SUPPLEMENTARY DATA

Cellular Fractionation of Mouse Small Intestine

Cellular fractionation of the mouse small intestine was performed as described (13). Briefly, the small intestine was removed, flushed with saline, opened and cut into 5-10 mm segments. The tissue was rinsed in calcium and magnesium-free Hank's balanced salt solution (HBSS-/-) containing 10 mM EDTA and then subjected to sequential digestion with collagenase XI (1 mg/ml HBSS-/-, Sigma-Aldrich Canada, Oakville, ON). Cells released after each digestion (referred to as 'fraction 1' and 'fraction 2') were recovered by centrifugation and used for quantitative RNA analysis.

Flow Cytometry

Freshly isolated Percoll-purified IELs were treated with Fc block (anti-mouse CD16/CD32 mAb, BD Biosciences Canada, Mississauga, ON) for 15 min at 4°C. Cell surface staining was then performed by incubation for 30 min at 4°C with fluorochrome-coupled anti-mouse CD45, CD3ε, CD19, CD4, CD8α, CD8β, TCRβ and TCRγδ mAbs obtained from BD Biosciences or eBioscience (San Diego, CA). A viability marker (DAPI or 7-AAD) was included to discriminate live cells. Stained cells were analyzed on a Gallios flow cytometer (Beckman Coulter Canada, Mississauga, ON). Data analysis was performed using Kaluza software (Beckman Coulter Canada). For FACS enrichment, pooled Percoll-purified IELs from 2-6 mice were labeled with the appropriate antibodies and sorted on a MoFlo Astrios instrument (Beckman Coulter Canada). Live untouched intestinal IELs were also obtained from pooled Percoll-purified IELs by sorting using forward and side scatter features in combination with viability staining. Post-sorting purity was consistently >96% and viability >97%.

Determination of Cytokine/Chemokine Levels

Protein levels of IFNγ, IL-1β, Il-2, Il-6, Il-13, TNFα and Cxcl1 were quantified in jejuna extracts, and media from cultured IELs using a Cytometric Bead Array assay kit (BD Biosciences). Jejunal extracts were homogenized in PBS containing 0.5% Triton X-100 and protease inhibitor cocktail (Sigma-Aldrich Canada). Homogenates were centrifuged at 16,000g for 20 min at 4°C, supernatants collected, and total protein content determined using a bicinchoninic acid (BCA) assay kit (Pierce, Rockford, IL).

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Supplementary Figure 1. (A) *Glp1r* expression segregates with markers of the villus epithelial compartment following cellular fractionation of the mouse small intestine. Fractions 1 and 2 correspond to cell fractions obtained following sequential collagenase XI digestion of the small intestine. Transcript levels for the different cell lineage markers are expressed relative to the corresponding jejunal mRNA levels and are mean±SD of 5 independent fractionation experiments. *iFabp*, intestinal fatty acid binding protein; *Clca3*, chloride channel calcium activated 3; *ChrA*, chromogranin A; *Crypt5*, cryptdin 5; *Uchl1*, ubiquitin carboxy-terminal hydrolase L1. (B) *Glp1r* expression along the mouse intestine correlates with the abundance of IEL markers. mRNA levels in duodenum (Duo), proximal (P), mid (M) and distal (D) jejunum (Jej), ileum (Ile) and colon are expressed relative to the corresponding transcript levels in the duodenum and are mean±SD (n=4 mice). (C) Contrasting sensitivity to forskolin among different murine lymphoid cell populations. Levels of cAMP were quantified in sorted untouched IELs, splenocytes, and thymocytes following incubation with either veh, Ex-4 or Fk. Data are expressed relative to the corresponding cAMP levels in veh-treated cells and are mean ±SD from two independent experiments with 2-3 replicates each (IELs) or from a single experiment performed in triplicate (splenocytes and thymocytes). (D) Exendin(9-39) antagonizes exendin-4 suppression of cytokine induction in activated IELs. Sorted untouched IELs were activated with anti-CD3/CD28 for 5 h in the presence of Ex-4 (3 nM) or vehicle (Veh) with or without exendin(9-39) (1 μM). mRNA levels of the indicated cytokines were assessed by qPCR. Data are mean±SD of a single experiment assayed in triplicate. (E) Exendin-4 suppresses cytokine protein levels in activated IELs from *Glp1r*$^{+/+}$ mice. Sorted untouched IELs were activated with anti- CD3/CD28 for 5 h (IFNγ, top panel) or 16 h (TNFα, bottom panel) in the presence of Ex-4 or vehicle (Veh). Levels of the indicated cytokines were assessed by cytometric bead assay. Data are mean±SD and representative of one out of three independent experiments. Values for the non-activated Veh-treated IELs were below the detection limit of the assay. ***= P, .001.

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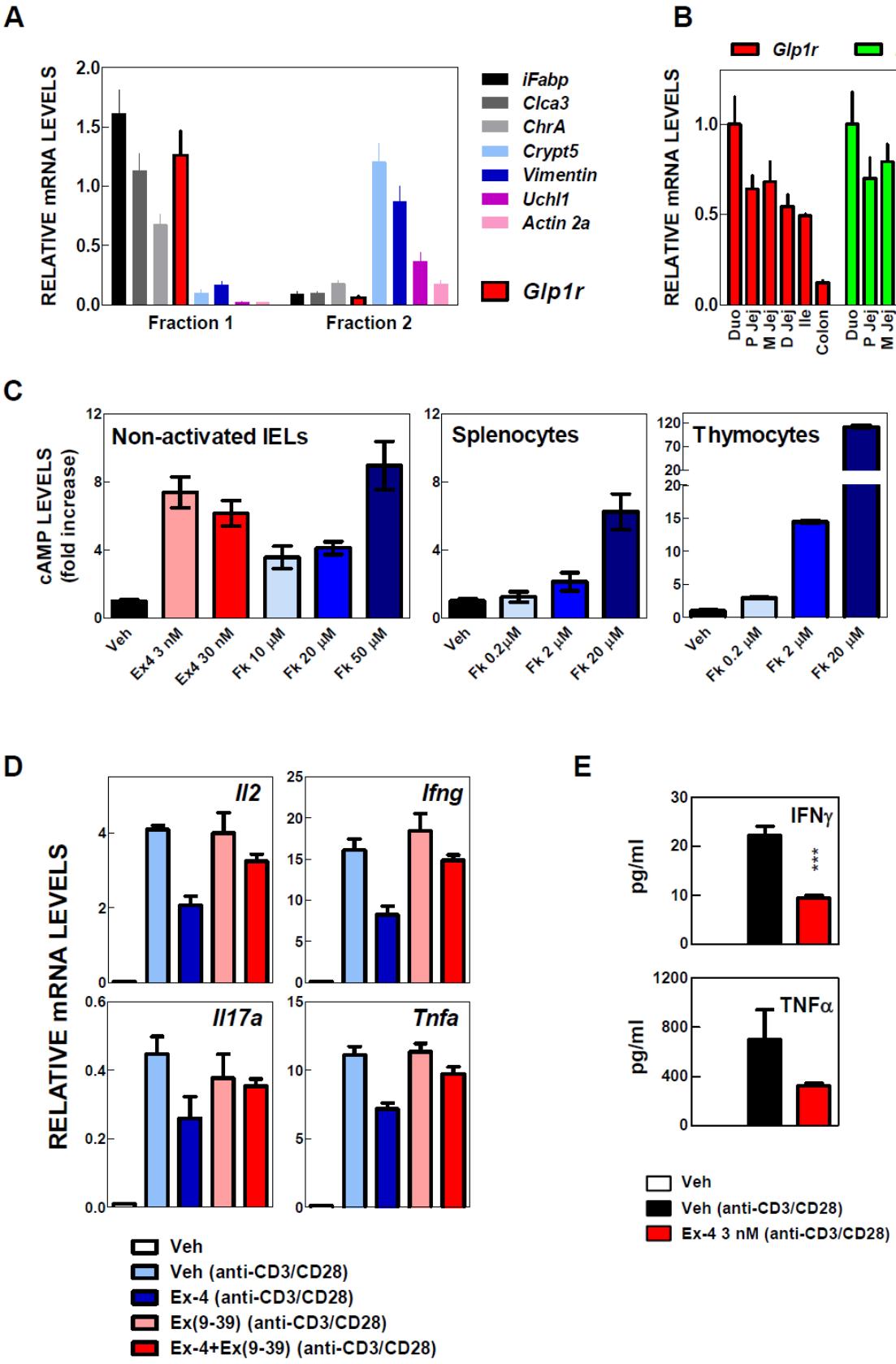
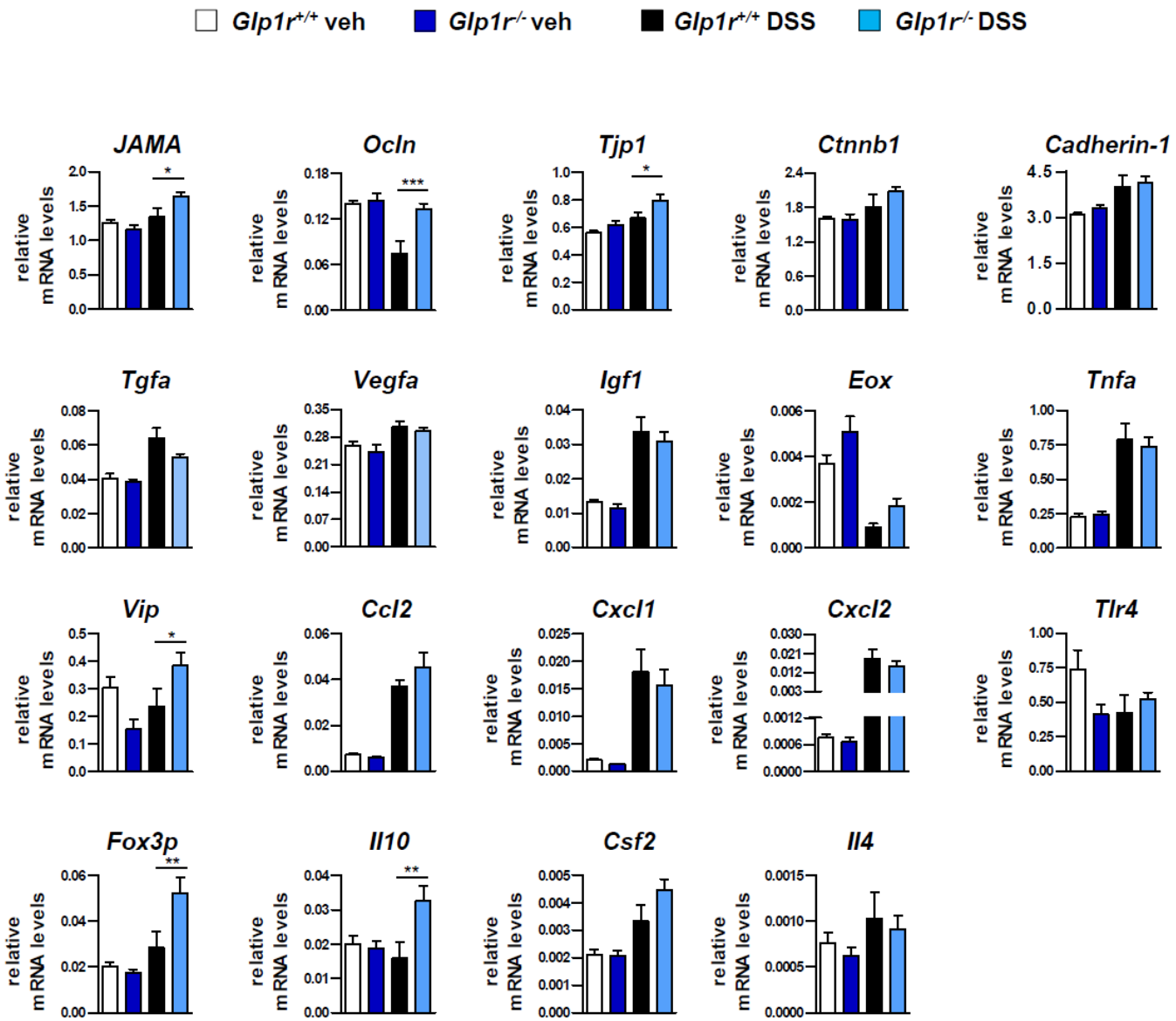


SUPPLEMENTARY DATA

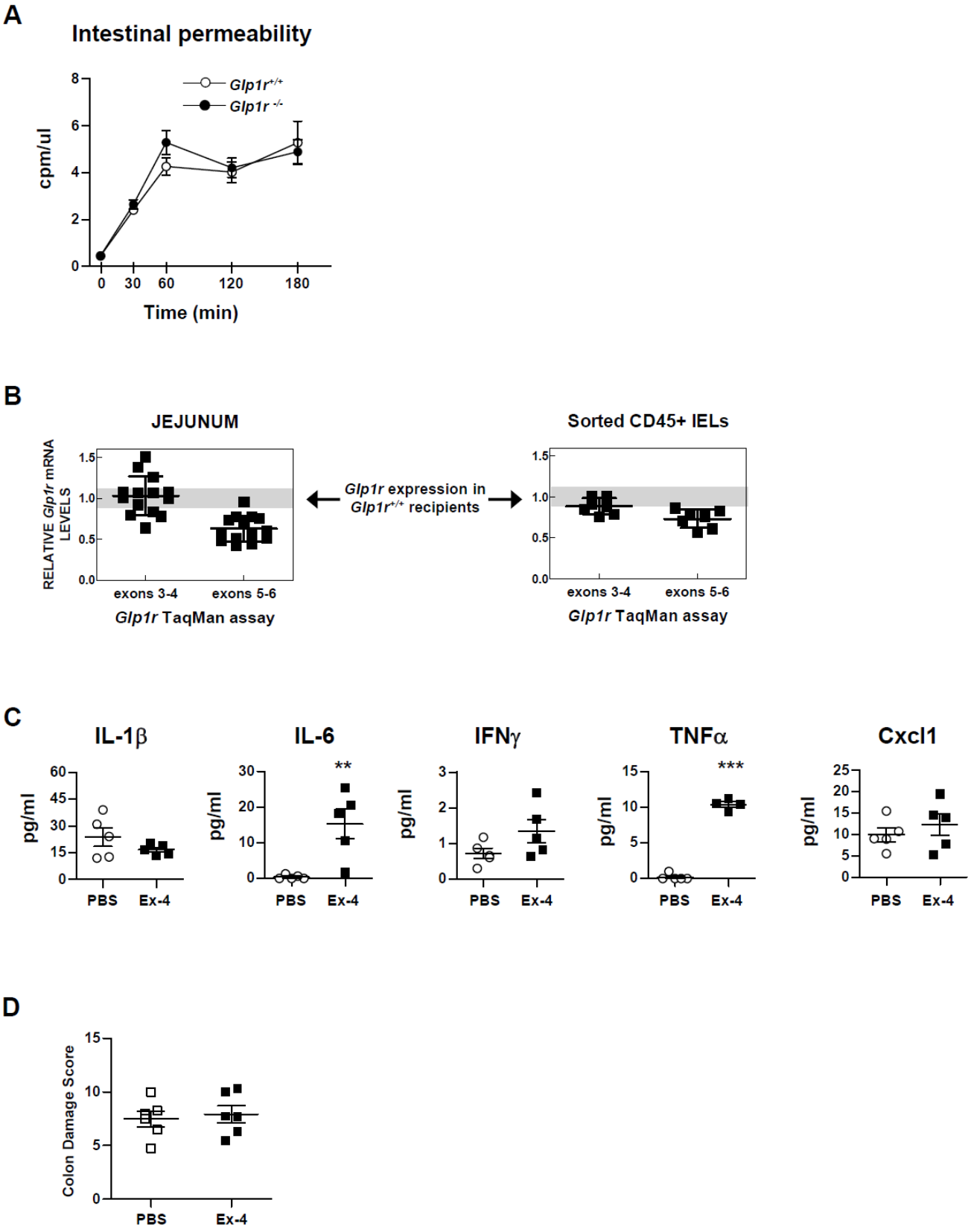
Supplementary Figure 2. Gene expression in colon samples of *Glp1r*$^{+/+}$ vs *Glp1r*$^{-/-}$ mice following 7 days of exposure to drinking water containing 3% DSS or regular drinking water (veh). n=9-12 mice/group. *, **, *** $p<0.05$, $p<0.01$ and $p<0.001$, respectively, for *Glp1r*$^{+/+}$ vs *Glp1r*$^{-/-}$ on DSS drinking water.



SUPPLEMENTARY DATA

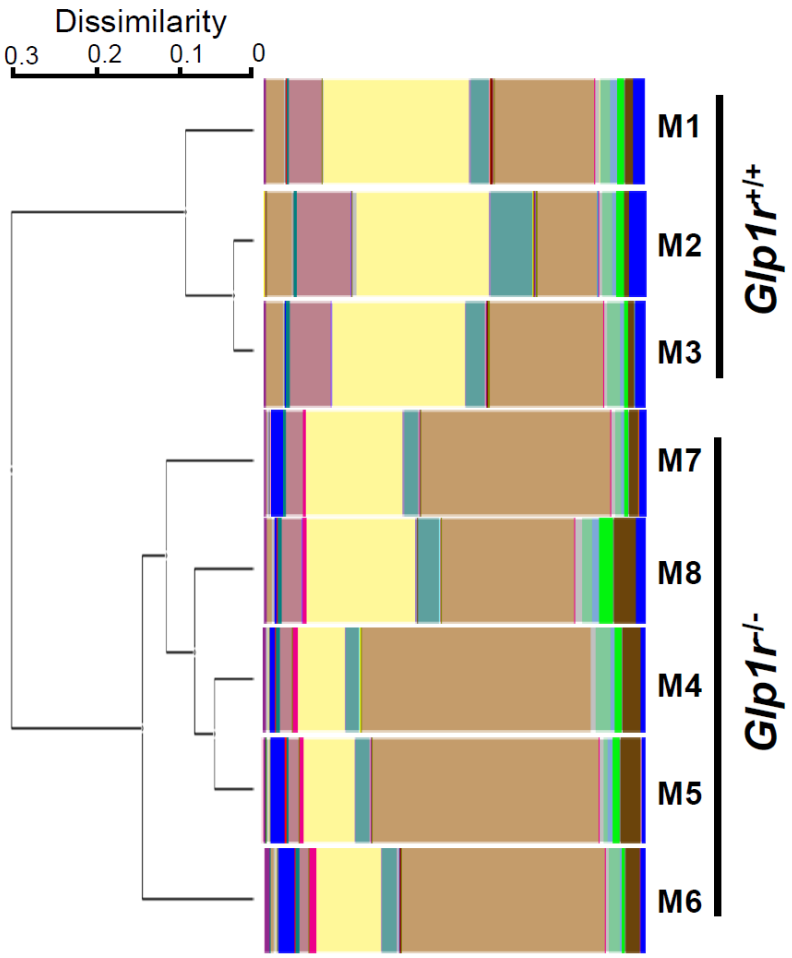
Supplementary Figure 3. (A) Small bowel permeability of 3-month-old *Glp1r*$^{+/+}$ and *Glp1r*$^{-/-}$ male mice. Mice were fasted overnight (16 h) and then given an oral gavage of 200 μl of PBS containing 0.4 μCi D-[1-14C]-mannitol (Perkin Elmer, Waltham, MA). Blood samples (50 μl) were collected from the tail vein after 0, 30, 60, 120 and 180 min and the amount of radioactivity in plasma determined by scintillation counting. (B) Quantitative assessment of *Glp1r* expression in jejunum (left panel) and sorted small intestinal IELs (right panel) from recipient *Glp1r*$^{-/-}$ female mice reconstituted with bone marrow from C57BL/6 donor male mice. The TaqMan expression assay targeting exons 3-4 of the murine *Glp1r* mRNA detects both wild-type and knockout *Glp1r* transcripts, whereas the assay targeting exons 5-6 only detects the wild-type *Glp1r* mRNA. *Glp1r* transcript levels are expressed relative to the *Glp1r* mRNA levels in *Glp1r*$^{+/+}$ recipient mice (grey horizontal stripe). Shown are combined data from 2 independent bone marrow transfer experiments. Each data point corresponds to one mouse. (C) Cytokine protein levels in jejunal extract samples from C57BL/6 mice that were given a single i.p. injection of Ex-4 (10 nmol/kg) or PBS and then sacrificed 4 h later. n=5 samples per treatment. **, ***$p<0.01$ and $p<0.001$, respectively for Ex-4- vs PBS-treated mice. IL-6 and TNFα protein levels for PBS-treated mice were below the detection limit of the assay and assigned a value of 0. (D) Colon damage score in C57BL/6 mice maintained on drinking water supplemented with 3% DSS for 4 days followed by 2 injections (12 h apart) of Ex-4 (10 nmol/kg) or PBS. n=6 mice/group.

SUPPLEMENTARY DATA



SUPPLEMENTARY DATA

Supplementary Figure 4. (A-B) Relative abundance of microbial communities in fecal samples from *Glp1r*$^{+/+}$ and *Glp1r*$^{-/-}$ mice. *Bacteroidetes* (light brown) and *Firmicutes* (yellow) represent the major bacterial populations in *Glp1r*$^{+/+}$ and *Glp1r*$^{-/-}$ mice. Each bar indicates the microbial composition of one mouse. MEtaGenome Analysis (MEGAN) was performed to determine microbial population similarities within and between groups of *Glp1r*$^{+/+}$ and *Glp1r*$^{-/-}$ mice. The cluster analysis tree was generated using UPGMA cluster analysis of Bray-Curtis dissimilarity coefficients.



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