Supporting Material

Methods

Human subjects

Ileal fluid samples were collected from patients with an ileostomy and seen in the surgical clinic at the Massachusetts General Hospital (MGH). Demographics and clinical characteristics of patients were obtained from the medical records. Human ileal fluid samples were homogenized in water (10 mg/ml), centrifuged (3,000 rounds/minute) and the supernatant collected to determine protein and IAP enzyme activity as previously described.[1] IAP activity was normalized for total protein levels (Coomassie Blue Protein Assay). The specific activity of the enzyme was expressed as picomoles pNPP hydrolyzed/min/µg of protein.

Intestinal alkaline phosphatase assay: The intestinal alkaline phosphatase (IAP) assay has been previously described[1-3]. Briefly, an individual stool or ileal fluid sample was homogenized in water (10 mg/mL) followed by incubation on ice for 30 min. Thereafter, the homogenates were centrifuged twice at 4 °C at 15,000 × g for 15 min, and the supernatants were collected to determine IAP activity as well as protein concentration. The Coomassie Blue Protein Assay (Bradford) kit from Fisher Scientific was used for protein quantification. For IAP assay, 25 µL of supernatant was mixed with 175 µL phosphatase assay reagent containing 5 mM of p-nitrophenyl phosphate (pNPP) followed by determining optical density at 405 nm. The specific activity of the enzyme is expressed as picomoles pNPP hydrolyzed/min/µg of protein. Protein concentration in a specific sample was determined using the protein assay reagents from Fisher Scientific.

IAP supplementation in WT mice: IAP supplementation was started in 10-month-old

WT C57BL/6 male mice. Mice were housed in a specific pathogen-free (SPF) environment at MGH (BL2) as described above. WT mice received a regular chow diet and autoclaved tap water *ad libitum.* At the age of 10 months, twelve WT mice were examined for physical health and assigned to 2 groups. One group received IAP supplementation and the other received vehicle. Each group contained 6 WT (*Mus musculus* C57BL/6) mice (3 animals per cage). The two groups were treated with either bovine intestinal alkaline phosphatase (IAP, New England Biolabs, Ipswich, MA or Synthetic Biologics, Bethesda, MD), or empty vehicle for IAP (50 mM KCl, 10 mM Tris-HCl (pH 8.2), 1 mM $MgCl_2$, 0.1 mM $ZnCl_2$, and 50% glycerol). IAP concentration was 100 units of IAP per ml. Water containing IAP or vehicle for IAP was replaced daily throughout the experimental period. Water bottles were refilled daily with 30ml of autoclaved water including 3000 units of IAP or vehicle. Stool samples were analyzed to document the increased intraluminal activity of IAP in the appropriate animals (Supplemental material). Body weight of each animal was regularly monitored. Blood samples were taken after 11 months of treatment at the age of 21 months. Frailty was scored before, after 8, and after 11 months of treatment.

Cytokine assays: TNFa, IL1β and IL6 were determined in serum and cell culture supernatant by ELISA kits following the manufacturer's instructions (eBioscience).

In-vivo intestinal permeability: Mice were gavaged with a phosphate buffer saline (PBS, pH 7.2) containing FITC-Dextran at a dose of 400 mg/kg body weight. Blood was collected 4 hours later from the facial vein, and serum was assayed for FITC-dextran concentration. Serum was diluted with an equal volume of PBS, and fluorescence intensity was measured by using a fluorospectrophotometer (PerkinElmer, Waltham, MA) with an excitation wavelength of 480 nm and an emission wavelength of 520 nm. Serum FITC-Dextran concentration was calculated from a

standard curve generated by serial dilution of FITC-Dextran in PBS.

Quantitative Real-Time PCR: Identical segments of liver tissue, small intestine and colon were resected and flushed with ice cold PBS. The tissue was then processed with TRIzol (Invitrogen) to isolate total RNA. Quantitative real-time PCR (qRT-PCR) was performed with a Mastercycler RealPlex instrument (Eppendorf) with a two-step qRT-PCR kit (Invitrogen). Primers for amplifying several pro-inflammatory cytokines were ordered from *MGH CCIB DNA Core.* Sequences for the primers used to amplify the various pro-inflammatory cytokines (Supplementary table 3). *Bactin* was used as the housekeeping gene internal control for normalization. Expression levels were calculated using the ΔΔCt method after correcting for differences in PCR efficiencies and expressed relative to control levels.

Histopathology: Mouse liver, colon and ileal samples were fixed in 10% formaldehyde and stained with Haemotoxylin and Eosin (H&E). Liver was additionally stained for neutral triglycerides and lipids using frozen sections (Oil Red O). Samples were examined by an independent pathologist blinded to animal group assignment. Grading of hepatic steatosis was based on macrosteatosis in each liver, assigned as follows: 0-5%: Grade 0; 5-33%: Grade 1; 33-66%: Grade 2; 66-100%: Grade 3.[4]

Limulus Amebocyte Lysate Assay (LAL): Portal and systemic serum LPS concentrations were measured with a commercial kit (GenScript), following the manufacturer's instructions. Briefly, samples were diluted 1:10–1:500 with endotoxin-free water, adjusted to the recommended pH, and heated for 10 min at 70 °C to minimize inhibition or enhancement by contaminating proteins. Limulus amebocyte lysate assay (LAL) reagents were added to each sample (in duplicate) and incubated at 37 °C for 45 min, and the absorbance was read at 545 nm. All samples were validated for the recovery and internal coefficient variation using known amounts of

LPS.

Target cell experiments: Systemic and portal sera of young and old IAP-KO and WT mice were added to cell culture media and incubated for 24 hours with primary mouse bone marrow-derived macrophages as target cells. After incubation for 24 hours, *Tnf* mRNA levels were quantified in the different groups using qRT-PCR, as previously described.

Metabolic profile: Serum total cholesterol, low- and high-density lipoprotein cholesterol (LDL-C and HDL-C), triglycerides, blood glucose, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured by the Clinical Pathology Laboratory at the Center for Comparative Medicine, MGH, Boston.

Fecal DNA extraction and 16S rRNA sequencing:

Fresh fecal samples were collected from mice prior to starting IAP supplementation, and then at 1- and 6-month timepoints after supplementation. Fecal samples were snapfrozen and stored at -80°C until analysis. DNA extracted from samples was amplified using Invitrogen's AccuPrime High Fidelity kit (catalogue # 12346094).

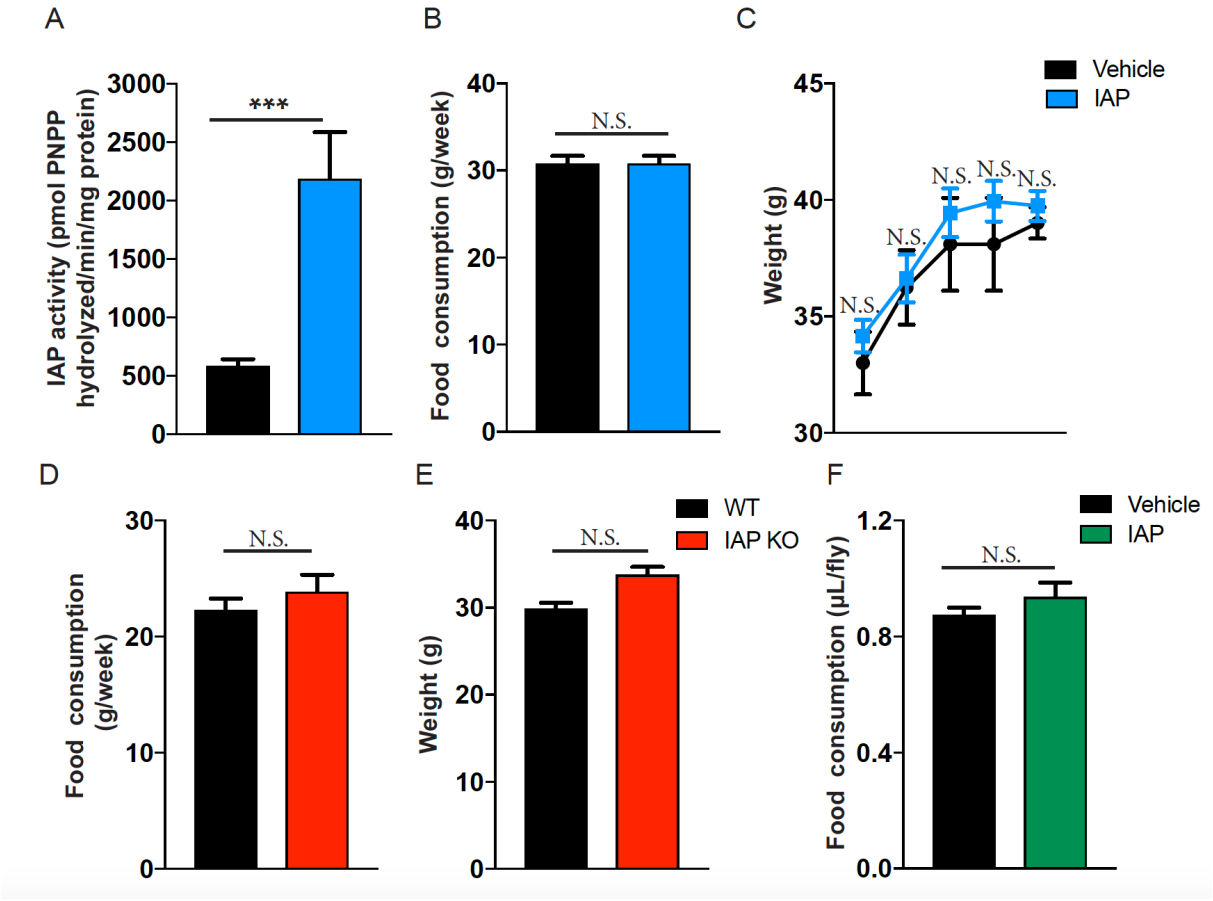
Each PCR reaction was prepared by combining 16 µl of the master mix (13.85 µl water + 2 µl 10 × reaction buffer + 1.5 µl *Taq* DNA polymerase), 2ul template DNA, and2 µl forward (515F) and reverse (806R) primers. The V4 hypervariable region was targeted using the 515 F and 806 R primers set which incorporated adapters enabling DNA sequencing of the amplified product using an Illumina MiSeq. The DNA samples were amplified using Hamilton On-Deck thermocycler under the following conditions: Initial denaturation at 95°C for 2 min followed by 33 amplification cycles of 20 s at 95°C, 45 s at 50°C and, 90 s at 72°C followed by final extension at 72°C for 10 min. The PCR product was purified using the QIAquick PCR purification kit (Catalogue# 28104) and the yield quantified using Invitrogen's Quant-iT Picogreen dsDNA assay kit (Catalogue

P7589). Amplicons were pooled and cleaned for 16S rRNA sequencing.

The 16S rDNA V4 region amplified by PCR was sequenced in the MiSeq platform (Illumina) using the 2x250 bp paired-end protocol yielding pair-end reads that overlap almost completely. The primers used for PCR amplification contain adapters for MiSeq sequencing and single-end barcodes allowing pooling and direct sequencing of PCR products.[5] The 16S rRNA gene pipeline data incorporates phylogenetic and alignment-based approaches to maximize data resolution. The read pairs were demultiplexed based on the unique molecular barcodes, and reads were merged using USEARCH v7.0.1090[6], allowing zero mismatches and a minimum overlap of 50 bases. Merged reads were trimmed at the first base with Q5. In addition, a quality filter was applied to the resulting merged reads; reads containing over 0.05 expected errors were discarded. 16S rRNA gene sequences were clustered into Operational Taxonomic Units (OTUs) at a similarity cutoff value of 97% using the UPARSE algorithm. OTUs were mapped to an optimized version of the SILVA Database[7,8] containing only the 16S v4 region to determine taxonomies. Abundances were recovered by mapping the demultiplexed reads to the UPARSE OTUs. A custom script constructed a rarefied OTU table from the output files generated in the previous two steps for downstream analyses of alpha-diversity, beta-diversity,[9] and phylogenetic trends. 16S rRNA gene sequencing methods were adapted from the methods developed for the NIH-Human Microbiome Project.[10,11] The pipeline used for 16S analysis leverages custom analytic packages and pipelines developed at the Alkek Center for Metagenomics and Microbiome Research (CMMR) to provide summary statistics and quality control measurements for each sequencing run, as well as multi-run reports and data-merging capabilities for validating built-in controls, and characterizing microbial communities across large numbers of samples or sample groups. The Kruskal-Wallis test was used as an exploratory tool to look for potential differences across time points in each

treatment group, and the Friedman Test and Dunn's multiple comparisons were used to confirm pair-wise differences.

Study approval: All human participants provided written informed consent and the study was approved by the MGH Institutional Review Board (Protocol Number: *2011P002755)*.



Supplementary figure 1. IAP supplementation or gene deletion has no significant effect on weight or food consumption in different animal models. (*A*) Oral IAP supplementation significantly increases IAP activity in stool (6 mice receiving IAP vs. 6 mice receiving empty vehicle). **(*B*)** Food consumption and **(C)** weight change of mice supplemented with vehicle or IAP. **(D)** Food consumption and **(E)** weight of WT and IAP-KO mice.(20 WT, 14 *IAP*-KO) **(F)** Food consumption of WT OreR *Drosophilae* supplemented with vehicle or IAP. (60 flies) Unpaired Student t-tests re used as statistical tests. *P*-values (**P* < 0.05; ***P* < 0.01; ****P* < 0.001) were considered statistically significant.

Supplementary table 1. Patient characteristics table.

Diagnosis	Number of patient	Age (year) (Mean+/-SD)	Gender (number) Male (M) Female (F)
Ulcerative Colitis (UC)	25	54 +/- 15	M: 17 F: 8
Crohn's Disease (CD)	13	45 +/- 15	M: 9 F: 4
Colon cancer	10	56 +/- 16	M: 7 F: 3
Motility disorder, Collagenous colitis, refractory constipation, Familial Amyloid Polyneuropathy (FAP)	12	59 +/- 14	M: 9 F: 3
Total	60	53 +/- 15	M: 42 F: 18

Supplementary table 2. Number of flies, median survival and range for each survival experiment in Drosophila.

	Number of flies tested (n)	Median survival (day)	Survival range (day)
Drosophilae, Drm-GAL4>YFP^{RNAi} control	120	48	(40-57)
Drosophilae , Drm-GAL4>CG5150^{RNAi}, CG10827^{RNAi}	114	40	(36-45)
Drosophilae , Drm-GAL4>CG5150^{RNAi}, CG10827^{RNAi} + IAP	112	46	(39-54)
Drosophilae, wild type OreR + Vehicle	172	50	(28-57)
Drosophilae, wild type OreR + IAP	180	60	(39-72)

Supplementary table 3. Oligonucleotides used in this manuscript.

Primer: mouse *Tnfa* Forward: 5' CCCAAAGGGATGAGAAGTT 3' Reverse: 5' CTCCTCCACTTGGTGGTTTG 3'
Primer: mouse *Il6* Forward: 5' TGGGAAATCGTGGAAATGAG 3' Reverse: 5' CCAGTTTGGTAGCATCCATCA 3'
Primer: mouse *Occludin* Forward: 5' CCCCATCTGACTATGTGGAAAGA 3' Reverse: 5' AAAACCGCTTGTCATTCACTTTG 3'
Primer: mouse *Zo-1* Forward: 5' GCTAAGA GCACAGCAATGGA 3' Reverse: 5' GCATGTTCAACGTTATCCAT 3'
Primer: mouse *Bactin* Forward: 5' AGC GAGCATCCCCCA AAGTT 3' Reverse: 5' GGGCACGGAGGC TCATCATT 3'
Primer: Drosophilae CG5150 Forward: 5' GCTGCTGCCAGGAGTTACATT 3' Reverse: 5' CTATCGGGGACTATTTTGTCCAC 3'
Primer: Drosophilae CG10827 Forward: 5' AAGTTGGCTTCTGAACCGAATAA 3' Reverse: 5' CCCGTGAAGGGGAAGTTTTC3'
Primer: Drosophilae E-cadherin Forward: 5' GAATCCATGTCGGAAAATGC 3' Reverse: 5' GTCACTGGCGCTGATAGTCA 3'
Primer: Drosophilae dlg1 Forward: 5' AACCTGGAGAACGTAACGCA 3' Reverse: 5' ATGCACCTGACTTTGGCTCT 3'
Primer: Drosophilae rp49 Forward: 5' AGCATACAGGCCCAAGATCG 3' Reverse: 5' TGTTGTCGATACCCTTGGGC 3'

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