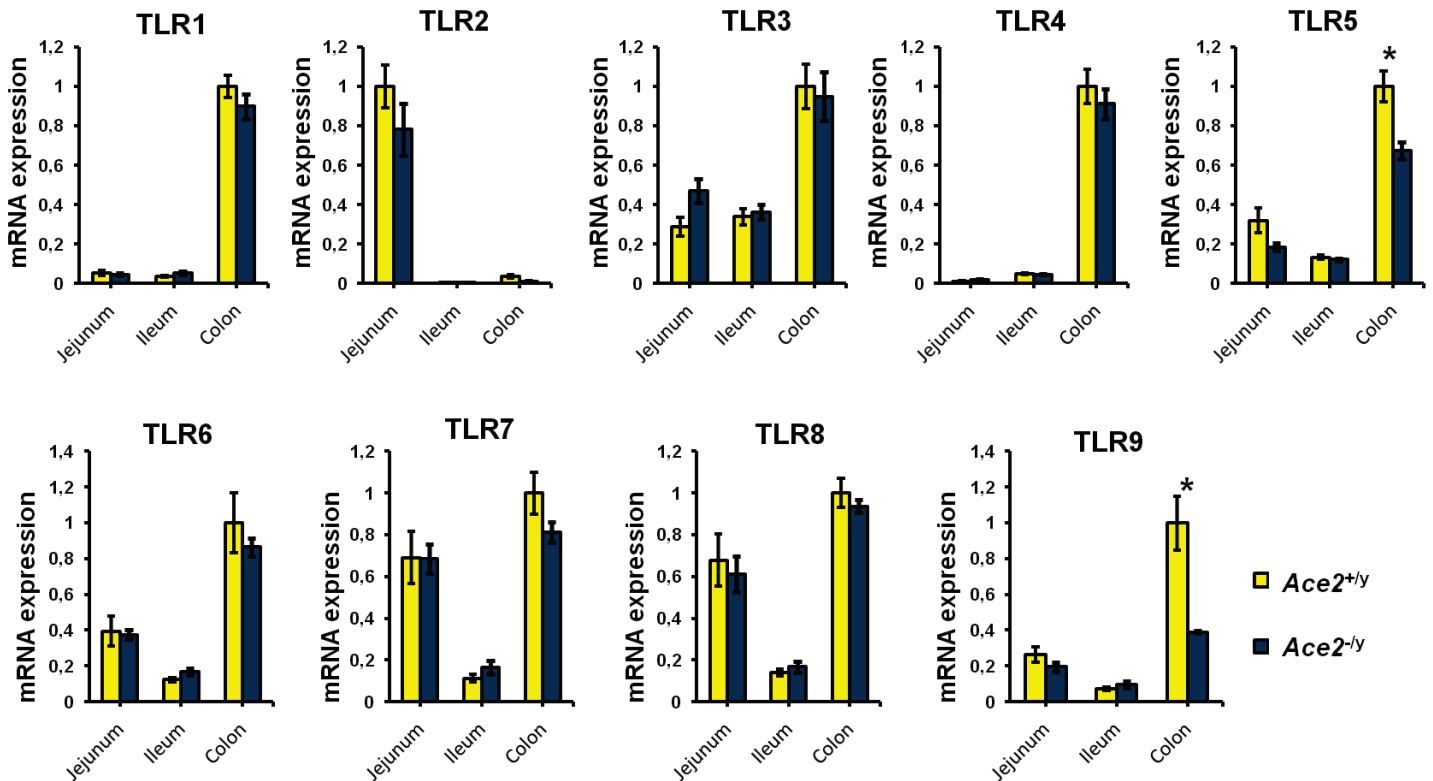
Supplementary Figure 5. DSS-induced colitis in *Apelin* mutant mice.

a, Colon histopathology (H&E staining; scale bar, 100µm), **b**, percent weight loss, **c**, diarrhea scores, **d**, colon length, **e**, hemocult scores, **f**, crypt scores, **g**, inflammation scores, and **h**, disease activity index in control and DSS-challenged *Apelin*^{+/-} and *Apelin*^{-/-} littermates. Note that, similar to *Ace2*, the *Apelin* gene is located on the X chromosome. Values are mean ± SEM of 6 mice per group. n.s., not significant (paired-t-test).



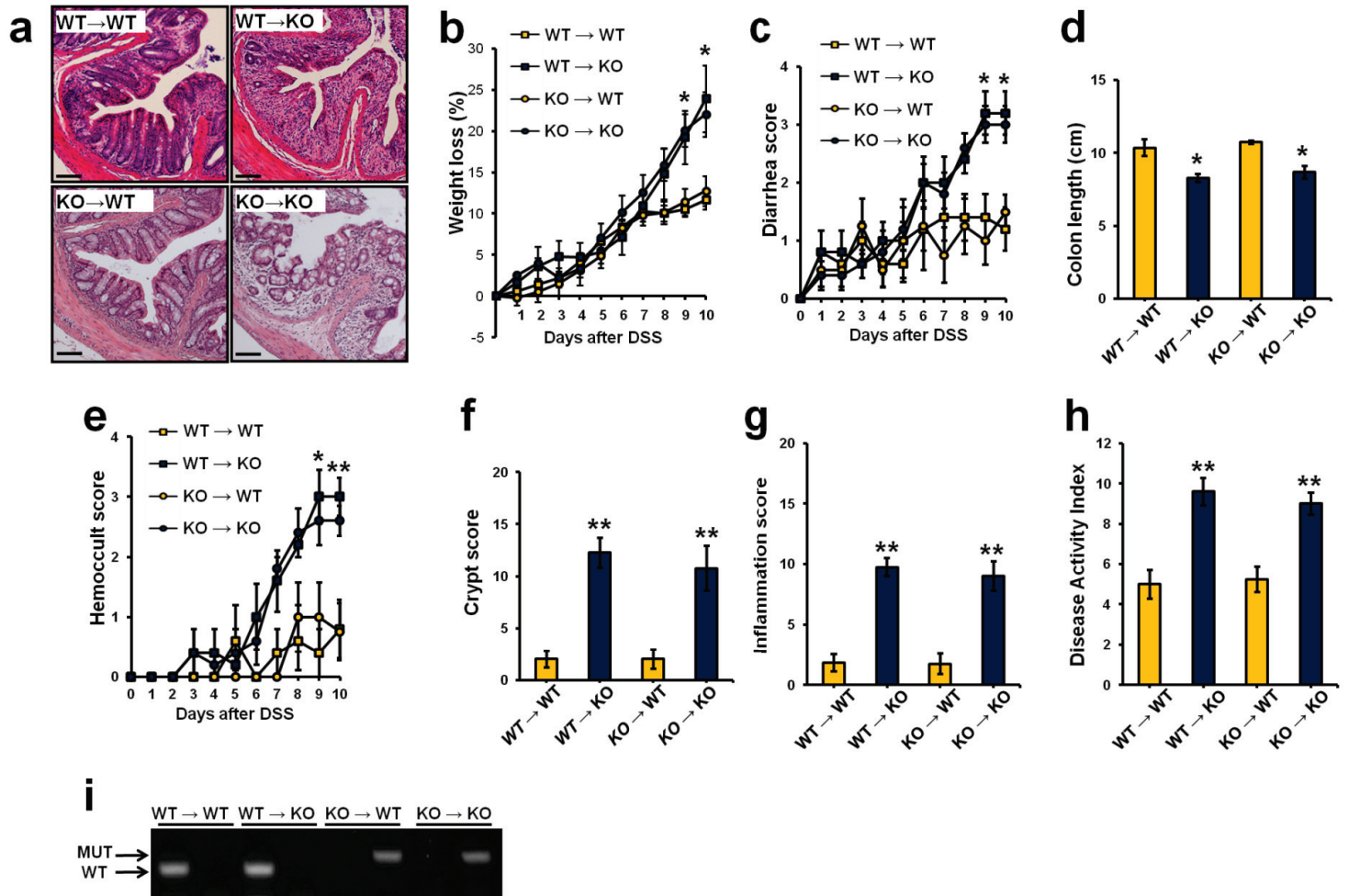
Supplementary Figure 6. *Apj* deficiency does not affect the severity of DSS-induced colitis.

a, Colon histopathology (H&E staining; scale bar, 100 μ m), **b**, percent weight loss, **c**, diarrhea scores, **d**, colon length, **e**, hemocult scores, **f**, crypt scores, **g**, inflammation scores, and **h**, disease activity index in control and DSS-challenged *Apj*^{+/+} and *Apj*^{-/-} littermates. Values are mean \pm SEM of 6 mice per group. n.s., not significant (paired-t-test).



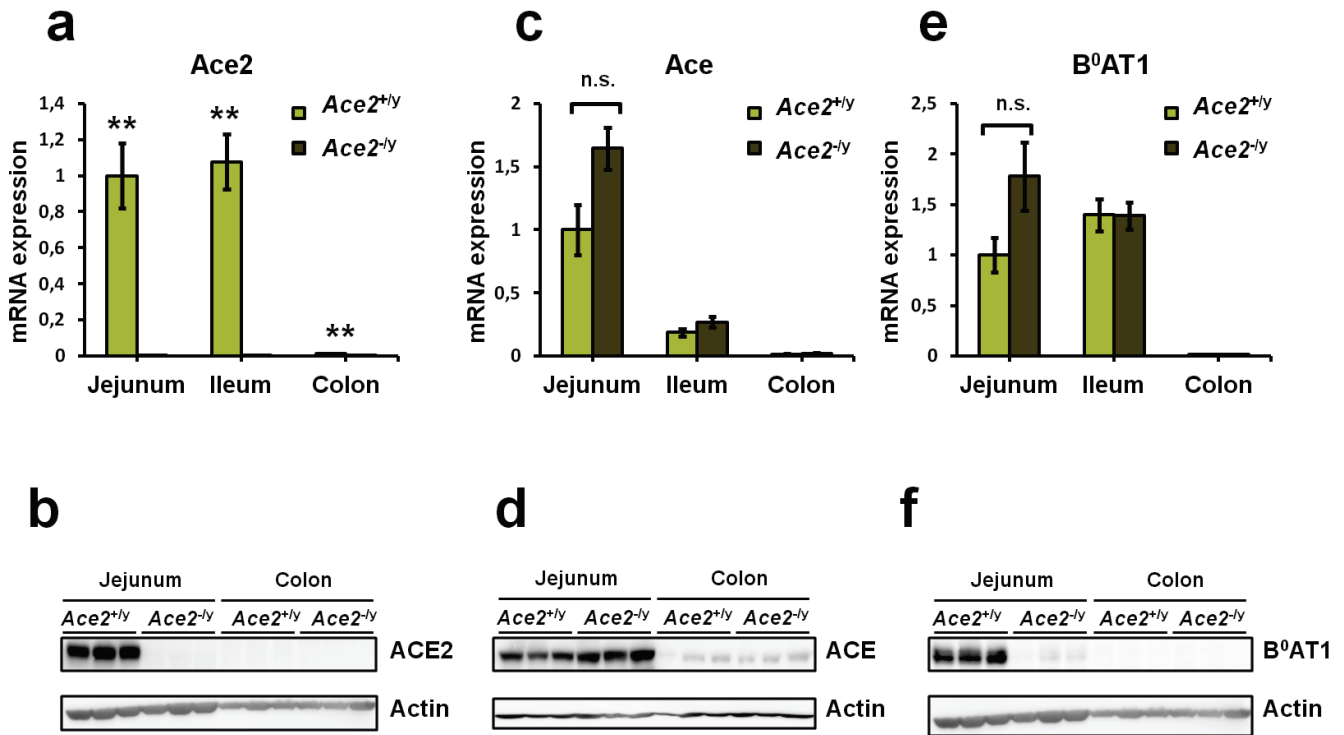
Supplementary Figure 7. Expression of Toll-like Receptors.

Relative mRNA expression levels of the indicated TLRs in jejunum, ileum, and colon mucosa isolated from 10-14 week old *Ace2*^{+y} and *Ace2*^{-y} mice. Values are mean \pm SEM of 3 mice per group. **P* < 0.05 comparing *Ace2*^{+y} vs. *Ace2*^{-y} mice (paired-t-test). Of note, in unchallenged *Ace2* mutant mice, we did not observe any apparent differences among macrophage and granulocyte numbers nor alterations in CD4, CD8 $\alpha\alpha$, CD8 $\alpha\beta$, TCR $\alpha\beta$, or TCR $\gamma\delta$ expressing intraepithelial (IEL) or lamina propria lymphocyte (LPL) populations of the colon and small intestine as compared to wild type littermates. Numbers of Foxp3⁺ Treg cells in the colon and small intestine were also comparable between control and *Ace2* mutant mice.



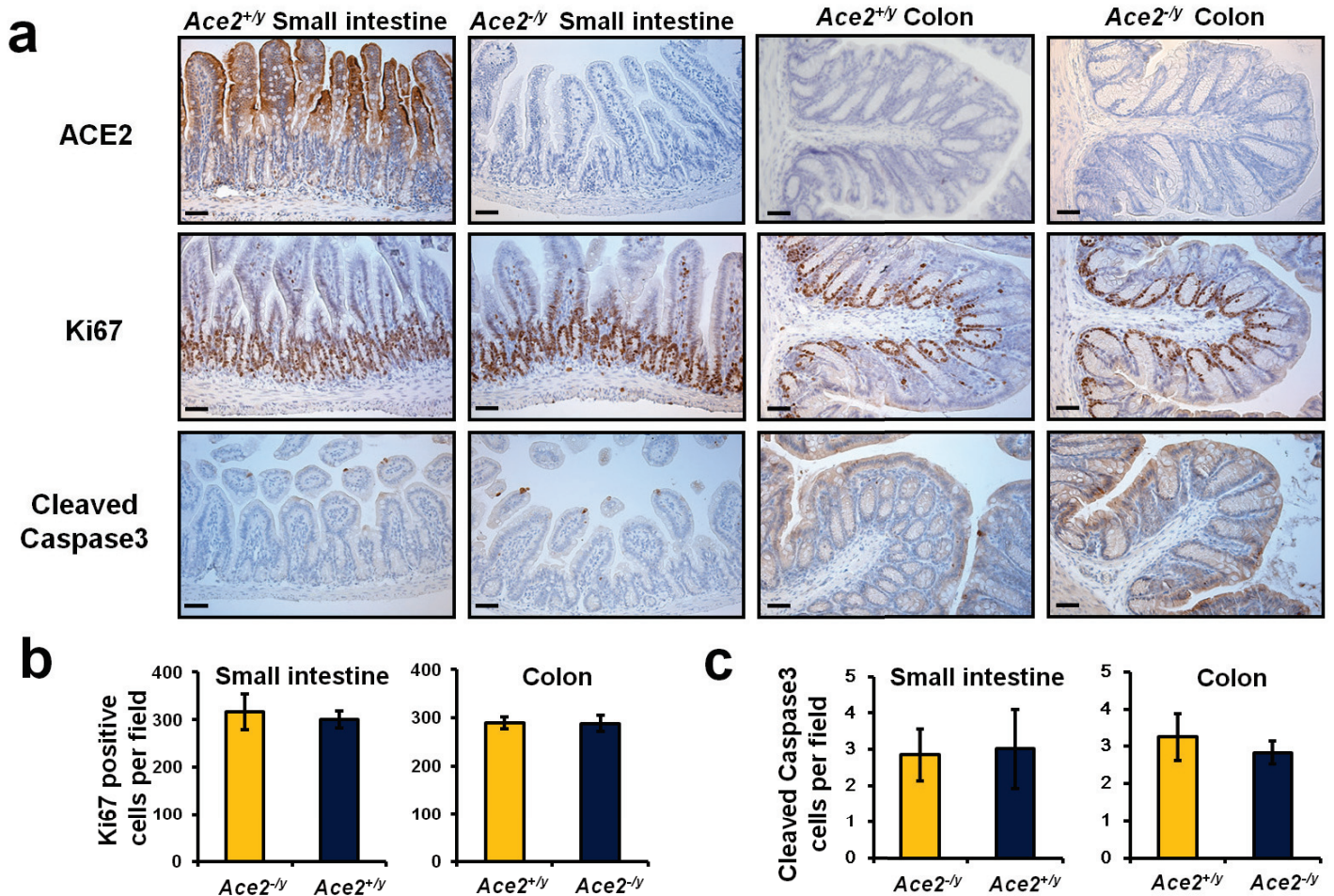
Supplementary Figure 8. ACE2 functions in non-haematopoietic cells.

a, Colon histopathology in control and DSS treated WT→KO, and KO→WT, WT→WT, and KO→KO bone marrow chimeric mice (H&E staining; scale bar, 100µm). **b**, percent weight loss, **c**, diarrhea scores, **d**, colon length, **e**, hemocult scores, **f**, crypt scores, **g**, inflammation scores, and **h**, disease activity index in control and DSS treated WT→KO, and KO→WT, WT→WT, and KO→KO bone marrow chimeric mice. Values are mean ± SEM of 5-10 mice per group. * $P < 0.05$, ** $P < 0.01$ comparing $Ace2^{+/y}$ vs. $Ace2^{-/y}$ mice (paired-t-test). **i**, Confirmation of bone marrow chimerism using PCR detection of the mutant (MUT) or wild type (WT) *Ace2* alleles.



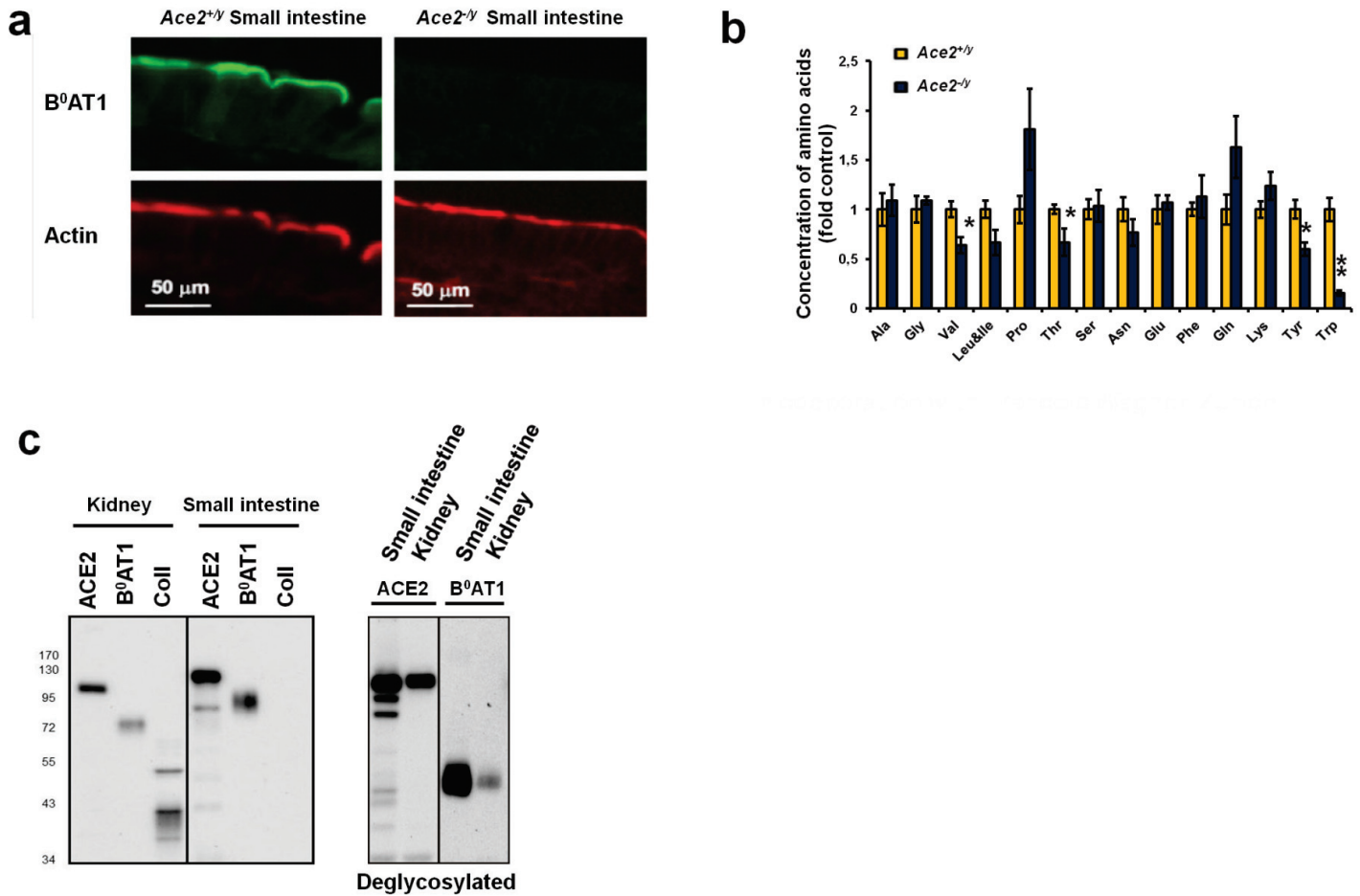
Supplementary Figure 9. Expression of ACE2, B⁰AT1, and ACE in gut.

Relative mRNA expression levels of **a**, *Ace2*, **c**, *Ace*, and **e**, B⁰AT1 mRNA in jejunum, ileum, and colon mucosa from *Ace2*^{+/-} and *Ace2*^{-/-} mice. Values are mean ± SEM of 3 mice per group. ***P*<0.01 comparing *Ace2*^{+/-} and *Ace2*^{-/-} mice; n.s., not significant (paired-t-test). Western blot analysis of **b**, ACE2, **d**, ACE, and **f**, B⁰AT1 in jejunum and colon mucosa membrane fractions from *Ace2*^{+/-} and *Ace2*^{-/-} mice. mRNA expression analyses of highly purified epithelial cell populations from the small intestine confirmed high expression of *Ace2* in differentiated cells whereas *Ace2* mRNA expression was lower, but still present in the stem cells as well as Paneth cells in the crypts. Data from individual mice are shown. β-actin is shown as a control for equal loading.



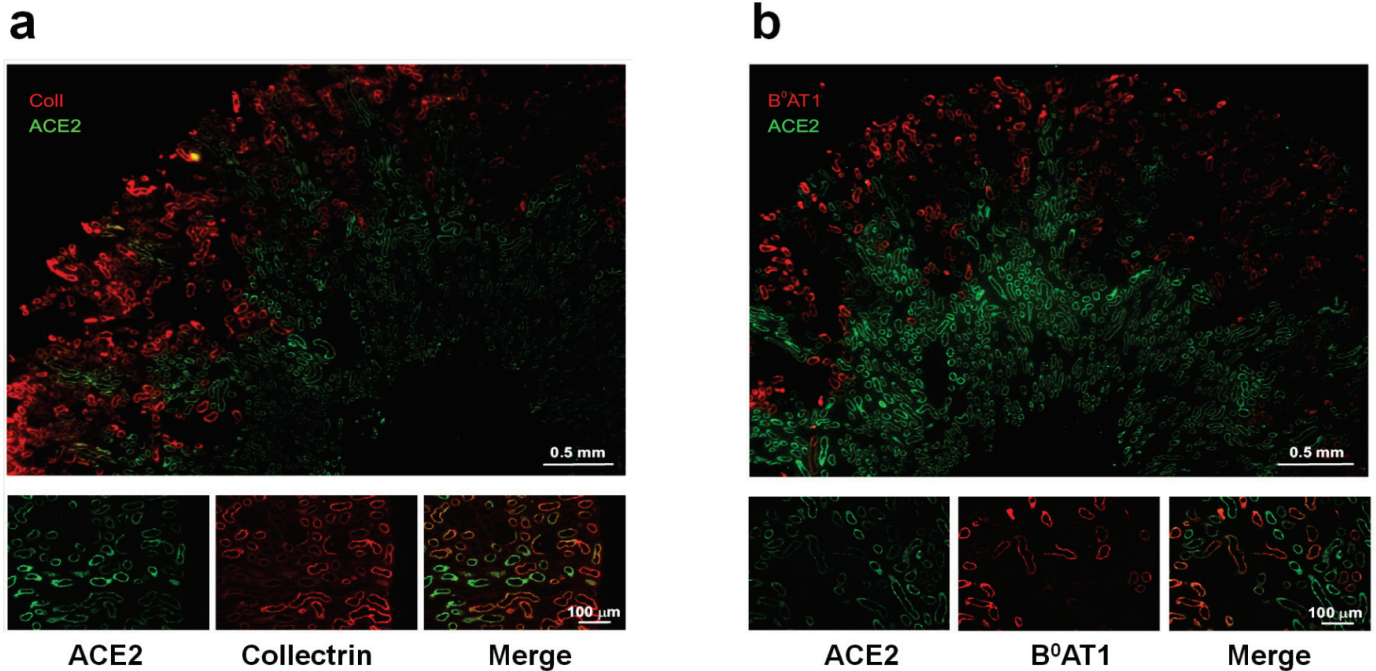
Supplementary Figure 10. ACE2 expression, proliferation, and cell death in gut epithelium.

a, Immunohistochemistry in the small intestine and colon of *Ace2^{+/-}* and *Ace2^{-/-}* mice to detect ACE2 protein expression and to visualise proliferation using Ki67 staining and apoptosis using cleaved Caspase3. Scale bars, 50 μ m. **b**, Quantification of Ki67 and **c**, cleaved Caspase3 staining in the small intestine and colon of *Ace2^{+/-}* and *Ace2^{-/-}* mice. Values are mean numbers of Ki67 or cleaved Caspase3 positive cells per visual field \pm SEM of at least 6 mice per group. For each mouse more than 3 visual fields were counted in total.

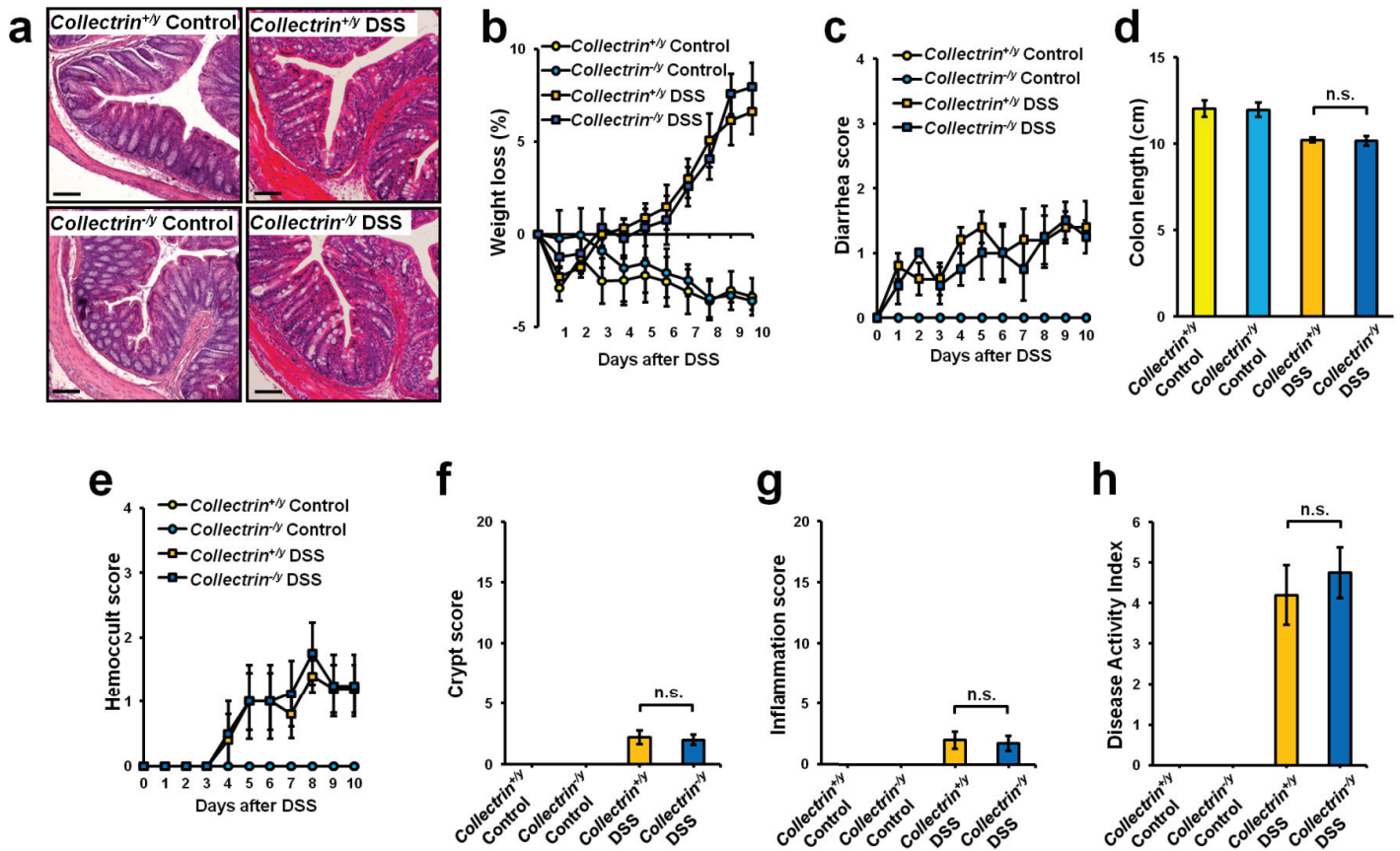


Supplementary Figure 11. Loss of intestinal B⁰AT1 expression and serum amino acid profiles.

a, Immunofluorescence staining to detect B⁰AT1 and, as a control, β-actin in the small intestine of *Ace2^{+/-}* and *Ace2^{-/-}* mice. Note that B⁰AT1 protein is completely absent in *Ace2^{-/-}* mice. Scale bars, 50μm. **b**, Serum amino acid levels in *Ace2^{-/-}* mice relative to that of *Ace2^{+/-}* littermates. Values are mean ± SEM of 5 mice per group. **P*<0.05, ***P*<0.01 comparing *Ace2^{+/-}* and *Ace2^{-/-}* mice (paired-t-test). Of note, we observed increased levels of proline (Pro) and glutamine (Gln); as observed in B⁰AT1 deficiency and in low protein diet in mice (Mariotta and Verrey, unpublished data), serum levels of non-essential amino acids can be increased due to an adaptation of amino acid metabolism. **c**, Western Blot analysis of ACE2, Collectrin (Coll), and B⁰AT1 protein expression in brush border membrane vesicles (BBMVs) from small intestine and kidney. Note that the Collectrin protein is not expressed in the small intestine, and that ACE2 and B⁰AT1 bands run at different sizes in kidney and small intestine due to differential glycosylation (right panels).

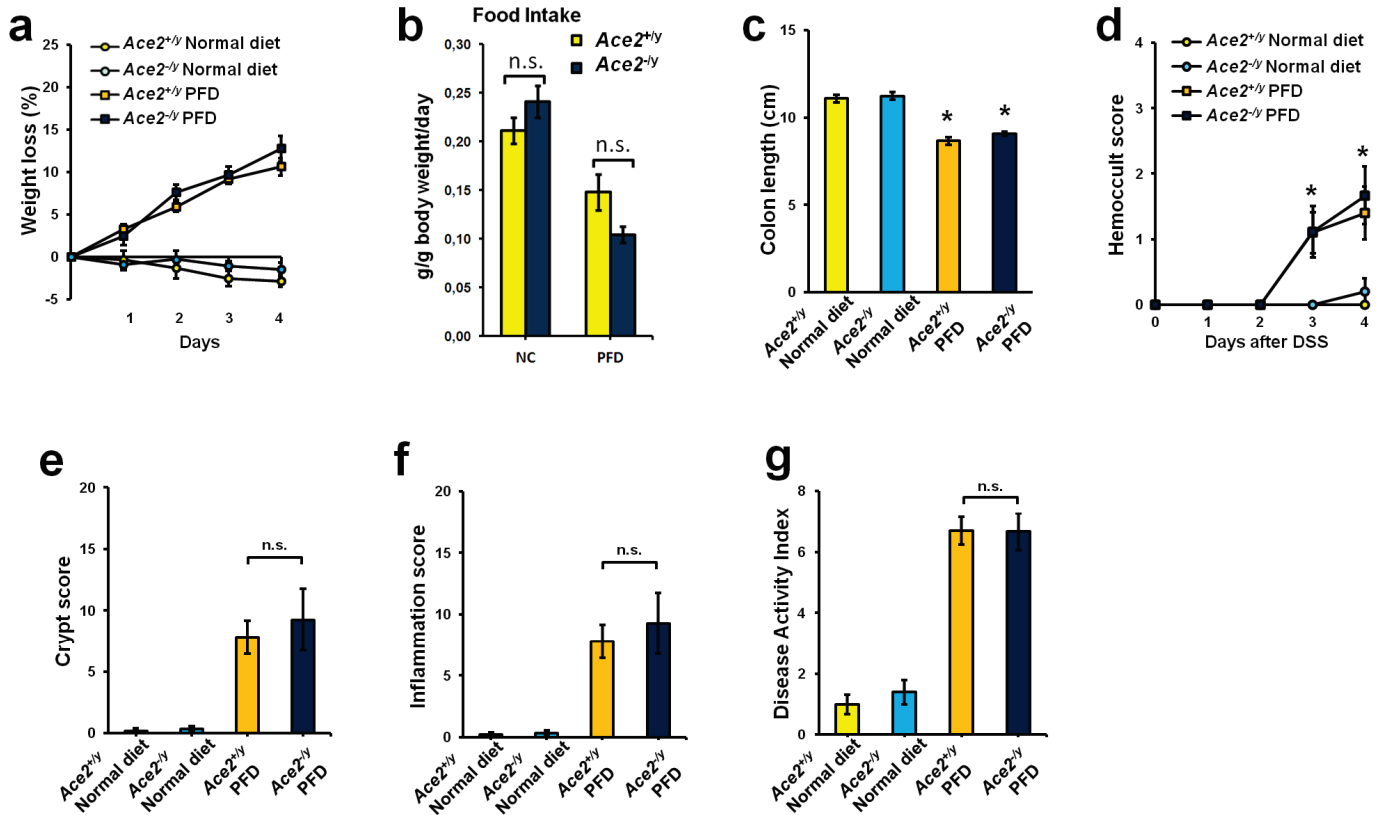


Supplementary Figure 12. ACE2 co-localisation with Collectrin and B⁰AT1 in the kidney. Immunofluorescence staining to detect ACE2 (green), Collectrin (red), and B⁰AT1 (red) in the kidney of wild type mice. Merged images are also shown. **a**, Immunofluorescence on mouse kidney sections shows that ACE2 (green) localises to the brush border membrane of the proximal tubule, with an axial gradient towards the later segments, as opposed to Collectrin (red) which localises to the earlier part of the proximal tubule. **b**, Immunofluorescence on mouse kidney sections shows that ACE2 (green) displays an axial gradient towards later segments along the proximal tubule in contrast to B⁰AT1 (red) that localises to earlier proximal tubule segments. Scale bars are 0.5mm and in the magnified panels 100μm.



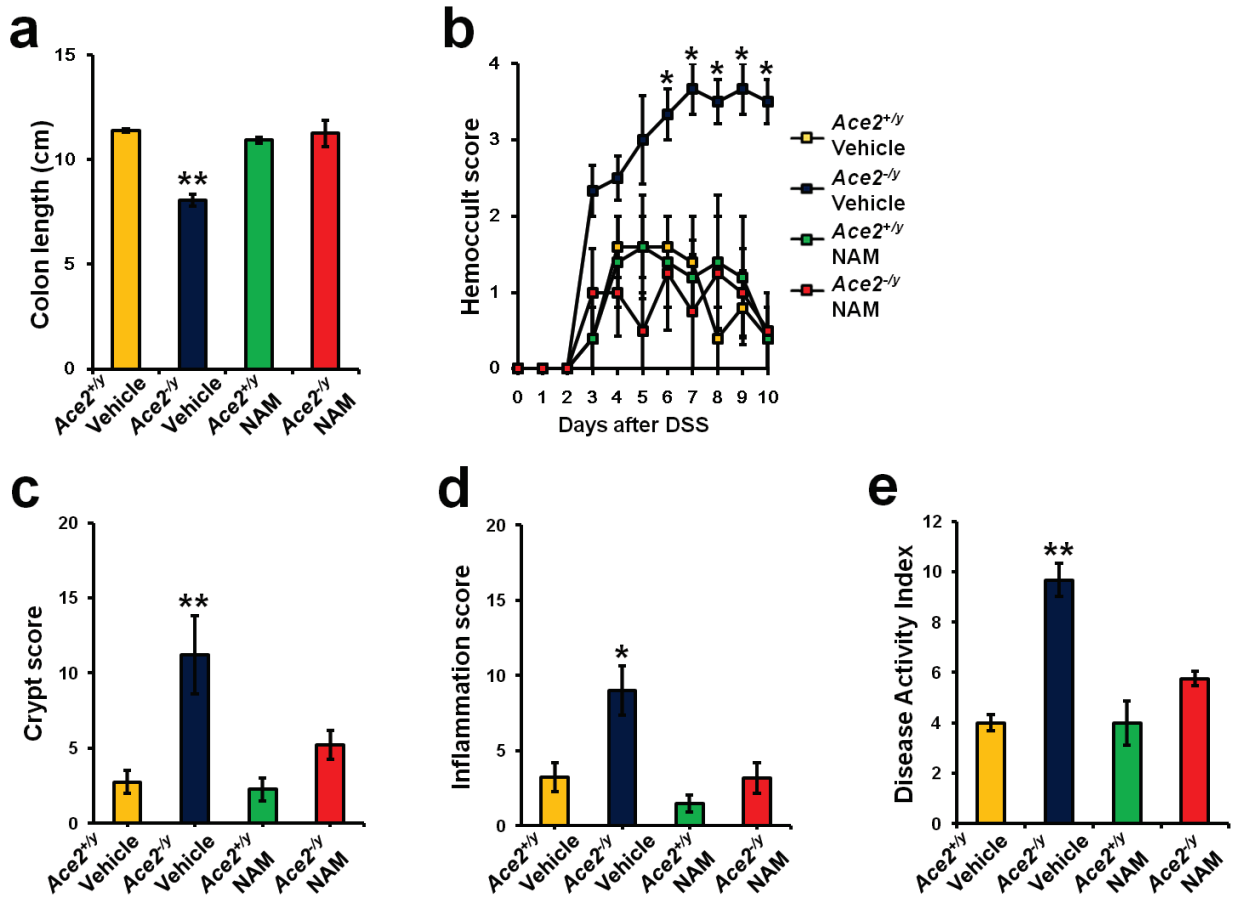
Supplementary Figure 13. *Collectrin* deficiency does not affect DSS-induced colitis.

a, Colon histopathology (H&E staining; scale bar, 100 μm), **b**, percent weight loss, **c**, diarrhea scores, **d**, colon length, **e**, hemocult scores, **f**, crypt scores, **g**, inflammation scores, and **h**, disease activity index in control and DSS-challenged *Collectrin*^{+/y} and *Collectrin*^{-/y} littermates. Note, similar to *Ace2* and *Apelin*, the *Collectrin* (*Tmem27*) gene is located on the X chromosome. Values are mean ± SEM of 6 mice per group. n.s., not significant (paired-t-test).



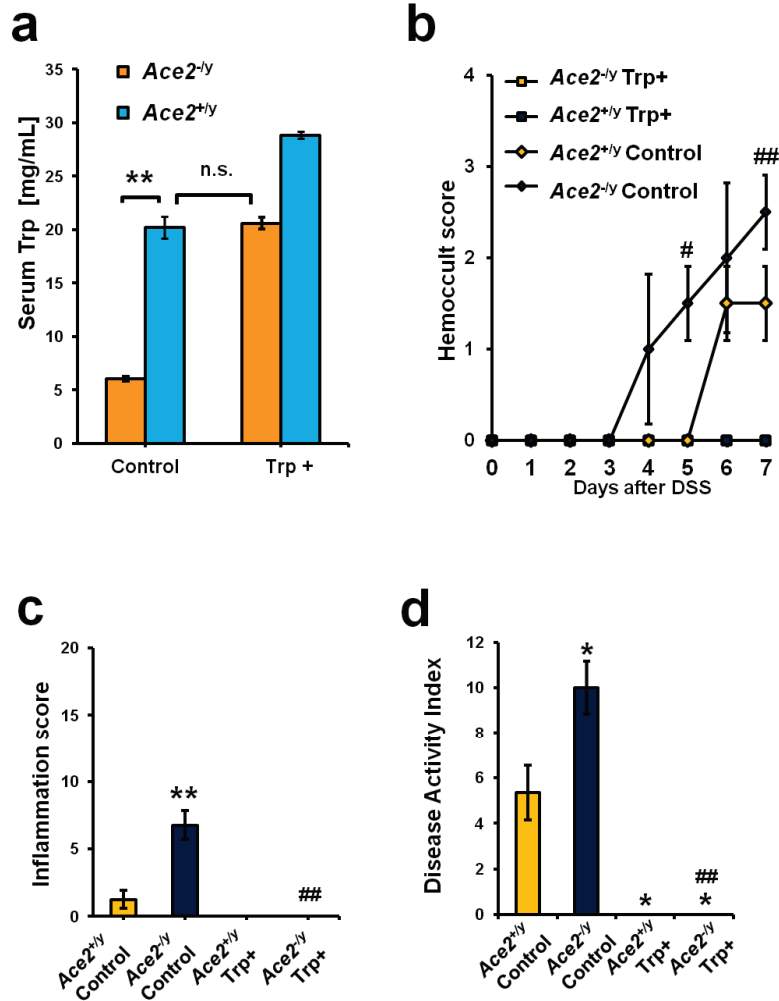
Supplementary Figure 14. Effects of protein free diet on colitis.

a, Percent weight loss in unchallenged $Ace2^{-/y}$ or $Ace2^{+/y}$ mice fed a protein free diet (PFD) or normal chow (Normal diet). **b**, Average food intake of unchallenged $Ace2^{-/y}$ and $Ace2^{+/y}$ mice was measured using indirect calorimetry. Data are shown as gramme normal chow (NC) or protein free diet (PFD) consumed per gramme body weight per day. Data in **a**, and **b**, are in the absence of DSS challenge. **c**, Colon length, **d**, hemocult scores, **e**, crypt scores, **f**, inflammation scores, and **g**, disease activity index in DSS-challenged $Ace2^{+/y}$ and $Ace2^{-/y}$ littermates on normal chow and isocaloric protein free diet (PFD). Note that for this experiment a low concentration of DSS (1%) was used to observe enhancement of the phenotype and that, due to the severe colitis in PFD fed mice, the experiment had to be stopped for ethical reasons on day 4. All data are shown as mean values \pm SEM of 7-10 mice per group. * $P < 0.05$, comparing $Ace2^{+/y}$ or $Ace2^{-/y}$ mice on normal diet vs. PDF; n.s., not significant (paired-t-test).



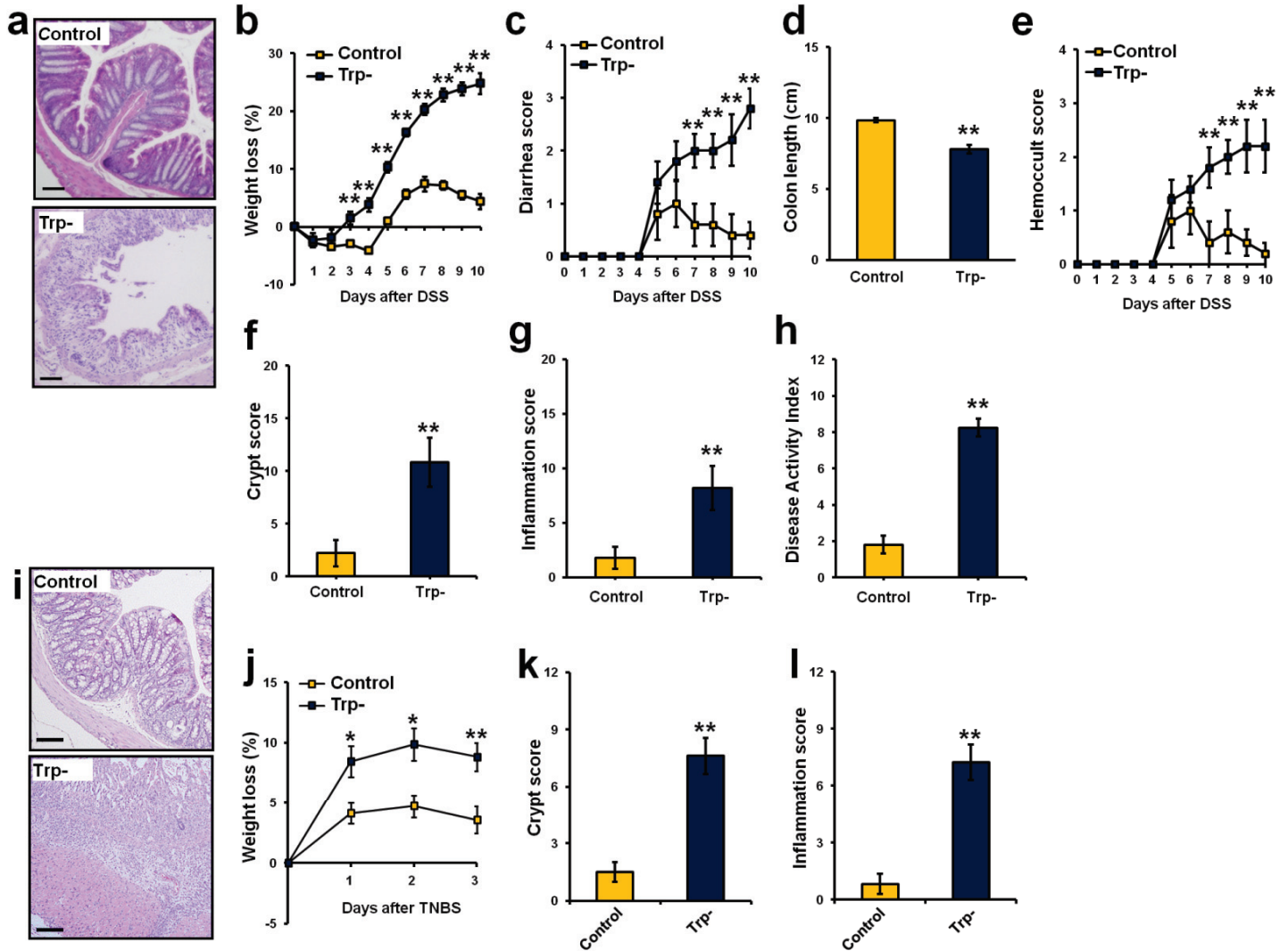
Supplementary Figure 15. Effects of nicotinamide on colitis.

a, Colon length **b**, hemocult scores, **c**, crypt scores, **d**, inflammation scores, and **e**, disease activity index in DSS-challenged *Ace2^{+/y}* and *Ace2^{-/y}* littermates that received either vehicle or nicotinamide (NAM) in their drinking water. NAM treatment was started 3 days before the first DSS challenge. Values are mean ± SEM of 7-10 mice per group. **P*<0.05, ***P*<0.01 comparing vehicle vs. NAM treated *Ace2^{-/y}* mice (paired-t-test).



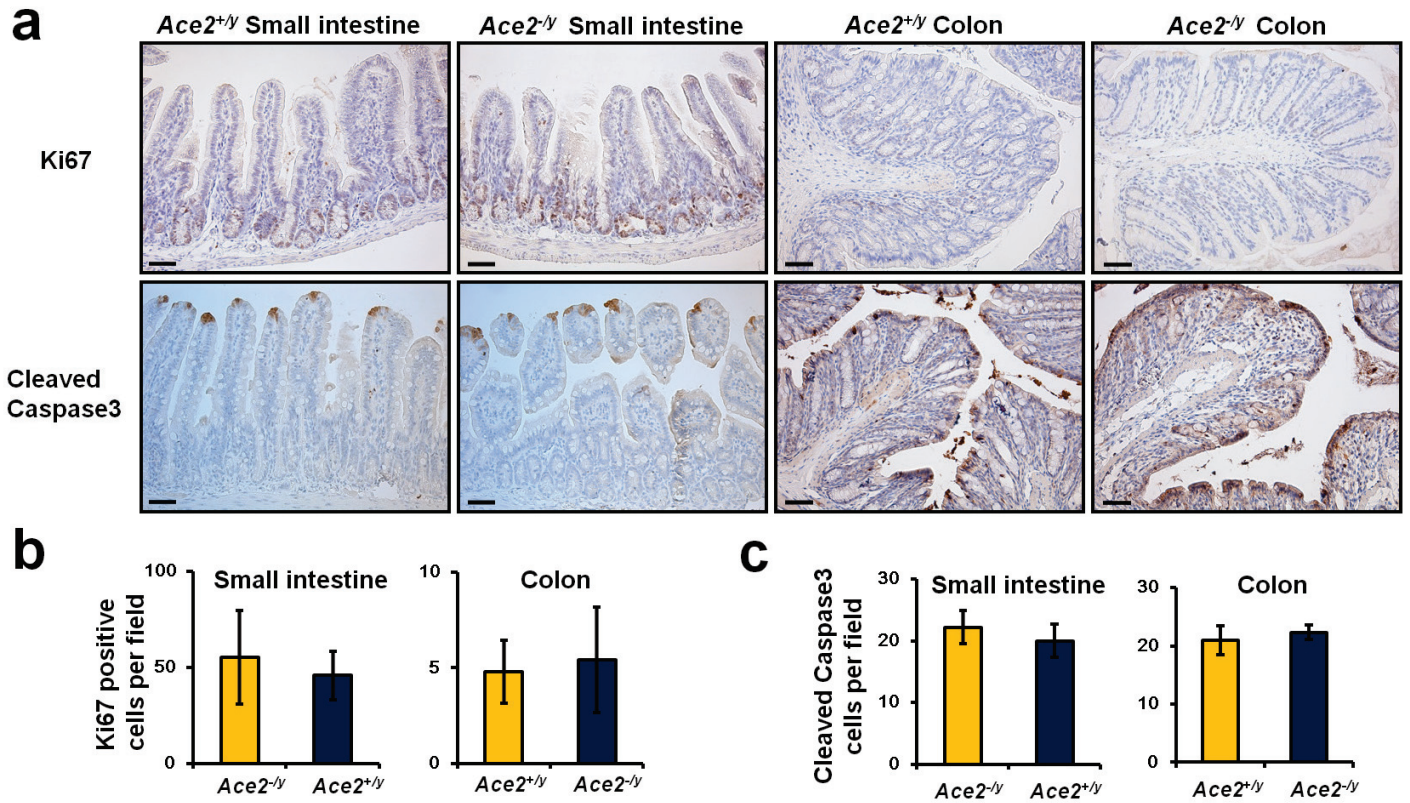
Supplementary Figure 16. Rescue of severe colitis in *Ace2^{-/-}* mice with tryptophan dipeptides.

a, Tryptophan levels in serum from non-challenged *Ace2^{+/-}* and *Ace2^{-/-}* mice fed a tryptophan dipeptide supplemented diet (Trp+) or normal chow (Control). Values are mean \pm SEM of 3 mice per group. **b**, Hemocult scores, **c**, inflammation scores, and **d**, disease activity index in DSS challenged *Ace2^{+/-}* and *Ace2^{-/-}* mice fed a tryptophan dipeptide supplemented diet (Trp+) or normal chow (Control). Values are mean \pm SEM of 3 mice per group. * P <0.05, ** P <0.01 comparing *Ace2^{+/-}* vs. *Ace2^{-/-}* mice. # P <0.05, ### P <0.01 comparing *Ace2^{-/-}* mice on tryptophan dipeptide supplemented diet versus normal chow; n.s., not significant (paired-t-test).



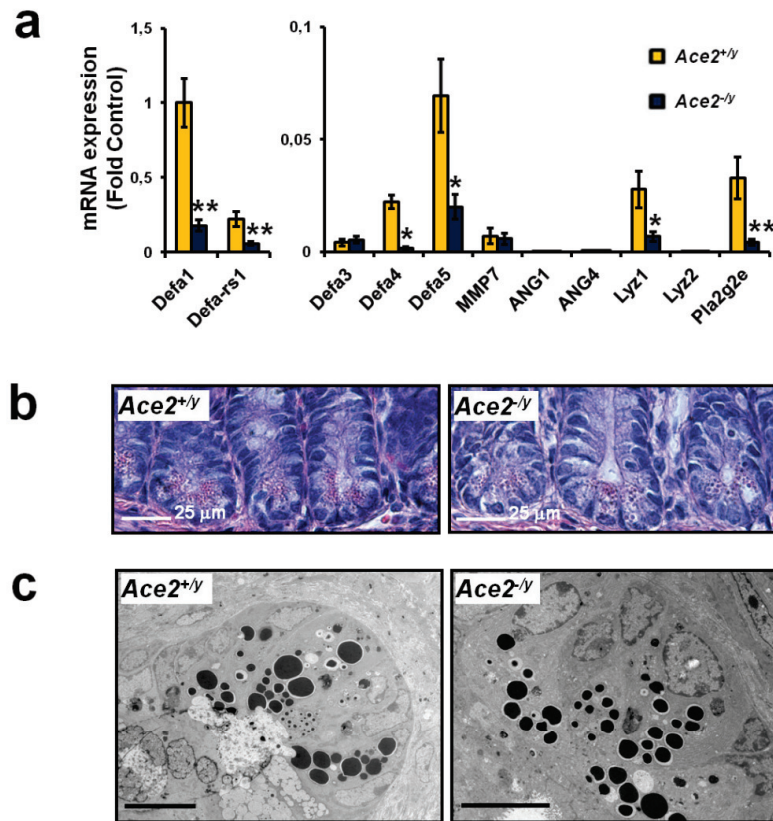
Supplementary Figure 17. Effects of tryptophan free diet on colitis.

a, Colon histopathology (H&E staining; scale bar, 100 μ m), **b**, percent weight loss, **c**, diarrhea scores, **d**, colon length, **e**, hemocult scores, **f**, crypt scores, **g**, inflammation scores, and **h**, disease activity index in DSS challenged *Ace2*^{+y} mice fed a tryptophan free, isocaloric diet (Trp-) or normal chow (Control). **i**, Colon histopathology (H&E staining; scale bar, 100 μ m), **j**, percent weight loss, **k**, crypt scores, and **l**, inflammation scores of TNBS treated *Ace2*^{+y} mice fed a tryptophan free, isocaloric diet (Trp-) or normal chow (Control). Values are mean \pm SEM of 6 mice per group. * P <0.05, ** P <0.01 (paired-t-test).



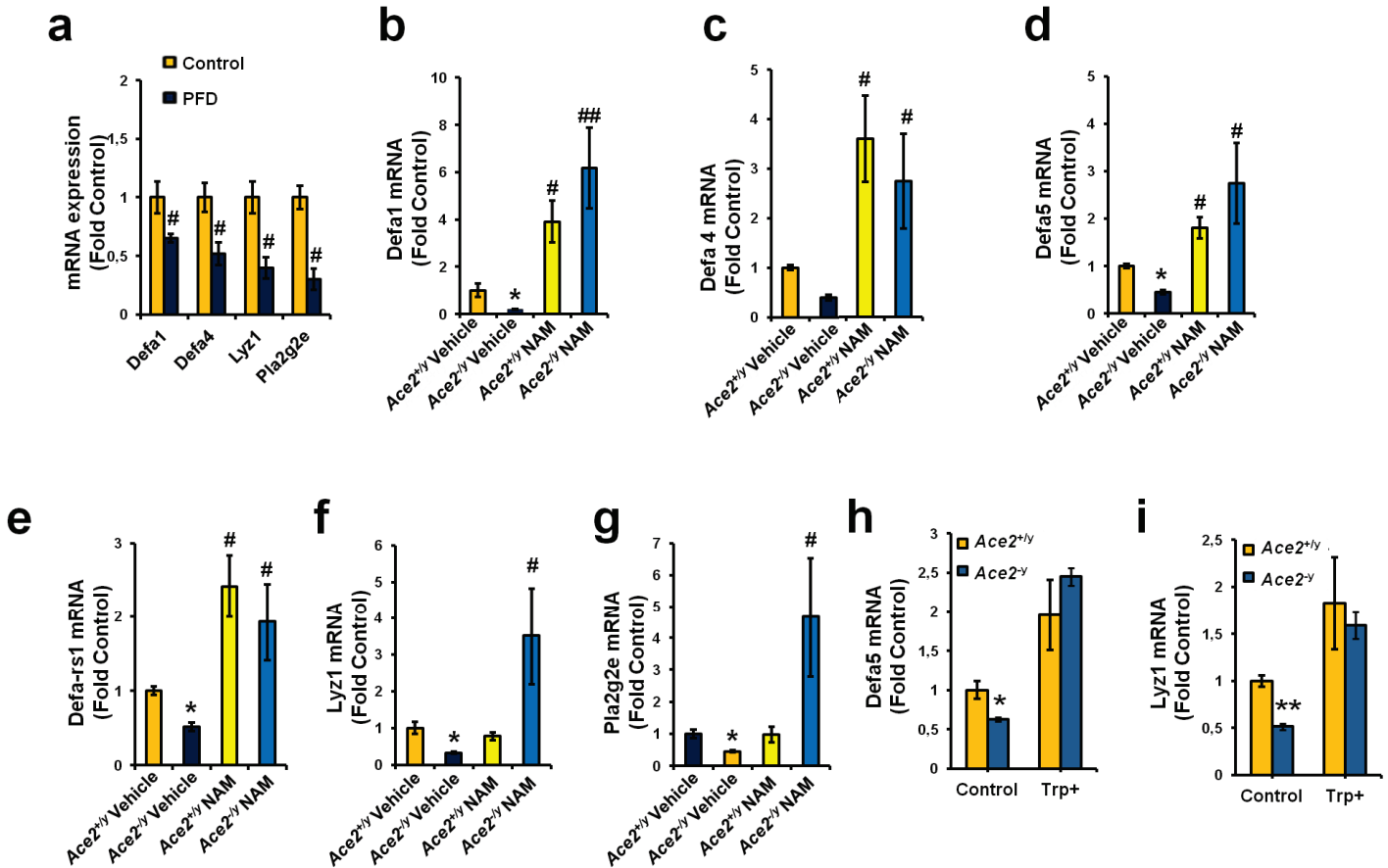
Supplementary Figure 18. Proliferation rates and cell death following DSS challenge.

a, Immunohistochemistry in the small intestine and colon of DSS-treated *Ace2^{+/y}* and *Ace2^{-/y}* mice to visualise proliferation using Ki67 staining and to detect apoptosis using cleaved Caspase3 as a read-out. Scale bars, 50µm. **b**, Quantification of Ki67 staining, and **c**, quantification of cleaved Caspase3 staining in the small intestine and colon of DSS challenged *Ace2^{+/y}* and *Ace2^{-/y}* mice. Values are mean numbers of Ki67 or cleaved Caspase3 positive cells per visual field ± SEM of at least 6 mice per group. For each mouse more than 3 visual fields were counted in total. The presented data are from day 5 after initial DSS challenge.



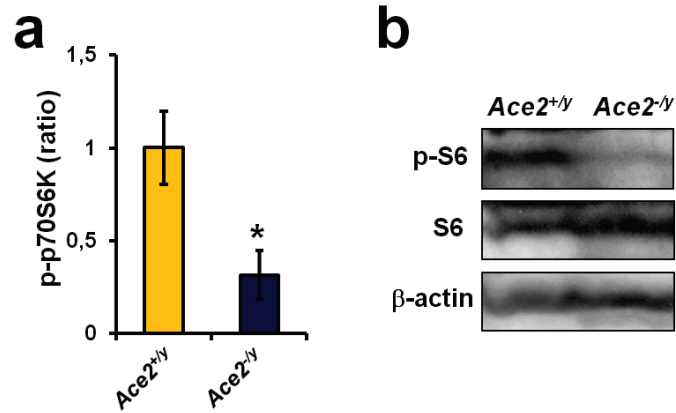
Supplementary Figure 19. Expression of antimicrobial peptides and normal Paneth cell morphology.

a, mRNA expression levels of the indicated antimicrobial peptides in epithelial cells isolated from the small intestine of unchallenged *Ace2*^{+/*y*} and *Ace2*^{-/*y*} littermates. * $P < 0.05$, ** $P < 0.01$ comparing *Ace2*^{+/*y*} vs. *Ace2*^{-/*y*} mice (paired-t-test). **b**, Histology (H&E staining; scale bars, 25 μm) and **c**, electron microscopy (scale bars, 10 μm) of Paneth cells. The distribution and structures of Paneth cells were comparable between unchallenged *Ace2*^{+/*y*} and *Ace2*^{-/*y*} mice.



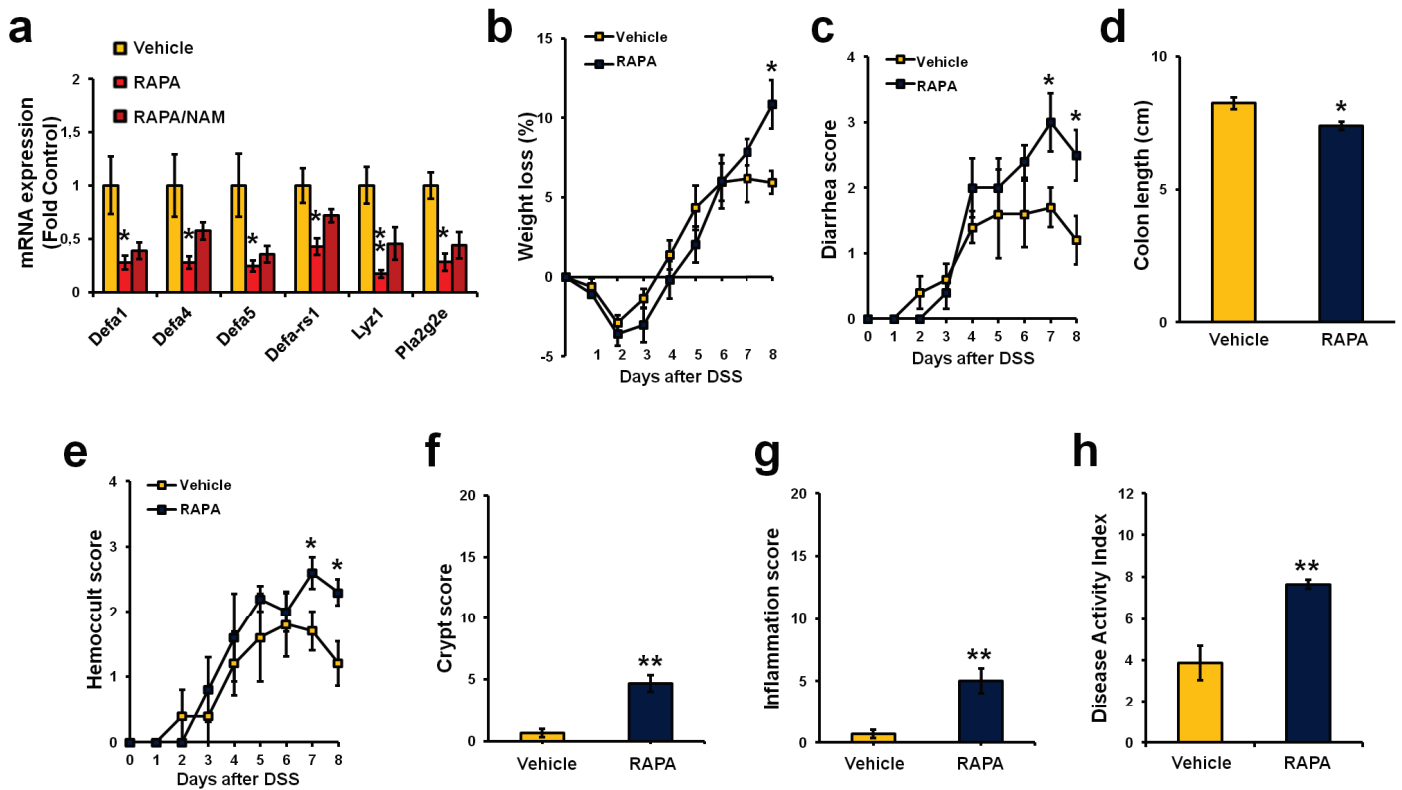
Supplementary Figure 20. Expression of antimicrobial peptide genes.

a, mRNA expression levels of the indicated antimicrobial peptides in epithelial cells isolated from the small intestine of unchallenged wild type mice fed a normal or protein free diet (PFD). **b-g**, mRNA expression levels of the indicated antimicrobial peptides in epithelial cells isolated from the small intestine of unchallenged *Ace2^{+y}* and *Ace2^{-y}* littermates that received vehicle or nicotinamide (NAM) in their drinking water for 4 days. **h-i**, mRNA expression levels of the indicated antimicrobial peptides in epithelial cells isolated from the small intestine of unchallenged *Ace2^{+y}* and *Ace2^{-y}* littermates that received tryptophan dipeptide supplemented diet (Trp+) or normal chow (Control). Values are mean \pm SEM of 3-6 mice per group. * $P < 0.05$, ** $P < 0.01$ comparing *Ace2^{+y}* vs. *Ace2^{-y}* mice. # $P < 0.05$, ## $P < 0.01$ comparing vehicle vs. NAM treated *Ace2^{+y}* or *Ace2^{-y}* mice or *Ace2^{+y}* mice on normal vs. protein free diet (paired-t-test).



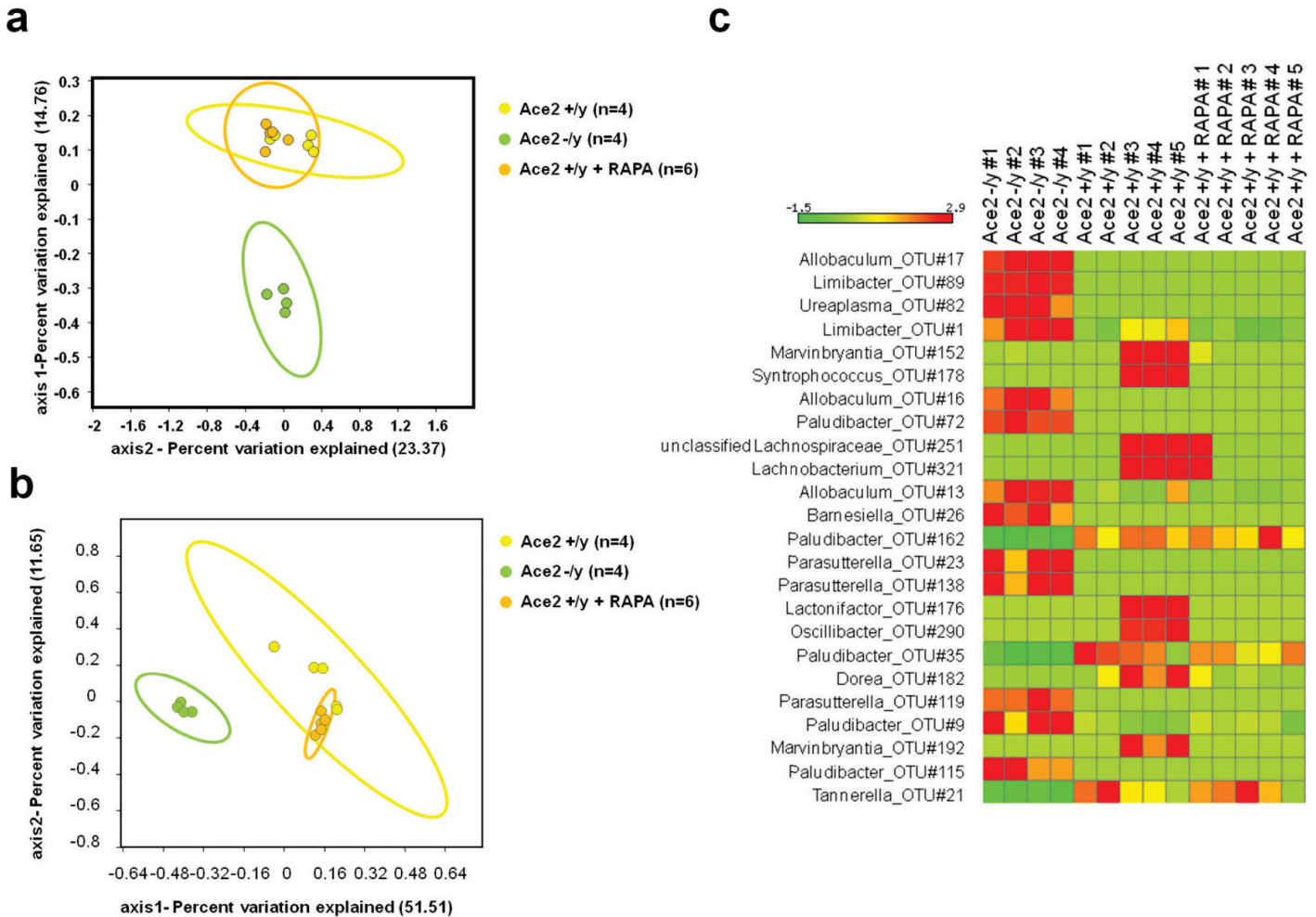
Supplementary Figure 21. mTOR pathway activity.

a, Expression levels of phosphorylated p70S6K in isolated small intestinal epithelial cells from unchallenged *Ace2^{+y}* and *Ace2^{-y}* littermates. Phosphorylated p70S6K levels were determined by ELISA. Values are mean \pm SEM of 5 mice per group. **b**, S6 phosphorylation and total S6 protein expression in isolated small intestinal epithelial cells from unchallenged *Ace2^{+y}* and *Ace2^{-y}* littermates. Representative Western blots are shown. β -actin is shown as loading control.



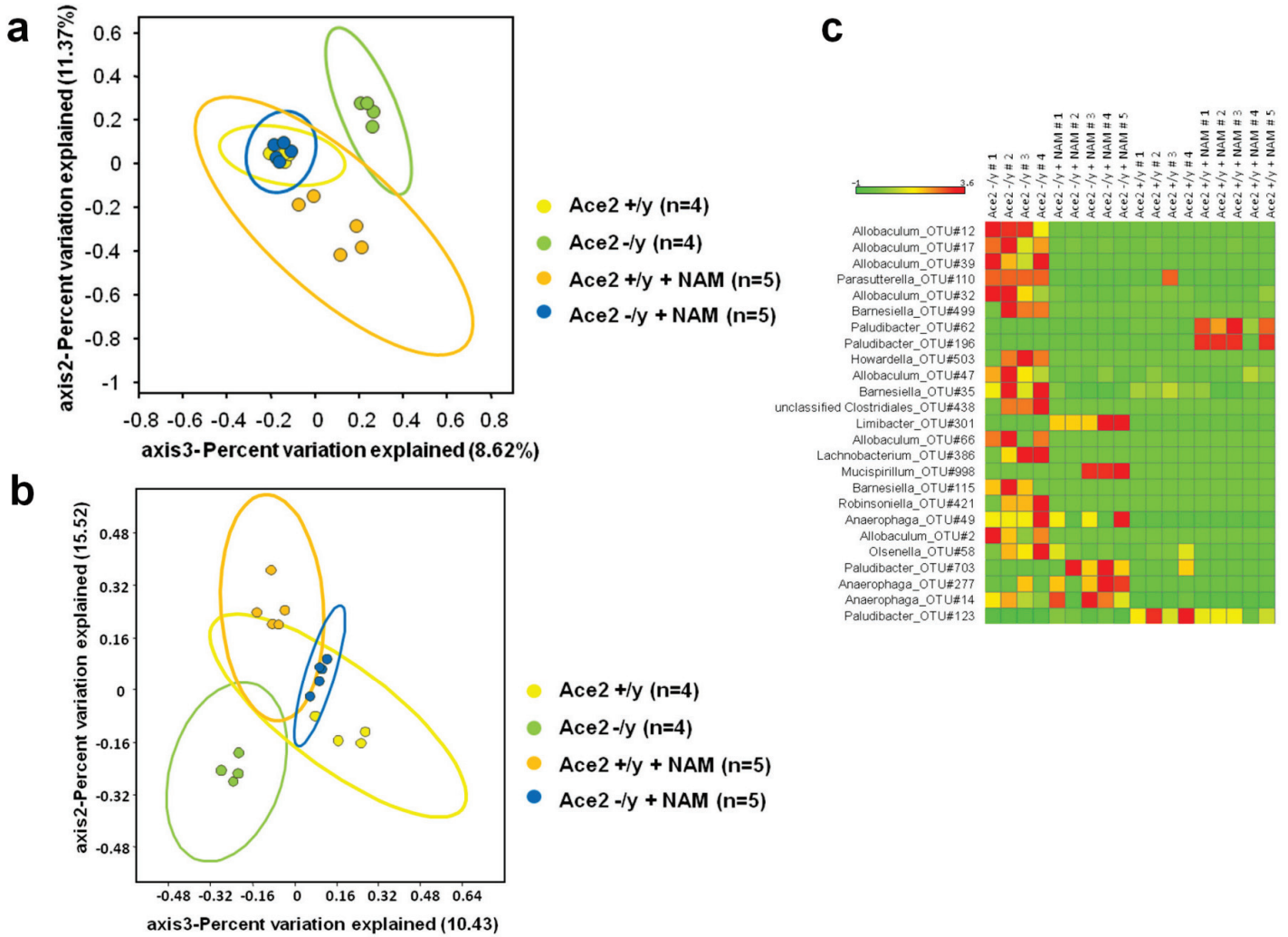
Supplementary Figure 22. Rapamycin pre-treatment worsens DSS induced colitis.

a, mRNA expression levels of the indicated antimicrobial peptides in epithelial cells isolated from the small intestine of unchallenged *Ace2^{+/y}* mice treated with rapamycin (RAPA; 2mg/kg/d) i.p. for 6 consecutive days in the presence or absence of NAM. Vehicle i.p. injected mice are shown as controls. **b**, Percent weight loss, **c**, diarrhea scores, **d**, colon length, **e**, hemocult scores, **f**, crypt scores, **g**, inflammation scores, and **h**, disease activity index in DSS-challenged wild type mice that received vehicle control or rapamycin (RAPA). Rapamycin treatment was started 6 days before the first DSS challenge. Values are mean \pm SEM of 5 mice per group. * $P < 0.05$, ** $P < 0.01$ comparing vehicle vs. NAM treated *Ace2^{+/y}* mice (paired-t-test).



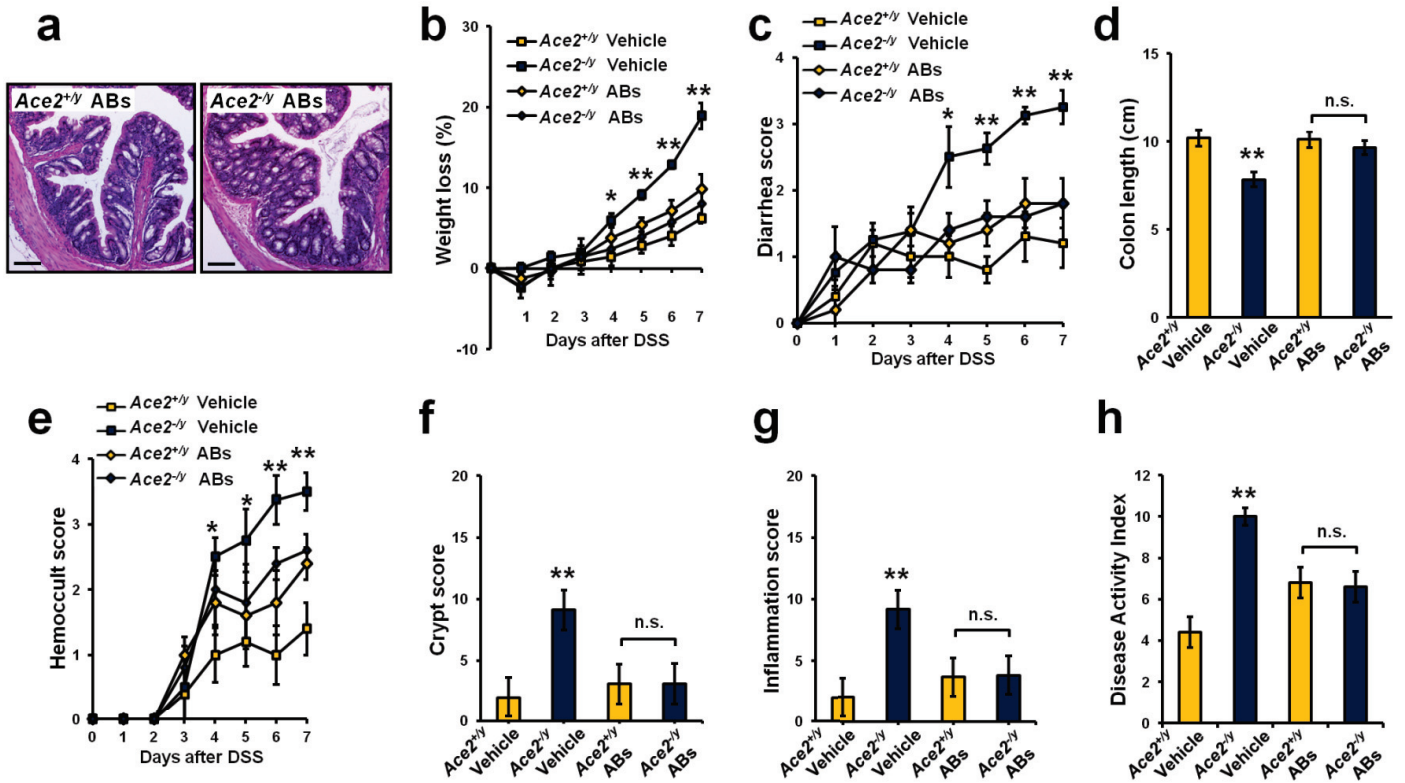
Supplementary Figure 23. Microbiota analysis of RAPA-treated mice.

a, Principal coordinate analysis (PCoA) plot based on distances calculated by unweighted UniFrac analysis showing similarity of ileocecal microbial composition from *Ace2*^{-/-} and *Ace2*^{+/-} mice with and without RAPA treatment. **b**, PCoA of weighted analysis based on Bray-Curtis similarity algorithm taking abundance into account. Only the two axis with high R² values are shown (axis 2, R² = 0.670; axis 3, R² = 0.693). **c**, Heat map of the top 40 species level OTUs contributing significantly to the axis shown in the PcoA plots. Experimental numbers of independent animals are given in the respective panels.



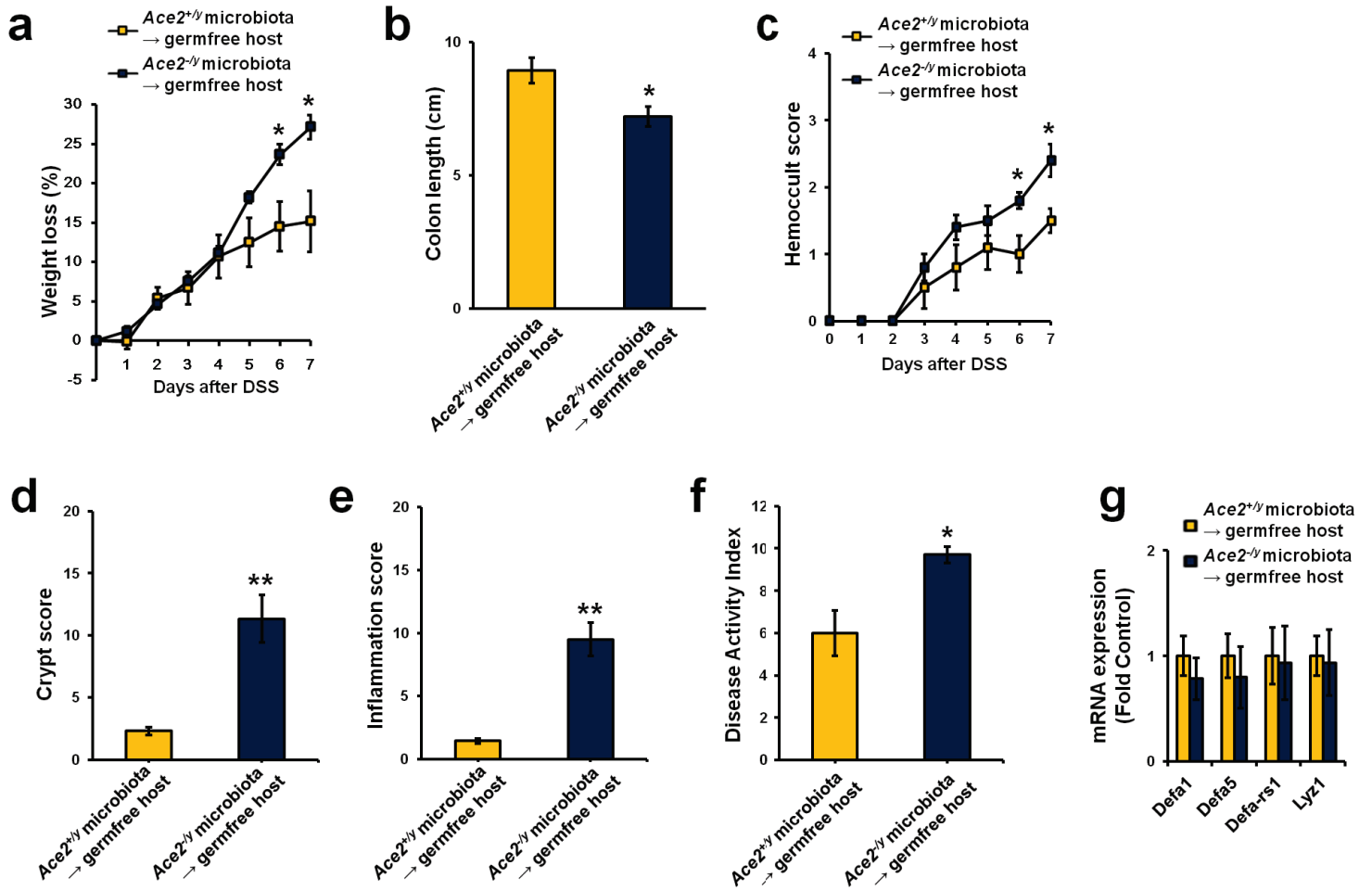
Supplementary Figure 24. Microbiota analysis of NAM-treated mice.

a, Principal coordinate analysis (PCoA) plot based on distances calculated by unweighted Unifrac analysis showing similarity of ileocecal microbial composition from *Ace2*^{-/y} and *Ace2*^{+/y} mice with and without NAM treatment. **b**, PCoA of weighted analysis based on Bray-Curtis similarity algorithm taking abundance into account. Only the two axis with high R² values are shown (axis 2, R² = 0.670; axis 3, R² = 0.693). **c**, Heat map of the top 40 species level OTUs contributing significantly to the axis shown in PcoA plots. Experimental numbers of independent animals are given in the respective panels.



Supplementary Figure 25. Antibiotic treatment alleviates severe colitis of $Ace2^{-/y}$ mice.

a, Colon histopathology (H&E staining; scale bars, 100µm), **b**, percent weight loss, **c**, diarrhea scores, **d**, colon length, **e**, hemocult scores, **f**, crypt scores, **g**, inflammation scores, and **h**, disease activity index in DSS-challenged $Ace2^{+/y}$ and $Ace2^{-/y}$ littermates treated with or without broad-spectrum antibiotics (ABs). Values are mean \pm SEM of 5 mice per group. * $P < 0.05$, ** $P < 0.05$ comparing vehicle vs. ABs treated $Ace2^{-/y}$ mice; n.s., not significant (paired-t-test).



Supplementary Figure 26. Communicable colitis.

a, Percent weight loss, **b**, colon length, **c**, hemocult scores, **d**, crypt scores, **e**, inflammation scores, and **f**, disease activity index in DSS-challenged germfree mice that were populated with the gut microbiota of *Ace2^{+/-}* or *Ace2^{-/-}* littermates. **g**, mRNA expression levels of the indicated antimicrobial peptides in epithelial cells isolated from the small intestine of germfree mice that were populated with the gut microbiota of *Ace2^{+/-}* and *Ace2^{-/-}* littermates without DSS-challenge. Values are mean ± SEM of 5 mice per group. * $P < 0.05$, ** $P < 0.01$ (paired-t-test).