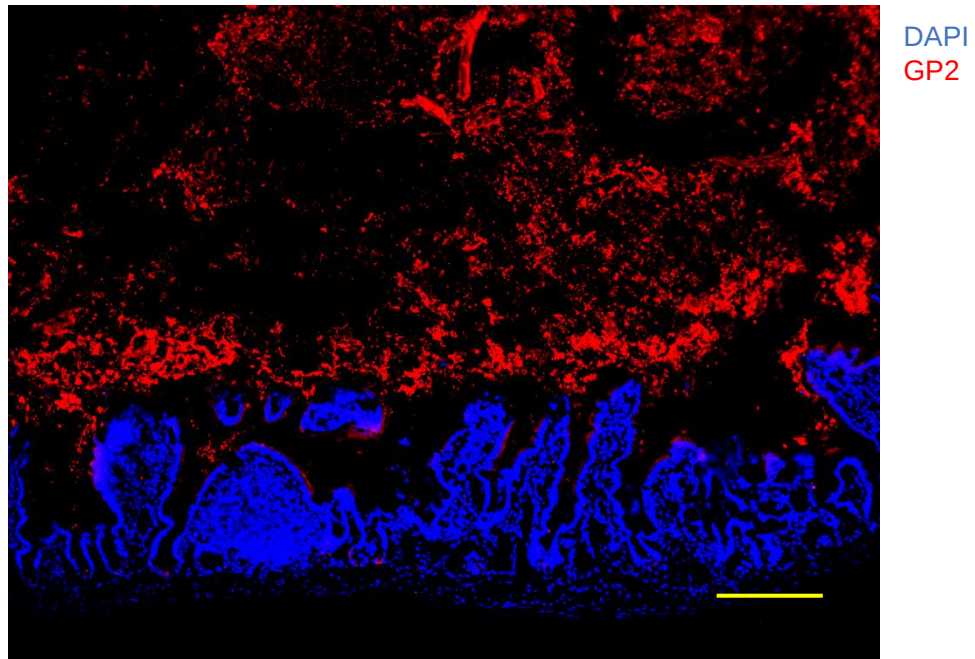


Supplementary Information for

**Pancreatic Glycoprotein 2 is a First Line of Defense for Mucosal
Protection in Intestinal Inflammation**

Kurashima Y et al

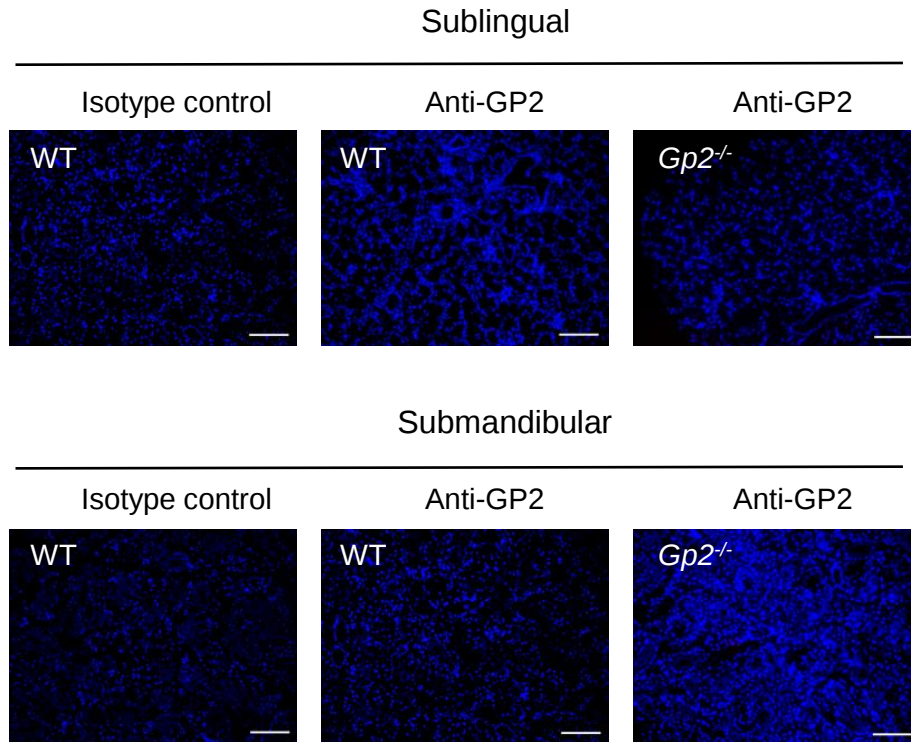
Supplementary Figure 1



Dissemination of GP2 in the intestinal lumen.

Immunohistochemical analysis of GP2 distribution in the gastrointestinal tract with luminal contents were shown. GP2 (red), DAPI (blue). Scale bars, 100 μm . Data are representative of at least three independent experiments.

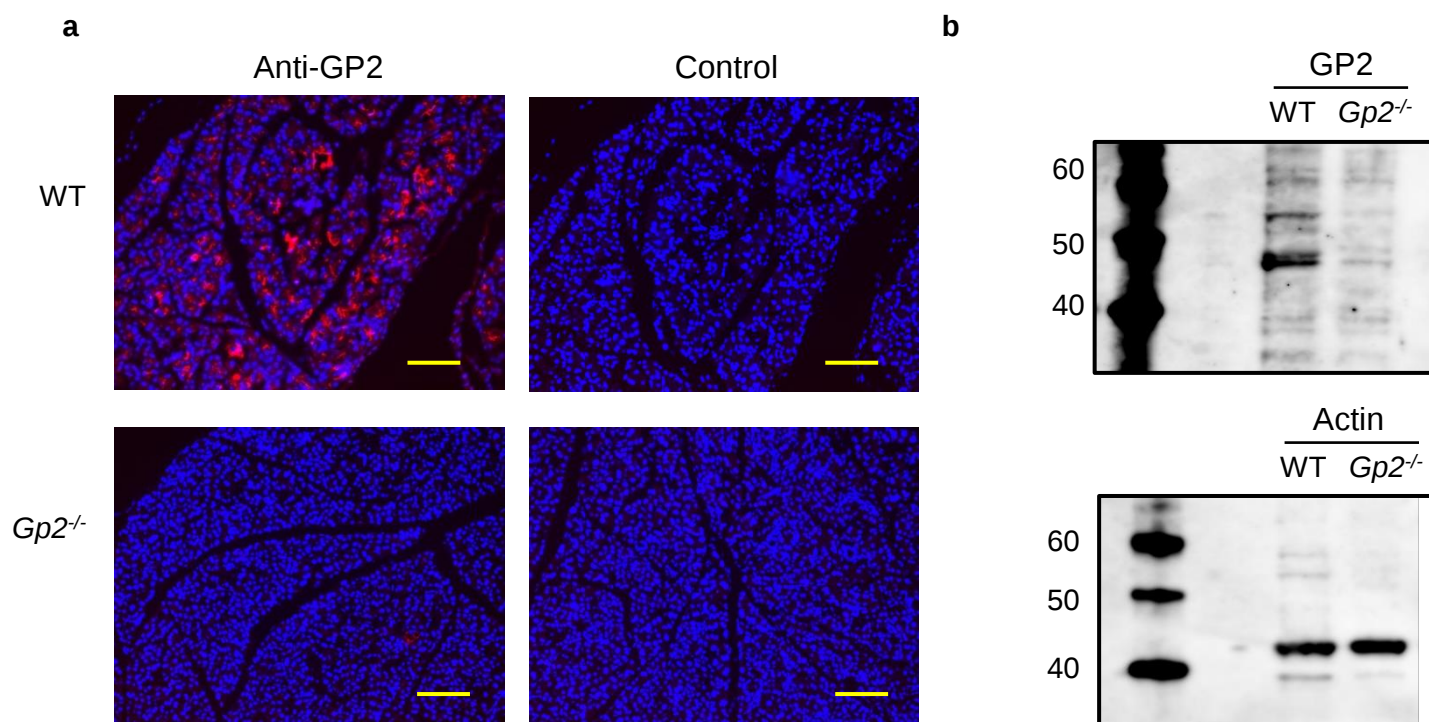
Supplementary Figure 2



No observation of GP2 in the salivary glands.

Immunohistochemical analysis of GP2 distribution in the sublingual and submandibular areas of an intact mouse. Representative data of three independent experiments were shown. GP2 (red) and DAPI (blue). Scale bars, 100 μ m.

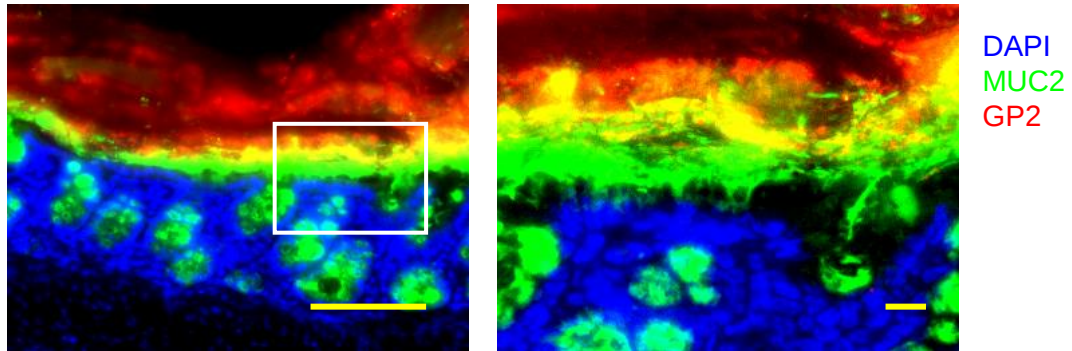
Supplementary Figure 3



Detection of pancreatic GP2.

a, Pancreas from WT and *GP2*^{-/-} mice were stained with or without anti-GP2 polyclonal antibody. Scale bars: 100 μ m. Data are representative of two independent experiments. **b**, Expression of GP2 (Thermo Fisher Scientific, polyclonal, #PA5-88542, 1:250) and actin (Merck Millipore, clone C4, #MAB1501R, 1:3000) in pancreas cells was examined by western blot analysis. Data are representative of two independent experiments.

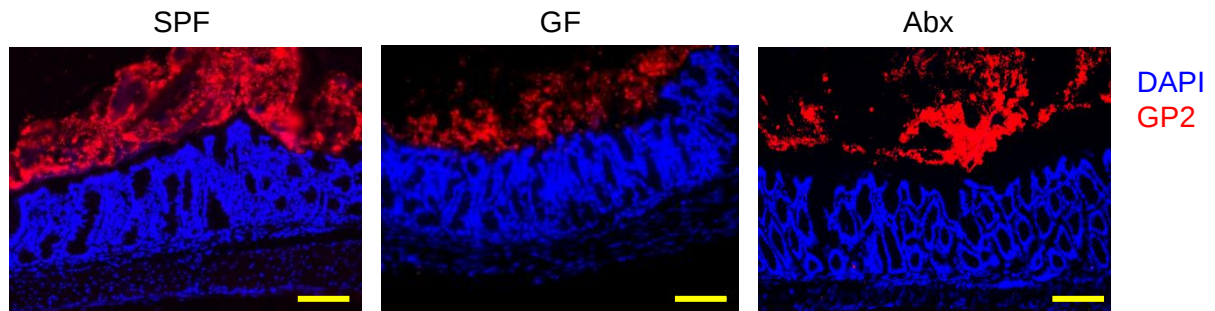
Supplementary Figure 4



Localization of pancreatic GP2 in the mucus layer.

Immunohistochemical analysis of GP2 distribution in the mucus layer was performed. Representative data of four independent experiments are shown. GP2 (red), MUC2 (green), and DAPI (blue). Scale bars, 100 μm (left panel) and 20 μm (right panel).

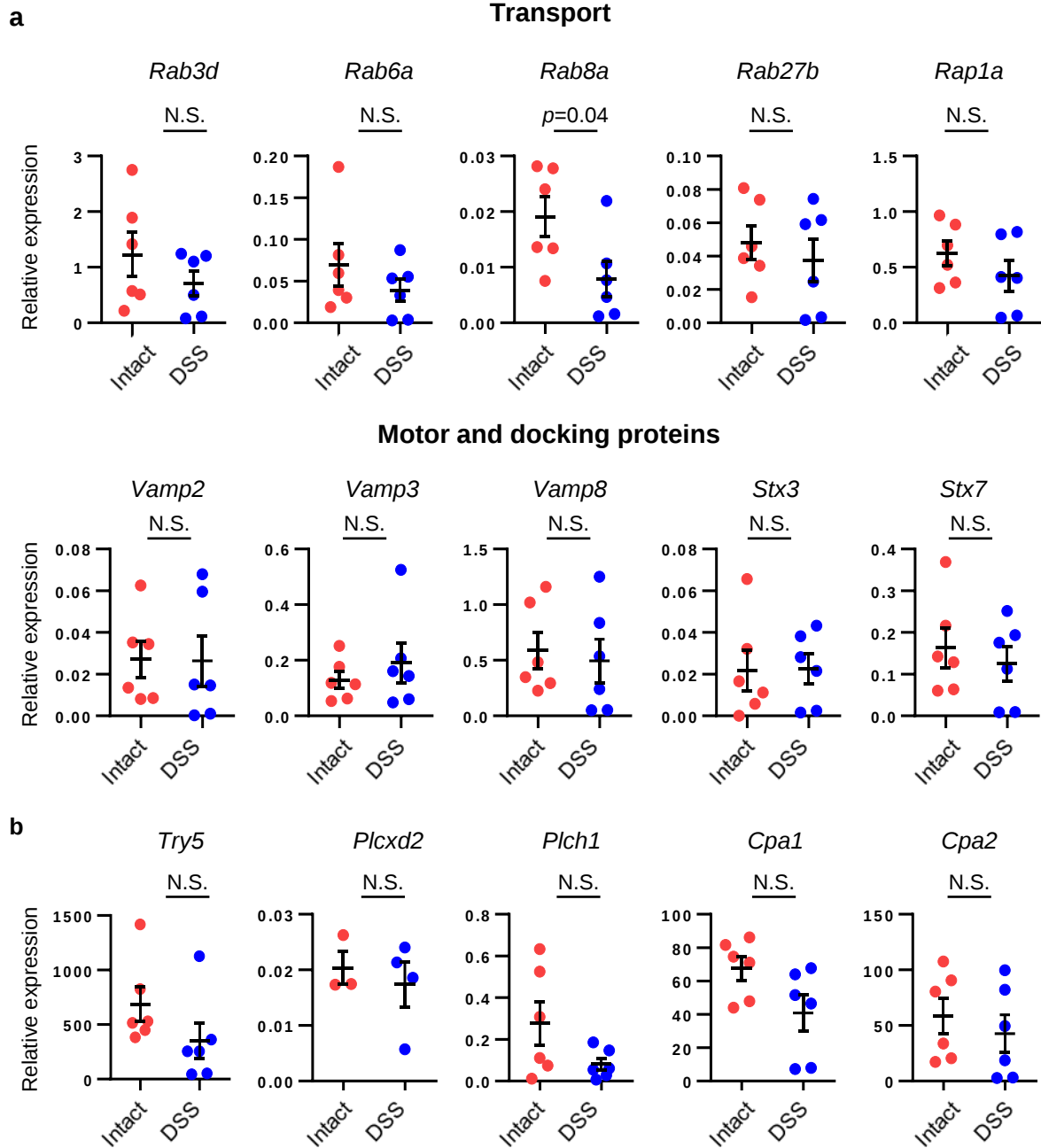
Supplementary Figure 5



Detection of luminal GP2 in germ free conditions.

GP2 distribution in the colon in SPF, germ-free (GF), antibiotics (Abx) treated mice are shown. Representative data of three independent experiments are shown. GP2 (red), DAPI (blue). Scale bars, 100 μm .

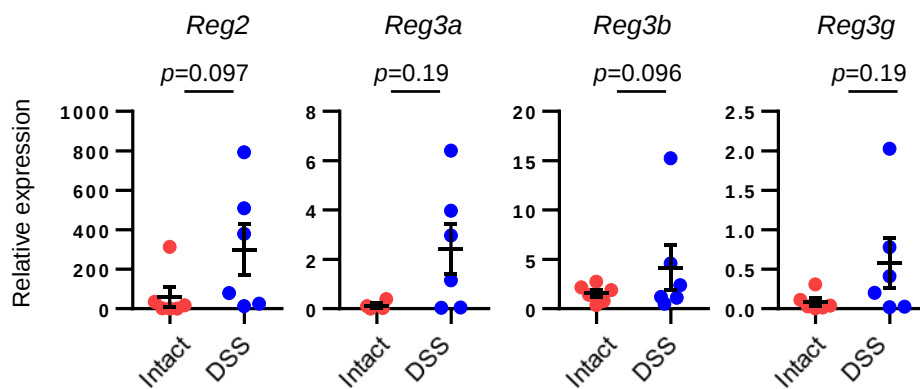
Supplementary Figure 6



Granular release of pancreatic GP2 in colitis.

a, Expressions of genes encoding granular transport proteins (*Rab3d*, *Rab61*, *Rab8a*, *Rab27b*, *Rap1a*) and motor and docking proteins (*Vamp2*, *Vamp3*, *Vamp8*, *Stx3*, *Stx7*) in the pancreas from intact and DSS treated mice were determined by qPCR (n=6). N.S. indicates not significant (two-tailed unpaired *t*-test). Data are presented as mean values $\pm$ SEM. **b**, Expressions of genes encoding enzymes involved in glycosylphosphatidylinositol anchor cleavage (*Try5*, *Plcxd2*, *Plch1*, *Cpa1*, and *Cpa2*) were determined by qPCR. Relative expression of mRNA was determined by quantitative RT-PCR and normalized to the expression of *Gapdh* (n=6). N.S. indicates not significant (two-tailed unpaired *t*-test). Data are presented as mean values $\pm$ SEM. Source data are provided as a Source Data file.

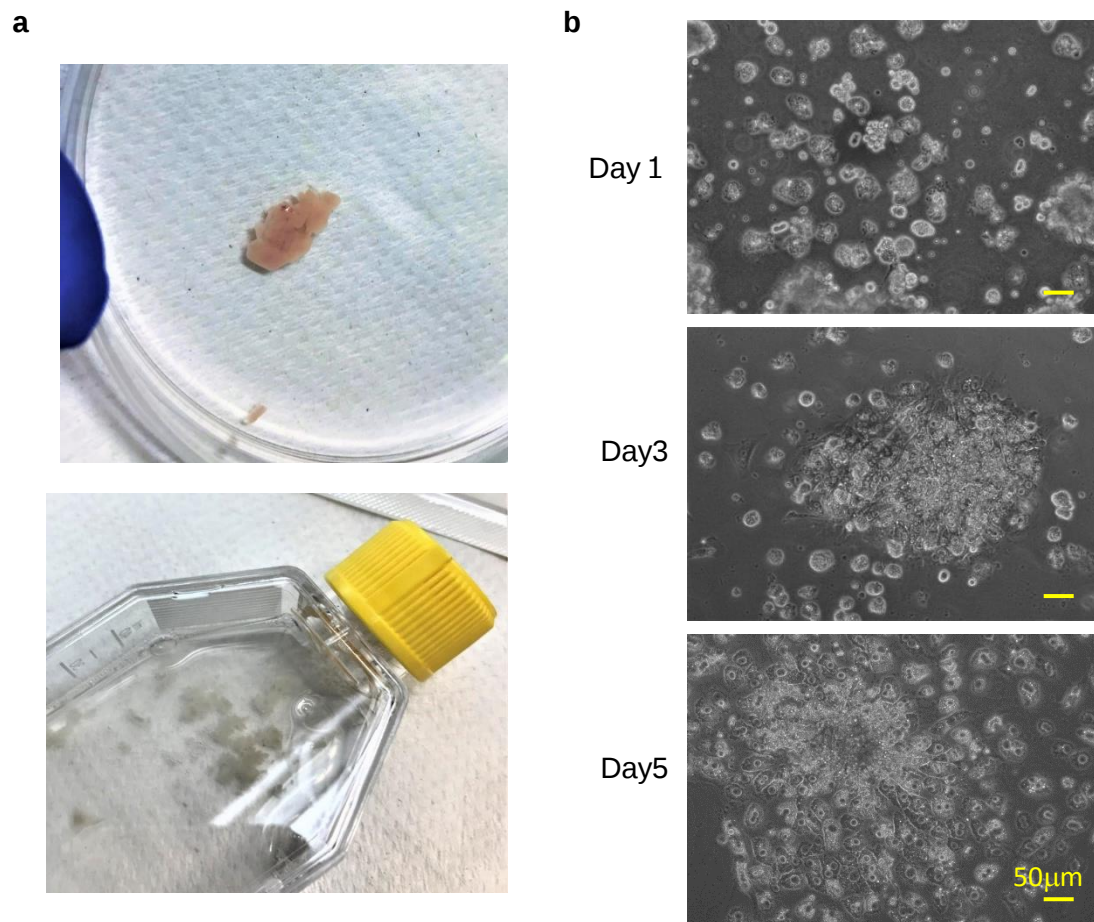
Supplementary Figure 7



Antibiotic protein expressions in pancreas.

a, Expressions of genes encoding Reg proteins (*Reg2*, *Reg3a*, *Reg3b*, *Reg3g*) in the pancreas from intact and DSS treated mice were determined by qPCR. Relative expression of mRNA was determined by quantitative RT-PCR and normalized to the expression of *Gapdh* (n=6). Data are presented as mean values +/- SEM. Source data are provided as a Source Data file.

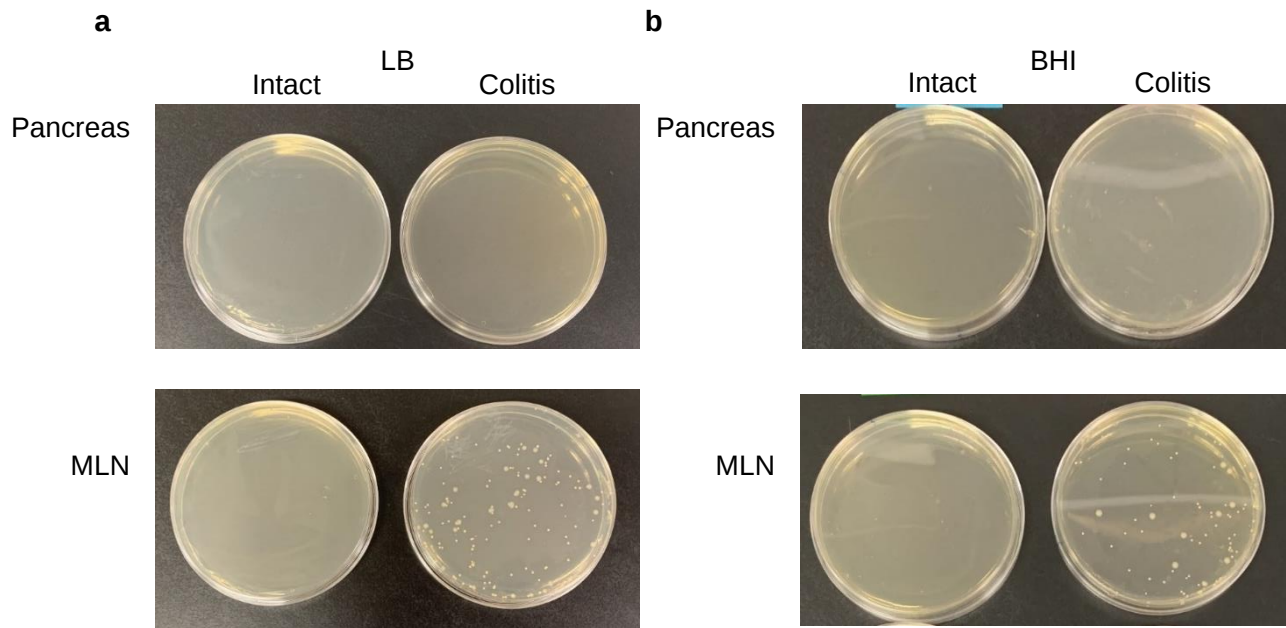
Supplementary Figure 8



***In vitro* culture of pancreatic acinar cells.**

a, Pancreas was isolated from mice (upper panel) and enzymatically digested as described in lower panel. **b**, Cultured acinar cells in day1, 3, 5 were shown. Data are representative of four independent experiments.

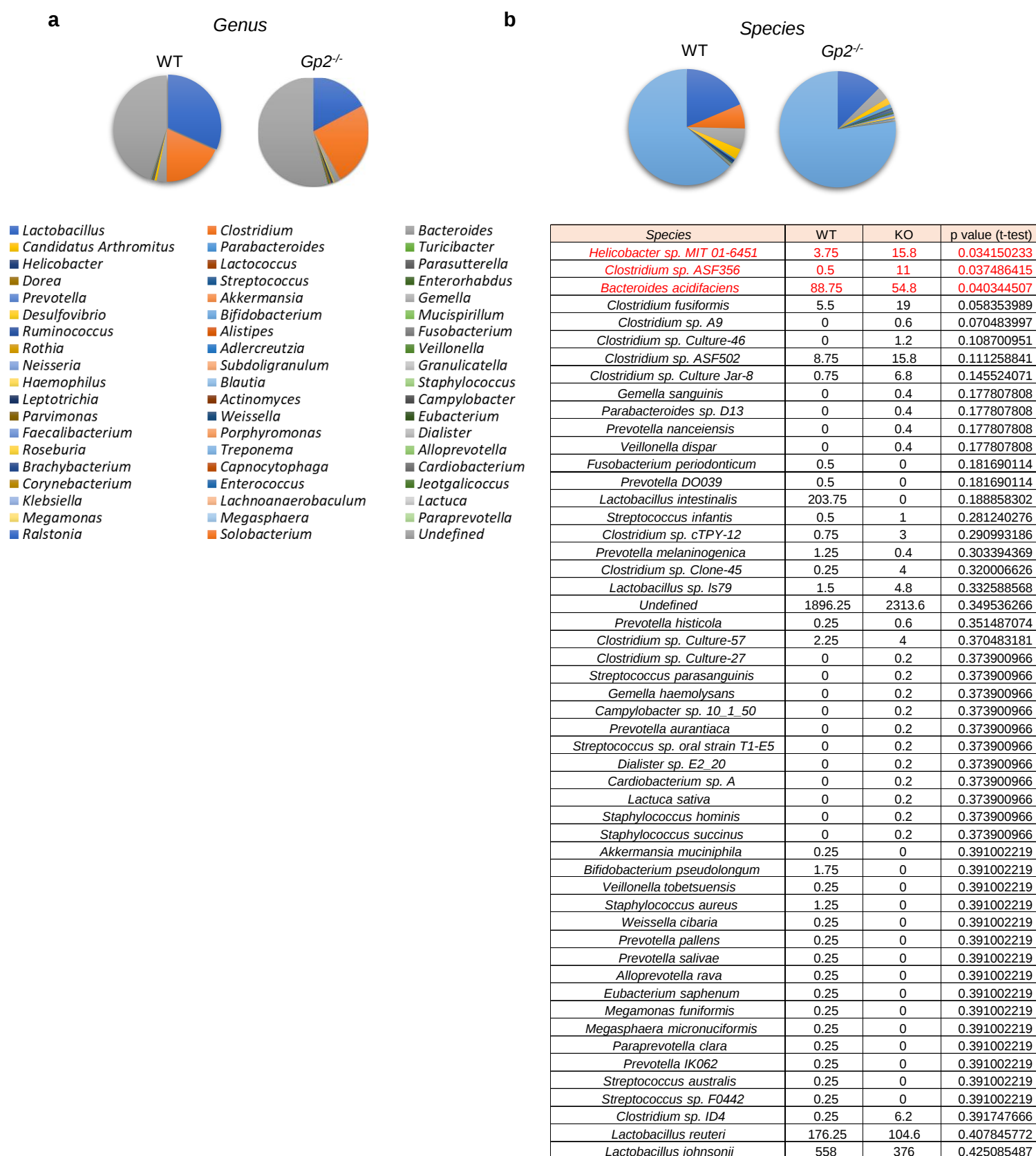
Supplementary Figure 9



Bacterial translocation in mesenteric lymph nodes and pancreas.

Mesenteric lymph nodes (MLN) and pancreas were isolated from intact and DSS-treated mice, enzymatically digested, and then cultured on LB (**a**) and BHI (**b**) plates. Representative photographs are shown.

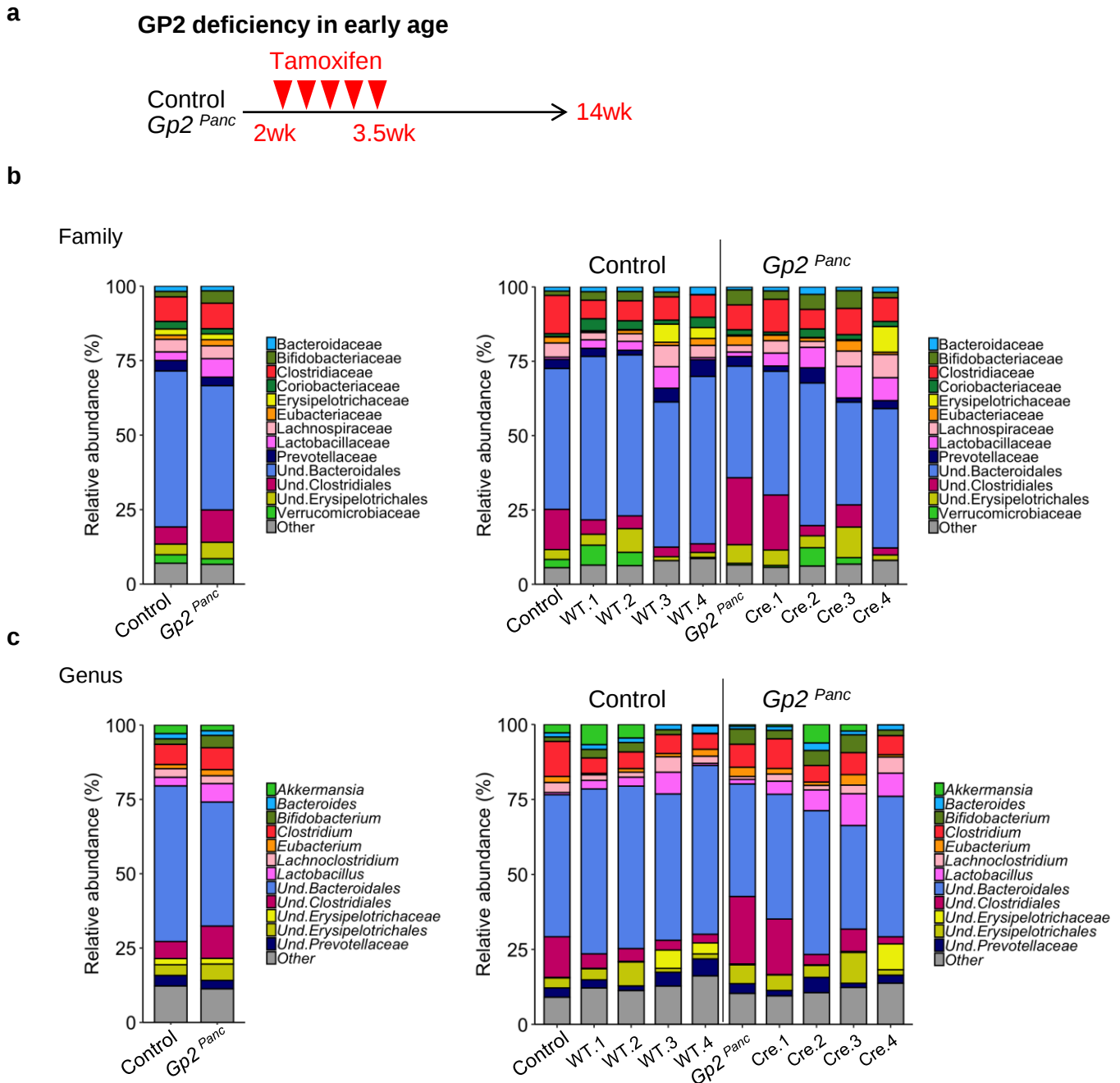
Supplementary Figure 10



Genus and species levels of 16S rRNA gene sequencing analysis.

(a and b) Results of 16S rRNA gene sequencing analysis of the feces of WT (n = 5) and *Gp2*^{-/-} (n = 4) mice. Bacterial species with significant differences are shown in red (unpaired two-tailed Student's *t*-test).

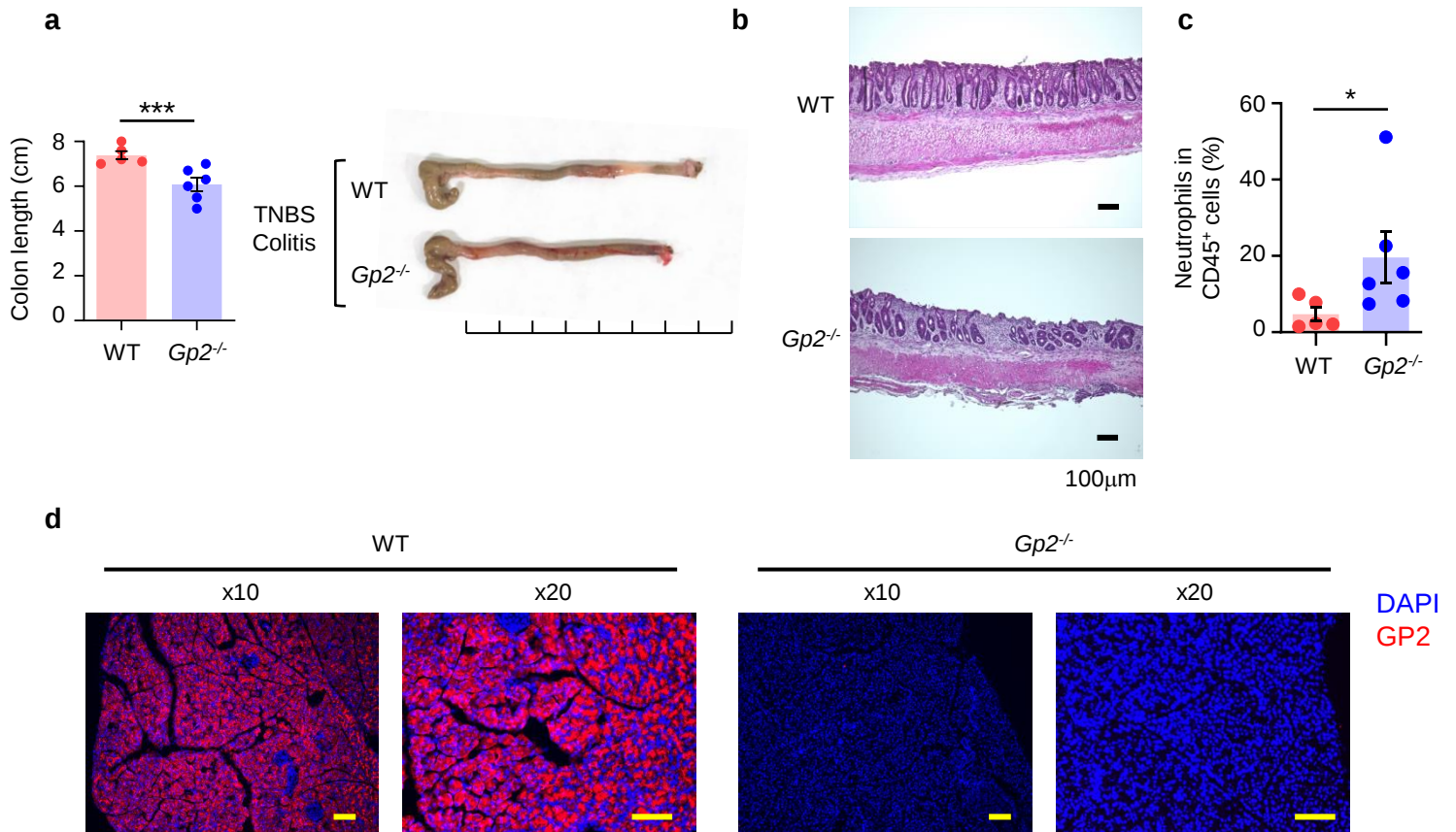
Supplementary Figure 11



No alteration of commensal microbiota by GP2-deficiency in the early age.

a, Experimental protocol for inducing GP2 deficiency in young mice (2–3.5 weeks old). Feces was collected at week 14. Family- (**b**) and genus-level (**c**) 16S rRNA gene sequencing data are shown as averages for the whole group (left) and for individual mice (right).

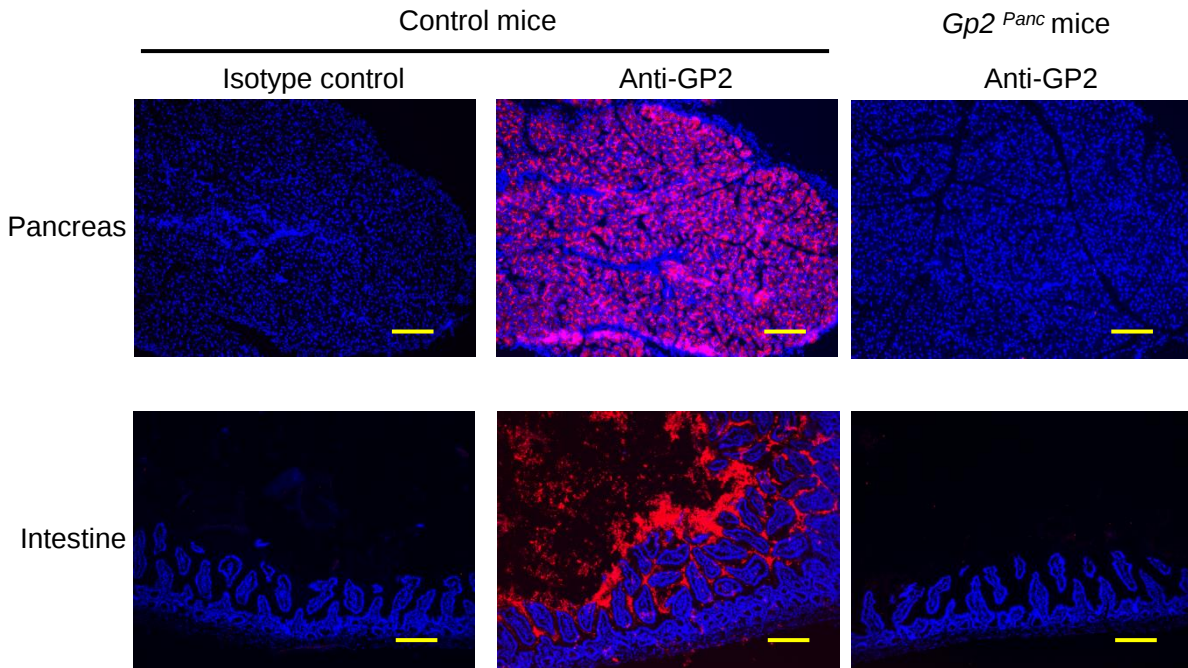
Supplementary Figure 12



Deletion of systemic GP2 results in severe TNBS-induced colitis.

a, Changes in colon length and photographs of representative colons of WT (n=5) and $Gp2^{-/-}$ (n=6) mice with TNBS-induced colitis. ***: $p = 0.0065$ (two-tailed Mann–Whitney U test). Data are presented as mean values $\pm$ SEM. **b**, Hematoxylin and eosin staining of colon at day 5 of TNBS treatment in wild-type (WT) and $Gp2^{-/-}$ mice. Data are representative of two independent experiments. **c**, Percentage of neutrophils in the CD45⁺ cell population in WT (n=5) and $Gp2^{-/-}$ (n=6) mice with TNBS-induced colitis was determined by flow cytometry. *: $p = 0.0303$ (two-tailed Mann–Whitney U test). Data are presented as mean values $\pm$ SEM. **d**, GP2 staining in TNBS-treated WT and $Gp2^{-/-}$ mice. Scale bars, 100 μ m. Data are representative of two independent experiments. Source data are provided as a Source Data file.

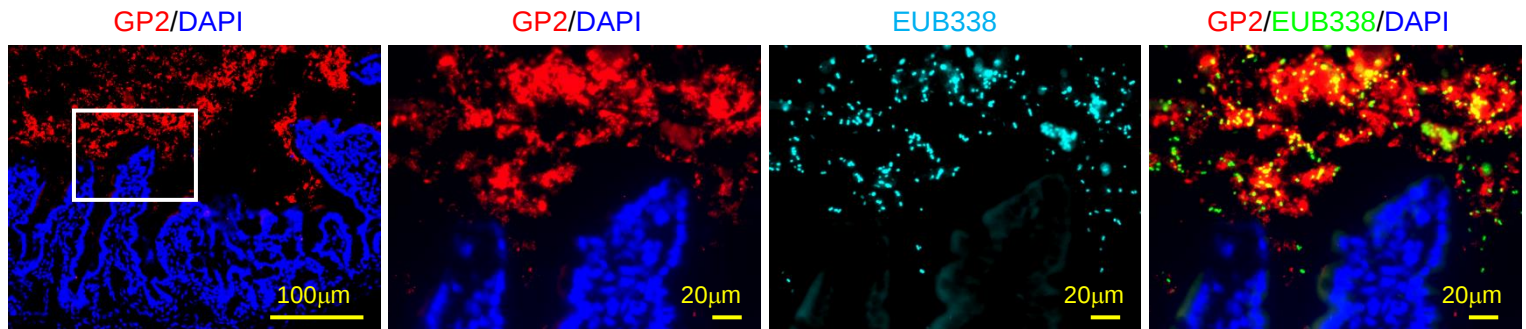
Supplementary Figure 13



The lack of pancreatic GP2 in tamoxifen treated *Ptfla-cre^{ETRM}-Gp2^{flx/flx}* (*Gp2^{Panc}*) mice.

Isotype control or GP2 stained in tamoxifen administered control or *Ptfla-cre^{ETRM}-Gp2^{flx/flx}* (*Gp2^{Panc}*) mice are shown. Representative pictures of pancreas and intestine are shown. Scale bars, 100 μm . Data are representative of three independent experiments.

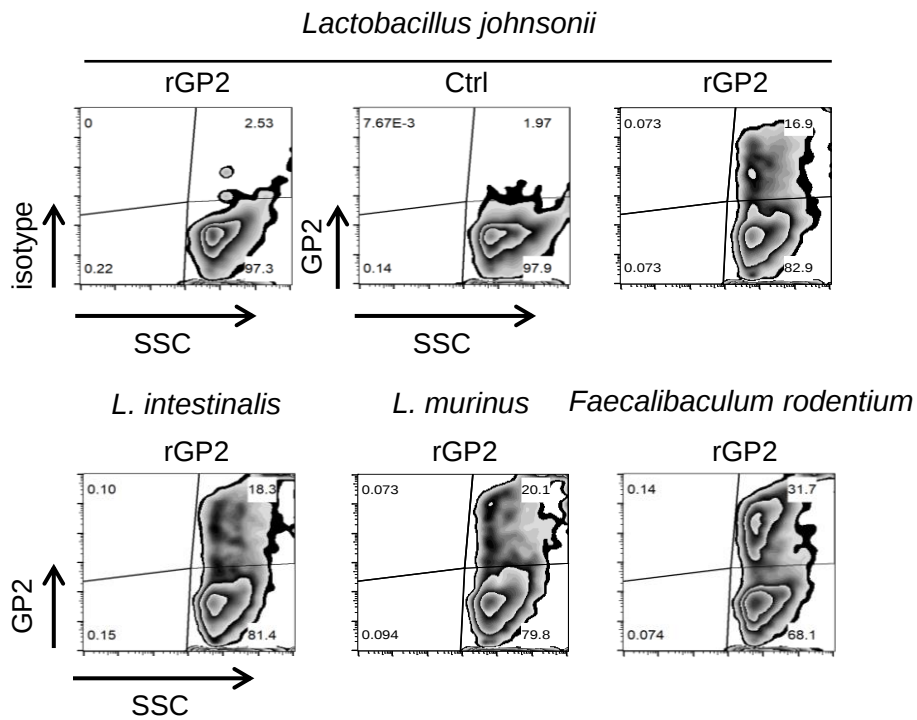
Supplementary Figure 14



Detection of luminal GP2 and bacteria in small intestine.

Luminal contents of small intestine were stained with GP2 (red) and EUB338 (blue or green). Representative pictures are shown. Data are representative of three independent experiments.

Supplementary Figure 15

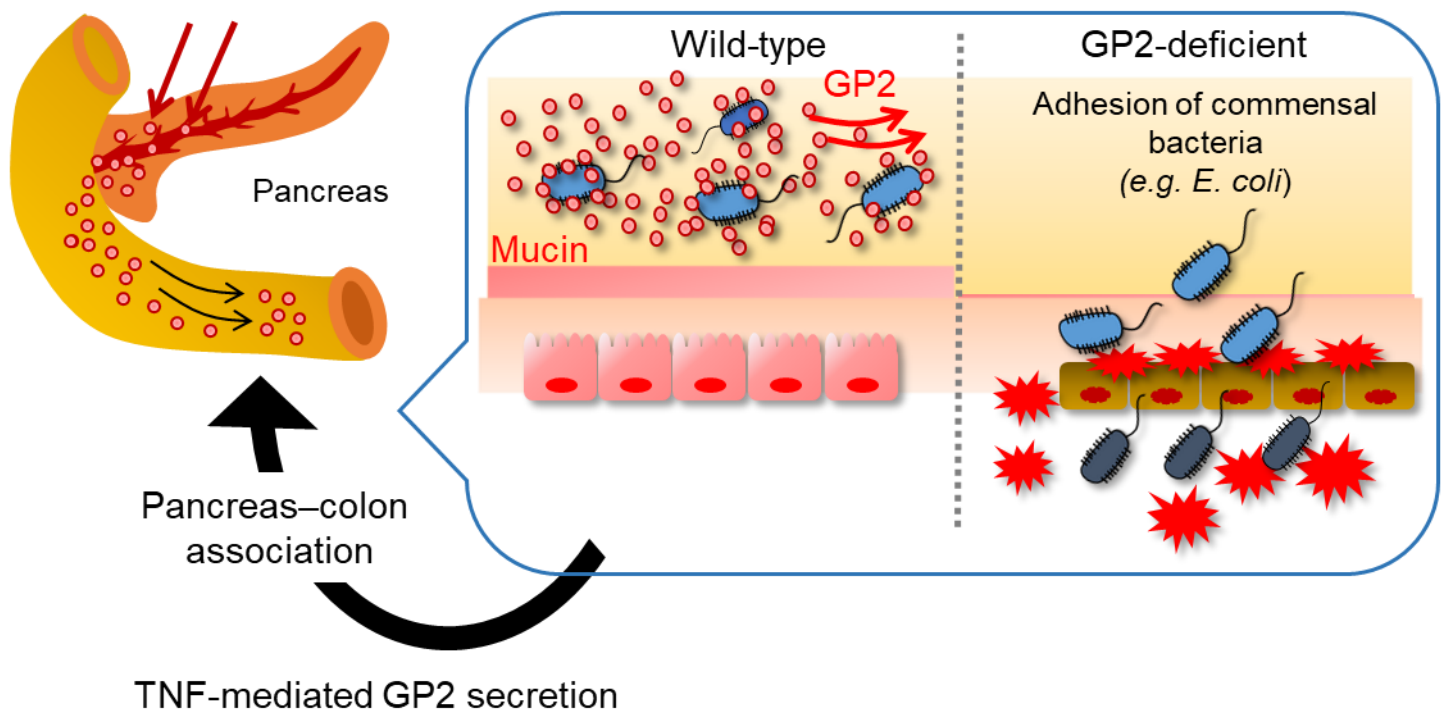


Characterization of GP2-bound commensal bacteria.

a, GP2-bound commensal bacteria were isolated by MACS. Isolated GP2-bound bacteria were cultured (1×10^6 CFU) with recombinant mouse GP2 (20 μ g) and examined by FACS. Data are representative of three independent experiments.

Supplementary Figure 16

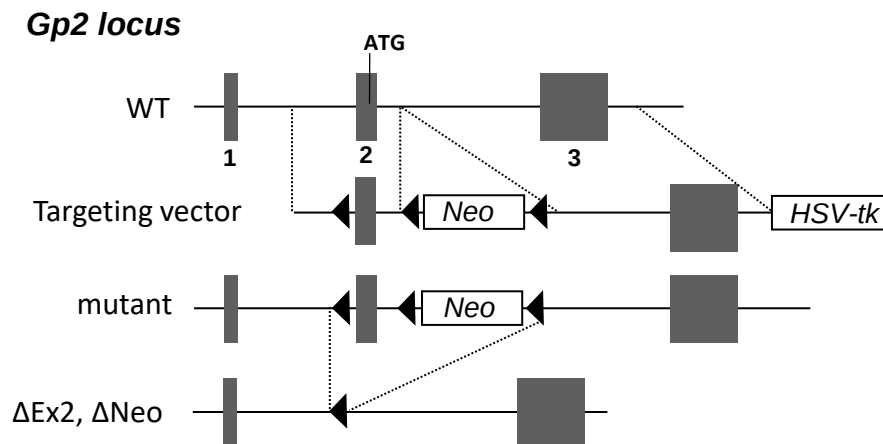
Exocrine pancreas derived GP2



Pancreatic Glycoprotein 2 as a First Line of Defense for Mucosal Protection in Intestinal Inflammation.

Exocrine pancreas derived GP2 bounds to luminal bacteria and regulates bacterial translocation. The scheme of current study has been shown.

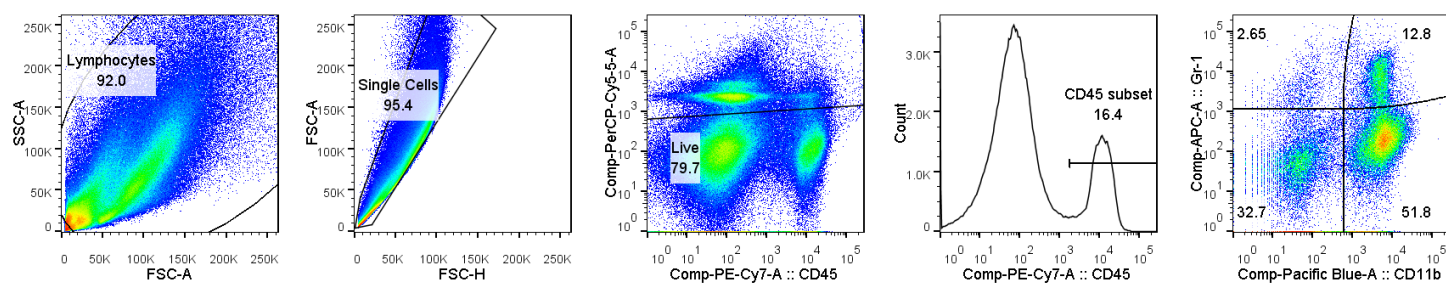
Supplementary Figure 17



Targeted disruption of the mouse *Gp2* gene by homologous recombination.

Schematic representation of the genomic structure of wild-type mouse *Gp2* gene exons 1-3, the targeting vector, the resultant mutant allele generated by homologous recombination (mutant), and the locus after removal of the region of the genome containing exons 2 and the neomycin resistant gene (Δ Ex2, Δ Neo). Exons are indicated by filled boxes.

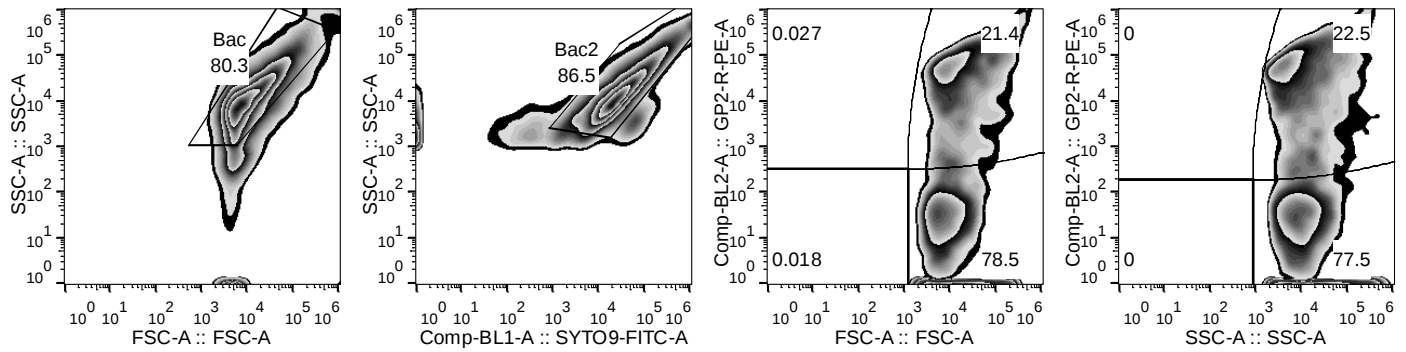
Supplementary Figure 18



Gating strategy of lamina propria cell flow cytometry.

The gating strategies for FACS analysis of mouse colon cells are shown.

Supplementary Figure 19



Gating strategy of bacterial flow cytometry.

The gating strategies for FACS analysis of isolated and luminal bacteria are shown.

Supplementary Table

List of primer sequences used for RT-PCR analysis in this study.

Primer Sequences

	Forward	Reverse
<i>Gapdh</i>	TGTCCGTCGTGGATCTGAC	CCTGCTTCACCACCTTCTTG
<i>Gp2</i>	GATACTTTGGGTTCTTCTCTTATGGT	GCAGCCTGCAGATTCTTCATA
<i>Pnlip</i>	CTGTGGACATTTGCAGTGCT	TGAAGCAGCCGAGTTTGTC
<i>Rab3d</i>	AAGTGTGACCTGGAAGACGAA	GCTGGCCTCAAAGAACTCAA
<i>Rab6a</i>	AATCCGCTGAGGAAAATTCAA	TGTCATACATGAATCGGGTGA
<i>Rab8a</i>	TCAAAGCAAAAATGGACAAAAA	TCCACTGTGATCTTGACTCCA
<i>Rab27b</i>	CGGGAAAAACGTGTGGTT	AAGCTGCAGATGTACCTTAAACG
<i>Rap1a</i>	CCCAACGATAGAAGATTCTACA	GCTCGGTTCTGCAGTGT
<i>Vamp2</i>	CCAAGCTCAAGCGCAAAT	GGGATTTAAGTGCTGAAGTAAACG
<i>Vamp3</i>	TCGCAGTTTGAAACAAGTGC	ATCCCTATCGCCACATCTT
<i>Vamp8</i>	CAGAATGTGGAGCGGATCTT	TGGGACGTTGTCTTGAAGTG
<i>Stx3</i>	CCGTTGGGCTGAAATAAGAG	AAGCTGGAGTGAAAGCTGGT
<i>Stx7</i>	GAGTTCGTTGCTCGAGTGC	TTCTTTTGAGCTGTCTTCAGGA
<i>Try5</i>	TCCCCTACCAGGTGTCTCTG	TCACTTGGATGCGGGTTT
<i>Plcx2</i>	AGGAGGCCTTTGGGAACA	GAAGGGACAGTGGTAGAAAATGA
<i>Plch1</i>	CTGAAGCAGCAAAAGACACG	GCTCCCTTTCTTCTGTCATCC
<i>Cpa1</i>	CGCAAACTCGATCACACAC	CTACTAGCTCCGGGCATCC
<i>Cpa2</i>	CGAGAGTGGGTTACGCAAG	TCCCATAATCAGACGCAATCT
<i>Reg2</i>	CTGCCAACCGTGGTTATTG	GGCTCTGAACTGCAGACAA
<i>Reg3a</i>	ATTGGGCTCCATGATCCA	AGATAATTCAGCACATCGGAGTT
<i>Reg3b</i>	TGGATTGGGCTCCATGAC	TCATCACGTCATTGTTACTCCA
<i>Reg3g</i>	ACCATCACCATCATGTCCTG	GGCATCTTTCTTGGCAACTT