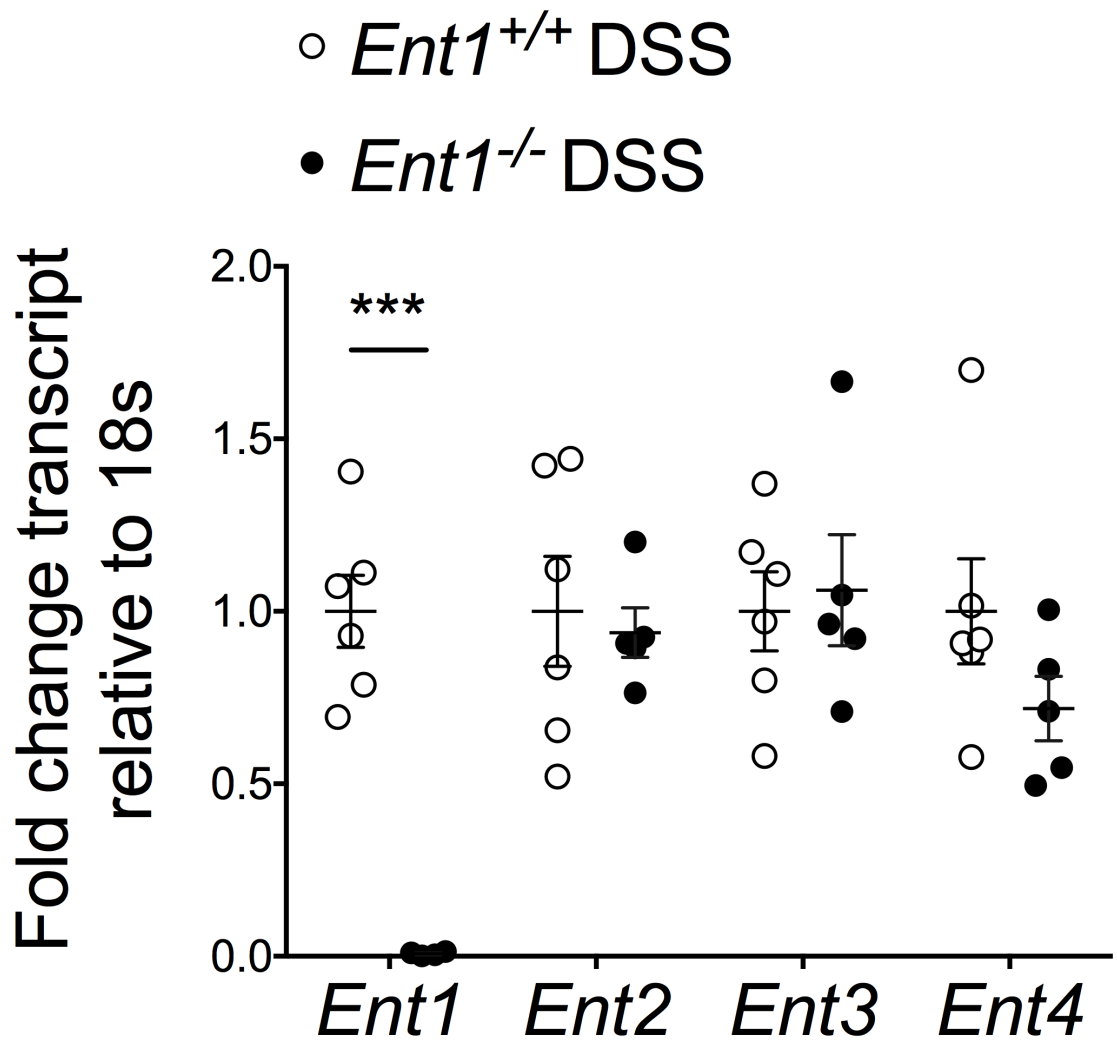
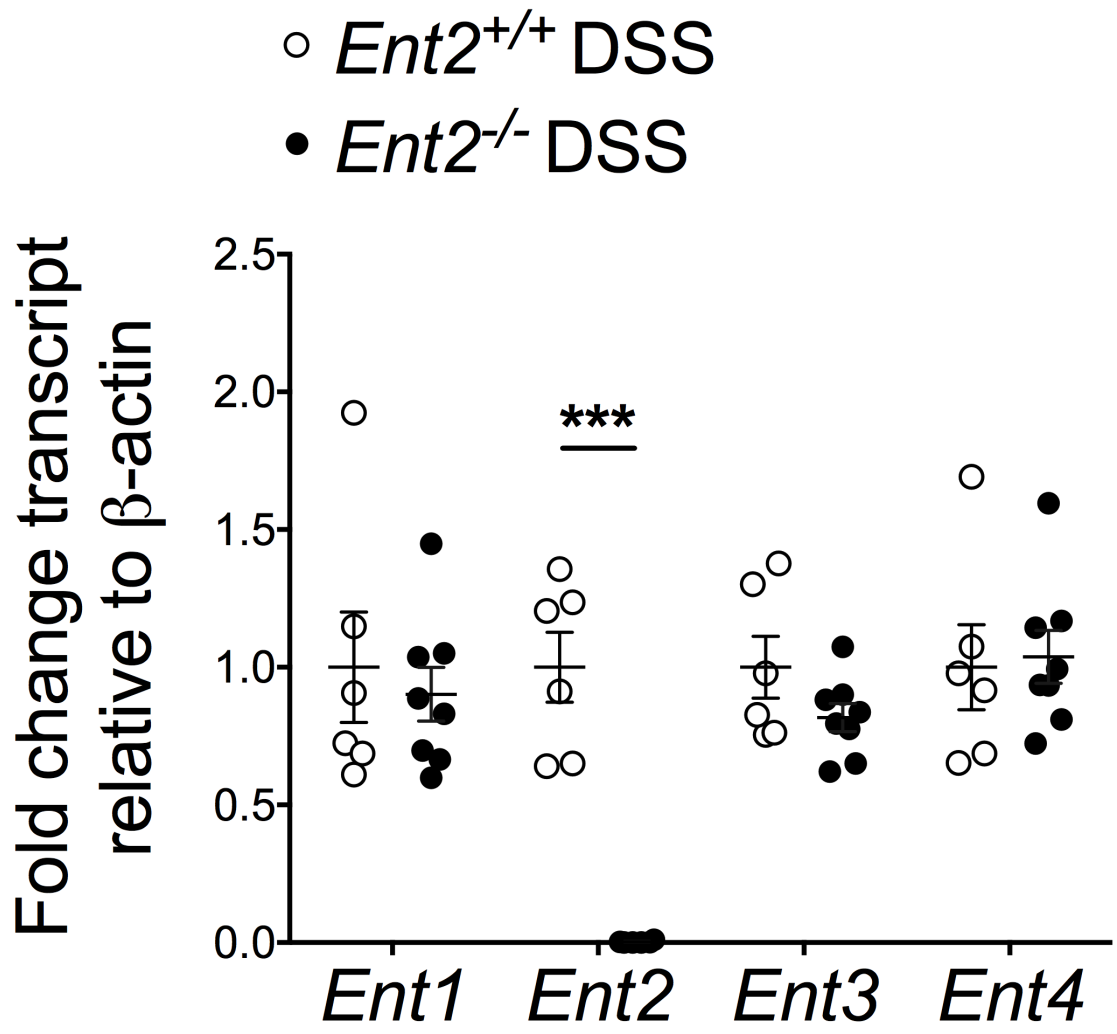
Supplemental Information

Supplemental Figure 1



Supplemental Figure 1. *Ent* expression in *Ent1* deficient mice during DSS. Gender, age and weight-matched *Ent1* deficient mice (*Ent1*$^{-/-}$) or C57BL/6 wildtype controls (*Ent1*$^{+/+}$) were exposed to DSS. Following 7 days whole colon was harvested and total RNA extracted. Taqman RT-PCR was performed. mRNA transcript levels were calculated relative to 18s and are expressed as the fold change compared to DSS treated wildtype mice. n=4-6 mice/group from 1 independent experiment. Statistical significance was determined by unpaired Student's *t*-test. ***P<0.0001.

Supplemental Figure 2



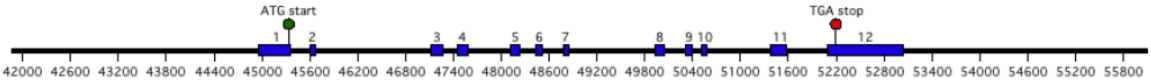
Supplemental Figure 2. *Ent* expression in *Ent2* deficient mice during DSS. Gender, age and weight-matched *Ent2* deficient mice (*Ent2*$^{-/-}$) or wildtype controls (*Ent2*$^{+/+}$) were exposed to DSS. Following 7 days whole colon was harvested and total RNA extracted. RT-PCR was performed using QuantiTect primers and mRNA transcript levels were calculated relative to β-actin. Data is expressed as the fold change compared to DSS treated wildtype mice. n=6-8 mice/group from 1 independent experiment. Statistical significance was determined by unpaired Student's *t*-test. ***P<0.0001.

Generation of a novel Ent2 flox mouse

In order to study potential tissue specific effects of Ent2 during intestinal inflammation we commissioned the generation of an Ent2 flox mouse (Ozgene). The flox strategy was designed by Ozgene using the following parameters. The Ent2 gene accession number NM_007854 was used to identify corresponding mRNA/cDNA sequences in GenBank with the following accession numbers BC048958, AK144451, AK010950, AF183397. The annotated genomic sequence was obtained from Ensembl Mouse Genome Server. The gene is located on chromosome 19 with the transcript ID of ENSMUSG00000024891. Intron/exon structure was confirmed by alignment of mouse mRNA sequence (BC048958) with the Ensembl chromosome 19 sequence. The Ent2 gene has 12 exons spread over 9kb (Supplementary Figure 3A). Following sequence analysis it was determined that exon 2 would be flanked with loxP sites to allow for Cre-mediated deletion (Supplementary Figure 3B). Deletion of exon 2 disrupts the signal peptide and a transmembrane helix thereby introducing a frameshift to produce an early stop codon in the Ent2 gene (Supplementary Figure 3B). PCR products for cloning were generated from C57BL/6 genomic DNA. An Ozgene proprietary plasmid was used to construct the targeting vector. Electroporation was used to introduce the linearised targeting vector into C57BL/6 Bruce4 ES cells. Surviving colonies were screened by Southern blot hybridization. Targeted clones were expanded and cells injected into mouse blastocysts. Injected embryos were implanted into pseudo-pregnant mice and pups were analyzed by Southern blot for verification of vector incorporation. Appropriate pups were delivered to our lab for genetic verification and breeding. Genotyping assays were designed and performed by GeneTyper Inc. First generation mice were heterozygous for Ent2 flox expression (Supplementary Figure 3C). Heterozygous mice were bred to generate mice with homozygous Ent2 flox expression (Supplementary Figure 3C). The Ent2 floxed mouse line is maintained as a homozygous line for breeding with Cre strains.

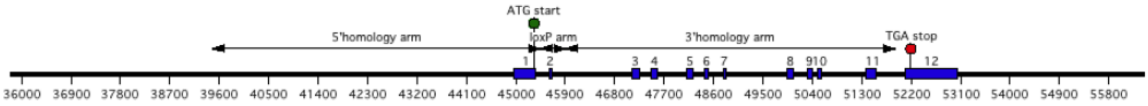
Supplemental Figure 3

A: Ent2 gene

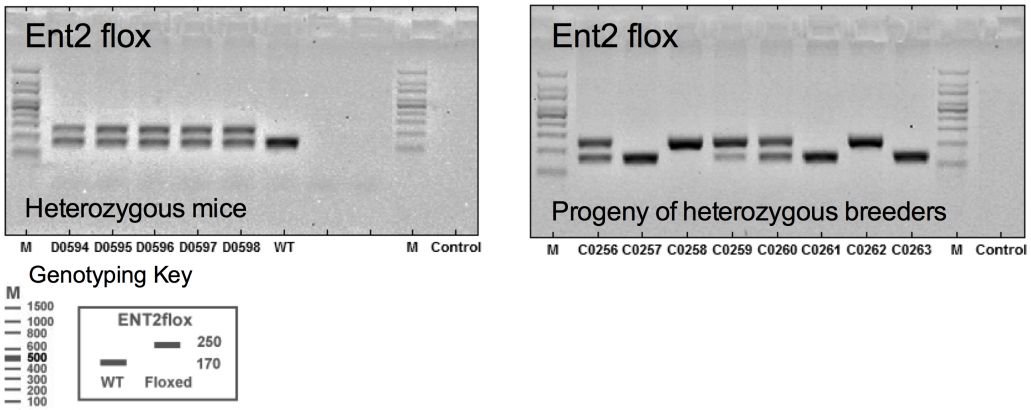


B: Ent2 flox strategy:

loxP sites flank exon 2

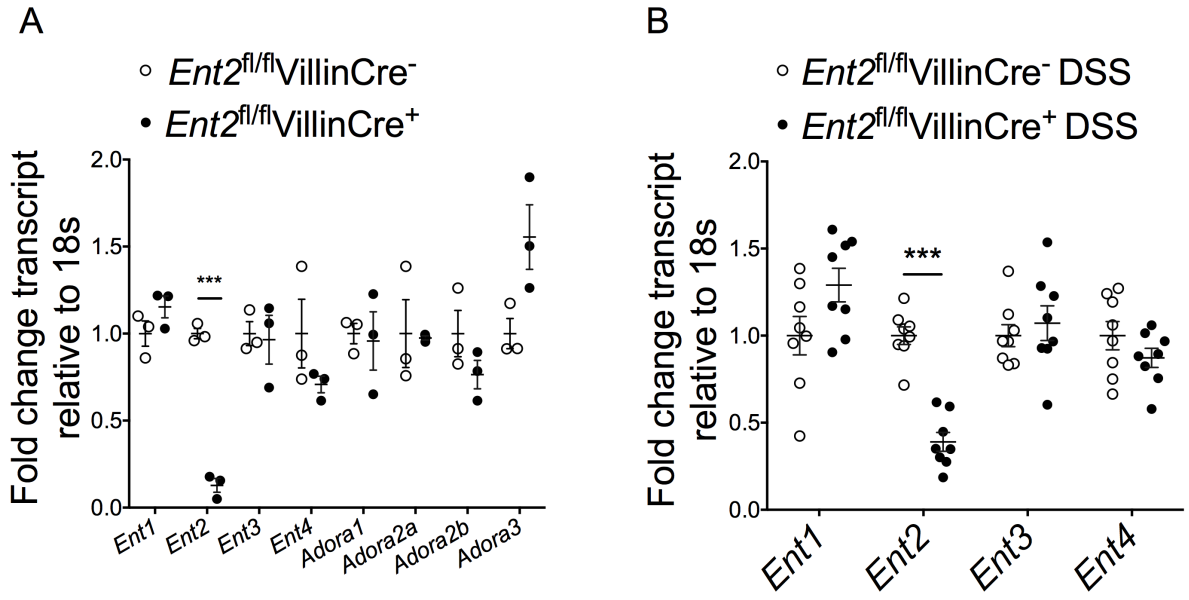


C: Ent2 flox genotyping



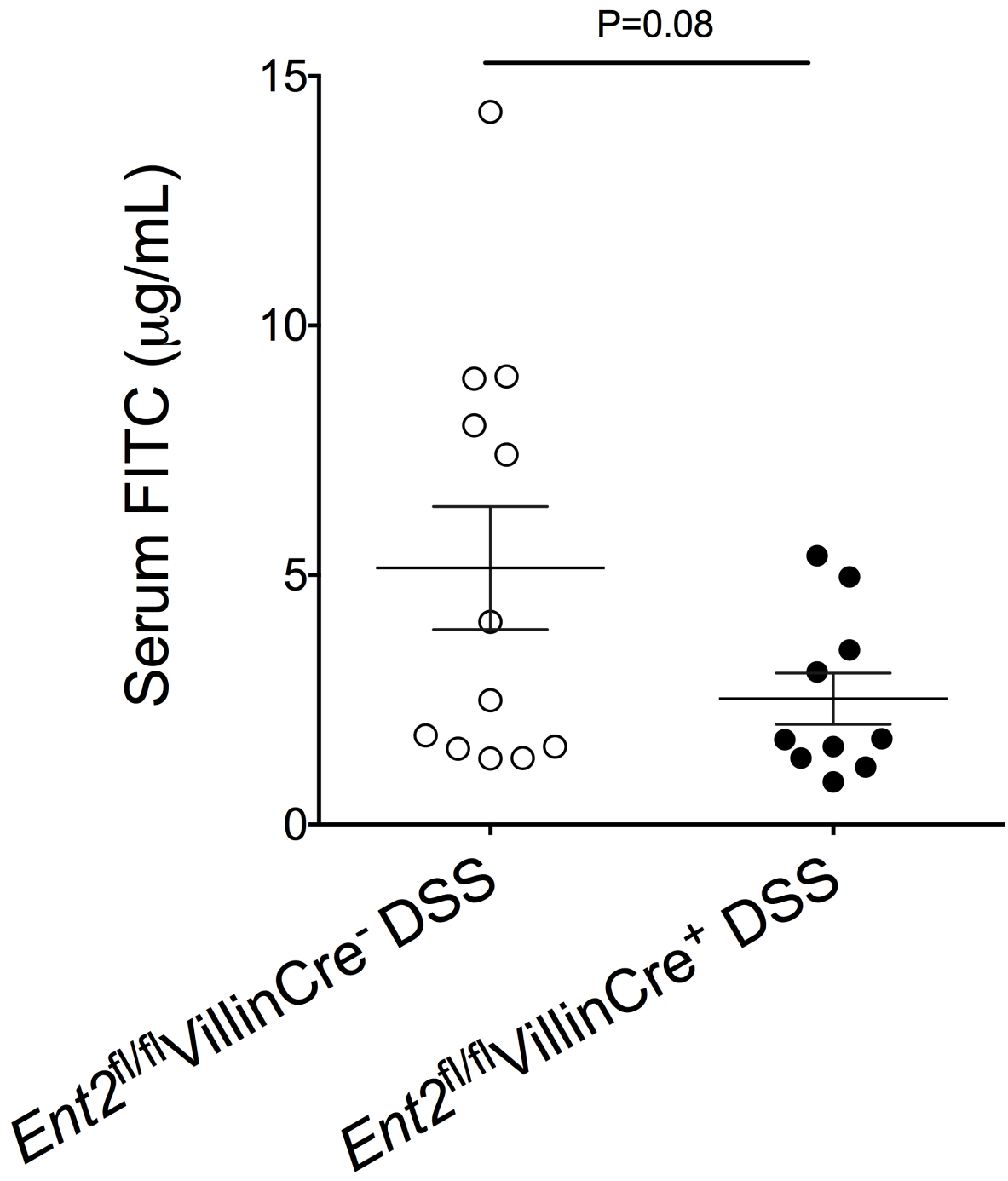
Supplemental Figure 3. Generation of a novel Ent2 flox mouse. The Ent2 flox strategy was designed by Ozgene. (**A**) The mouse Ent2 mRNA sequence (BC048958) was aligned with the Ent2 gene sequence on chromosome 19 (Ensembl) confirming that the Ent2 gene has 12 exons over 9kb. (**B**) The flox strategy was designed to flank exon 2 with loxP sites. (**C**) Pups produced following insertion of the Ent2 targeting vector into ES cells, expansion of viable colonies, injection of appropriate cells into mouse blastocysts and implantation into pseudo-pregnant mice were provided by Ozgene. Tails were clipped litters and sent for genotyping PCR (GeneTyper Inc.). Pups provided were determined to be heterozygous for Ent2 flox. Heterozygous mice were bred and the resulting pups were genotyped showing generation of homozygous flox mice. This line is maintained using homozygous flox breeding pairs.

Supplemental Figure 4



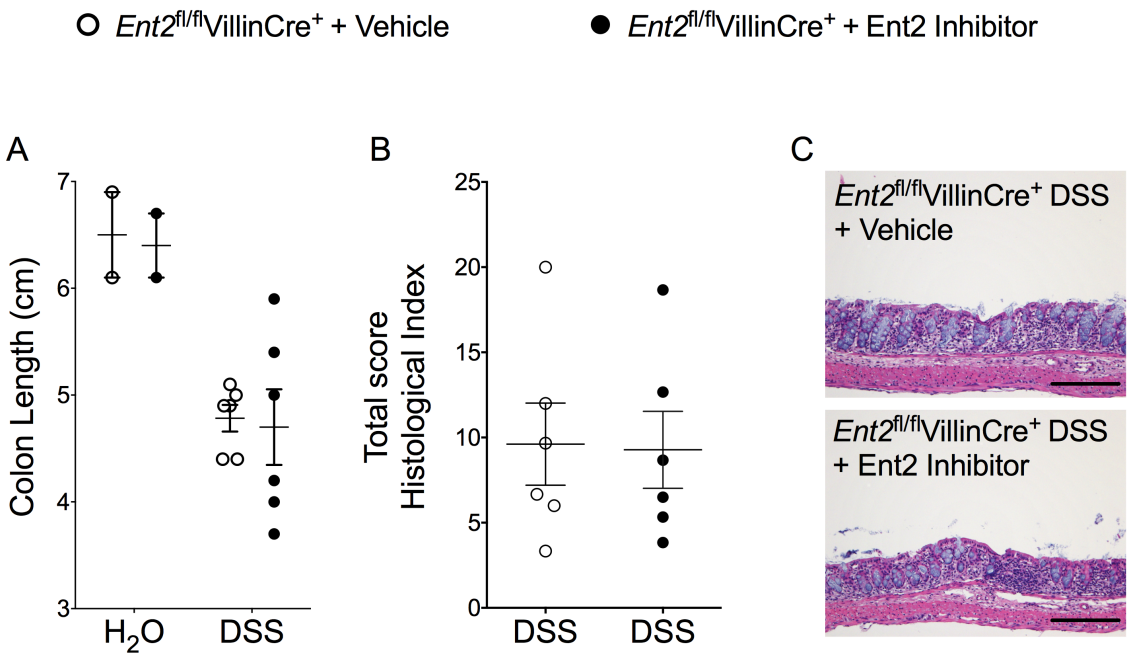
Supplemental Figure 4. *Ent* expression in *Ent2*$^{fl/fl}$VillinCre mice. (**A**) Whole colon was removed from gender and age matched mice with *Ent2* deletion in the intestinal epithelium (*Ent2*$^{fl/fl}$VillinCre^{+}) or wildtype littermate controls (*Ent2*$^{fl/fl}$VillinCre^{-}) for profiling of Ent and adenosine receptor expression. Total RNA was extracted and Taqman RT-PCR was performed with specific primers for Ent1-4 and adenosine receptors (A1: *Adora1*, A2A: *Adora2a*, A2B: *Adora2b* and A3: *Adora3*). mRNA transcript levels were calculated relative to 18s. Data is expressed as the fold change compared to wildtype mice (*Ent2*$^{fl/fl}$VillinCre^{-}). n=3 mice/group. (**B**) Gender, age and weight-matched mice with *Ent2* deletion in the intestinal epithelium (*Ent2*$^{fl/fl}$VillinCre^{+}) or matched wildtype littermate controls (*Ent2*$^{fl/fl}$VillinCre^{-}) were given DSS. Following 7 days whole colon was harvested and total RNA extracted. Taqman RT-PCR was performed and mRNA transcript levels were calculated relative to 18s. Data is expressed as the fold change compared to DSS treated wildtype mice. n=8 mice/group. In all cases, statistical significance was determined by unpaired Student's *t*-test. ***P<0.0001.

Supplemental Figure 5



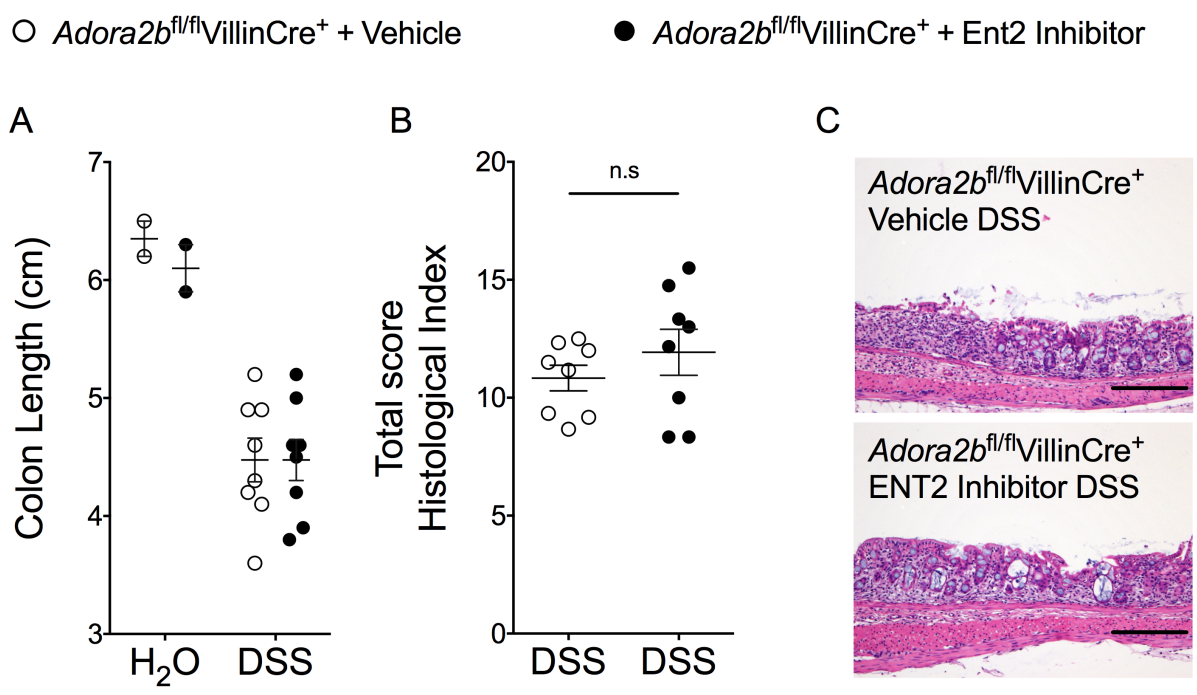
Supplemental Figure 5. Ent2 expression on the intestinal epithelium increases epithelial permeability in experimental colitis. Mice with Ent2 deletion in the intestinal epithelium (*Ent2*$^{fl/fl}$VillinCre^{+}) or matched wildtype littermates (*Ent2*$^{fl/fl}$VillinCre^{-}) were given water or DSS. FITC-dextran was orally gavaged (0.6mg/g at 80mg/mL) prior to sacrifice on day 7 post DSS. Serum was harvested and fluorescence measurement used to determine FITC concentration. Results are from 2 independent experiments. n=10-12 mice/group.

Supplemental Figure 6



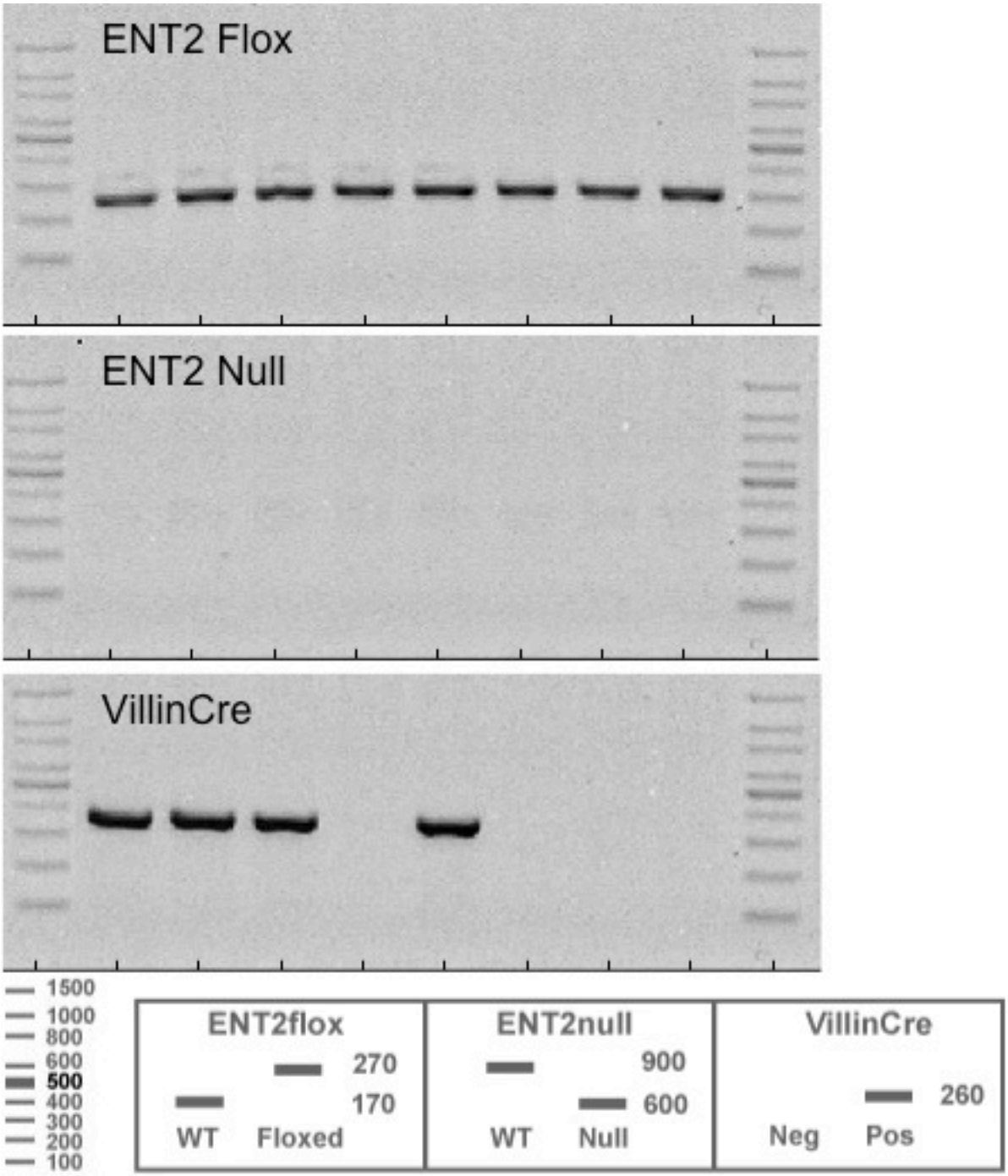
Supplemental Figure 6. Specific pharmacological inhibition of Ent2 requires Ent2 expression on the intestinal epithelium to mediate protection during experimental colitis. Matched mice with Ent2 deletion on the intestinal epithelium (*Ent2*$^{fl/fl}$VillinCre^{+}) were treated with soluflazine (Ent2 inhibitor, 7.7µg/kg, Alzet pump) or vehicle 1 day prior to exposure to DSS or water. (**A**) Following sacrifice on day 7 colons were harvested and measured. (**B**) Bar graph of histological scores for each group provided by a pathologist blinded to the groups and the study. (Bar represents 200µm; images acquired at 10X). (**C**) Representative histological sections from whole colon harvested on day 7 after DSS. Results are displayed as mean±SEM (n=2-6 mice/group from 1 independent experiment). Two way ANOVA with post-hoc Tukey's multiple comparison was used to determine statistical change in colon length, in all other cases Student's *t*-test was used.

Supplemental Figure 7



Supplemental Figure 7: Specific pharmacological inhibition of Ent2 requires A2B receptor expression on the intestinal epithelium to mediate protection during experimental colitis. Matched mice with A2B receptor deletion on the intestinal epithelium (*Adora2b*$^{fl/fl}$VillinCre^{+}) were treated with soluflazine (Ent2 inhibitor, 7.7µg/kg, Alzet pump) or vehicle 1 day prior to exposure to DSS or water. (**A**) Following sacrifice on day 7 colons were harvested and measured. (**C**) Bar graph of histological scores for each group provided by a pathologist blinded to the groups and the study. (**D**) Representative histological sections from whole colon harvested on day 7 after DSS (Bar represents 200µm; images acquired at 10X). Results are displayed as mean±SEM (n=2-8 mice/group from 1 independent experiment). Two way ANOVA with post-hoc Tukey's multiple comparison was used to determine statistical change in colon length, in all other cases Student's *t*-test was used.

Supplemental Figure 8



Supplemental Figure 8. Genotyping PCR on *Ent2*$^{fl/fl}$VillinCre mice. Upon weaning, tails were clipped from *Ent2*$^{fl/fl}$VillinCre mouse litters and sent for genotyping PCR (GeneTyper Inc.). PCR was performed to test for the presence of Ent2flox, off target Ent2 deletion (Null, no band when targeted Ent2 deletion achieved) and Cre expression (VillinCre).