SUPPLEMENTAL MATERIAL

Schmidt et al., https://doi.org/10.1084/jem.20171696

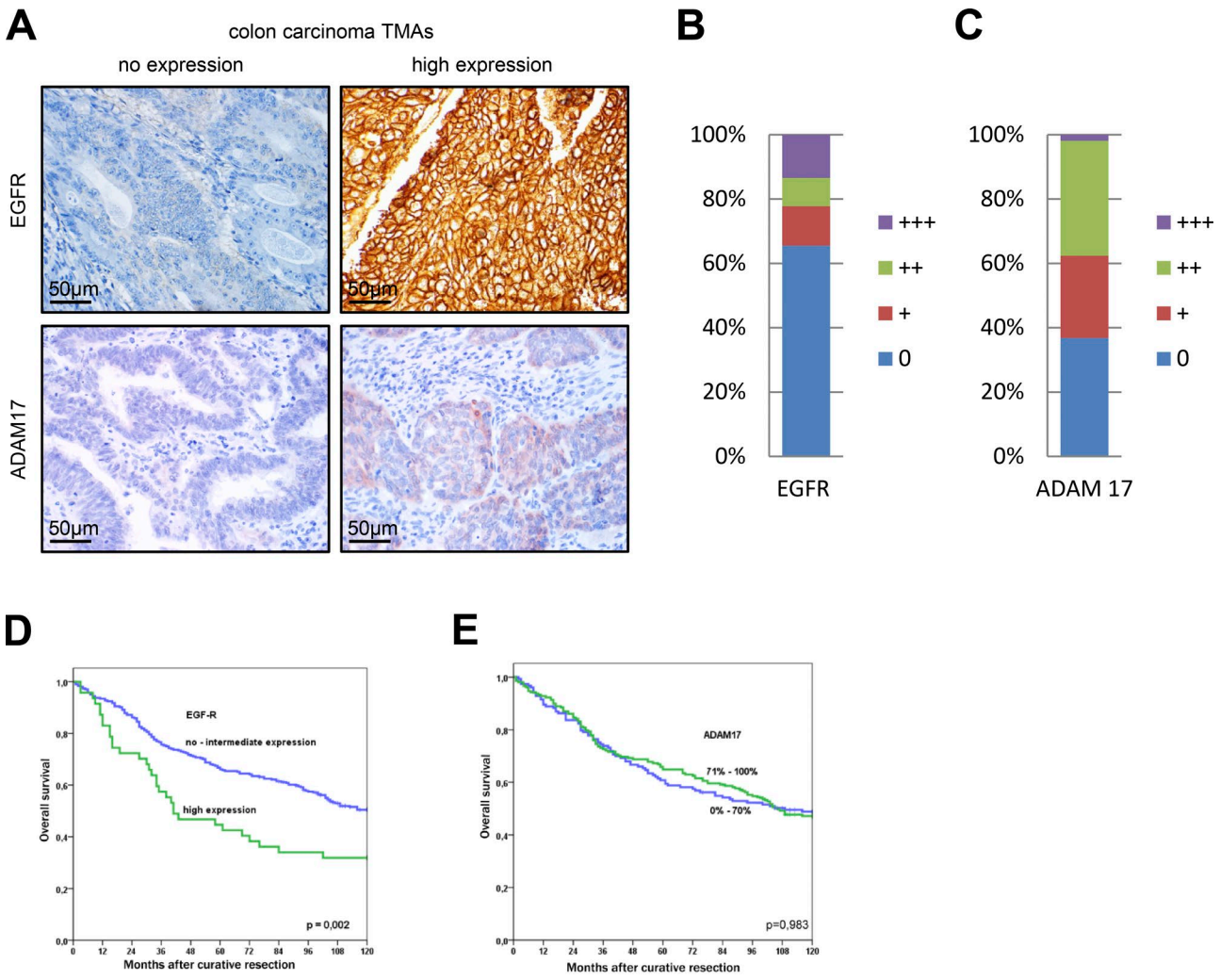

Figure S1. **EGF-R but not ADAM17 expression intensity on tissue microarrays is correlated with survival of colorectal carcinoma patients. (A)** IHC staining patterns depicting no and high expression of EGF-R and ADAM17. **(B and C)** Distribution of staining intensity of ADAM 17 (n = 361) and EGF-R (n = 330); 0 = no expression, + = weak expression, ++ = intermediate expression, +++ = high expression. **(D)** Overall survival (OS) of patients with no to intermediate EGF-R expression (0 to ++) and patients with high EGF-R expression (+++); P = 0.002. **(E)** OS of patients with no to intermediate (0 to ++) ADAM17 expression or high (+++) ADAM 17 expression; P = 0.387. We have rated the EGF-R and ADAM17 expression as IHC score of +++ (n = 45 and 7), ++ (n = 29 and 129), + (n = 41 and 92), and negative (n = 215 and 133). A difference was considered significant at P < 0.05 by Fisher's exact test.

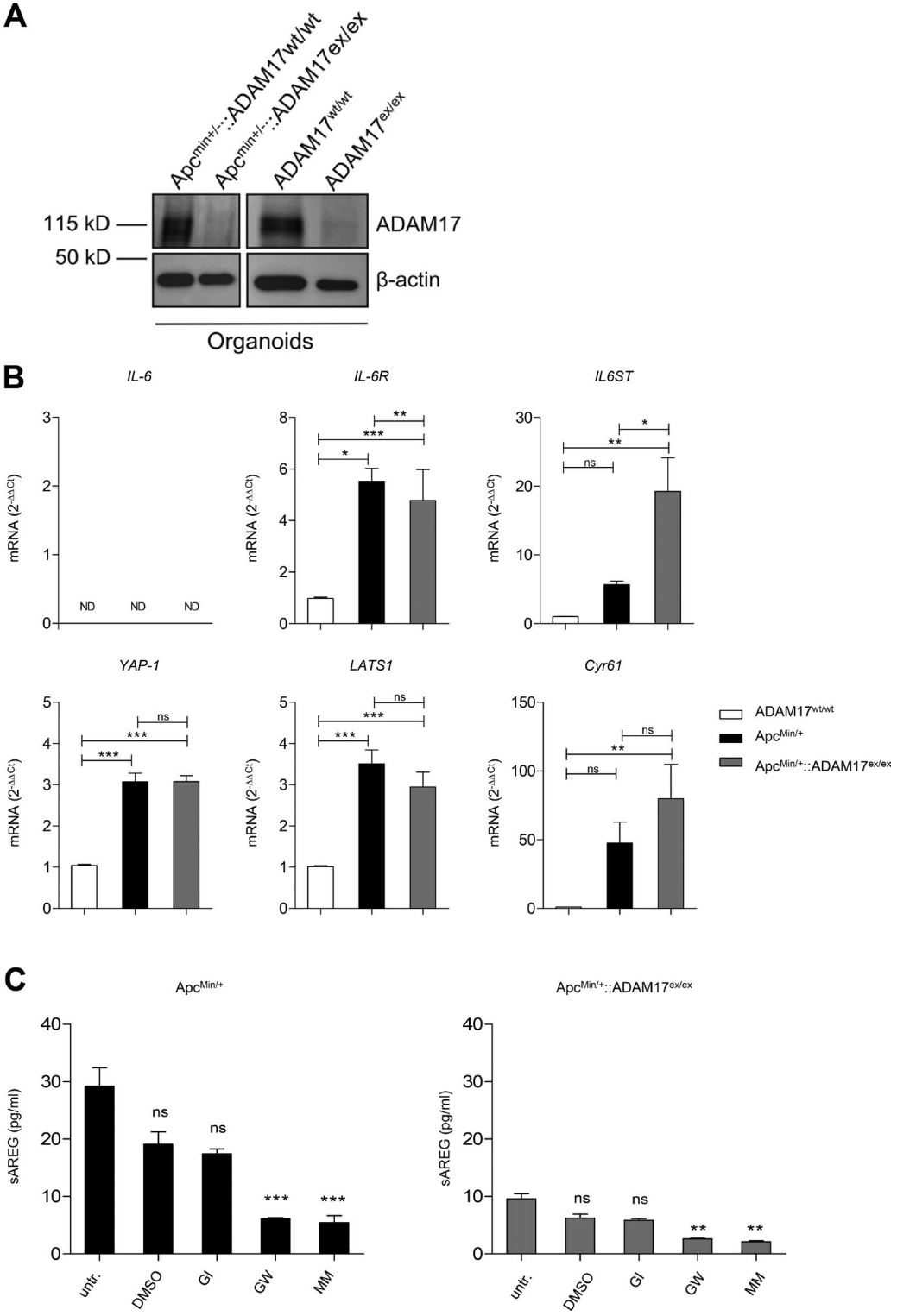

Figure S2. **Gene expression analysis of intestinal organoids from $Apc^{Min/+}$ and $Apc^{Min/+}$::$ADAM17^{ex/ex}$ mice. (A)** ADAM17 protein expression in organoids obtained from $Apc^{Min/+}$ mice and $Apc^{Min/+}$::$ADAM17^{ex/ex}$ mice were compared with organoids from WT mice. Four independent experiments were performed, and one representative experiment is shown. **(B)** qRT-PCR analysis of mRNA expression of IL-6, IL-6 receptor (IL-6R), IL6ST (also known as gp130), yap-1, lats1, and cyr61 expression in indicated organoids from four independent experiments ($n = 4$). qRT-PCR data were normalized to GAPDH. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ns, not significant by unpaired *t* test with Welch's correction. **(C)** Organoids from $Apc^{Min/+}$ and $Apc^{Min/+}$::$ADAM17^{ex/ex}$ mice were cultured for 72 h in APC tumor medium (untr.) or mixed with DMSO for control as well as in the presence of GI (GI254023X, ADAM10-selective inhibitor, 30 µM), GW (GW280264X, ADAM10- and ADAM17-selective inhibitor, 30 µM), or Marimastat (MM; pan-metalloprotease inhibitor; 100 µM). Supernatants from organoids were measured by ELISA for soluble amphiregulin. Values were normalized to organoid number per well. **, $P < 0.01$; ***, $P < 0.001$ by Kruskal–Wallis test.

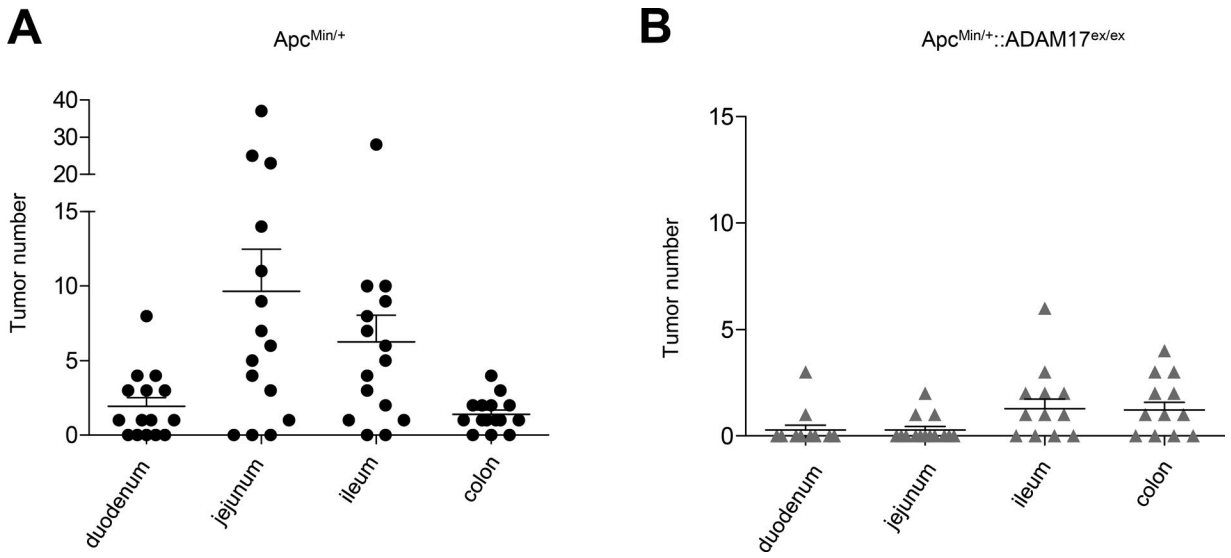

Figure S3. **Tumor distribution in the intestine of $Apc^{Min/+}$ and $Apc^{Min/+}$::$ADAM17^{ex/ex}$ mice.** Distribution of dysplasias within the small intestine from $Apc^{Min/+}$ mice and $Apc^{Min/+}$::$ADAM17^{ex/ex}$ mice. **(A and B)** The number of dysplasias was determined macroscopically within the small intestine (duodenum, jejunum, ileum) and colon of $Apc^{Min/+}$ mice (n = 15; A) and $Apc^{Min/+}$::$ADAM17^{ex/ex}$ mice (n = 14; B).

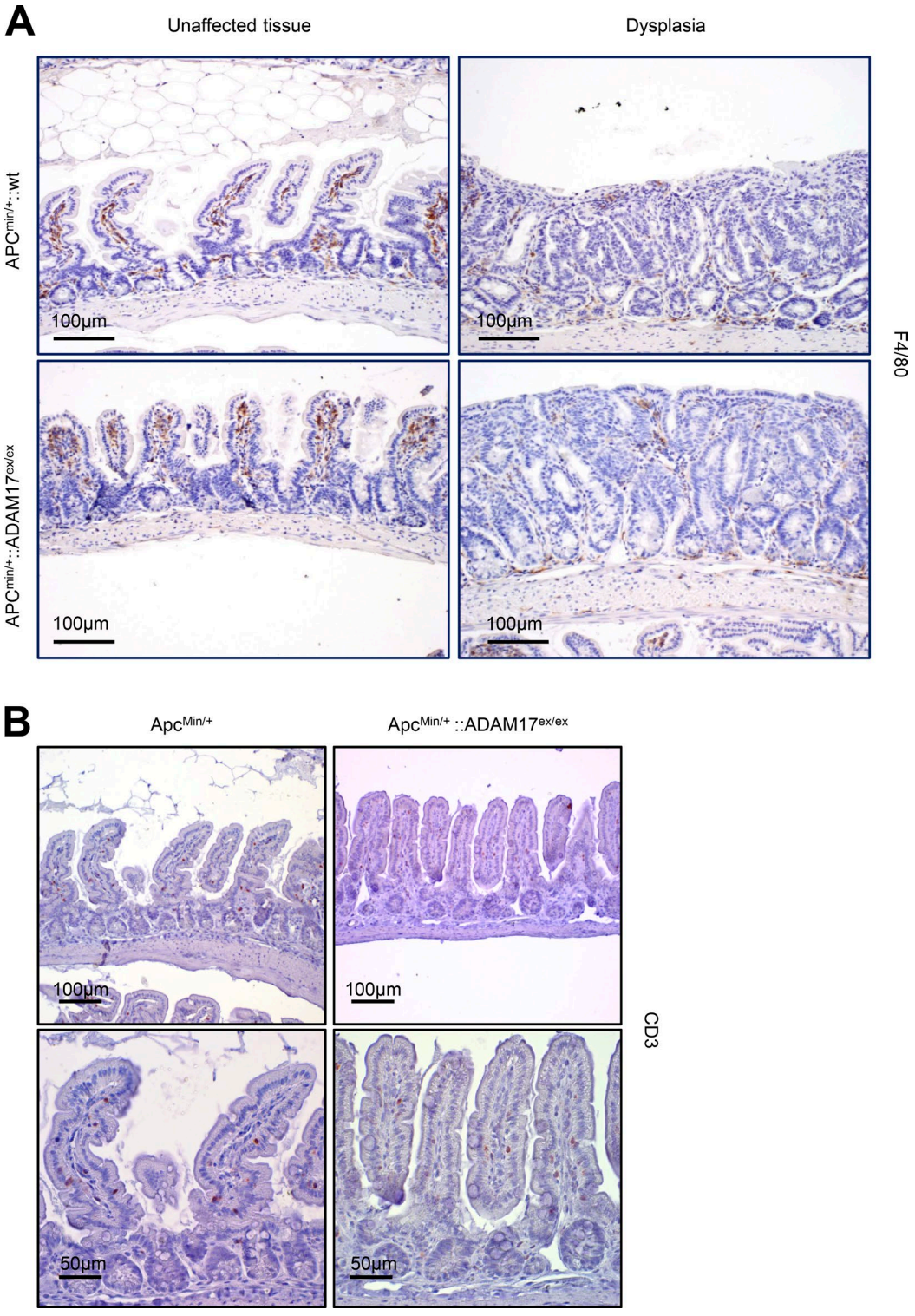

Figure S4. **Infiltration of macrophages and T cells in the intestine of $Apc^{Min/+}$ and $Apc^{Min/+}$::$ADAM17^{ex/ex}$ mice. (A)** Representative IHC staining of unaffected tissue and dysplasia in the small intestine of WT and $Apc^{Min/+}$ and $Apc^{Min/+}$::$ADAM17^{ex/ex}$ mice with antibodies for F4/80. Bars, 100 µm. **(B)** Representative IHC staining of unaffected tissue of the small intestine of $Apc^{Min/+}$ and $Apc^{Min/+}$::$ADAM17^{ex/ex}$ mice with anti-CD3 antibodies. Bars: (top) 100 µm; (bottom) 50 µm.

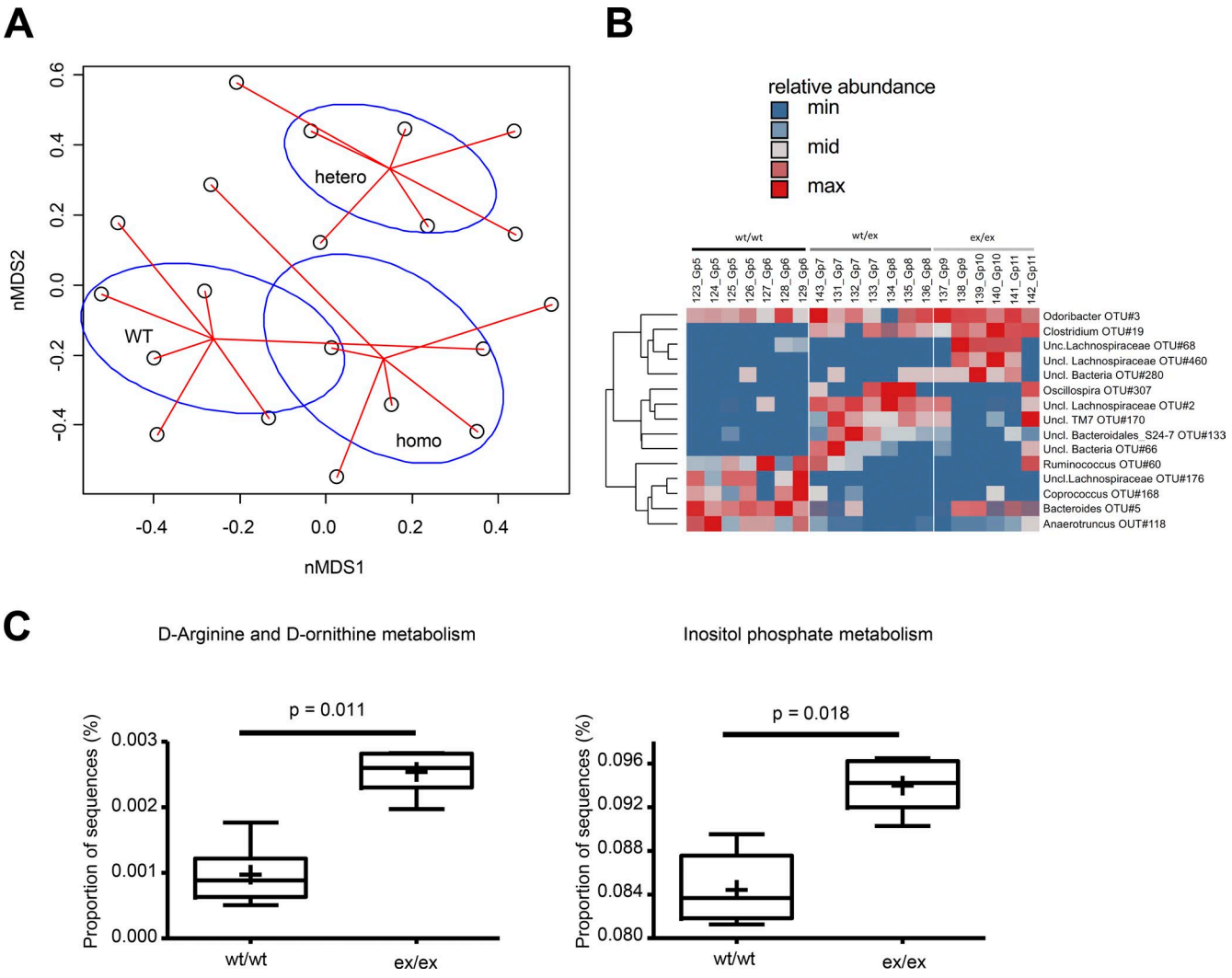

Figure S5. **Loss of ADAM17 alters the gut microbiome. (A)** Nonmetric multidimensional scaling (nMDS) plot of unweighted Unifrac distances (16S rDNA V3/V4) of fecal microbial communities. Note that ADAM17 wt/wt (wt), wt/ex (hetero), and ex/ex (homo) group separation was confirmed by multivariate statistics (ANOSIM and AMOVA). **(B)** Indicator OTU analysis of the respective genotypes depicted by heat map. Clustering of *z*-score normalized OTU abundances was performed using unweighted pair-group method with arithmetic mean (UPGMA). ADAM17 wt/wt mice (n = 10), ADAM17wt/ex mice (n = 10), ADAM17ex/ex mice (n = 12). **(C)** Box plot showing inferred functions of KEGG categories significantly different in WT and ADAM17$^{ex/ex}$ (ex/ex) mice. Boxes represent relative proportion of sequences assigned to predicted functions. Whiskers denote 5th–95th percentiles. Line within box represents median values with mean as a plus sign. The significances of difference denoted in the figure were obtained by White's nonparametric test. Benjamin–Hochberg false discovery rate was used to correct for multiple testing.