Methods and Supplementary Figures

Methods

Generation of floxed Orai1 mouse: The targeting construct, in which exons 2 and 3 are flanked by loxP sites, was made by modifying a bacteria artificial chromosome clones using recombineering as shown in Supplementary Figure S1. The anticipated recombination of exon1 and 4 in the presence of Cre recombinase would create an out-of-frame fusion, rendering Orai1 loss of function. The targeting construct was electroporated into ES cells and G418 resistant clones were selected and picked for determination of targeted homologous recombination. To facilitate the PCR screen of ES clones with targeted homologous recombination, a control plasmid with neomycin expression cassette and Orai1 genomic sequence that extends the short homology arm was electroporated into ES cells and genomic DNA from these ES cells was used as positive control in PCR screen. Positive ES clones that were confirmed with homologous recombination were injected into blastocyst to generate chimeric mice that were later bred with C57BL/6 to confirm germline transmission of the desired genomic modification resulting in Orai1$^{fl/fl}$ mice. The deletion of exons 2 and 3 of Orai1 gene was confirmed by genomic PCR and RT-PCR.

Generation of pancreatic acinar cells specific Orai1 knockout mice: Orai1$^{fl/fl}$ mice were bred with Tamoxifen inducible elastase promoter driven Cre transgenic mice, the characterization of which is described in (Desai et al., 2007; Ji et al., 2008). The Orai1$^{fl/fl}$ were bred with Orai1$^{fl+/fl-}$/Cre (from the first generation) and 25% of the offspring were Orai1$^{fl/fl}$/Cre and were used to generate knockout mice and the Orai1$^{fl/f}$ littermates were used as wild-type controls. Orai1$^{fl/fl}$/Cre mice were injected/gavaged with 5mg/40g BW Tamoxifen in sunflower oil for 5 consecutive days. The mice were used between 5-30 days after the last injection. All the mice were on C57/BL6J background. Compared groups were always fed from the same batch of diet, and Orai1f/f mice were always cohoused with Orai1$^{-/-}$ mice. Mice were all housed in the same facility.

Q-PCR: RNA was extracted from the cells and tissues using TRIZOL reagent and the mRNA levels were determined by quantitative PCR. In brief, isolated mRNA was reverse-transcribed into cDNA by the cDNA synthesis kit from Life Technologies. The primers for quantitative RT-PCR for Orai1 (Life technologies, Mm07734349_g1) and GAPDH (Life technologies, Mm99999915_g1) were purchased from Applied Biosystems. The fold change in transcript levels was calculated by normalizing the Ct values from WT and Orai1$^{-/-}$ tissues (threshold values) to GAPDH.

Preparation of pancreatic acini: Dispersed acinar clusters and cells were prepared as previously described in(Kim et al., 2009). In brief, the pancreas was removed and finally minced in a solution containing (in mM) 140 NaCl, 5 KCl, 1 $MgCl_2$, 1 $CaCl_2$, 10 Hepes (pH 7.4 with NaOH), 10 glucose mM (solution A) and supplemented with 1mg/ml sodium pyruvate, soybeans trypsin inhibitor 0.15mg/ml (solution B0and 1 mg/ml BSA. After a wash the minced pancreas was digested with collagenase P added to solution B, washed with solution A and kept on ice until use.

Ca^{2+} imaging: The freshly prepared pancreatic acini were loaded with Fura-2 by incubation with 5 μM Fura2 for 30 min at room temperature, washed and placed in a perfusion chamber. The acini were continuously perfused with PSA (solution B) at (37 ^{o}C). Agonists were added to the perfusate. Fluorescence images were captured with a TILL photonics system at excitation wavelength 340 and 380 nm and colleting the light at emission wavelength above 500 nm. Image analysis was off line by the TILL system and Origin software.

Measurement of Amylase activity: Stimulated amylase release was measured using phaebdas tablets and according to the protocol reported in(Kim et al., 2009). Briefly, the isolated pancreatic acini were stimulated with 100pM CCK or 25μM CPA +1 μM PMA and the supernatant was collected at 0, 5, 15 and 30 min intervals to measure amylase activity. A

sample of the acini was used to measure total amylase by lysing the acini to release the amylase and results are reported as amylase release relative as % of total amylase content. Blood samples were collected retro-orbitally from the mice and left at room temperature to clot for 15-30 and spun down at 1000g to collect the serum. The serum was analyzed for amylase activity using the phaebdas tablets.

Measurement of total Lipase, Trypsin and MPO activity: The pancreas of Orai1$^{fl/fl}$ and from the Orai1$^{-/-}$ mice was removed and homogenized to gently release the digestive enzyme content. The lysates were centrifuged at 1500xg for 5 min and the supernatant containing the digestive enzymes were used to measure lipase activity according to the manufacturer's protocol using lipase activity assay kit from Sigma-Aldrich (MAK046) and trypsin activity assay kit from abcam (ab102531). The MPO activity to determine the neutrophil infiltration in the pancreas was measured in homogenized pancreas using the colorimetric MPO activity kit from Sigma (MAK068). The serum samples from Orai1$^{fl/fl}$ and Orai1$^{-/-}$ mice were used for analyzing Lipase activity as described.

Chymotrypsin activity: *In vivo* digestive enzyme secretory function was estimated by measuring chymotrypsin activity in feces. Feces 15–30 mg were suspended in a solvent containing 0.1% Triton X-100, 0.5 M NaCl and 100 mM $CaCl_2$, sonicated 5 times and centrifuged at 20,000xg for 10 min at 0 °C. The cleared supernatant was used for fluorometric analysis of chymotrypsin activity using 5 μM of the substrate AMC-(Suc-Ala_2-Pro-Phe) (Bachem). The initial rates of substrate hydrolysis were calculated and are expressed in arbitrary fluorescence units per min and then converted to U mg^{-1} using purified chymotrypsin to generate standard curve. The activity was normalized to fecal weight.

Intestinal permeability and transit time: To measure solid and liquid food transit time the different mice lines were gavaged with a suspension of AIN-76 from Bioserv mixed with fluorescein 8K dextran (solid food) or 200μl (liquid food). The feces were collected every hour, suspended in PBS, centrifuged and fluorescence of the supernatant was measured. The fecal samples were collected until fluorescence was detected. To measure intestinal permeability, 150 μl containing 80mg/ml fluorescein dextran 100,000 (Sigma, St. Louis, MO) was gavaged for each mouse and blood samples were collected for serum analysis every hour for 3 hours. The blood was centrifuged at 2000xg to collect the serum that was diluted 100 fold in PBS and fluorescence was measured at excitation wavelength of 488 nm and emission wavelength of 530 nm.

Measurement of Satiety Hormones and Inflammatory markers: Serum samples from Orai1$^{fl/fl}$ and Orai1$^{-/-}$ mice were collected after 5, 10 and 30 days after gavage and the levels were measured using Luminex assay kit from EMD Millipore for inflammatory cytokines (Mcytomag-70K) and satiety hormones (MMHMAG-40K) according to the manufacturer instructions and read on Biorad, Bio-plex 200 system.

Liquid and Solid Diets: The Orai1$^{fl/fl}$ and Orai1$^{-/-}$ mice were fed the AIN-76 purified liquid diet or the white round pelleted solid food with identical caloric protein, fat, soluble/insoluble fiber, and carbohydrate content from Bioserv (pelleted sold diet: F0076, solid diet for pancreatic enzyme replacement experiments: F0761, Liquid diet: F1268SP). Mice were fed for 48 h prior to induction of the Cre with Tamoxifen and the diets were continued after induction until the mice were euthanized if not died before. The liquid diet was in the form of powder and was re suspended in water according to the manufacturer's instructions. The diet was administered in special graduated glass tubes in steel holders. The round white pellets weighing 1g/pellet were used for solid diet and fed in glass tubes and holders from Bioserv. The round pellets and liquid diet in the graduated tubes were used to calculate food intake by the mice.

Digestive enzyme supplement: Pancreatic enzymes supplement was administered to the mice in two forms. PancrePlus (#50408) powder containing lipase, protease, amylase, vitamin A, Vitamin D_3 and E from NIH pharmacy was mixed with powdered diet from Bioserv

(F0761, 0.9g/50g of powdered diet) in special glass containers and fed to the mice. Alternatively, enzyme supplement was solubilized in drinking water at the amount of 1mg/ml and the mice had free access to the water. The enzyme supplement was given one week prior to deletion of acinar cells Orai1 and continued to the end of the experiment.

Treatment with antibiotics: A mixture of broad-spectrum antibiotics were added to the drinking water and the water was changed daily. The antibiotic cocktail consists of Neomycin (1g/L), Valinomycin (0.5g/L) and Primaxin (0.5g/L). The mice were acclimated to antibiotics for one week prior to start of Tamoxifen gavage to delete Orai1 and continued in this manner until the end of the experiments.

Treatment with CRAMP and scrambled peptides: The CRAMP peptide LL-37 sequence is LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES and the scrambled peptide sequence is GLKLRFEFSKIKGEFLKTPEVRFRDIKLKDNRISVQR (Sun et al., 2015). Orai1$^{-/-}$ mice on solid diet were gavaged with 100µg in 200ul of PBS, 6 days before start of TX application, the 100µg peptides were gavaged 4 hours prior to the first two TX applications on days 6 and 7. Finally, peptides were added by gavage on days 13 and 14. The mice were euthanized at day 21 and the cecal contents were collected for 16S rRNA analysis.

Feeding and weight curves: Orai1$^{fl/fl}$ and Orai1$^{-/-}$ mice fed with liquid diet in graduated feeding tubes or the special feeding tubes for solid round pellets were used to calculate the food intake by the mice 48 h prior to gavage and continued through the experiments. The mice were weighed on the day of gavage and then weighed twice weekly. The amount of food intake was calculated daily by subtracting the leftover diet from the volume of liquid diet given or the number of solid round pellets. The total solid round pellets and the leftover pellets were weighed in addition to counting the number of pellets to verify food intake. The total food intake was divided is expressed relative to body weight.

Cecal gavage: Orai1$^{-/-}$ mice fed clear liquid diet (6 weeks of age, gavaged with tamoxifen) were treated with antibiotic cocktail consisting of Neomycin (1g/L), Valinomycin (0.5g/L) and Primaxin (0.5g/L) in drinking water for 2 weeks. Cecal contents from donor Orai1$^{fl/fl}$ and Orai1$^{-/-}$ mice on solid diet were collected aseptically in a laminar flow bench and diluted in 100µl of sterile PBS, aliquoted and frozen at -80ºC. The cecal samples were thawed on ice after 48h and diluted 1:50 in sterile PBS and were used to inoculate the Orai1$^{-/-}$ mice on liquid diet. Mice were orally gavaged either with 0.15 ml of diluted cecal contents (Ellekilde et al., 2014).

Western blots: The pancreas from Orai1$^{fl/fl}$ and Orai1$^{-/-}$ mice were harvested 5 days after the last Tamoxifen gavage and minced in solution A, centrifuged and re suspended inRIPA buffer with protease inhibitors. The minced pancreas was homogenized in glass homogenizers (10 strokes on ice) and centrifuged for 30 min at 10,000xg. The supernatant was collected and used to separate proteins by SDS-PAGE. The blots were probed with several antibodies as follows: the anti Cathelicidin (CRAMP) antibodies were from Lifespan biosciences, cat# LSB6696 and were used at a dilution of 1:250, the anti Vamp8 antibodies (V7514-200UL) were from Sigma and used at a 1:1000 dilution and the anti Syntaxin-3 antibody from Syntaxin systems, cat # 110 033 were used at a dilution of 1:500.

Histological analysis: The pancreas and the intestinal samples were collected aseptically and snap frozen in OCT or embedded in paraffin and sectioned in cryostat at 8 µm thickness. For lipid staining with oil red O (Sigma-Aldrich Japan), sections were air dried for 30 minutes. Oil red O solution was prepared freshly by dissolving 300 mg oil red O in 100 ml 99% isopropanol, diluting 40 ml of this solution with 60 ml of distilled water and filtering to remove any crystals. Slides were stained by incubation for 10 minutes and rinsed once in distilled and once in tap water. The slides were counterstained with hematoxylin and mounted using pure glycerol (Matsuda et al., 2014). Trichrome-Masson's (Hensley et al., 2015) staining was used to assess fibrosis and pancreatic cell injury. The sections were fixed in Bouin's solution at 56ºC for an hour and rinsed in running tap water for 5-10 minutes to remove the yellow color. The slides were stained in Weigert's iron hematoxylin solution for 10 minutes and rinsed in running warm tap

water for 10 minutes. Then, the sections were stained in Biebrich scarlet-acid fuchsin solution for 15 minutes and washed in distilled water. The color was differentiated in phosphomolybdic-phosphotungstic acid solution for 15 minutes and transferred directly to aniline blue solution for 10 minutes. The sections were rinsed briefly in distilled water and differentiate in 1% acetic acid solution for 5 minutes and washed in distilled water. The sections were dehydrated very quickly by immersion in 95% ethyl alcohol, absolute ethyl alcohol and cleared in xylene. The slides were mounted with Permount from electron microscopy sciences. Paneth cells were stained using the Phloxine B/tartrazine stain as in (Steenwinckel et al., 2009). In brief, seven µm sections were cut, dewaxed at 60ºC, and rehydrated. Nuclei were stained with hematoxylin for 45 s and destained in running tap water for 10 min. Sections were stained in phloxine solution (0.5 g of phloxine B and 0.5 g of calcium chloride in 100 ml of distilled water) for 20 min, successively rinsed in water and in Cellosolve (2-ethoxy ethanol, Aldrich), and differentiated with a saturated tartrazine solution (2.5 g of tartrazine in 100 ml of Cellosolve) for 5 min. The slides were rinsed in Cellosolve for 3 min, dehydrated in xylene twice for 1 min and mounted in Permount. Pancreatic and duodenal sections from paraffin-embedded blocks were used for Haematoxylin and Eosin staining and the damage was analyzed using the Image J software.

Immunofluorescence: Pancreatic acini or pancreatic sections were fixed in cold methanol (-20%), washed in PBS and blocked in 5% goat serum for an hour. The sections/cells were incubated with the primary antibodies raised against (Claudin-2 (Invitrogen#516100), Orai1 (Generated by Thermo fisher Scientific using the peptide reported in Gwack *et al*, 2008), Zo1 (Sigma# SAB1306492-40TST), IP_3R3 (BD BioSciences #610312), Amylase (Sigma, A8273-1VL), and Rab27 (BD Pharmingen #558532) overnight at 4ºC. In some experiments sections were fixed in 4% PFA , washed in PBS, blocked in glycine buffer, washed and blocked for an hour in 5% goat serum in PBS, incubated with primary antibodies overnight at 4ºC and washed. All samples were stained with FITC or Rhodamine-tagged secondary antibodies. The paraffin embedded sections from the duodenum were deparaffinized and rehydrated in water and then stained for IgA (Sigma, F9384), RegIIIγ (santa cruz biotechnology # sc377038), T lymphocytes (CD3 antibody, abcam%ab16669) and Lysozyme (abcam #108508) using the above described protocol. In some experiments the section were also counterstained with DAPI and/or Phalloidin (Invitrogen) for an hour. Fluorescent intensity and the area stained for Claudin-2 and other proteins were calculated using Volocity software.

Pancreatic and intestinal antibacterial secretion: To assay pancreatic antimicrobial secretion, $Orai1^{fl/fl}$ and $Orai1^{-/-}$ acini from a whole pancreas were stimulated with 100 pM CCK in 1 ml of solution A for 30 min at 37ºC. The supernatant was collected by centrifugation at 1000xg for 5 min. The samples were concentrated and diafiltered using 4K cut-off centrifugal filter units from Millipore to 100 µl in solution A supplemented with protease inhibitors cocktail tablet from Roche. Intestinal antimicrobial secretion was evaluated using equal blocks of duodenum from $Orai1^{fl/fl}$ and $Orai1^{-/-}$ mice on solid and liquid diets were dissected and minced in solution A. The minced duodena were incubated with 100 µM carbachol for 45 min at 37ºC in solution A. The supernatants were collected by brief centrifugation at 1000xg to remove the tissue, concentrated and treated as described above for pancreatic secretion. Anti-bacterial activity was evaluated using DH10B strain of *E.coli* from Life Technologies that was grown in LB broth to an optical density of 0.2-0.3, an early log phase. Then, 100 µl of the *E.coli* cells were incubated with either solution A with protease inhibitors or 150-300 µg of the concentrated supernatants. The optical density was measured at the indicated times at 600 nm to construct growth curve time course.

Bacterial staining: Frozen sections from duodenum, jejunum and colon of $Orai1^{fl/fl}$ and $Orai1^{-/-}$ mice collected aseptically and were stained for bacteria using the Brown and Brenn's Method staining gram-positive and gram-negative bacteria. Briefly, the frozen sections were thawed, rinsed with PBS to remove loss bacteria. The sections were air dried at room temperature and fixed in 10% buffered neutral formalin, air-dried and gram-stained for bacteria. The sections were stained in 1% aqueous solution of crystal violet with 5 drops of 5% aqueous

sodium bicarbonate for 1 min, flooded with 1% iodine for 1 min, rinsed with water and dried on a filter paper. The slides were decolorized with 1:1 solution of acetone: alcohol. The slides were then dipped in Basic Fuschin working solution for 1 min and washed in water. The slides were quickly differentiated in acetone and differentiated further in 0.1% picric acid-acetone solution to yellowish-pink color. After quick rinse in acetone, then acetone-xylene, 3-4 changes in xylene, the slides were mounted in Permount. The sections were analyzed using light microscope at 40x magnification. Between 5-6 images were taken from different areas of each section, with 5 images /mouse were obtained from 3 mice at each condition. The number of bacterial cells were counted using Metamorph. Metamorph calculates the total area of the image and divides the image with a grid. The bacteria were counted in each of the sections and divided by the area for each image.

Analysis of cecal microbiome: The Orai1$^{fl/fl}$ and Orai1$^{-/-}$ mice maintained on solid and liquid diets were gavaged with vehicle or TX for 5 days and the cecal contents were collected 5 days after the last gavage before the mice on solid diet died. Cecal content was collected aseptically using autoclaved surgical instruments and laminar flow bench. To collect cecal content the GI tract was isolated by grabbing the colon with forceps, cutting with scissors, and carefully pulling out the GI tract and laying it on a surgical table, which was lined with clean towels. The fecal content in the cecum was squeezed out into a 2 mL cryovial and snap frozen in liquid N_2. These samples were stored at -80 until further processing. DNA was extracted using the QiaAMP Fast DNA Stool Mini kit (Qiagen) for analysis of bacterial load and the rest of the samples were sent for microbiome analysis at UC Davis Host-Microbe Systems Biology Core by 16S gene variable region (V1-V3 or V3-V4 directed primers) sequencing by Titanium 454 (3000 avg seq read depth) of cecal contents. Sequences were processed through the QIIME analysis pipeline to taxonomically classify them and to assess alpha and beta diversity of the community.

Bacterial translocation assay: After euthanasia, mouse skin was sterilized with 70% ethanol before opening the abdomen. Sterile organs (spleen, liver, and pancreas) were removed under aseptic conditions in a safety vertical laminar flow hood. Each organ was weighed in a sterile pre-weighted plastic bag and homogenized in 9 parts of PBS containing 0.1% Tween20 per g of tissue. Tryptone soy agar plates were inoculated with serial dilutions of the resulting homogenate (100□L of diluted homogenate per plate) and cultured at 37^{o}C under 5% CO_2 atmosphere for 24-72h, until colony forming units (CFUs) were evident. The viable bacterial counts were expressed as the log10 mean±s.e.m of the number of CFU per gram of tissue. Statistical analysis was performed in Graph Pad Prism using Mann-Whitney nonparametric t-test.

Analysis of Duodenal inflammation by scoring: Histopathological scores of duodena were assigned in a blinded manner by study investigators (M.A. and D.M.S.) using established methods (Erben et al., 2014). Histology was scored as follows: inflammatory cell infiltrate (I): 0, none; 1, mild mucosal and sometimes submucosal; 2, moderate, mucosal and sometimes submucosal; 3, marked, mucosal and submucosal, sometimes transmural; 4 marked, transmural; intestinal architecture (A): 1, mild villous blunting; 2, mild epithelial hyperplasia and moderate villous blunting; 3, moderate epithelial hyperplasia and goblet cell loss, moderate villous blunting and broadening with some atrophy; 4, marked epithelial hyperplasia and goblet cell loss, villous atrophy and ulcerations. Total histology score is given as I+A. The histological score was calculated from observation of $\geq$10 different fields per gut region at x200 magnification from $\geq$5 mice in each group.

Immune cells collection: Pancreatic leukocytes were prepared as follows. Pancreata were extracted and minced with scissors, then incubated in digestion buffer (DMEM with Type IV collagenase 1mg/ml (Sigma Aldrich), DNAse I 10U/mL (Sigma Aldrich), 1% BSA, trypsin

inhibitor 150□g/mL) for 20 minutes at 37ºC with continuous shaking (100 cycles/min). The suspension was then vortexed at low speed for 20 s and centrifuged, and the cell pellet was resuspended in DMEM and filtered through at 40□M nylon strainer. The suspension was centrifuged and the pellet was resuspended in DMEM and filtered again through a 40□M nylon strainer. The suspension was centrifuged again and the cell pellet was resusupended in red blood cell lysing buffer (Quality Biological) for 90 seconds. The cells were spun down, washed twice with PBS with 2% bovine calf serum, and used for surface staining.

For B cell and T cell isolation from spleen, spleens and lymph nodes were extracted from WT and Orai1$^{-/-}$ mice. CD3+ T cells and B cells were isolated using negative selection (StemCell Technologies Inc., Vancouver, BC). Cells were centrifuged, washed, and resuspended in Trizol for RNA extraction.

Antibodies and flow cytometry: For surface staining, murine cells were stained with the following antibodies: FITC-CD11b (BD Biosciences 553310, 1:250), PercpCy5.5-Ly6G (ebiosciences 45-5931-80, 1:400), PECy7-F4/80 (BioLegend 123114, 1:500), eF450-CD4 (ebiosciences 45-5931-80, 1:200), APC-TCR (BioLegend 109212, 1:300), APCeF780-CD45.2 (ebiosciences 47-0454-82, 1:900). Dead cells were excluded from analysis using Live/Dead Fixable Aqua stain (Invitrogen L34957, 1:500). Flow cytometry data collection was performed on a FACSVerse (BD Biosciences) and analyzed using FlowJo software (Treestar Inc.).

SCFA analysis: Sample preparation: Mouse fecal pellets were homogenized in a bullet blender (no beads), in 400 µL of a solution of 30 mM HCl plus isotopically-labeled acetate (0.125 mM), butyrate (0.125mM), and hexanoate (0.0125mM.). Then 250 µL of Methyl tert-butyl ether (MTBE) was added to each sample, and the mixture was vortexed for 10 seconds to emulsify, incubated at 4 °C for 5 mins, and vortexed again for 10 seconds. Samples were centrifuged for 1 minute to separate the solvent layers and MTBE was then removed to an auto-sampler vial for GC-MS analysis. 10 µl of MTBE was removed from each sample and pooled in a separate auto-sampler vial for quality control purposes. A series of calibration standards were prepared along with the samples to quantify the metabolites. **GC-MS analysis**: GC-MS analysis was performed on an Agilent 69890N GC -5973 MS detector with the following parameters: 1µL samples were injected with a 1:10 split ratio on a ZB-WAXplus, 30m x0.25mmx0.25um (Phenomenex Cat#7HG-G013-11) GC column, with He as the carrier gas at a flow rate: 1.1ml/min. The injector temperature was 240 °C, and the column temperature was isocratic at 310 °C. **Data analysis:** Data were processed using Mass Hunter Quantitative analysis version B.07.00. SCFAs were normalized to the nearest isotope labeled internal standard and quantitated using 2 replicated injections of 5 standards to create a linear calibration curve with accuracy better than 80% for each standard.

Phylogenetic analyses of sequencing data: Read count data for OTUs defined in the QIIME analysis were exported as BIOM tables and analyzed and visualized using the 'phyloseq' package (version 1.14) (McMurdie and Holmes, 2013) distributed as part of the Bioconductor (version 2.30) (Huber et al., 2015) repository for the R statistical programming language (R version 3.2.2). Of the 33 samples sequenced, four were identified to be outliers based on the ROBPCA algorithm (Hubert et al., 2005) and were excluded from further analysis. For principal coordinates analysis (PCoA), a minimum read count of 10 (i.e. OTUs with greater than 10 reads total across samples) was applied before coordinate transformations, and statistical significance was calculated using adonis function in the 'vegan' package (version 2.4-1) (https://cran.r-project.org/web/packages/vegan/index.html). Top OTU abundances were computed as the sum of read counts across replicate animal samples in each condition.

Differential expression analysis of OTUs: After application of a filtering step as described in the previous section, the raw, non-rarified read counts were prepared for

differential expression testing. For a discussion on the effect of rarefying on microbiome data, see(McMurdie and Holmes, 2014). The count data were analyzed for differential expression due to genotype in the solid diet condition using the DESeq2 package (version 1.12.4)(Love et al., 2014) where statistical significance was calculated based on the Wald test using the Benjamini-Hochberg correction for multiple comparisons. Plotting and visualization was performed using built-in functions provided by phyloseq and modified using the 'ggplot2' package (version 2.2.0)(Wickham, 2009).

Statistical analysis: All the experiments were repeated at least three times, and the results are given as means±s.e.m. For bacterial translocation assays, pancreatic leukocyote counts, and histology scores, statistical analysis was performed in GraphPad Prism using Mann-Whitney nonparametric unpaired t-test. Statistical analysis for Survival plots was done using JMP statistical software version 12.0. Log Rank statistics was computed to test the homogeneity of the estimated survival function across groups. The test determines the assumption that all survival functions were equal. The statistical analysis for time-derived data was done by Repeated measures ANOVA. The group, time, and group-time interaction were used as fixed effects and individual subjects within a group were treated as random effects. Pairwise comparisons between groups was done by Least Square Means conducted using Tukey-HSD test. Differences between the groups for rest of the experiments were analyzed for statistical significance using unpaired Student's t-test. In all cases, $P<0.05$ or better was considered statistically significant.

Supplementary Figures

Supplementary Figure 1: Generation of the Orai1$^{fl/fl}$ mice and expression of Orai1, ZO1 and IP$_3$R3 in Orai1$^{fl/fl}$ and Orai1$^{-/-}$ acini. Related to Figure 1.

(a, b) Generation of the Orai1$^{-/-}$ mice (see methods for detail). **(c)** Expression of Orai1 mRNA in the pancreas and several other tissues at 2 and 30 days after TX gavage. **(d-g)** Sections (d, f) and isolated acini (e, g) from Orai1$^{fl/fl}$ (d, f) or Orai1$^{-/-}$ mice (e, g) were stained for Orai1. Note in (f) the preserved staining of Orai1 in a pancreatic duct and blood vessel (BV). **(h-k)** Acini from Orai1$^{fl/fl}$ (h, i) or Orai1$^{-/-}$ mice maintained on solid diet (j, k) were stained for the tight junction ZO1 (h, j) or the IP$_3$Rs receptor type 3 (i, k)

Supplementary Figure 2: Expression of amylase and Rab27 in Orai1$^{fl/fl}$ and Orai1$^{-/-}$ acinar cells. Related to Figure 1.

(a-d) Sections from Orai1$^{fl/fl}$ (a, b) or Orai1$^{-/-}$ mice maintained on solid diet (c, d) were stained for Amylase (a, c, red) or Rab27 (b, d, red). Green is actin and blue is DAPI. Higher magnification images of the sections marked by the squares are shown to the right of the main images. **(e)** Intensity of amylase staining in 9 images obtained from 3 mice of each phenotype. The results are mean±s.e.m.

Supplementary Figure 3: Satiety hormone in Orai1$^{fl/fl}$ and Orai1$^{-/-}$ mice. Related to Figure 1.

(a-d) The level of the satiety hormones PYY (a), Leptin (b), Insulin (c) and Glucagon (d) was measured in the serum of 3 Orai1$^{fl/fl}$ (black) and 5 Orai1$^{-/-}$ mice maintained on solid diet (red) 5 day (a, b) or 10 days (a-d) after end of Tamoxifen gavage. The results are mean±s.e.m.

Supplementary Figure 4: Multiple assays of pancreatic damage in Orai1$^{fl/fl}$ and Orai1$^{-/-}$ mice. Related to Figure 3.

(a) Sections obtained from Orai1$^{fl/fl}$ (left image) or Orai1$^{-/-}$ mice on solid diet (all other images) were stained for Cluadin2. The middle images show example of intracellular staining and the right image example of lack of Claudin2 staining. **(b)** stained area in Orai1$^{fl/fl}$ relative to Orai1$^{-/-}$ acini in 9 and 15 images obtained from 3 Orai1$^{fl/fl}$ and 5 Orai1$^{-/-}$ mice. Results are mean±s.e.m. **(c)** Relative staining intensity in the same images as in (b). **(d)** H&E staining of pancreatic sections obtained from Orai1$^{fl/fl}$ and Orai1$^{-/-}$ mice on solid or liquid diet. The columns on the right summaries (mean±s.e.m) the damage in 3 mice with each line and 5 fields in each pancreas (15 images). Damage was evaluated by determining area with edema as in (Kim et al., 2009). **(e)** Oil red (lipids) staining in sections of Orai1$^{fl/fl}$ and Orai1$^{-/-}$ pancreas maintained on solid (middle) or liquid diet (right). The columns are mean±s.e.m of 12, 15 and 12 images, respectively. **(f)** Trichrome Mason's staining (collagen) staining in sections of Orai1$^{fl/fl}$ and Orai1$^{-/-}$ pancreas maintained on solid (middle) or liquid diet (right). The columns are mean±s.e.m of 18, 21 and 18 images, respectively. **(g)** Serum amylase and **(h)** Serum lipase were measured in 3 mice of each line. **(i)** Blood cells were harvested from the pancreata of the indicated number of Orai1$^{fl/fl}$ and Orai1$^{-/-}$ mice maintained on solid diet and analyzed by flow cytometry.

Supplementary Figure 5: Number of spores, transit time and bacterial adherence in Orai1$^{fl/fl}$ and Orai1$^{-/-}$ mice. Related to Figures 4, 5 and 6.

(a) Average number of Bacteria/field in the indicated intestinal region of the three mice lines (n=5 mice, 15 images). **(b)** Solid food transient time was measured in Orai1$^{fl/fl}$ (black), and Orai1$^{-/-}$ mice maintained on solid (red) and liquid diets (blue). **(c)** Liquid food transient time was measured in Orai1$^{fl/fl}$ (black), and Orai1$^{-/-}$ mice maintained on solid (red) and liquid diets (blue). **(d)** Adherence of bacteria to the intestine of Orai1$^{fl/fl}$ mice. The images of Orai1$^{-/-}$ mice on solid diet are reproduce from Figure 4 for comparison. **(e)** Solid food consumption by untreated Orai1$^{fl/fl}$ (black, blue) and Orai1$^{-/-}$ mice (red, green) maintained on solid diet or treated with wide-spectrum antibiotics (blue, green) for one week before and during the experiment provided in drinking water.

Table S1: Phenotype of Orai1$^{fl/fl}$ and Orai1$^{-/-}$ mice maintained on the indicated diet. Related to all Figures.

The mice were submitted for necropsy by the investigator to a veterinary pathologist. Pancreatic damage was determined by the pathologist based on fibrosis. Hepatic lipidosis was determined by the pathologist based on presence of fatty replacement. Intestinal inflammation, hepatitis, and gastritis were determined by the pathologist based on cellular infiltration. Systemic infection was determined based on histologic evidence of bacterial infiltration in organs that should be sterile (e.g. heart, CNS, liver). *2 mice with pancreatic damage also have intestinal inflammation. **3 mice with intestinal inflammation also have pancreatic damage.

References

Desai, B.M., Oliver-Krasinski, J., De Leon, D.D., Farzad, C., Hong, N., Leach, S.D., and Stoffers, D.A. (2007). Preexisting pancreatic acinar cells contribute to acinar cell, but not islet beta cell, regeneration. The Journal of clinical investigation *117*, 971-977.

Ellekilde, M., Selfjord, E., Larsen, C.S., Jakesevic, M., Rune, I., Tranberg, B., Vogensen, F.K., Nielsen, D.S., Bahl, M.I., Licht, T.R*., et al.* (2014). Transfer of gut microbiota from lean and obese mice to antibiotic-treated mice. Sci Rep *4*, 5922.

Erben, U., Loddenkemper, C., Doerfel, K., Spieckermann, S., Haller, D., Heimesaat, M.M., Zeitz, M., Siegmund, B., and Kuhl, A.A. (2014). A guide to histomorphological evaluation of intestinal inflammation in mouse models. Int J Clin Exp Pathol *7*, 4557-4576.

Hensley, M.T., de Andrade, J., Keene, B., Meurs, K., Tang, J., Wang, Z., Caranasos, T.G., Piedrahita, J., Li, T.S., and Cheng, K. (2015). Cardiac regenerative potential of cardiosphere-derived cells from adult dog hearts. Journal of cellular and molecular medicine *19*, 1805-1813.
Huber, W., Carey, V.J., Gentleman, R., Anders, S., Carlson, M., Carvalho, B.S., Bravo, H.C., Davis, S., Gatto, L., Girke, T.*, et al.* (2015). Orchestrating high-throughput genomic analysis with Bioconductor. Nat Methods *12*, 115-121.
Hubert, M., Rousseeuw, P.J., and Vanden Branden, K. (2005). ROBPCA: A new approach to robust principal component analysis. Technometrics *47*, 64-79.
Ji, B., Song, J., Tsou, L., Bi, Y., Gaiser, S., Mortensen, R., and Logsdon, C. (2008). Robust acinar cell transgene expression of CreErT via BAC recombineering. Genesis *46*, 390-395.
Kim, M.S., Hong, J.H., Li, Q., Shin, D.M., Abramowitz, J., Birnbaumer, L., and Muallem, S. (2009). Deletion of TRPC3 in mice reduces store-operated Ca2+ influx and the severity of acute pancreatitis. Gastroenterology *137*, 1509-1517.
Love, M.I., Huber, W., and Anders, S. (2014). Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol *15*, 550.
Matsuda, A., Makino, N., Tozawa, T., Shirahata, N., Honda, T., Ikeda, Y., Sato, H., Ito, M., Kakizaki, Y., Akamatsu, M.*, et al.* (2014). Pancreatic fat accumulation, fibrosis, and acinar cell injury in the Zucker diabetic fatty rat fed a chronic high-fat diet. Pancreas *43*, 735-743.
McMurdie, P.J., and Holmes, S. (2013). phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PloS one *8*, e61217.
McMurdie, P.J., and Holmes, S. (2014). Waste not, want not: why rarefying microbiome data is inadmissible. PLoS Comput Biol *10*, e1003531.
Steenwinckel, V., Louahed, J., Lemaire, M.M., Sommereyns, C., Warnier, G., McKenzie, A., Brombacher, F., Van Snick, J., and Renauld, J.C. (2009). IL-9 promotes IL-13-dependent paneth cell hyperplasia and up-regulation of innate immunity mediators in intestinal mucosa. Journal of immunology *182*, 4737-4743.
Sun, J., Furio, L., Mecheri, R., van der Does, A.M., Lundeberg, E., Saveanu, L., Chen, Y., van Endert, P., Agerberth, B., and Diana, J. (2015). Pancreatic beta-Cells Limit Autoimmune Diabetes via an Immunoregulatory Antimicrobial Peptide Expressed under the Influence of the Gut Microbiota. Immunity *43*, 304-317.
Wickham, H. (2009). ggplot2: Elegant Graphics for Data Analysis. Use R, 1-212.

Figure S1, Ahuja et al

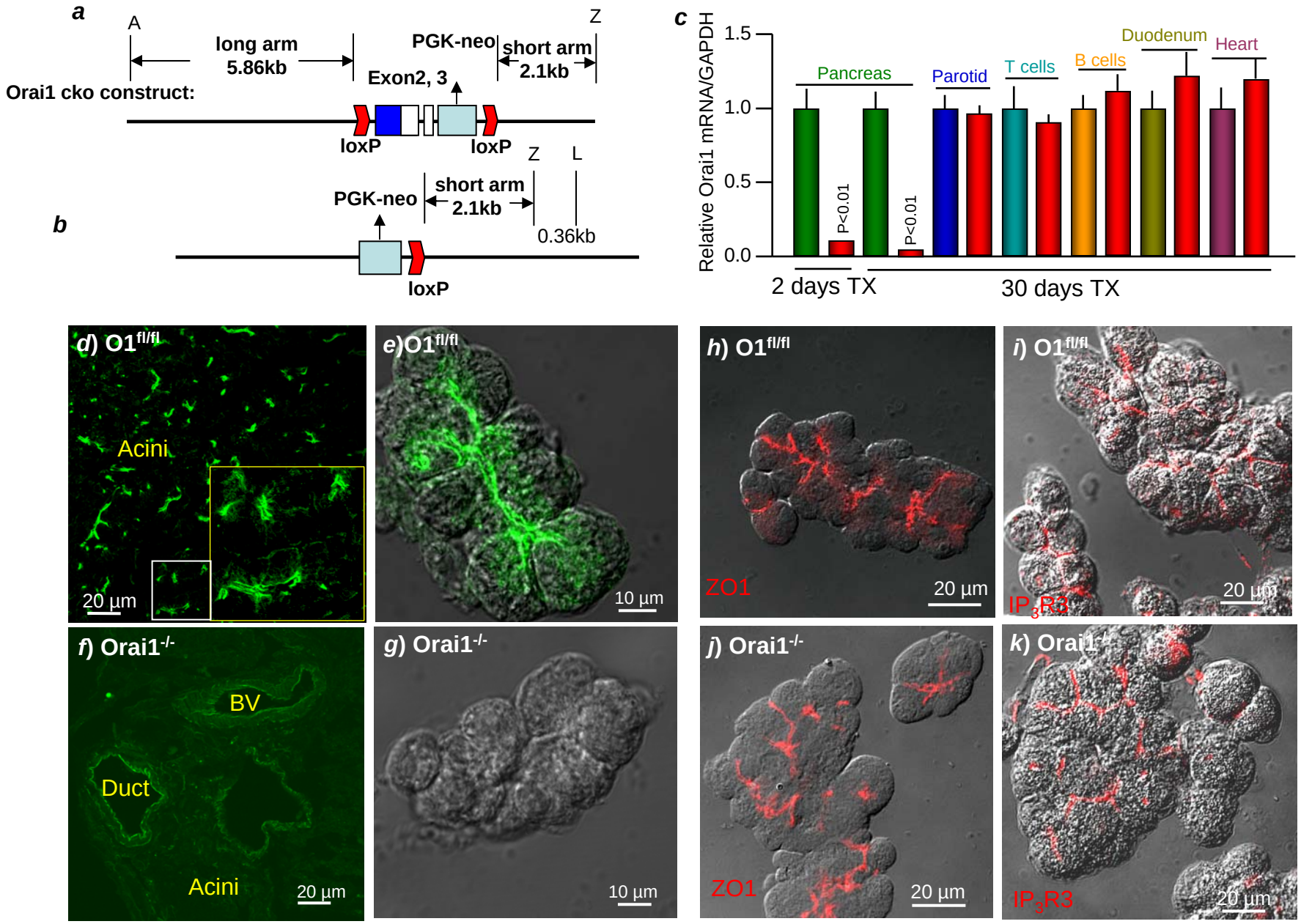

Figure S2, Ahuja et al

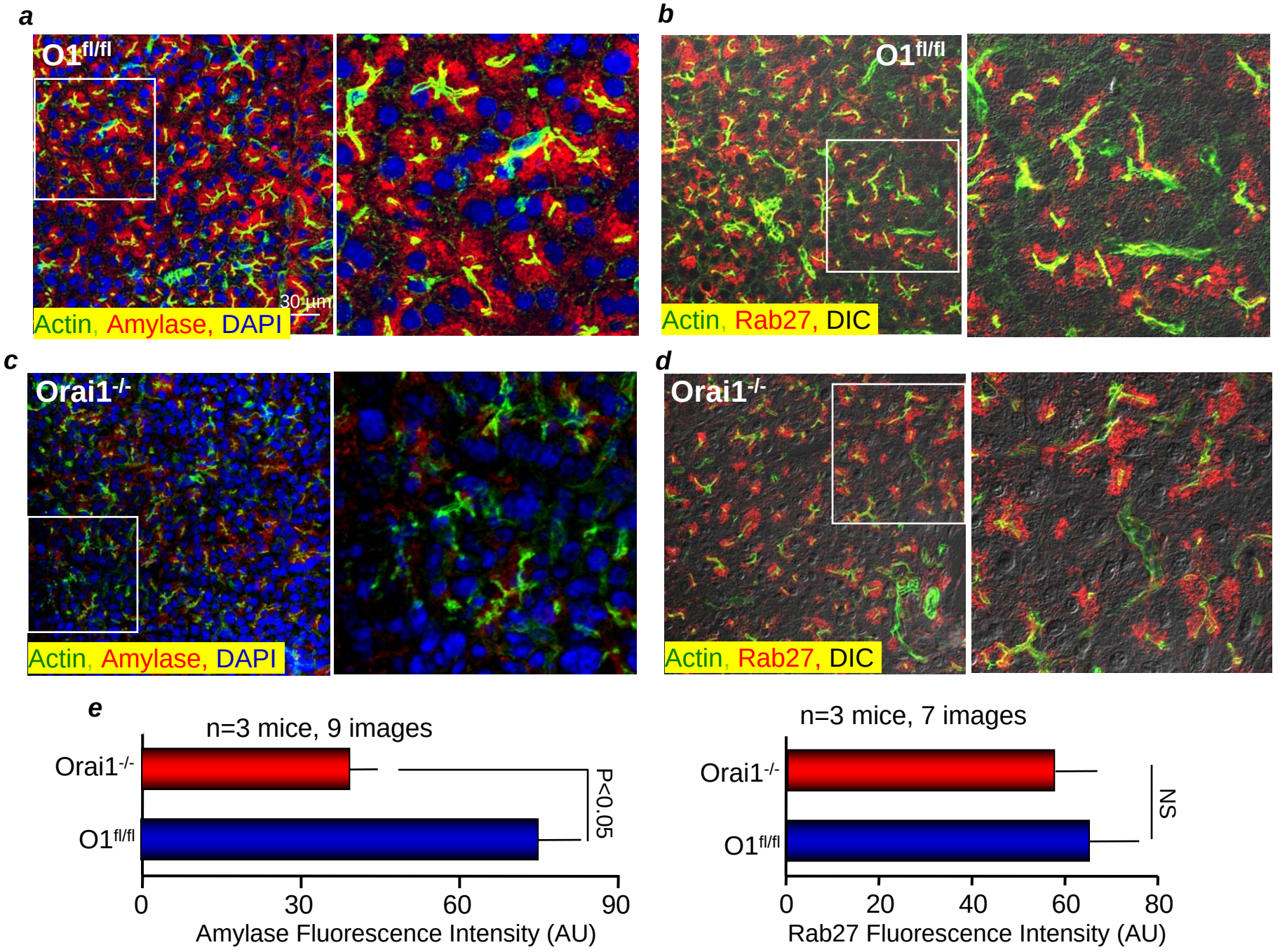

Figure S3, Ahuja et al

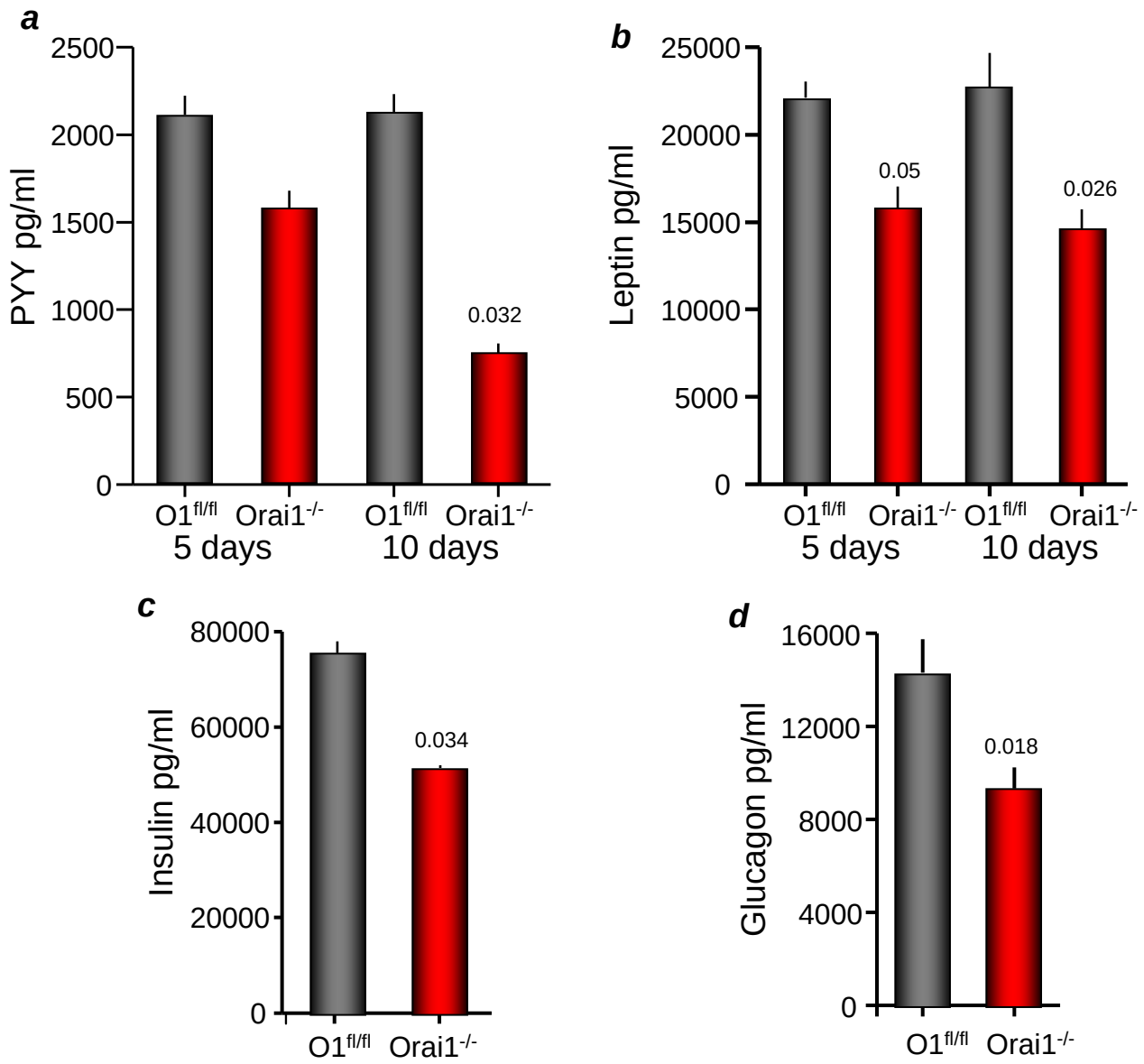

Figure S4, Ahuja et al

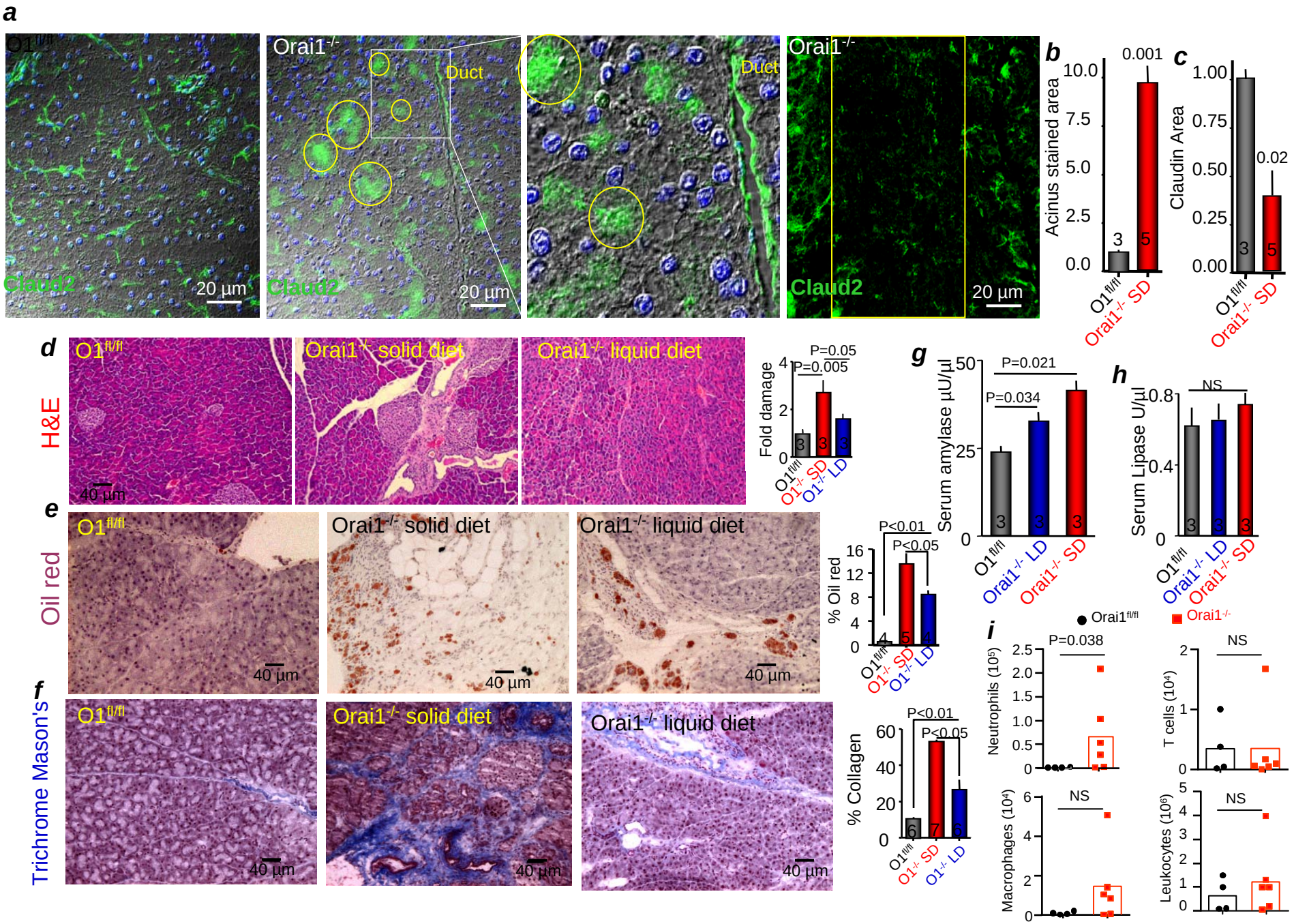

Figure S5, Ahuja et al

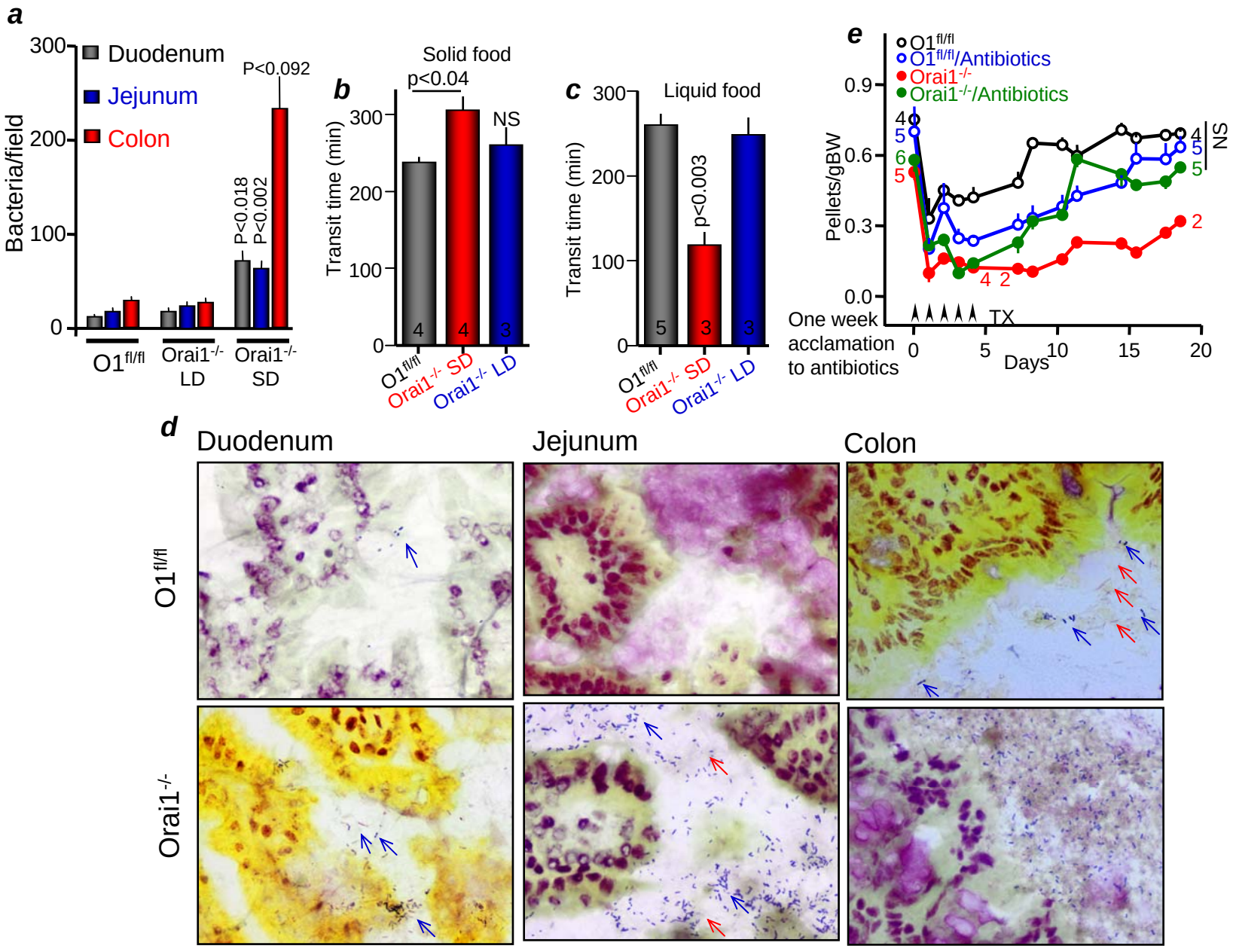

Table S1, Ahuja et al

	$O1^{f/f}$ SD (n=7)	$O1^{-/-}$ SD (n=13)	$O1^{f/f}$ LD (n=5)	$O1^{-/-}$ LD (n=5)
Very lean, ruffled coat	0	13	0	1
Pancreatic damage	1	5*	0	2
Gastric pH range	7.5-8	7.5-8	5-6	5-7
Gastritis	2	5	0	2
Intestinal inflammation	0	6**	1	1
Systemic infection	0	4	0	0
Liver mild lipidosis mild hepatitis	 1 1	 3 1	 0 0	 0 0