Supplemental Information Appendix

Epithelial delamination is protective during pharmaceutical-induced enteropathy

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Supplemental Methods

Zebrafish lines and animal husbandry

All zebrafish experiments were performed in accordance with NIH guidelines and protocols approved by the Duke University Medical Center Institutional Animal Care and Use Committee (protocol number A115-16-05). Wild type Tübingen zebrafish and the following engineered lines were used: *Tg(-4.5fabp2:DsRed)*^{pd1000} (1), *Tg(lyz:EGFP)*^{nz117Tg} (2), *Tg(NFkB:EGFP)*^{nc1Tg} (1), *Tg(CMV:GFP-LC3)*^{zf155Tg} (3), *Tg(elf1a:xbp1-d-GFP)*^{mb10Tg} (4), *Tg(mpeg1:Gal4;UAS:NTR-mCherry)*^{gl24Tg} (5) [referred to as *Tg(mpeg1:UNM)*], *cdh1-tdTomato*^{xt18} (6), *Gt(ctnna1-citrine)*^{ct3a} (7), *Gt(jup-citrine)*^{ct520a} (7), and *TgBAC(tnfa:GFP)*^{pd1028Tg} (8). *myd88* mutant zebrafish were kindly provided by Karen Guillemin (9). Adult zebrafish were maintained in a recirculating aquaculture system on a 14/10h light/dark cycle. From 5 to 14 dpf, larvae on the system were fed Zeigler AP100 <50 μm larval diet (Pentair, LD50-AQ) twice daily and Skretting Gemma Micro 75 (Bio-Oregon, B5676) powder once daily. Beginning at 12-14 dpf, larvae were fed Artemia (Brine Shrimp Direct, BSEACASE) twice daily supplemented with a daily feed of Skretting Gemma Micro 75. From 28 dpf, Gemma Micro 75 diet was replaced with Gemma Micro 300 (Bio-Oregon item no. B2809). At the onset of sexual maturity, adult fish were fed twice daily, once with Artemia and once with a 1:1 mixture of Skretting Gemma Micro 500 (Bio-Oregon, B1473) and Skretting Gemma Wean 0.5 (Bio-Oregon, B2818). Larval zebrafish are of indeterminate sex. Unless otherwise noted, embryos were collected from natural matings and maintained in embryo media [0.3%(w/v) crystal sea salt , 0.75 mM $CaCO_3$, 0.45 mM $NaHCO_3$, methylene blue] at a density of <1 larva / mL at 28.5ºC on a 14/10h light/dark cycle until 3 dpf, at which time they were sorted and randomly assigned to treatment groups.

Generation of *Tg(ubb:seca5-tdTomato)*^{xt24} transgenic zebrafish

The ubb:seca5-tdTomato transgene was created by multisite Gateway recombination according to Kwan et al. (10), using pENTR5'_ubi (11) (a kind gift from Leonard Zon, Addgene plasmid #27320), pME secA5 and p3E-tdTomato (12) into pDestTol2pA2 (10). pME secA5, containing a *C. elegans* secretion signal followed by the human Annexin V coding sequence, was made by Gateway BP recombination of pBH secA5 (13) (a kind gift of Randall Peterson, Addgene plasmid #32359) with pDONR221. Tol2 transposase mRNA was transcribed using an mMessage mMachine kit (ThermoFisher Scientific) from T3TS-Tol2 (14).

Transgenic zebrafish lines were generated by injecting single-cell embryos with ~1 nL of transgenesis mixture containing 50 ng/μL ubb:seca5-tdTomato transgenesis construct, 25 ng/μL Tol2 transposase mRNA, 0.3% phenol red and 1x Tango buffer (ThermoFisher). Transgene positive larvae were selected by fluorescence microscopy and raised to adulthood. Mosaic founders were outcrossed to generate the *Tg(ubb:seca5-tdTomato)*^{xt24} line.

Generation of *(cdh1-YFP)*^{x17} knock-in zebrafish

cdh1-YFP knock-in zebrafish were generate essentially as described, except the tdTomato open reading frame was substituted with YFP in the donor plasmid (6).

Serial Glafenine exposure

For serial Glafenine exposure in conventionally raised larvae, eggs were collected from natural matings in embryo media. For all experiments, at least two clutches were pooled. At 3 dpf, larvae were randomly assigned to treatment groups, transferred to either 10 cm petri dishes or 6-well (non-tissue-culture treated) plates, maintained at a density of 1 larva/mL in gnotobiotic zebrafish media (GZM) (15), and exposed to 30 μM Glafenine or isovolumetric amounts of DMSO. Media was changed at 24h intervals (50% v/v). Embryos and larvae were maintained on a 14/10h light/dark cycle at 28.5°C.

Pharmacological manipulations

Unless otherwise noted (**Table S1**), stock solutions of drugs were prepared in ACS-grade DMSO (Fisher Scientific, D136), aliquoted and stored at -20°C. In the absence of previous reports using pharmacological agents in zebrafish, concentrations used were based both on reported efficacy in other model systems and solubility in GZM. Preliminary toxicity testing was performed from 3-6 dpf, with re-exposure at 24h intervals in accordance with the Glafenine dosing paradigm. Initially, ≥3 concentrations were tested. 2D chemical structures were drawn with ChemDraw Professional v16.0 (PerkinElmer). For *in vivo* SAR, compounds with chemical structures similar to Glafenine were identified via PubChem and EMBL ChEBI. *In silico* similarity assessment was performed using the ChemMine Workbench to compute both Tanimoto coefficients (Tc) and maximum common substructure (MCS) scores (16) (**Dataset S1**).

Serial Glafenine exposure in gnotobiotic zebrafish

Derivation of germ-free (GF) zebrafish was performed as described,(15) with the following modifications: gentamycin (Sigma, G1254, final concentration 50 μg/mL) was included in the GZM solution containing antibiotics (AB-GZM). At 1 dpf, sterility was assessed by inoculating media into brain heart infusion (BHI) broth and spotting on tryptic soy agar (TSA), followed by aerobic, static incubation at 28.5°C (absence of microbial growth was indicative of sterility). At 2 dpf, embryos were transferred to 6-well plates. At 3 dpf, media was removed, and either 5 mL (for GF groups) or 2.5 mL (for CV groups) of sterile GZM was added to each well. CV groups were inoculated with 2.5 mL system water collected from our aquaculture facility (passed through a 5 μm filter (Millipore, SLSV025LS) to remove large debris). Subsequently, 5 mL GZM with 60 μM Glafenine (or DMSO) was added to each well, for a final concentration of 30 μM. At 24h intervals, 50% (v/v) media changes were performed as described above.

Acridine Orange staining, imaging and quantification

To quantify IEC shedding, larvae were stained with Acridine Orange (1 μg/mL, Sigma, A8097) in GZM for 30 minutes in the dark at room temperature with gentle agitation, rinsed 3x with GZM (50% volume), and anesthetized with 0.75 mM MS222. Larvae were embedded in 3% methylcellulose (w/v in GZM) and imaged with a Leica M205 FA stereomicroscope equipped with a 1x objective, metal-halide light source, Leica DFC365FX or Hamamatsu Orca Flash4.0LT camera, and mCherry filter. Acquisition was performed with Leica LAS X software, and exposure time and magnification held constant. Images were opened in Fiji and a line was drawn down the middle of the intestine along the anterior-posterior axis from the portion of the intestine immediately posterior to the swim bladder to the cloaca. CSV files of position (X) and grayscale value (Y) along the line were exported using the Fiji Plot Profile function, and data were position-normalized to account for variation in the length of measured regions. Of note, we observe similar responses in non-transformed data. Data were imported into MATLAB R2017a (64bit, Mathworks Inc., Natick, Massachusetts), and trapezoidal numerical integration was performed on grayscale values versus position using the *trapz* function, to yield "Integrated Intestinal Acridine Orange Fluorescence" (Supplementary **Figure S1A-D**). While the absolute values varied between experiments,

the magnitude of change was consistent. The same quantification method was used for analysis of *Tg(ubb:seca5-tdTomato)*^{xt24} larvae.

In vivo confocal microscopy

In vivo confocal microscopy was performed with a Zeiss 780 upright microscope equipped with a GaAsP array detector and a 20x water dipping objective (NA 1.0, WD 1.8 mm). Larvae were anesthetized in 0.75 mM MS222 and immobilized in 1% LMP Agarose (w/v in GZM, supplemented with 0.75 mM MS222), and GZM containing 0.75 mM MS222 was used as immersion medium. For imaging delamination, 4D (*xyzt*) imaging experiments were performed by collecting z-stacks (0.5 μm step size) over time (interval = 0) for 30-45 minutes. Two intestinal ROIs were imaged: ventral to the swim bladder, and immediately posterior to the swim bladder. Movies were generated using Fiji.

Vibratome, immunofluorescence, and EdU labeling

Larvae were fixed in 4% PFA in PBS (overnight, 4°C, shaking), rinsed 3x with PBS, then embedded in 4% LMP Agarose (w/v in PBS, Fisher Scientific, BP165) in cryo-molds (Tissue-Tek, 4566). Sections were cut (200 μm thickness) with a Leica VT1000S vibratome and either directly mounted on glass slides with Vectashield (H-1000, Vector Labs) or transferred to 24-well plates and immersed in 1x PBS.

For immunofluorescence staining, sections were washed 3x with 1x PBS, permeabilized with 0.1% Triton x-100 in PBS (PBS-T), then blocked (5% normal donkey serum in PBS-T, 2h, room temperature). Sections were incubated in primary antibodies (overnight, 4°C) diluted in blocking solution (**Supplemental Table 3**). Sections were washed 3x with 1x PBS, then incubated for 2h with secondary antibodies, and Hoechst (ThermoFisher) diluted in PBS-T. Sections were washed 3x with 1x PBS, then mounted with Vectashield on slides and cover slipped.

For proliferation analysis, larvae were incubated with EdU (10 μM) for 24h, euthanized, fixed, and processed for vibratome sectioning as described above. EdU incorporation was detected using the Click-IT EdU Alexa Fluor 647 Imaging Kit (ThermoFisher) following the manufacturers protocol. Following click-detection, sections were processed for indirect immunofluorescence labeling with antibodies (rabbit anti RFP and/or mouse anti 4E8, **Supplemental Table 3**) as described above.

Imaging was performed with a Zeiss 780 upright confocal microscope equipped with a GaAsP array detector and 63x oil immersion objective (WD 0.19 mm, NA 1.4). Z-stacks were collected at 0.25 μm intervals with a 1024x1024 frame size and averaging set to 4. For proliferating cell quantification, lsm files were opened with Fiji, lookup tables applied, channels overlaid, and z-slices ≥5 μm apart were analyzed. The total number of epithelial cells, sub-epithelial cells, EdU^+ epithelial cells and EdU^+ sub-epithelial cells were enumerated. To quantify LC3 punctae, lsm files were opened in Fiji, lookup tables applied, and channels corresponding to DAPI, *Tg*(*fabp2*:*DsRed*) and *Tg(CMV:GFP-LC3)* merged. Z-slices ≥5 μm apart were analyzed and for a given z-slice, enterocytes with and without GFP-$LC3^+$ punctae were enumerated. E-cadherin and ZO-1 localization and intensity was quantified along line measurements using the "segmented line" and "plot histogram" functions in Fiji. Measurements were performed along 3 non-adjacent cell borders per z-slice, from ≥5 z-slices per samples (at least 10 μm apart).

Larval intestine explant culture

For intestinal explant culture, larvae were euthanized with 3 mM MS222 then transferred to agarose dissecting pads in minimal volumes of media and the intestines dissected using fine forceps and a tungsten wire. Care was taken remove skin, liver and other tissues. Each intestine was resuspended in 5 μL cold Matrigel (Corning, 356231) and transferred to a 96-well black-walled thin bottom plate (Greiner, 655090) with a glass capillary (1 intestine / well). The Matrigel was allowed to polymerize at 28.5°C for 30 minutes. DMSO or Glafenine were diluted (0.03% or 30 μM, respectively) in RPMI1640 (phenol-free) supplemented with 10% heat-inactivated FBS and 10 mM HEPES. Intestines were overlaid with 100 μL media and immediately imaged with a Zeiss AxioObserver Z1 microscope equipped with an Xcite 120Q

light source (Lumen Dynamics), MRm camera (Zeiss), and 20x objective lens (WD 7.9 mm, NA 0.4). Multi-position 4D imaging was performed using Zen Blue 2012 edition (Zeiss) by collecting z-stacks (5 slices / sample, 20 μm step size) at 30 minute intervals. Image processing and quantification was performed with Fiji.

Intestinal barrier function

Intestinal permeability was assayed as described (17). Texas Red-conjugated dextran (ThermoFisher, 10 kDa, 0.5% w/v) in sterile 1x PBS with 0.05% phenol red tracer was microgavaged into the intestinal bulb of 5 or 6 dpf larvae using microforged borosilicate glass needles and a Picospritzer III injection apparatus (Parker, 051-0600-200-003). Following incubation (28.5°C, 30 minutes, in the dark) animals were imaged with a Leica M205 FA stereomicroscope equipped with a Leica DFC365FX camera. To induce barrier leakage, 20 μM EDTA was also included in the gavage solution for control larvae. Fluorescence was measured in an ROI posterior to the swim bladder, immediately dorsal to the intestine but ventral to the notochord using Fiji, and background subtracted prior to graphing and statistical analyses.

Media dot blot

Media was mixed by gentle pipetting and collected at indicated time points in 15 mL conical tubes and stored at -80°C until further analysis. Aliquots were thawed on ice and 1 mL of media / sample was subjected to acetone precipitation, then resuspended in 250 μL RIPA buffer (Sigma) supplemented with protease inhibitor cocktail (cOmplete Mini EDTA-free, Roche). Samples were applied to nitrocellulose membranes using a vacuum dot blot manifold (S&S Minifold I). Membranes were washed with TBS-T, blocked in 5% BSA/TBS-T, and probed with anti 4E8 (Abcam) at 4°C overnight. Membranes were subsequently washed, and incubated with secondary antibody (2h, room temperature), washed with TBS and imaged with a Li-Cor Odyssey scanner. Quantification was performed with Fiji.

Flow cytometry and FACS

Flow cytometry and FACS were performed as described with minor modifications (18). Briefly, pools of larvae (20 / replicate) were euthanized with 3 mM MS222 and washed for 5 minutes at room temperature with gentle agitation with deyolking buffer (55 mM NaCl, 1.8 mM KCl, 1.25 mM $NaHCO_3$). Buffer was removed, and larvae were transferred to gentleMACS "C" tubes (Miltenyi Biotec, 130-096-334) containing 5 mL Buffer 1 [HBSS supplemented with 5% heat-inactivated FBS (HI-FBS, Sigma) and 10 mM HEPES]. Larvae were subsequently dissociated using a combination of enzymatic and mechanical disruption. Following addition of Liberase (Roche, 05 401 119 001, 5 μg / mL final), DNaseI (Sigma, D4513, 2 μg/mL), Hyaluronidase (Sigma, H3506, 6 U/mL final) and Collagenase XI (Sigma, C7657, 12.5 U/mL final), samples were dissociated using pre-set program C_01 on a gentleMACS dissociator (Miltenyi Biotec), then incubated at 30°C on an orbital platform at 75 RPM for 10 minutes. The disruption-incubation process was repeated 4 times, after which 400 μL ice-cold 120 mM EDTA was added to stop enzyme activity. Following addition of 10 mL Buffer 2 (HBSS supplemented with 5% HI-FBS, 10 mM HEPES and 2 mM EDTA) samples were filtered through 30 μm cell strainers. Filters were washed with 10 mL Buffer 2, and cells were pelleted by centrifugation (1800 rcf, 15 minutes, room temperature), then gently resuspended in 500 μL Buffer 2. For CellROX labeling, cells were transferred to a 24-well plate, CellROX Green (ThermoFisher) was added to a final concentration of 1 μM, and samples were incubated in the dark at 28.5°C on a tilting platform for 45 minutes. Samples were transferred to FACS tubes, and DNaseI (10 μg/mL final) and 7-AAD (5 μg/mL final) were added. FACS was performed at the Duke Cancer Institute Flow Cytometry Shared Resource with either a Beckman Coulter MoFlo XDP, Becton-Dickinson DIVA, or Beckman Coulter Astrios instrument. Gating and compensation were performed with single and dual color controls, and viable cells identified as $7\text{-}AAD^-$. Data were analyzed with FloJo v.10 (TreeStar, CA).

Gene expression analysis

For gene expression analysis of digestive tissues, larval zebrafish digestive tracts were dissected with fine forceps under a stereomicroscope (15-20 guts / replicate), and total RNA isolated using TRIzol (Invitrogen) and a PureLink RNA Mini Kit (ThermoFisher). RNA was quantified with a Nanodrop ND-1000 spectrophotometer, and 50 or 100 ng was DNaseI-treated (NEB) and used for cDNA synthesis (iScript, Bio-Rad).

For gene expression analysis of FACS-isolated enterocytes, 3,000 - 6,000 7-AAD$^-$ *fabp2:DsRed*$^+$ cells were collected into 750 µL TRIzol LS (ThermoFisher). Following extraction with 200 µL chloroform, an equal volume of 70% Ethanol (prepared in DEPC-treated H_2O) was added to the aqueous phase and mixed. RNA was isolated using the RNA Cleanup and Concentrate Micro-Elute kit (Norgen Biotek) following the manufacturer's instructions. RNA was eluted in 10 µL, and 8 µL was subsequently DNaseI-treated and used for cDNA synthesis (iScript, Bio-Rad). Quantitative PCR was performed with gene-specific primers (**Table S2**) and 2x SYBR Green Master Mix with ROX (PerfeCta, Quanta Bio) on an ABI StepOnePlus instrument. Data were analyzed with the *ΔΔCt* method.

***xbp1* splicing assay**

To monitor *xbp1* splicing, 1 µL cDNA (from dissected digestive tracts of 6 dpf larvae, n=20 / replicate) was used as template in a 20 µL PCR reaction containing 2x GoTaq Green master mix (Promega) and 500 nM primers (*xbp1*-RT-F and *xbp1*-RT-R; Supplementary **Table S2**). PCR products were resolved on either 3% Agarose or 6% PAGE gels (ThermoFisher) run in 1x TBE buffer, post-stained with ethidium bromide and imaged. Densitometry was performed with Fiji.

Prostaglandin E metabolite ELISA

Pools of 20 larvae were euthanized and transferred to Eppendorf tubes. After removal of media, samples were snap frozen in liquid nitrogen and stored at -80°C. Samples were thawed on ice, resuspended in 300 µL 0.1 M phosphate buffer (pH 7.4) with 1 mM EDTA, and transferred to 2 mL screw top tubes containing ceramic beads (Precellys 2 mL Soft Tissue Homogenizing Ceramic Beads Kit). Samples were homogenized in a Precellys 24 homogenizer (6500 rpm, 2 x 30 second pulses). An aliquot of the homogenate was reserved for total protein quantification by BCA Assay (ThermoFisher). Proteins were precipitated by addition of 4 volumes of ethanol, and centrifugation (8,000 rcf, 10 minutes, 4°C). The supernatant was transferred to a new Eppendorf tube, and evaporated using a Speed Vac (Savant, SC110A). The residue was dissolved in 500 µL acetate buffer, pH 4.0 and applied to a pre-equilibrated C18 column (Cayman Chemical). Columns were washed, then samples eluted with 5 mL 5% methanol in ethyl acetate into glass screw-top tubes. The solvent was evaporated under nitrogen, and the residue dissolved in 500 µL ELISA buffer. The Prostaglandin E Metabolite (PGEm) ELISA Assay (Cayman Chemical, Cat# 514531) was performed following the manufacturer's protocol. After addition of Ellman's reagent, absorbance was measured at 420 nm with a BioTek Synergy^2 plate reader. PGEm concentration was calculated from a standard curve and normalized to input protein concentration.

Caspase activity assays

Caspase activity was measured using a modified version of a reported protocol (19). Larvae (20 / replicate) were snap frozen in liquid nitrogen and stored at -80°C. Samples were resuspended in 300 µL lysis buffer [20 mM HEPES-KOH (pH 7.5), 250 mM sucrose, 50 mM KCl, 2.5 mM $MgCl_2$, 1 mM DTT] and transferred to 2 mL screw top tubes containing ceramic beads (Precellys 2 mL Soft Tissue Homogenizing Ceramic Beads Kit). Following homogenization (6500 rpm, 2 x 20 seconds) in a Precellys 24 homogenizer, samples were incubated on ice for 30 minutes, transferred to Eppendorf tubes and centrifuged to remove debris (15,000 rcf, 15 minutes, 4°C). Supernatants were stored at -80°C. An aliquot was used for total protein concentration determination (Coomassie Protein Assay Reagent, Pierce). Samples were diluted 1:10 in lysis buffer, and 100 µL was added to white walled solid bottom assay plates in triplicate (PerkinElmer). An equal volume of Caspase Assay Reagent (Promega G8090 or

G8200) was added to each sample, plates were sealed with foil and incubated for 2h at room temperature on an orbital shaker. Luminescence was measured with a BioTek Synergy^2 plate reader.

Genotyping

Following imaging at experimental endpoints, larvae from *myd88*$^{+/m}$ in-crosses were collected in 50 μL of 50 mM NaOH. Samples were heated at 95°C for 20 minutes, then 5 μL of 1M Tris-HCl (pH 8.0) was added, followed by vortexing. PCR was performed with 2x GoTaq Green master mix, using 1 μL template and primers myd88-F (5'-GGGGTAACGCGGAGATATAC-3') and myd88-R (5'-TTGAGCTCGGATATGACATCTTTC-3'). Purified PCR products were digested with BslI (NEB) for 2h at 55°C, then subjected to electrophoresis.

Fish-free Glafenine experiments

GZM (2.5 mL) was added per well of 6-well non-tissue-culture treated plates. System water was collected from our aquaculture facility, passed through a 5 μm syringe filter to remove large particulate, and 2.5 mL was added to each well as described for the conventionalization protocol. Finally, 5 mL of 60 μM Glafenine or 0.06% DMSO was added to each well, for a final concentration of 30 μM. Plates were maintained at 28.5°C on 14/10 hour light/dark cycle, and 50% (v/v) media changes were performed as described for the serial Glafenine exposure.

Quantification of bacterial load

For enumeration of gut bacteria by CFU plating, digestive tracts from individual larva were dissected with sterile forceps under a stereomicroscope and disrupted in 500 μL sterile PBS using a Tissue-Tearor (BioSpec Products, 985370) (30 seconds, maximum speed). Serial dilutions were prepared in sterile 1x PBS and 10 μL was spotted on TSA in duplicate. Media samples were similarly diluted in sterile 1x PBS and spotted on TSA. Plates were incubated aerobically at 28.5°C overnight.

Bioinformatic Analysis

Generation of Ire1α *phylogenetic tree:* a phylogenetic tree based on Ire1 amino acid sequences of various vertebrates was constructed and visualized using default settings in CLC Sequence Viewer (Qiagen). All sequences were downloaded from NCBI GenBank except for cod, stickleback and tetraodon, which were acquired from Ensembl: [accession numbers XP_016879837 (human), XP_016788240.1 (chimpanzee), XP_023100373.1 (cat), NP_076402.1 (mouse), XP_027327436.1 (duck), XP_015135376.1 (chicken), XP_014342576 (coelacanth), ENSGMOT00000012342.1 (cod), ENSGACT00000015505.1 (stickleback), ENSTNIT00000003430.1 (tetraodon), and NP_001018366 (zebrafish)].

Domain Prediction: protein domain prediction of the zebrafish Ire1α amino acid sequence was performed using the default configuration for the ProSite web-server (20).

Structural Modeling: protein structure modeling was performed by aligning the zebrafish Ire1α amino acid sequence to the human Ire1α crystal structure (PDB 5hgi) using the default settings in SwissModel (21-23). The human aa sequence was also aligned for comparison.

16S rRNA gene sequencing and analysis

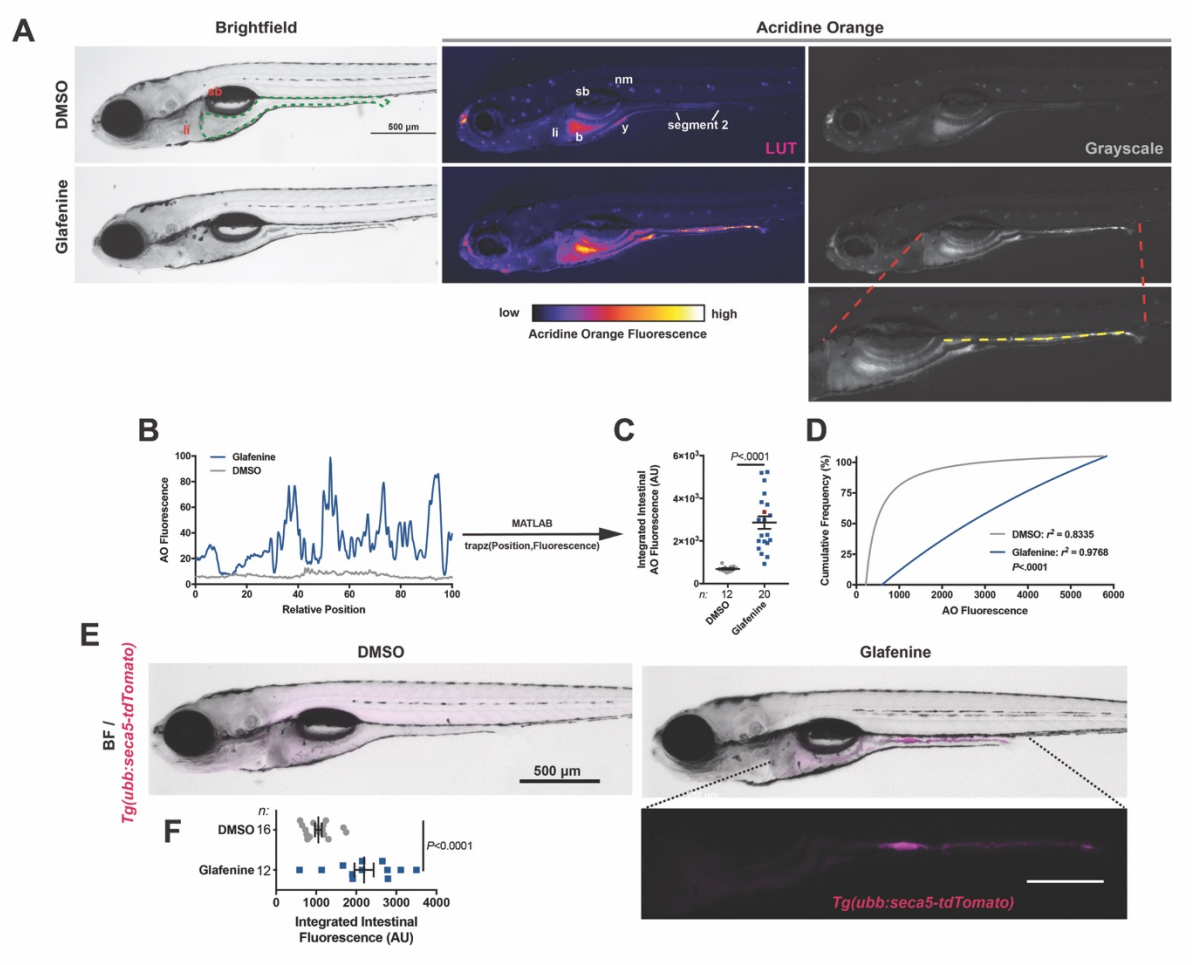
Glafenine or vehicle-treated larvae were euthanized with filter-sterilized MS222 at 6 dpf, and their digestive tracts were dissected and pooled (n = 4 digestive tracts / replicate, 6 replicates / condition), applied to sterile filter paper in Eppendorf tubes, and snap frozen in liquid nitrogen. For the conventionalized experiment, media was collected from plates that contained zebrafish as well as plates treated with DMSO or Glafenine that had never contained zebrafish. Media aliquots (1 mL) were snap frozen in liquid nitrogen. Zebrafish and media samples were stored at -80°C until further processing.

DNA isolation and sequencing were performed by the Duke Microbiome Shared Resource. Bacterial DNA was isolated using a MagAttract PowerSoil® DNA EP Kit (Qiagen, 27100-4-EP) in 96

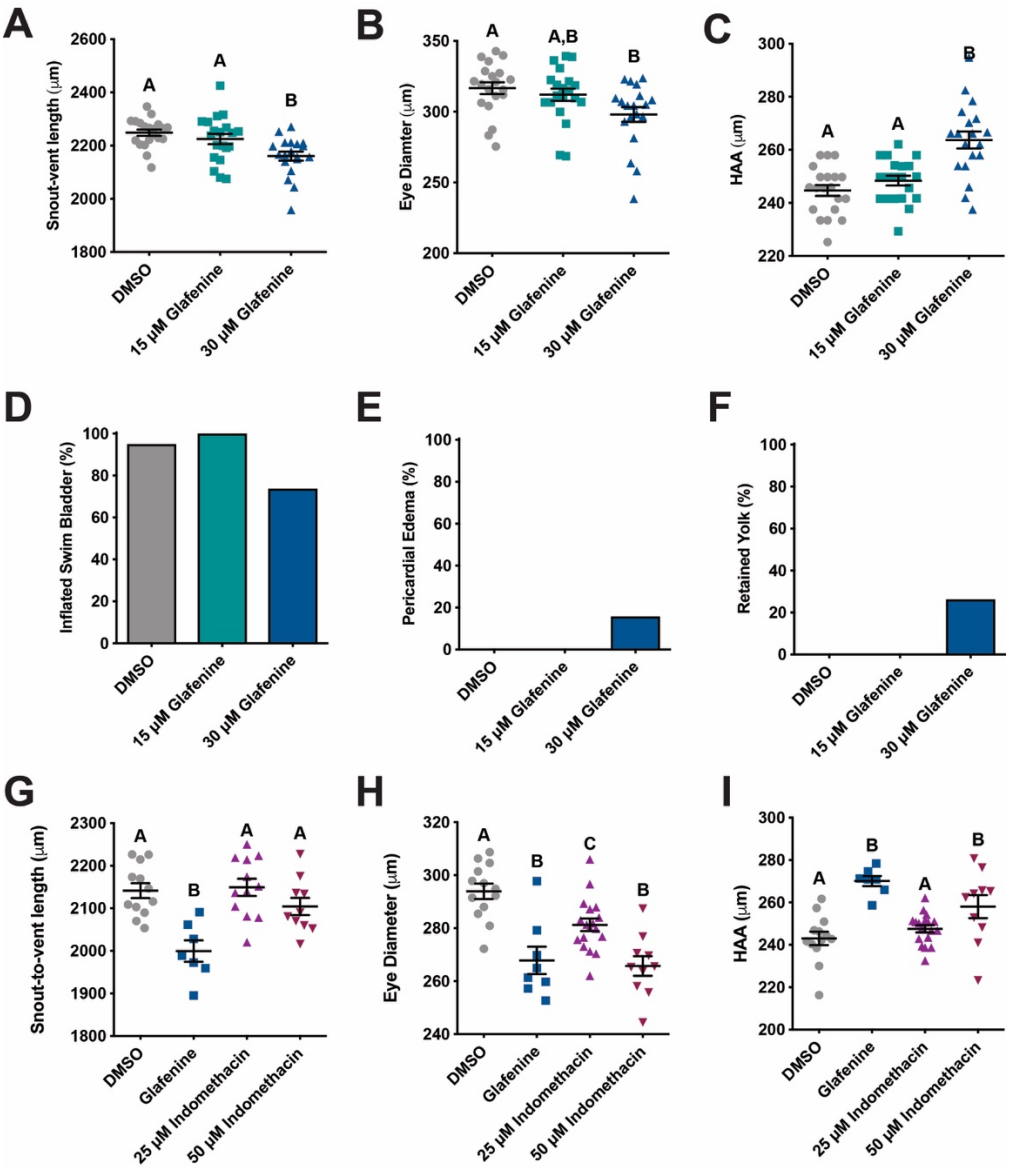
well plate format using a Retsch MM400 plate shaker. For media samples, DNA was isolated from 200 µL of each sample. DNA was quantified with a PerkinElmer Victor plate reader and Qubit dsDNA HS assay (ThermoFisher, Q32854).

Bacterial community composition in isolated DNA samples was characterized by amplification of the V4 variable region of the 16S rRNA gene by polymerase chain reaction using barcoded forward (515F) and reverse primers (806R) following the Earth Microbiome Project protocol (http://www.earthmicrobiome.org/). Equimolar amounts of 16S rRNA PCR products were quantified and pooled for sequencing. Sequencing was performed by the Duke Sequencing and Genomic Technologies shared resource on an Illumina MiSeq sequencer configured for 150 base-pair paired-end sequencing runs.

Subsequent analysis was conducted in QIIME2 (https://qiime2.org) (24). Paired reads were demultiplexed with qiime demux emp-paired, and denoised with qiime dada2 denoise-paired (25). Taxonomy was assigned with qiime feature-classifier classify-sklearn, using a naive Bayes classifier, trained against the 99% clustered 16S reference sequence set of SILVA, v. 1.19 (26). A basic statistical diversity analysis was performed using qiime diversity core-metrics-phylogenetic, including alpha- and beta-diversity, as well as relative taxa abundances in sample groups. For graphical presentation of relative abundance, the following filters were applied: for a given taxonomic level, RSVs shown were present in ≥2 samples at ≥1% relative abundance. LEfSe (Linear discriminant analysis Effect Size) (27) was used to identify significantly differential taxa in sample groups. Sequence data have been deposited in the NCBI Short Read Archive (SRA): accession number PRJNA512913.



Supplemental Figure 1. Quantification of Acridine Orange stained lumenal material and response of *Tg(ubb:seca5-tdTomato)* larvae to Glafenine exposure.
(*A*) Brightfield and fluorescence images (range indicator lookup table and grayscale) of DMSO and Glafenine treated larvae from a representative experiment (li =liver; sb = swim bladder; b = intestinal bulb; y = yolk; nm = neuromast).
(*B*) Representative AO line scan fluorescence traces for larvae shown in (*A*).
(*C*) Scatter dot plot of integrated intestinal AO from the same experiment in a [the points colored red correspond to the larvae shown in (*A*)]. Significance determined by unpaired two-sided Student's t-test.
(*D*) Cumulative frequency distribution plot of data from (*C*) fit with 3-parameter least squares regression (significance was determined by extra-sum-of-squares F-test, rejecting the null hypothesis that one curve would fit both datasets).
(*E,F*) Images of *Tg(ubb:seca5-tdTomato)*^{xt24} DMSO- and Glafenine-treated 6 dpf larvae and quantification (each dot corresponds to an individual larva). Significance was determined by unpaired two-sided Student's *t*-test.

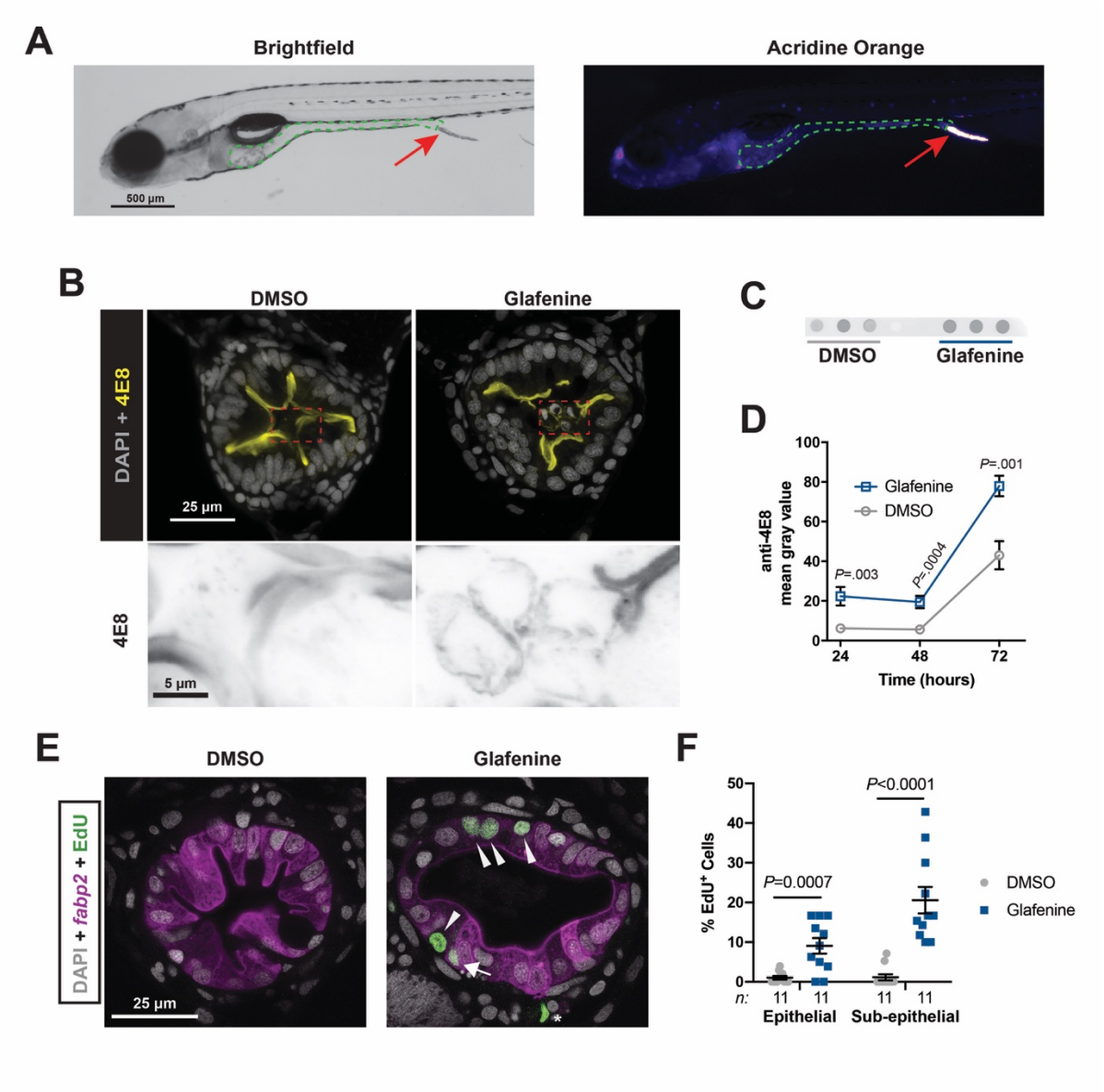


Supplemental Figure 2. Developmental metrics of control and Glafenine-treated 6 dpf zebrafish larvae.

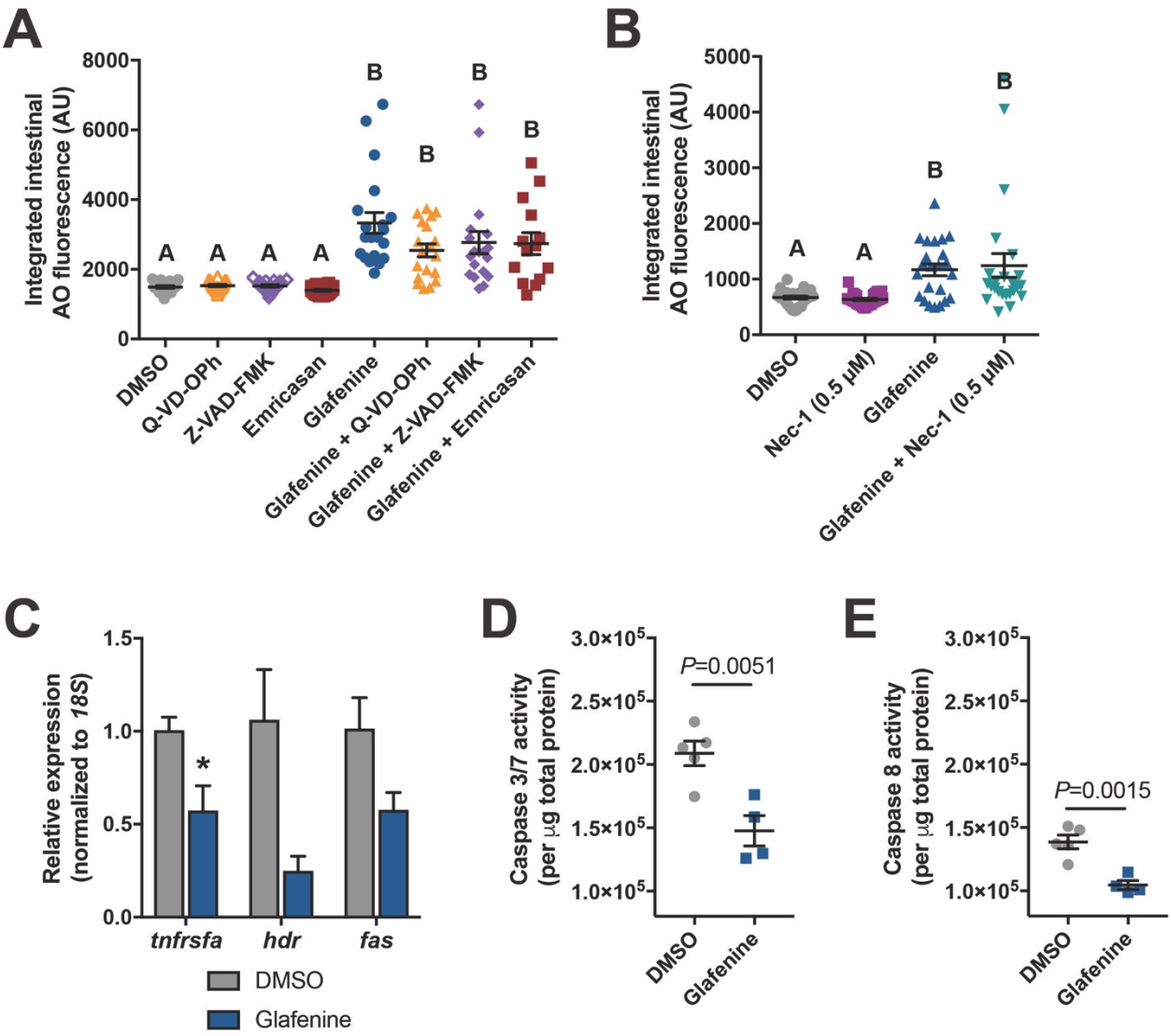
(*A*) Eye diameter, (*B*) HAA (height at anterior of anal fin), and (*C*) snout to vent length were measured from brightfield images of larvae treated as indicated.

Relative penetrance of (*D*) delayed yolk resorption, (*E*) pericardial edema, and (*F*) swim bladder inflation.

(*G-I*), Quantification of the indicated developmental metrics from larvae treated with DMSO, 30 μM Glafenine or 25 μM or 50 μM Indomethacin. Data shown are from a representative experiment, ≥20 larvae per condition. Significance determined by one-way ANOVA with Tukey's post-test; letters indicate groups determined to be statistically different.



Supplemental Figure 3. Expelled mass of apoptotic cells contains enterocytes, and Glafenine treated larvae exhibit increased intestinal proliferation.
(*A*) Representative brightfield and AO fluorescence images of a 6 dpf Glafenine-treated zebrafish larva excreting AO^+ IEC mass.
(*B*) Representative confocal micrographs of transverse sections immunostained with the IEC brush border antibody 4E8 (merge with DAPI above, inverted 4E8 below).
(*C,D*) Representative media dot blot of one sample pair probed with anti-4E8 and quantification (n = 4 samples / condition / time point, blotted in triplicate).
(*E,F*) Analysis of epithelial proliferation in Tg(fabp2:DsRed) DMSO- and Glafenine-treated larvae (arrow heads mark EdU^+ enterocytes, arrow denotes EdU^+ sub-epithelial cell, * EdU^+ extra-intestinal cell; significance determined by unpaired two-sided Student's *t*-test).



Supplemental Figure 4. Caspase and Ripk1 activity are dispensable for Glafenine-induced IEC delamination, and death receptor expression as well as Caspase activity are suppressed in Glafenine-treated larvae.

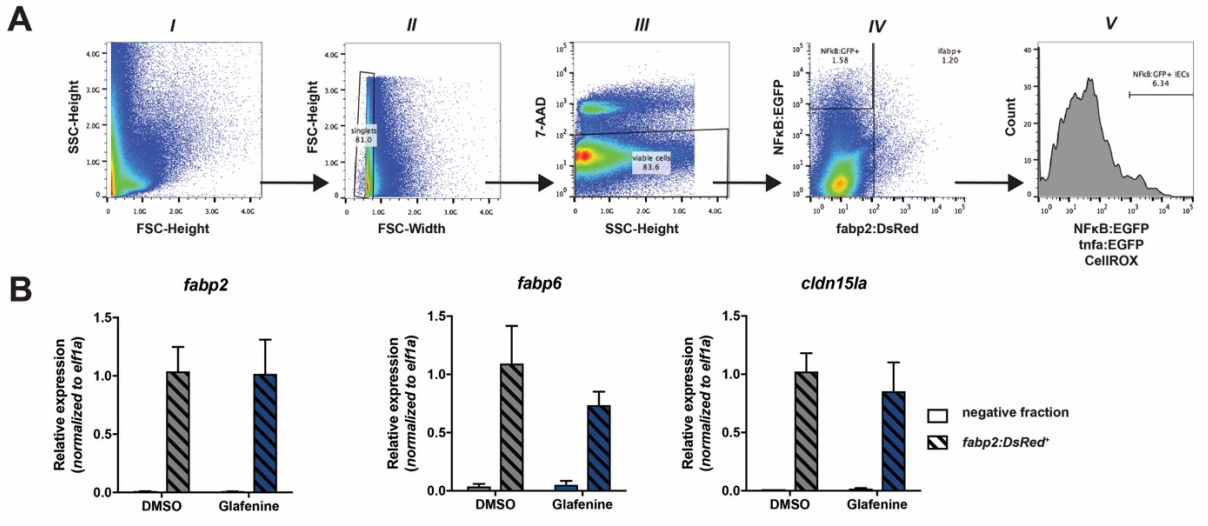
(*A*) Co-exposure with pan-caspase inhibitors (10 μM Q-VD-OPh, 10 μM Z-VAD-FMK, 10 μM Emricasan) or

(*B*) the Ripk1 inhibitor Nec-1 does not alter IEC loss after 72 hours of Glafenine treatment (intestinal AO quantification; each point corresponds to an individual larva).

(*C*) qRT-PCR analysis of death receptor mRNA levels in isolated *fabp2:DsRed*$^{+}$ enterocytes from DMSO- and Glafenine-treated larvae (n = 4 / group).

(*D,E*) Caspase activity assays from whole-larvae homogenates reveals decreased activity for Caspase-3/7 and Caspase-8 following Glafenine treatment (each point corresponds to a pool of 20 larvae).

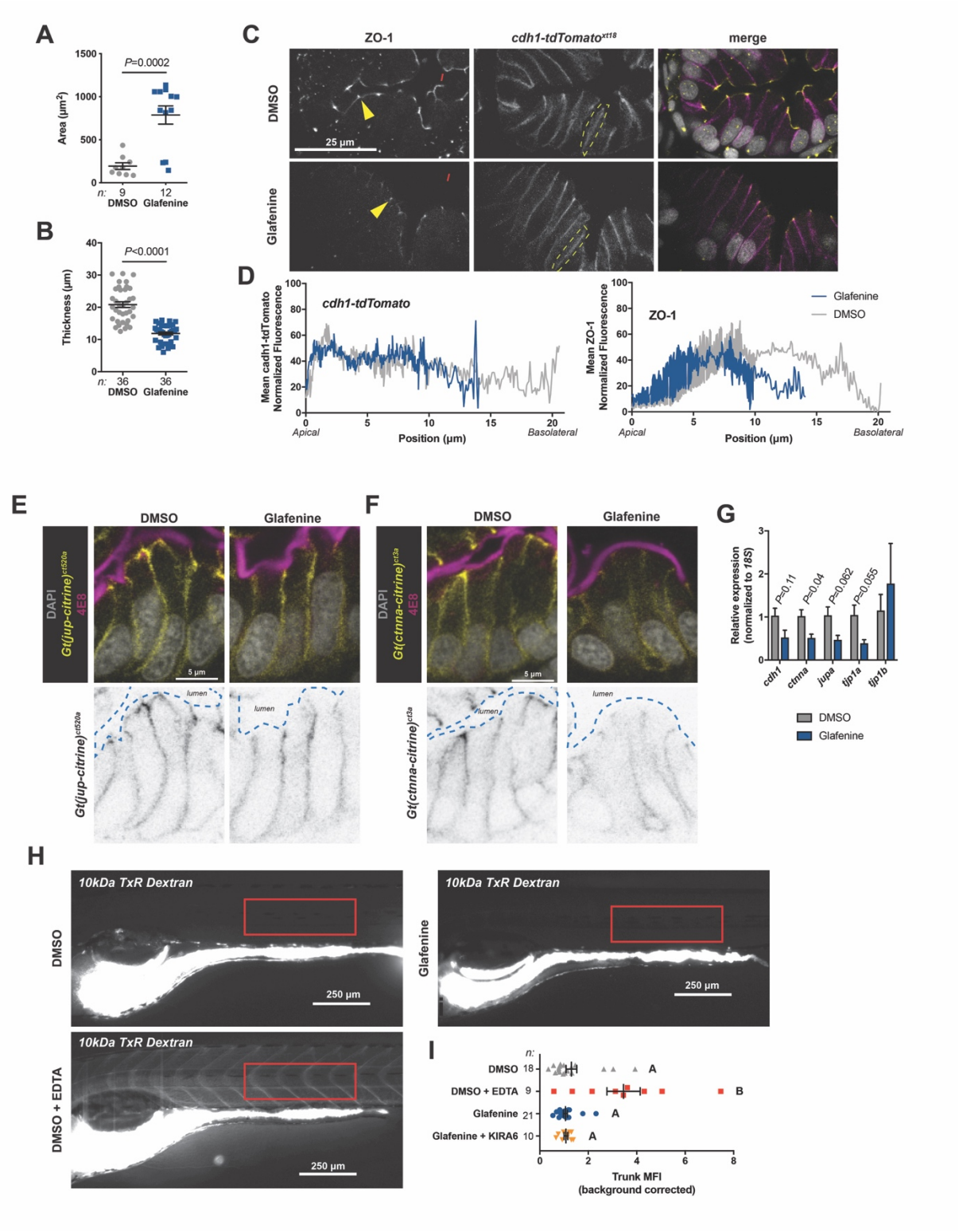
Statistical testing was performed by one-way ANOVA with Tukey's post-test (*A,B*) or unpaired two-sided Student's *t*-test (*C*, *D*, and *E*) (letters indicate groups determined to be statistically different; * $P < 0.05$).



Supplemental Figure 5. FACS gating scheme and validation of isolated enterocytes from 6 dpf zebrafish larvae.

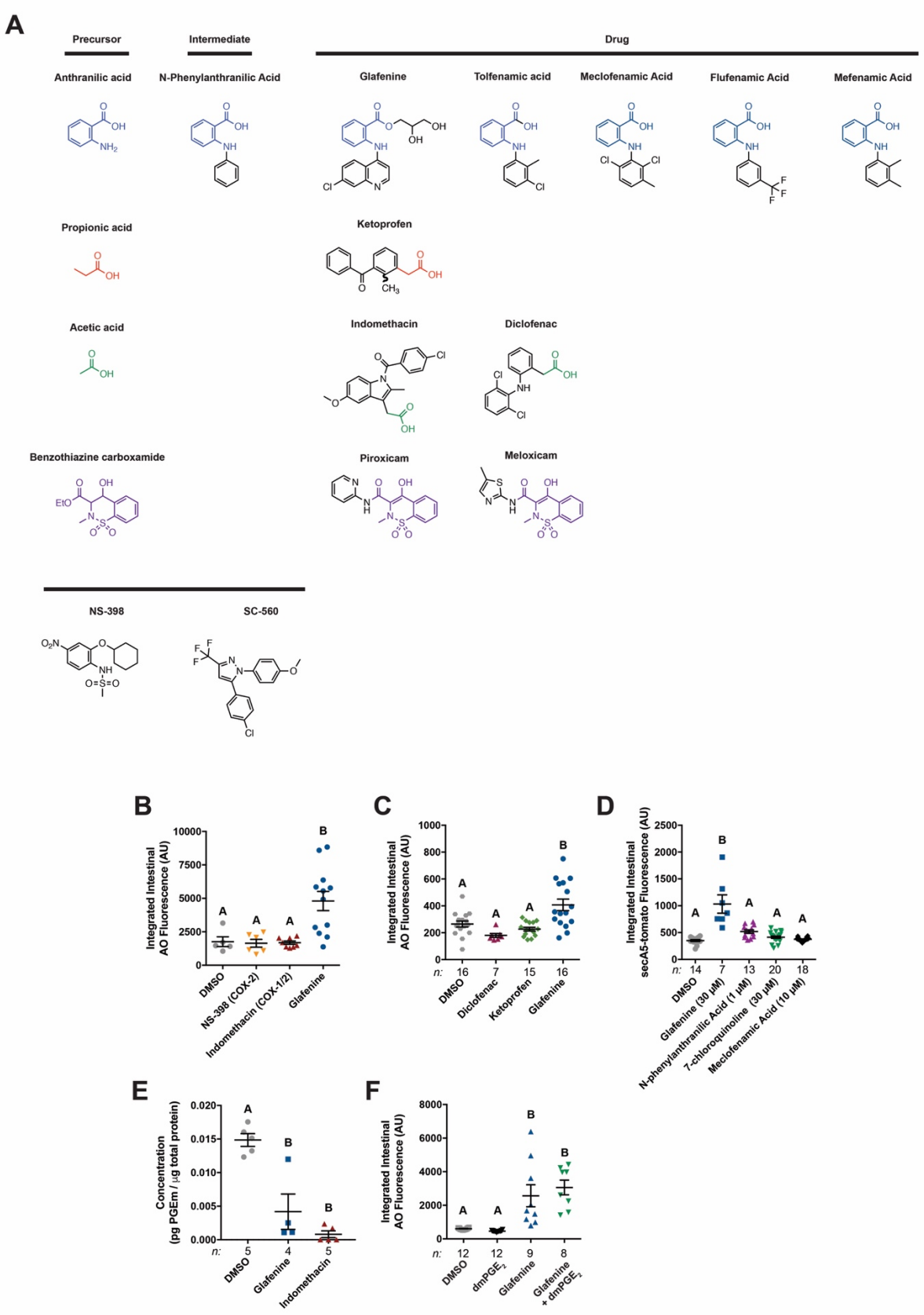
(*A*) Gating strategy for FACS isolation and flow cytometry analysis of enterocytes from *Tg(fabp2:DsRed)* larvae.

(*B*) qRT-PCR analysis of IEC-specific mRNAs in isolated *fabp2:DsRed*$^{+}$ cells compared to cells from the negative fraction from DMSO- and Glafenine-treated larvae [for (*B*), n = 4 samples / replicate, ≥5,000 *fabp2:DsRed*$^{+}$ cells and 5,000 cells from the negative fraction].

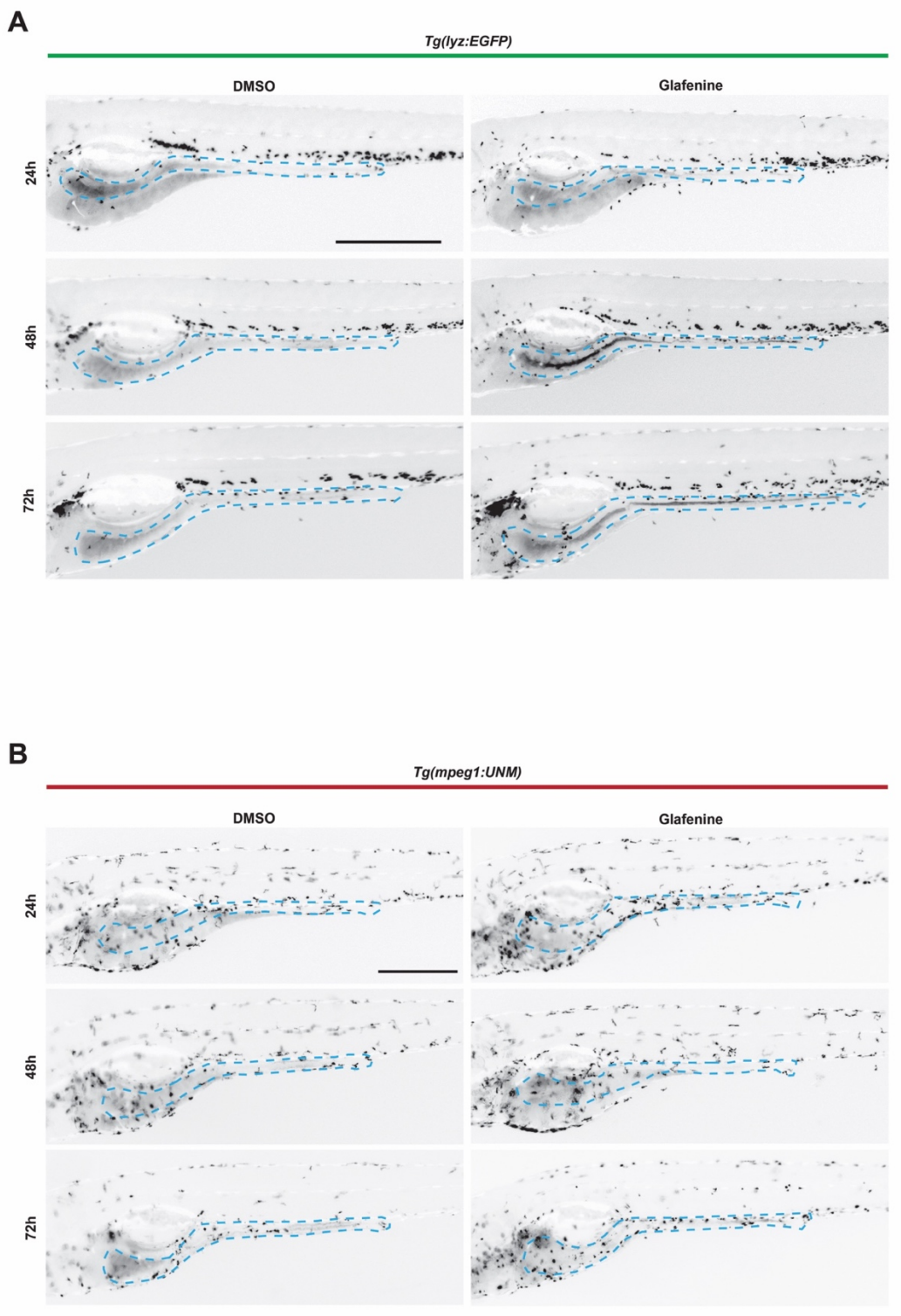


Supplemental Figure 6. Epithelial junctional organization is overtly normal and barrier function is preserved in Glafenine-treated zebrafish larvae.

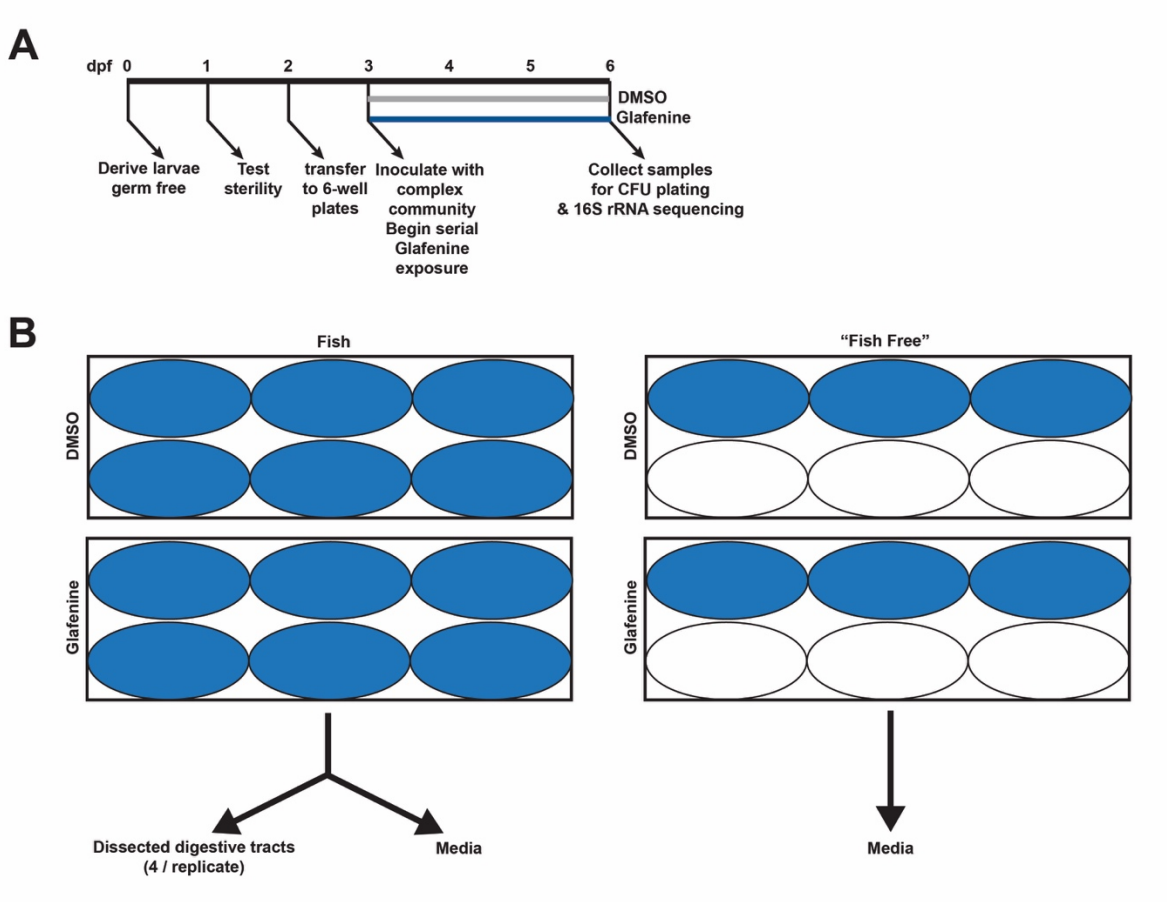
(*A,B*) Morphometric analysis of intestinal lumen area and epithelial thickness from transverse sections of 6 dpf larvae. Significance determined by unpaired two-sided Student's *t*-test.
(*C*) Confocal micrographs of transverse vibratome sections from DMSO and Glafenine-treated *cdh1-tdTomato*^{xt18} (magenta) larvae stained with anti ZO-1 (yellow) and DAPI (gray). Yellow arrowheads point to apical ZO-1 staining and yellow dashed ovals denote representative basolateral patterning of Cdh1-tdTomato.
(*D*) Representative traces of Cdh1-tdTomato and ZO-1 localization patterns from vibratome sections of larvae treated as indicated.
(*E,F*) Representative confocal images of vibratome sections from 6 dpf DMSO and Glafenine treated *Gt(jup-citrine)*^{ct520a} and *Gt(ctnna-citrine)ct3a* larvae (upper panels show merged images including DAPI and the brush border marker 4E8, lower panels show inverted grayscale images of indicated gene trap lines).
(*G*) qRT-PCR analysis of indicated epithelial junctional components from FACS-isolated *fabp2:DsRed*$^{+}$ enterocytes. Significance determined by unpaired two-sided Student's *t*-test.
(*H*) Representative images of larvae 30 minutes post gavage with 10 kDa Texas Red dextran (DMSO larvae co-gavaged with EDTA were used as a positive control group for barrier permeability).
(*I*) Quantification of fluorescence in the trunk from larvae treated as indicated (example ROI shown in red boxes in *H*). Significance determined by one-way ANOVA with Tukey's multiple comparison's test; letters indicate groups determined to be statistically different.


A
Precursor
Intermediate
Drug
Anthranilic acid
N-Phenylanthranilic Acid
Glafenine
Tolfenamic acid
Meclofenamic Acid
Flufenamic Acid
Mefenamic Acid
Propionic acid
Ketoprofen
Acetic acid
Indomethacin
Diclofenac
Benzothiazine carboxamide
Piroxicam
Meloxicam
NS-398
SC-560
B
Integrated Intestinal AO Fluorescence (AU)
DMSO
NS-398 (COX-2)
Indomethacin (COX-1/2)
Glafenine
C
Integrated Intestinal AO Fluorescence (AU)
n: 16 7 15 16
DMSO
Diclofenac
Ketoprofen
Glafenine
D
Integrated Intestinal secA5-tomato Fluorescence (AU)
n: 14 7 13 20 18
DMSO
Glafenine (30 μM)
N-phenylanthranilic Acid (1 μM)
7-chloroquinoline (30 μM)
Meclofenamic Acid (10 μM)
E
Concentration (pg PGEm / μg total protein)
n: 5 4 5
DMSO
Glafenine
Indomethacin
F
Integrated Intestinal AO Fluorescence (AU)
n: 12 12 9 8
DMSO
dmPGE2
Glafenine
Glafenine + dmPGE2

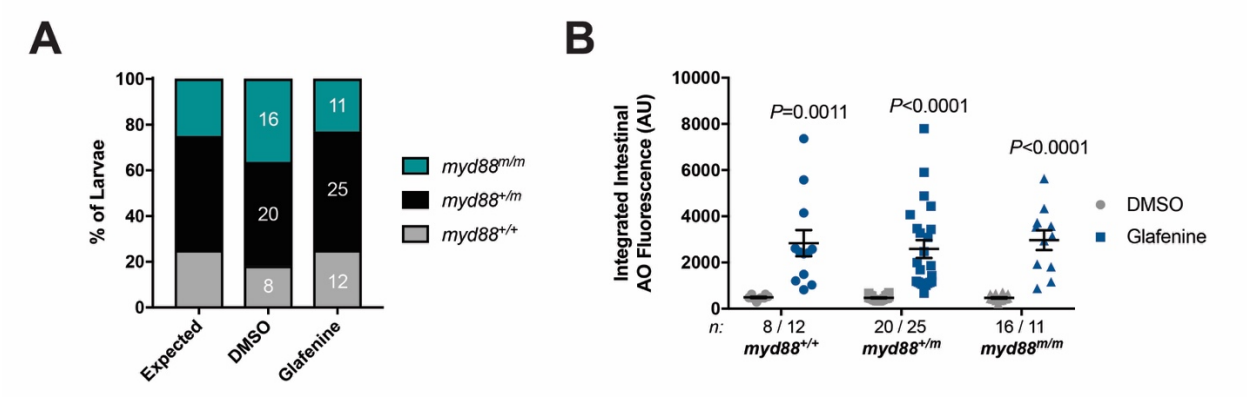
Supplemental Figure 7. Structures and *in vivo* activity of Glafenine, other NSAIDs, COX inhibitors, as well as precursors and parent compounds.
(*A*) Structures of NSAIDs derived from anthranilic acid, propionic acid, acetic acid, and benzothiazine carboxamide, as well as structures of selective COX1 (SC-560) and COX2 (NS-398) inhibitors.
(*B-D*) Intestinal AO quantification in larvae treated with the indicated selective and non-selective NSAIDs.
(*E*) Glafenine reduces PGE metabolite levels in larvae (each point corresponds to a pool of 20 larvae).
(*F*) Intestinal AO quantification in control and Glafenine-treated larvae ± PGE_2.
For (*B-F*), significance was determined by one-way ANOVA with Tukey's multiple comparison's test; letters indicate groups determined to be statistically different.



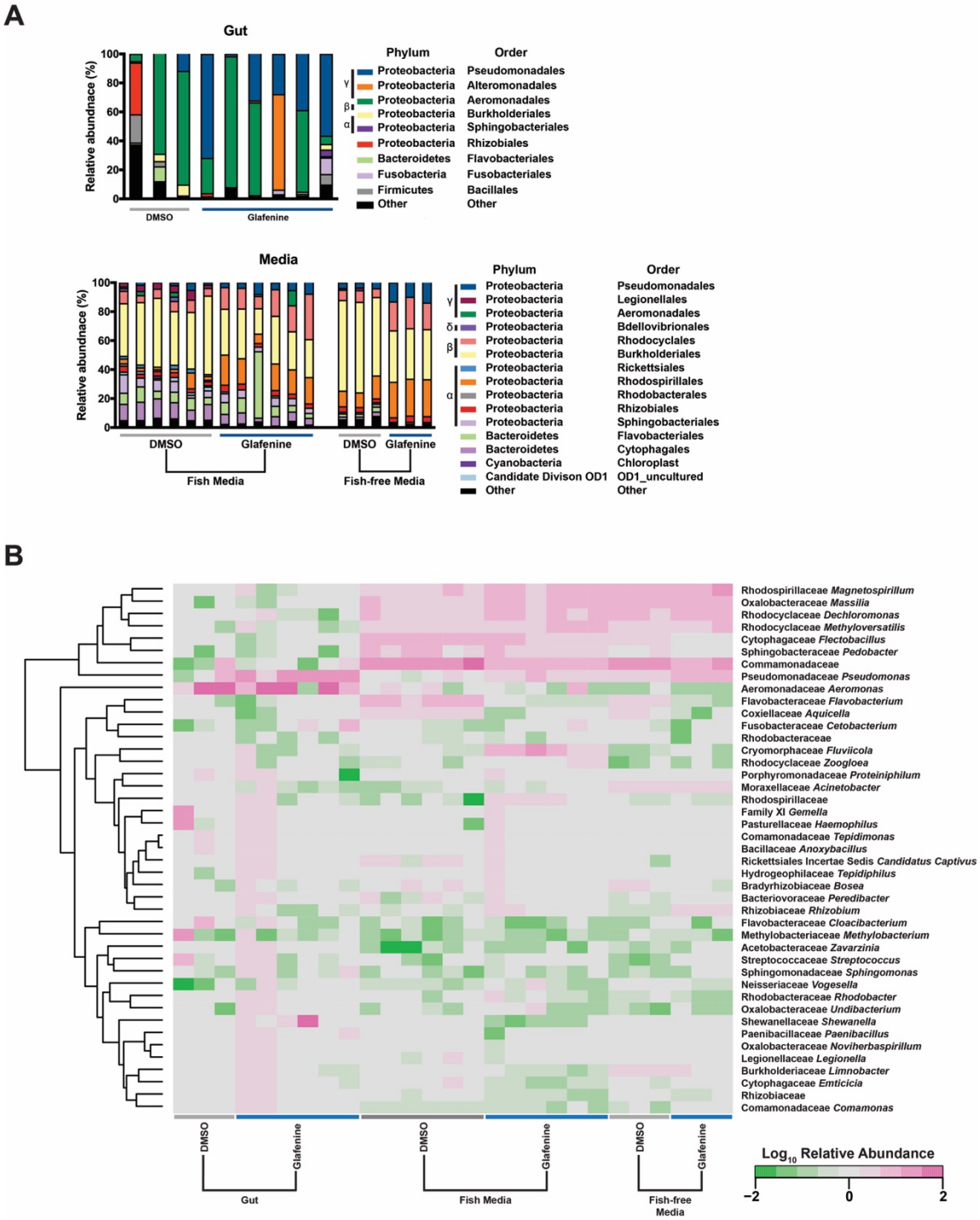
Supplementary Figure 8. Fluorescent stereomicroscopy of intestine-associated leukocytes. (*A,B*) Representative 8-bit images of DMSO and Glafenine-treated *Tg(lyz:GFP)* (*A*) and *Tg(mpeg1:UNM)* (*B*) larvae at the indicated times during the serial exposure regimen (inverted for presentation). The intestine in each image is outlined with a dashed blue line.



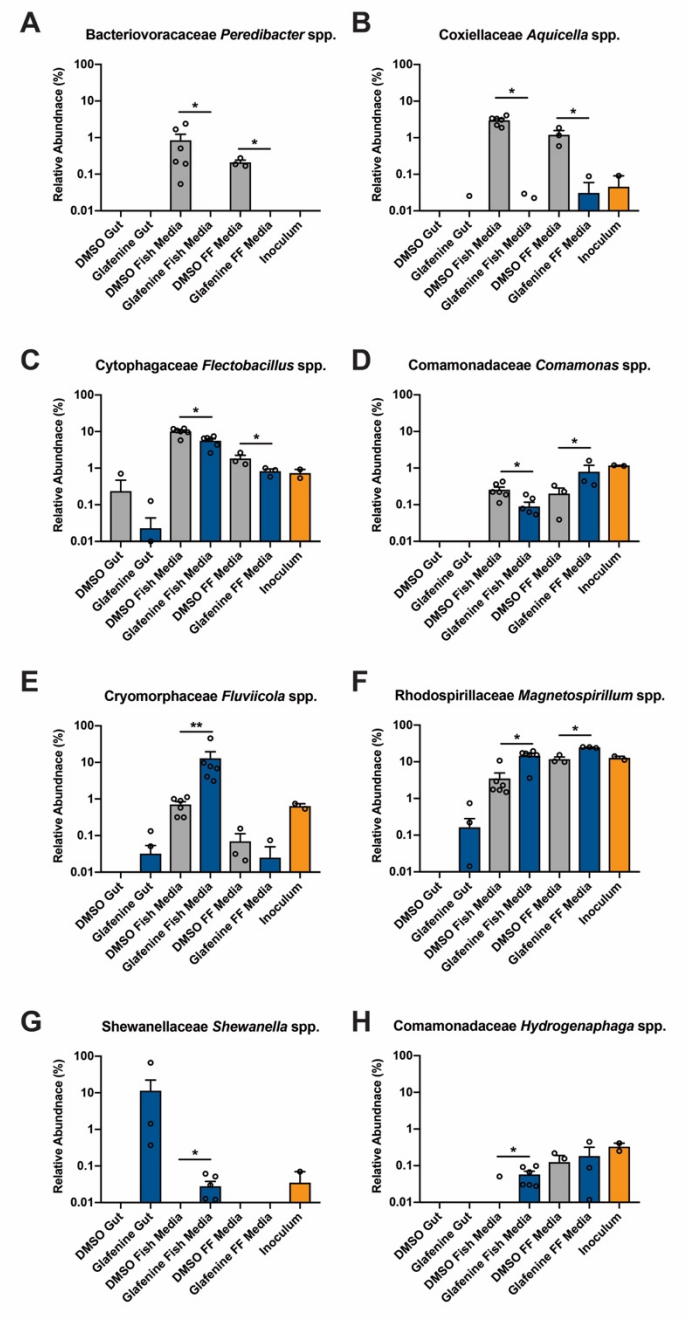
Supplemental Figure 9. Experimental design of 16S rRNA sequencing study.
(*A*) Experimental design timeline for gnotobiotic serial Glafenine exposure.
(*B*) Experimental design schematic for 16S rRNA gene sequencing study.



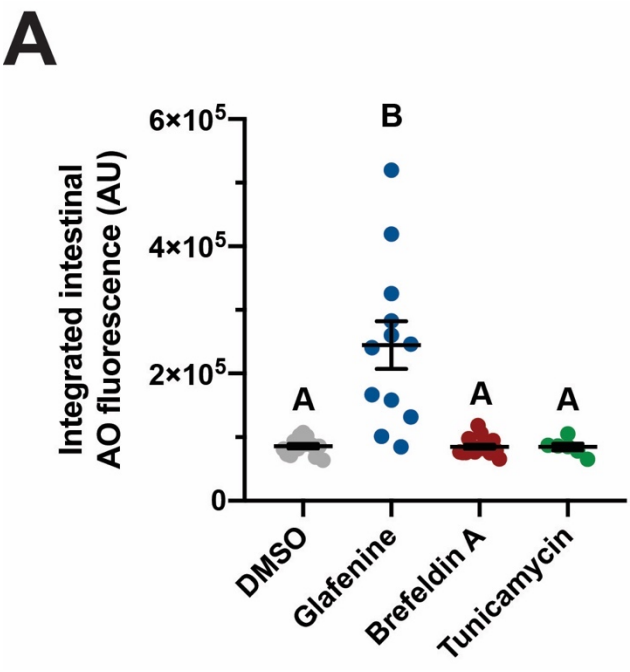
Supplemental Figure 10. The adapter protein MyD88 is dispensable for Glafenine-induced IEC delamination.
(*A*) Genotype distributions of larvae from *myd88*$^{+/m}$ in-cross treated with DMSO or Glafenine.
(*B*) Intestinal AO quantification from DMSO- and Glafenine-treated larvae of the indicated *myd88* genotypes. Within genotype significance testing performed by unpaired two-sided Student's *t*-test.



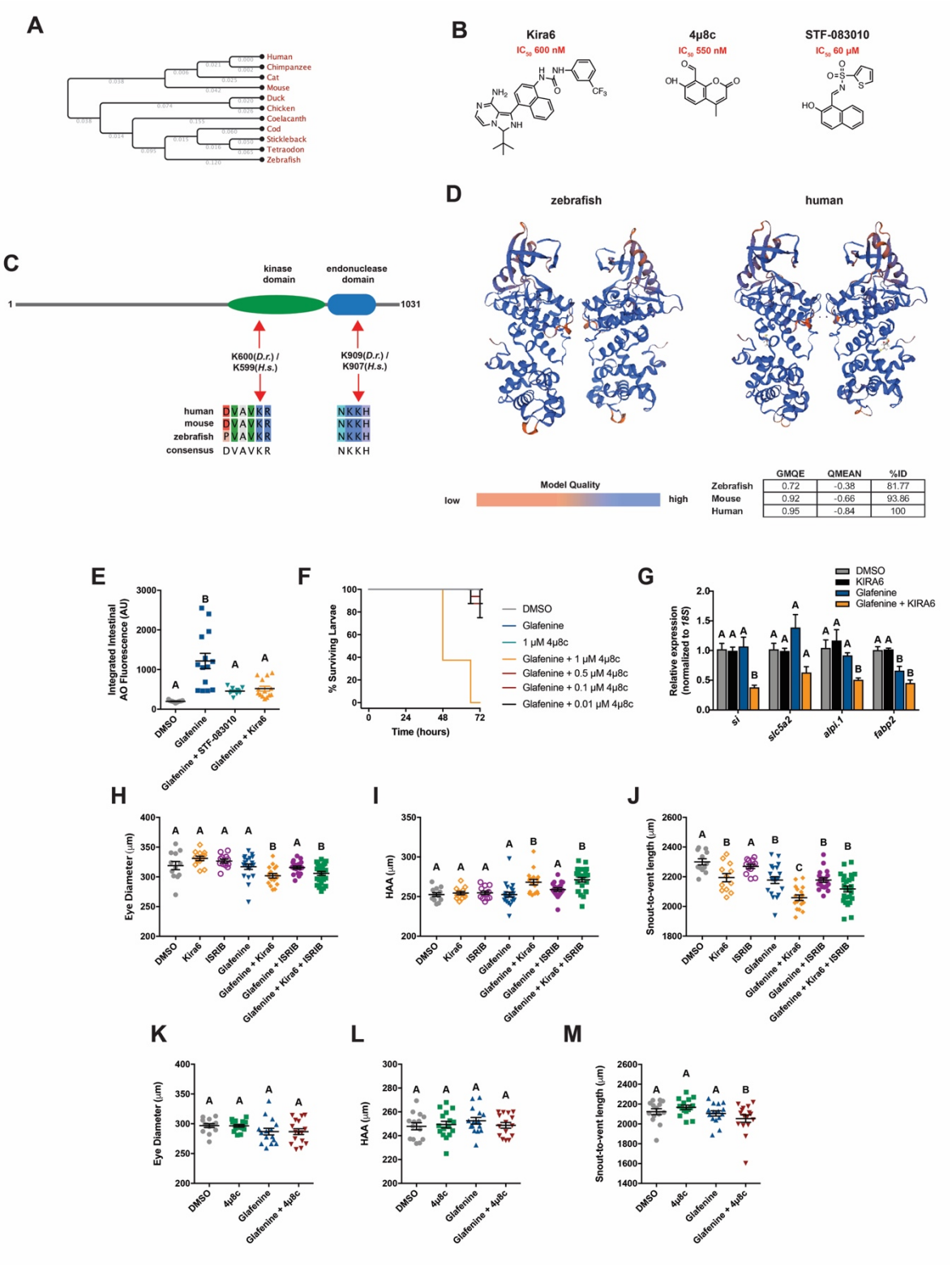
Supplemental Figure 11. 16S rRNA gene sequencing analysis identifies changes in host-associated and environmental microbial community composition following Glafenine treatment.
(*A,B*) Bacterial order-level relative abundance bar plots for the indicated groups and samples (bacterial orders plotted were present in ≥1 sample at ≥1% relative abundance).
(*C*) Heatmap of $\log_{10}$ relative abundance for genera detected in 16S rRNA sequencing (% ≥1 relative abundance in ≥1 sample).



Supplemental Figure 12. Analysis of relative genera abundance.
(*A-H*) Relative abundance bar plots of select genera from digestive tracts, fish media, fish-free media and inoculum samples (significance determined by LEfSe).



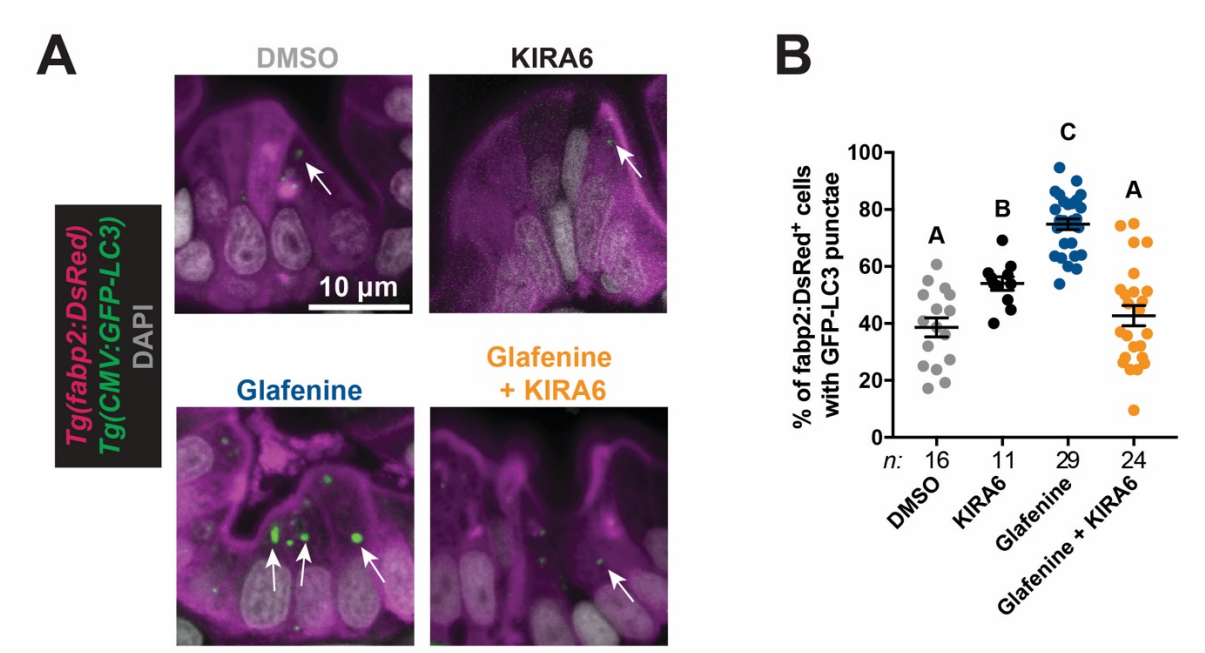
Supplemental Figure 13. Chemical induction of ER stress is not sufficient to induce IEC-shedding. (*A*) Other known inducers of endoplasmic reticulum stress [Brefeldin A and Tunicamycin (28)] do not induce IEC shedding at concentrations with known *in vivo* efficacy (2 μg/mL and 1 μg/mL, respectively). Significance was determined by one-way ANOVA with Tukey's multiple comparison test; letters indicate groups determined to be statistically different.



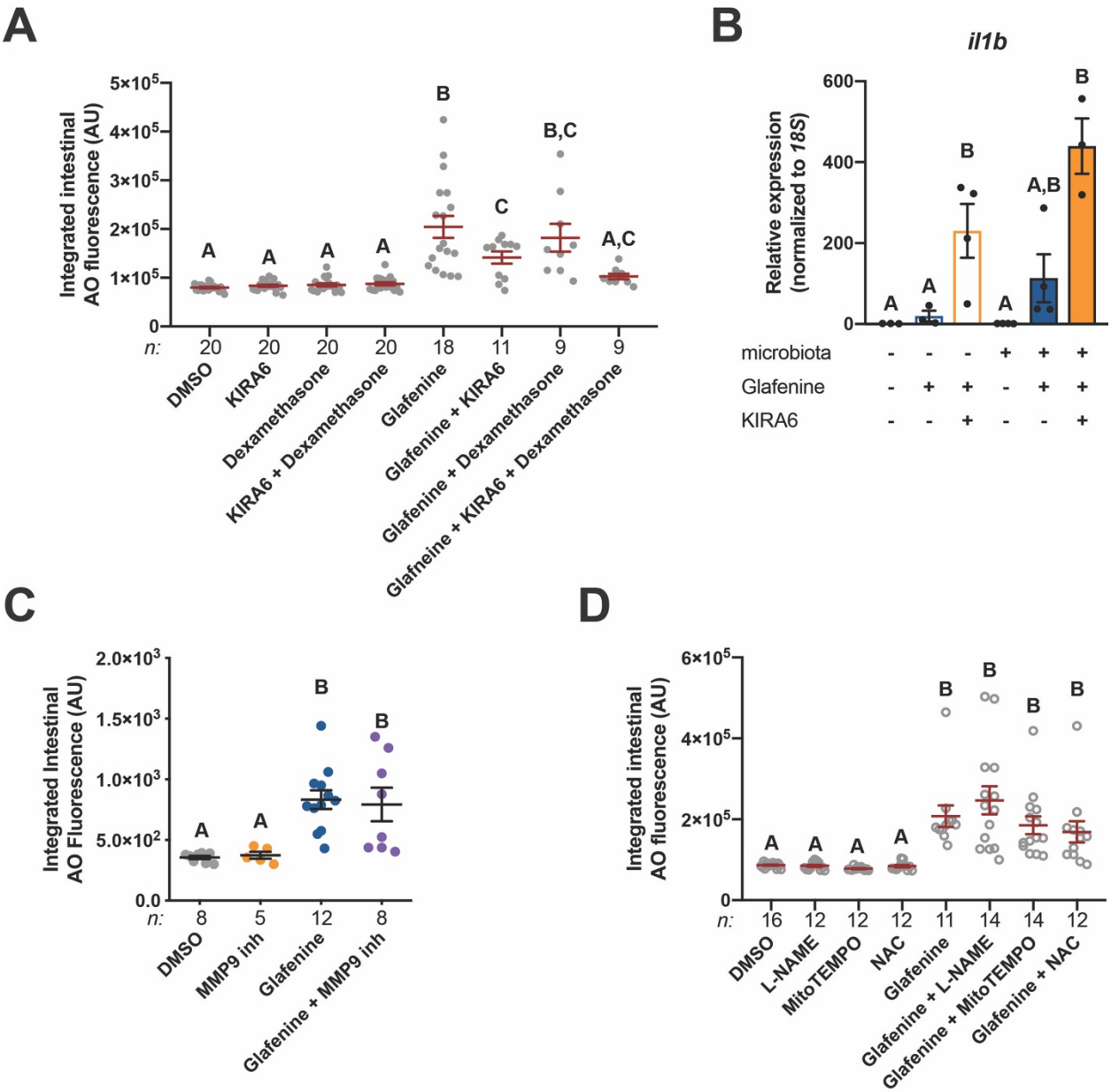
Supplemental Figure 14. Comparative analysis and effects of pharmacological inhibitors of Ire1α on Glafenine-treated larvae.

(*A*) Phylogenetic tree based of Ire1a based on amino acid (aa) sequences.

(*B*) Structures and reported IC_{50} values for Ire1α inhibitors.
(*C*) PROSITE (20) domain predictions from zebrafish Ire1a aa sequence; sequence alignment with mouse and human Ire1α highlights conservation of residues essential for inhibitor activity (K599 and K907).
(*D*) SwissModel (21, 23) results from zebrafish (left) and human (right) amino acid sequence corresponding to the Ire1a cytosolic domains with modeling summary shown below (modeled against PDB 5hgi (22)).
(*E*) Intestinal AO quantification from larvae treated with Glafenine and Ire1α inhibitors.
(*F*) Survival curve of larvae treated with Glafenine and increasing concentrations of 4μ8c.
(*G*) qRT-PCR analysis of brush border components from dissected digestive tracts of larvae treated as indicated (n = 20 dissected digestive tracts / replicate, 5 replicates / condition).
(*H-J*) Developmental morphometry of 6 dpf larvae treated with KIRA6, ISRIB, and Glafenine alone or in combination.
(*K-M*) Developmental morphometry of 6 dpf larvae treated with 0.5 μM 4μ8c and Glafenine alone or in combination.
For (*E*), and (*G-M*), significance determined by one-way ANOVA with Tukey's multiple comparisons test (each dot corresponds to an individual larva); letters indicate groups determined to be statistically different.



Supplemental Figure 15. Glafenine-induced autophagy in enterocytes is mediated by Ire1α signaling.
(*A*) Representative confocal images of vibratome sections of *Tg(fabp2:DsRed)*;*Tg(CMV:GFP-LC3)* larvae. Arrows point to GFP-LC3^{+} structures.
(*B*) Quantification of LC3^{+} autophagic punctae in enterocytes from larvae treated as indicated. Significance was determined by one-way ANOVA with Tukey's multiple comparison test; letters indicate groups determined to be statistically different.



Supplemental Figure 16. The anti-inflammatory glucocorticoid dexamethasone does not alter IEC delamination in larvae treated with Glafenine or Glafenine+KiRA6, and failed delamination associated with Ire1α inhibition is sufficient to induce intestinal inflammation in GF larvae
(*A*) Quantification of intestinal Acridine Orange (AO) staining in larvae treated with all possible combinations of Glafenine, KIRA6, and Dexamethasone (0.75 μM). These data indicate that alleviating inflammation by treating larvae with the anti-inflammatory glucocorticoid Dexamethasone does not restore the reduced IEC delamination observed in Glafenine+KIRA6 treated larvae, suggesting that elevated inflammation does not suppress IEC shedding.
(*B*) qRT-PCR analysis of dissected digestive tissues from 6 dpf gnotobiotic larvae treated with DMSO, Glafenine, or Glafenine+KIRA6 demonstrates induction of *il1b* mRNA in GF larvae treated with both Glafenine and KIRA6, supporting a model wherein failed IEC delamination (following Ire1α blockade) leads to an augmented inflammatory response (perhaps due to a local increase in the concentration of DAMPs).

(*C*) Co-exposure with an MMP-9 inhibitor does not alter IEC delamination in Glafenine-treated larvae at 6 dpf.
(*D*) Co-exposure with the antioxidants L-NAME, MitoTEMPO or N-Acetyl Cysteine (NAC) does not ameliorate Glafenine-induced IEC delamination in 6 dpf larvae.
For (*A*-*D*), significance was determined by one-way ANOVA with Tukey's multiple comparison's test; letters indicate groups determined to be statistically different

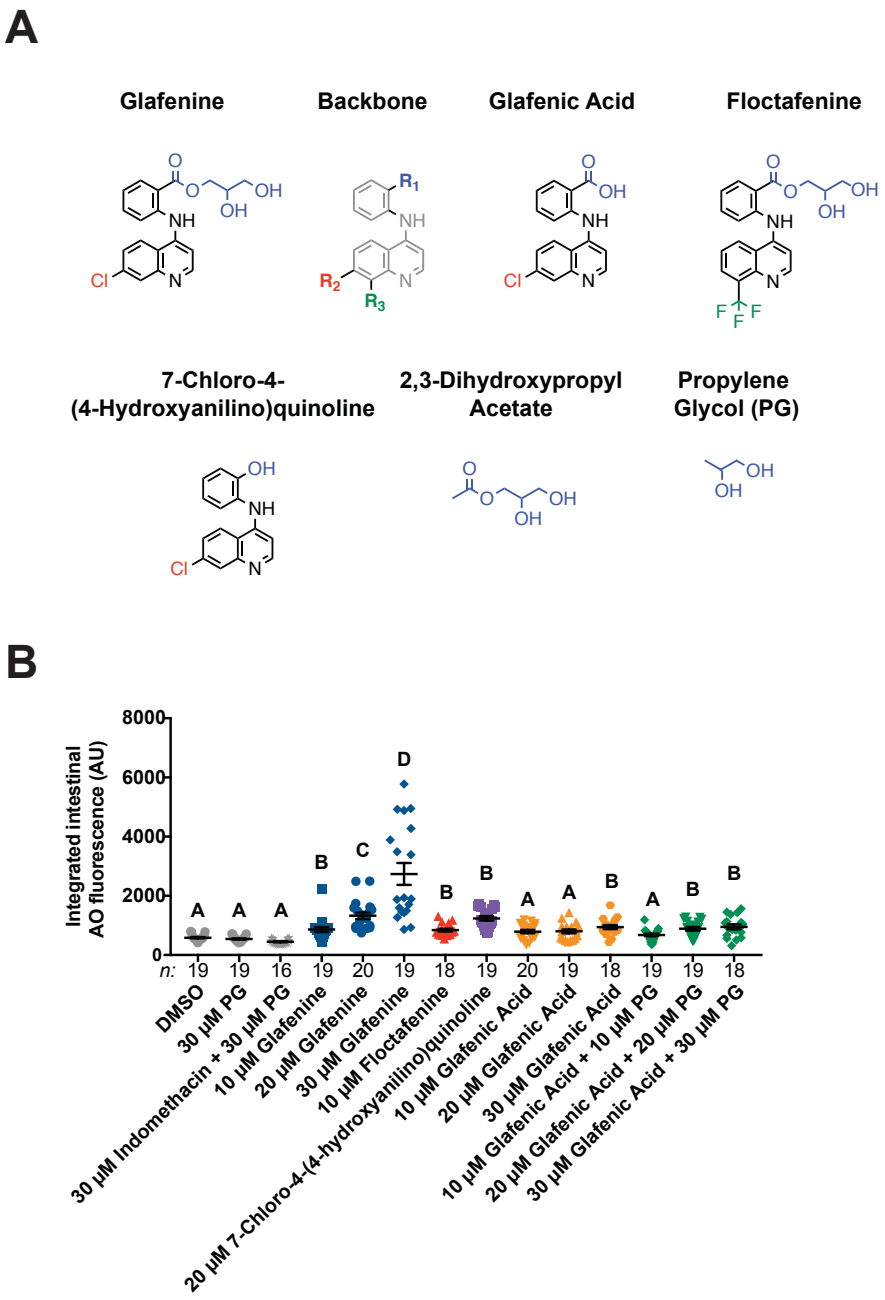
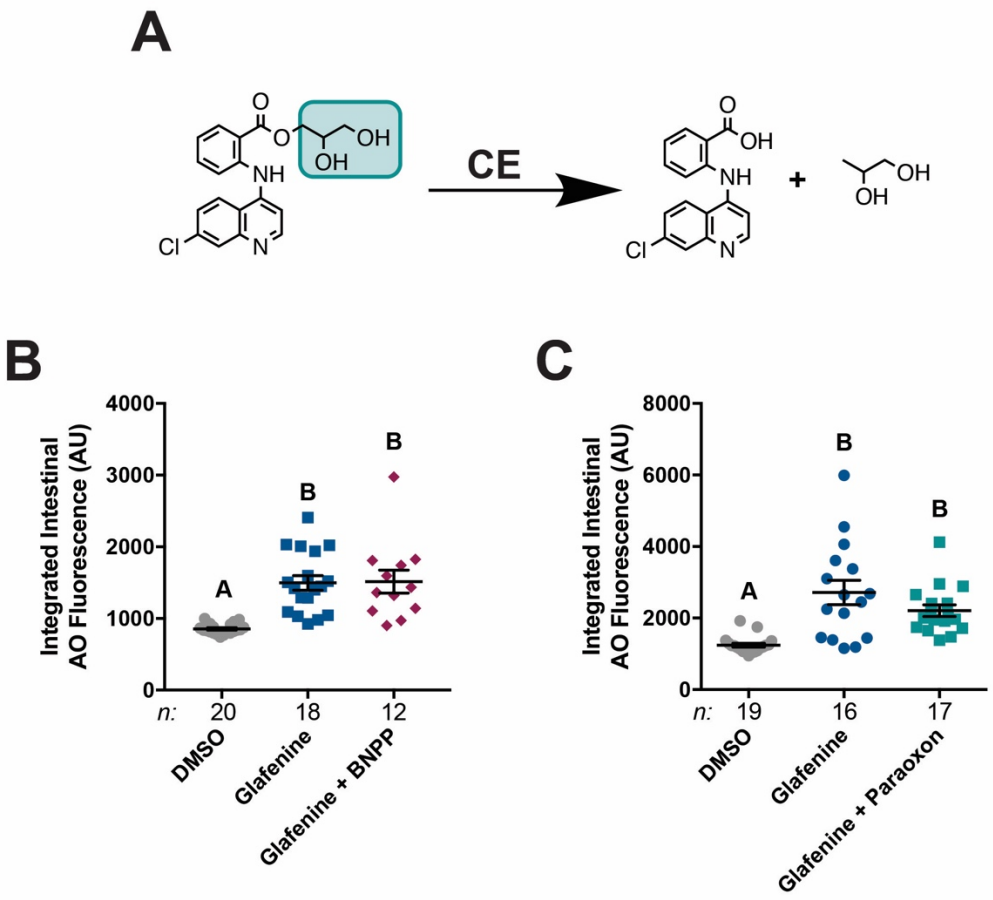


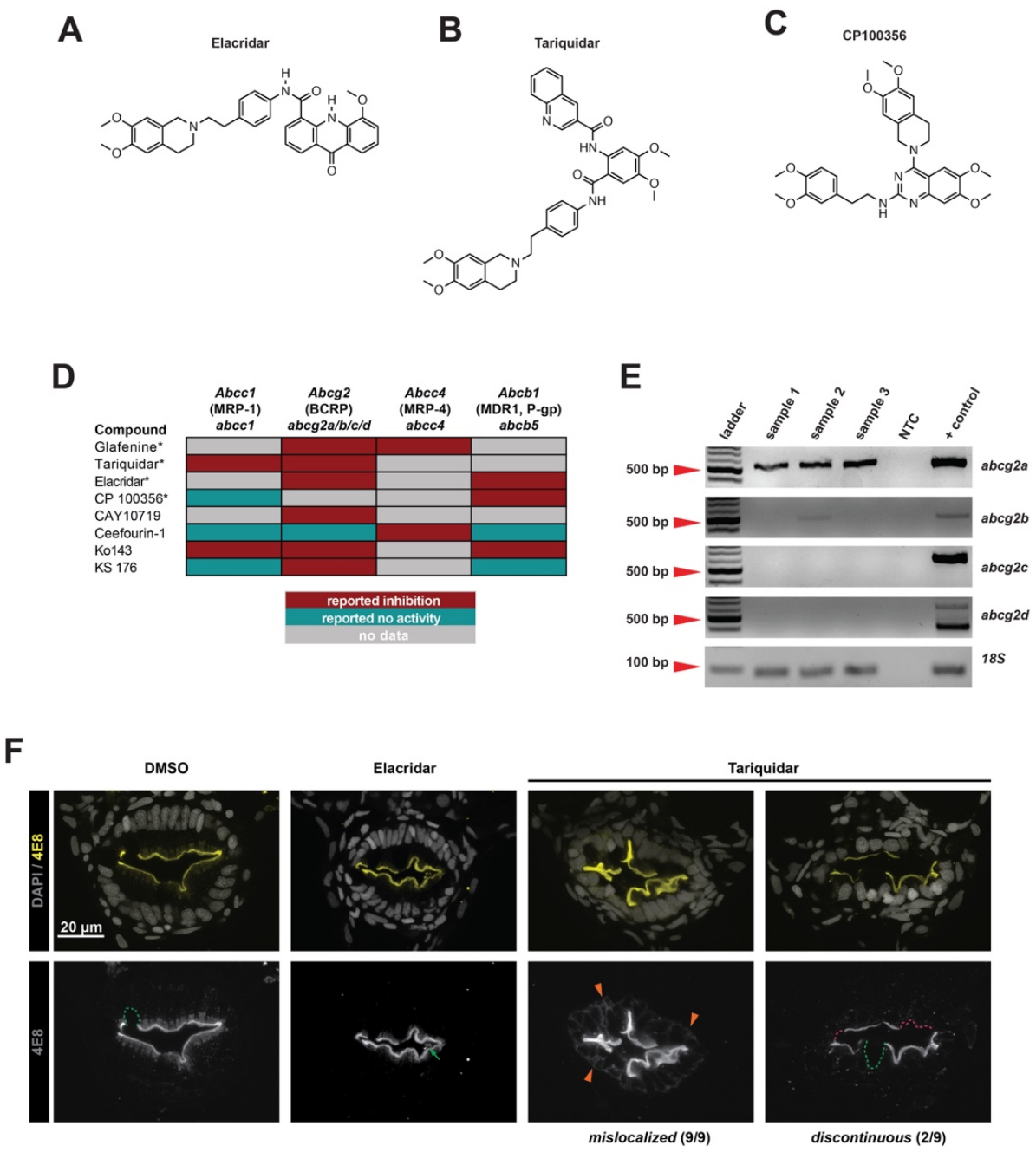
Figure S17. Structure-activity relationship (SAR) analysis identifies essential molecular features required for Glafenine-induced IEC shedding

(*A*) Chemical structures of Glafenine and structurally-related compounds.

(*B*) Quantification of intestinal AO from larvae treated with the compounds shown in (*A*) (each dot corresponds to an individual larva; significance determined by one-way ANOVA with Tukey's multiple comparison test; letters indicate groups determined to be statistically different).



Supplemental Figure 18. Co-exposure with carboxylesterase (CES) inhibitors does not alter Glafenine induced IEC shedding.
(*A*) Predicted CES-mediated Glafenine catabolism.
(*B,C*) Treatment with neither of the carboxylesterase inhibitors BNPP (*B*) nor paraoxon (*C*) alters accumulation of intestinal AO^+ material in Glafenine-treated larvae.
For (*B*) and (*C*), significance was tested by one-way ANOVA with Tukey's multiple comparison's test (each dot corresponds to an individual larva). Letters indicate groups determined to be statistically different.



Supplemental Figure 19. Structures, known targets of MDR efflux pump inhibitors, and effects on intestinal morphology in zebrafish larvae.
(*A*-*C*) Structures of the indicated compounds.
(*D*) Specificities of MDR efflux pump inhibitors [mouse gene name listed on top (italicized), protein name in the middle, zebrafish ortholog(s) on bottom (italicized); compounds marked with an * induce IEC shedding]
(*E*) RT-PCR analysis of *abcg2* gene family member expression in FACS-isolated *fabp2:DsRed*$^{+}$ enterocytes from 6 dpf zebrafish larvae (samples 1-3 correspond to 3 separate pools of 20 larvae; ≥5,000 cells / sample; dissected digestive tissue was used as a positive control).

(*F*) Confocal micrographs of transverse sections from 6 dpf larvae treated with DMSO, Elacridar or Tariquidar stained with DAPI and the brush-border reactive antibody 4E8. Lumenal debris labeled with 4E8 can be observed in the image from an Elacridar-treated larva (green arrow). Tariquidar treatment is associated with increased incidence of abnormal 4E8 staining patterns, including basolateral mis-localization (orange arrows; observed in 9/9 animals), as well as discontinuous staining at the apical surface (observed in only 2/9 animals). In the lower right panel, the magenta dashed lines highlight regions of unusual discontinuity, while normal discontinuity due to a goblet cell is denoted by the green dashed line.

Video S1: Labelling of lumenal material in a Glafenine-treated *Tg(ubb:seca5-tdTomato)* larva

Video S2: *In vivo* confocal microscopy documents enterocyte delamination in a Glafenine-treated *Tg(fabp2:DsRed)* larva (anterior) (time)

Video S3: *In vivo* confocal microscopy documents delaminated enterocytes in the intestinal lumen of a Glafenine-treated *Tg(fabp2:DsRed)* larva, posterior to swim bladder (z)

Video S4: *In vivo* confocal microscopy of a DMSO-treated *Tg(fabp2:DsRed)* larva (time)

Video S5: *In vivo* confocal microscopy of a DMSO-treated *Tg(fabp2:DsRed)* larva (z)

Video S6: Larval intestinal explant – DIC (top), Cdh1-YFP (middle), seca5-tdTomato (bottom, range indicator) – DMSO (time)

Video S7: Larval intestinal explant – DIC (top), Cdh1-YFP (middle), seca5-tdTomato (bottom, range indicator) - Glafenine (time)

Video S8: Live imaging of an Elacridar treated *Tg(fabp2:DsRed)* larva (z) reveals delaminated fabp2:DsRed+ enterocytes in the intestinal lumen [upper panel *Tg(fabp2:DsRed)*, lower panel *Tg(fabp2:DsRed)* + DIC merge]

Dataset S1: SMILES, Tanimoto Coefficient and MCS for Glafenine and related compounds

Dataset S2: Alpha diversity metrics and pairwise Kruskal-Wallis p-values

Dataset S3: Beta diversity pairwise PERMANOVA p-values

Dataset S4: Genus level relative abundance

Dataset S5: LEfSe Log_{10} LDA

Table S1: Drugs and chemicals

Table S2: Zebrafish RT-PCR primers

Table S3: Antibodies, dyes and fluorescent probes

Table S1: Drugs and chemicals

Chemical	Supplier	Catalog Number	Stock Concentration	Stock Solvent	Final Concentration	Duration of exposure	Route of expsoure
2,3-dihydroxy propyl acetate	BOC	106-61-6	neat	N/A	≤ 30 µM	72 hours	immersion
4µ8c	Calbiochem	412512	100 mM	DMSO	≤ 10 µM	72 hours	immersion
7-chloroquinoline	Sigma Aldrich	CDS014905	100 µM	DMSO	≤ 30 µM	72 hours	immersion
7-Chloro-4-(4-Hydroxyanilino)Quinoline	Sigma Aldrich	S770043	100 mM	DMSO	≤ 20 µM	72 hours	immersion
Bis(4-nitrophenyl) phosphate	Sigma Aldrich	123943	50 mM	H2O	≤ 250 µM	72 hours	immersion
Brefeldin A	Calbiochem	203729	2 mg/mL	DMSO	≤ 2 µg/mL	72 hours	immersion
CAY10719	Cayman Chemical	20469	4 mM	DMSO	≤ 30 µM	72 hours	immersion
Ceefourin-1	TOCRIS	5867	100 mM	DMSO	≤ 30 µM	72 hours	immersion
CP100356	TOCRIS	4193	20 mM	DMSO	≤ 10 µM	72 hours	immersion
Dexamethasone - water soluble	Sigma Aldrich	D2915	25 mM	H2O	0.75 µM	72 hours	immersion
Diclofenac	Cayman Chemical	70680	100 mM	DMSO	≤ 10 µM	72 hours	immersion
EdU (5-ethynyl-2'-deoxyuridine)	ThermoFisher	C10340	10 mM	DMSO	10 µM	24 hours	immersion
Elacridar	Cayman Chemical	18128	1 mM	DMSO	≤ 3 µM	72 hours	immersion
Emricasan	Selleckchem	S7775	50 mM	DMSO	≤ 10 µM	72 hours	immersion
Floctafenine	TRC	F401700	100 mM	DMSO	≤ 10 µM	72 hours	immersion
Flufenamic Acid	Cayman Chemical	21447	100 mM	DMSO	≤ 30 µM	72 hours	immersion
Glafenine•HCl	Sigma Aldrich	G7020	100 mM	DMSO	≤ 30 µM	72 hours	immersion
Glafenic Acid	TRC	G406493	100 mM	DMSO	≤ 30 µM	72 hours	immersion
Indomethacin	Sigma Aldrich	I7378	24 mM	DMSO	≤ 50 µM	72 hours	immersion
ISRIB	Sigma Aldrich	SML0843	10 mM	DMSO	≤ 10 µM	72 hours	immersion
Ketoprofen	Cayman Chemical	10006661	50 mM	DMSO	≤ 10 µM	72 hours	immersion
Kira6	Calbiochem	532281	50 mM	DMSO	≤ 10 µM	72 hours	immersion
Ko 143	TOCRIS	3241	50 mM	DMSO	≤ 10 µM	72 hours	immersion
KS 176	TOCRIS	4169	100 mM	DMSO	≤ 30 µM	72 hours	immersion
L-NAME	Sigma Aldrich	N5751	50 mg / mL	H2O	≤ 1 mM	72 hours	immersion
Meclofenamic Acid	Cayman Chemical	70550	50 mM	DMSO	≤ 30 µM	72 hours	immersion
Meloxicam	Cayman Chemical	14906	50 mM	DMSO	≤ 30 µM	72 hours	immersion
MitoTEMPO	Sigma Aldrich	SML0737	10 mg/mL	DMSO	≤ 50 µM	72 hours	immersion
MMP-9 inhibitor	Calbiochem	444278	10 mg/mL	DMSO	≤ 1 µM	72 hours	immersion
N-acetyl cystein	Sigma Aldrich	A8199	100 mg/mL	DMSO	≤ 200 µM	72 hours	immersion
N-phenylanthranilic Acid	Sigma Aldrich	144509	100 mM	DMSO	≤ 10 µM	72 hours	immersion
Necrostatin-1 (Nec1)	Cayman Chemical	11658	50 mM	DMSO	≤ 1 µM	72 hours	immersion
NS-398	Cayman Chemical	70590	60 mM	DMSO	≤ 10 µM	72 hours	immersion
Paraoxon ethyl	TRC	P191850	10 mM	DMSO	≤ 1 µM	72 hours	immersion
Piroxicam	Cayman Chemical	13368	50 mM	DMSO	≤ 30 µM	72 hours	immersion
PGE2	Cayman Chemical	14010	100 mM	DMSO	≤ 10 µM	72 hours	immersion
Propylene Glycol	Fisher	P355	neat	N/A	≤ 30 µM	72 hours	immersion
Q-VD-OPh	Selleckchem	S7311	50 mM	DMSO	≤ 50 µM	72 hours	immersion
SC-560	Cayman Chemical	70340	60 mM	DMSO	≤ 10 µM	72 hours	immersion
STF-083010	Calbiochem	412510	10 mM	DMSO	≤ 30 µM	72 hours	immersion
Tariquidar	TOCRIS	5757	100 mM	DMSO	≤ 3 µM	72 hours	immersion
Thapsigargin	Calbiochem	586005	25 mM	DMSO	≤ 1 µM	72 hours	immersion
Tolfenamic Acid	Cayman Chemical	70480	25 mM	DMSO	≤ 30 µM	72 hours	immersion
TUDCA	Calbiochem	580549	100 mM	H2O	≤ 100 µM	72 hours	immersion
Tunicamycin	Calbiochem	654380	10 mg/mL	DMSO	≤ 1 µg/mL	72 hours	immersion
Z-VAD-FMK	Selleckchem	S7023	50 mM	DMSO	≤ 10 µM	72 hours	immersion

Table S2: Zebrafish RT-PCR Primers

Target	Ensembl Gene ID	P1 (5' – 3')	P2 (5' – 3')
18S	ENSDARG00000100392	CACTTGTCCCTCTAAGAAGTTGCA	GGTTGATTCCGATAACGAACGA
abcg2a	ENSDARG00000100075	CATCGGACTCAATGGCATCATG	CCATCACTACATCATCCTGCACTAC
abcg2a-RT	ENSDARG00000100075	ATCGCAGAGAGGAAACTGTTTGTACATG	TCATGTGAACTTCTTAATGAAGCGCAG
abcg2b	ENSDARG00000079361	GTAGTACAGGATGATGTCCTGATG	CTTATAAACTCAGTACCAATCTTGGTGTC
abcg2b-RT	ENSDARG00000079361	CATTAATGAGCGAGCAATTTTTGTGCATG	CCTGCGTAACTGTATGTATGCAAATGTC
abcg2c-RT	ENSDARG00000058574	AGCGAGAGAGAGCTCTTCATCCAT	GACACAACTGCACATACGCCAGAA
abcg2d-RT	ENSDARG00000016818	GCATACAAAACAGGCGAGTTGATCAC	CTACATGAGGACGTGTGCAGCAG
atf4	ENSDARG00000111939	TTAGCGATTGCTCCGATAGC	GCTGCGGTTTTATTCTGCTC
atf6	ENSDARG00000012656	CTGTGGTGAAACCTCCACCT	CATGGTGACCACAGGAGATG
bip	ENSDARG00000103846	AAGAGGCCGAAGAGAAGGAC	AGCAGCAGAGCCTCGAAATA
cdh1	ENSDARG00000102750	GGCAAAGTGACTTTCAATAACTGTGATG	CAGGGACACGCTCAACTCTGACAG
cldn15la	ENSDARG00000016081	CACCACATCGACCCTGTATG	TACCGGCTATTCTGCCTTTG
ctnna1	ENSDARG00000102441	CCCAGGTCACCACACTTGTAAAC	CATCTTCATGGATTCTCCTTGCTTGCG
cyp7a1	ENSDARG00000069018	GCAGGCGTGCCAATGTG	CAGCTCGTTGAAGGTAGATAGTGTGT
ddit3	ENSDARG00000059836	AAGGAAAGTGCAGGAGCTGA	TCACGCTCTCCACAAGAAGA
defbl1	ENSDARG00000075161	AGGATGCAGCCTCATTCTCTTT	TGAAGCCCCAGAGCATATTTATC
defbl3	ENSDARG00000097181	TGGTTATCGTGGCTGCCC	CGCAAATGTTAAAATCGCAAAG
dnajc3a	ENSDARG00000041110	TCCCATGGATCCTGAGAGTC	CTCCTGTGTGTGAGGGGTCT
DsRed		CCCCGTAATGCAGAAGAAGA	TCTTGGCCTTGTACACGGTC
duox	ENSDARG00000062632	CGTGGCCTTTGGTTATCATGTGTG	GCCGTCACGTGATTCACCTGTG
edem1	ENSDARG00000025094	ATCCAAAGAAGATCGCATGG	TCTCTCCCTGAAACGCTGAT
eif2ak3	ENSDARG00000062139	CCGCGGGGCAACAGAGT	GGTGGCAGCGATACAGAAGAAGAT
elf1a	ENSDARG00000020850	CTTCTCAGGCTGACTGTGC	CCGCTAGGATTACCCTCC
ern1	ENSDARG00000013997	ATGGCGTGGGGAGTGTGC	GTATTCTGTGCGGCCAAGGTAAA
fabp2	ENSDARG00000006427	TCAACGGGACCTGGAAAGTC	CCCATTTGTTCCATGAACTTCTC
fabp6	ENSDARG00000044566	CTCCGCTCAATCAACACCAA	TGAGATTCGGTTTCCCACTTG
fabp10a	ENSDARG00000038439	TCACCACCATGGACGGCAAG	CTGTCAGCGTCTCCACCATT
GFP		GAAGAAGTCGTGCTGCTTCA	CCTGAAGTTCATCTGCACCA
grp94	ENSDARG00000003570	TCCCGCACCGATGACGA	GTAGAAGCCCACACCGAACTGAC
hnf4a	ENSDARG00000021494	CAGTGTCGGTACTGCAGACTAAAG	GTGAGCTCGCAGTAAAGCCACCTG
il1b	ENSDARG00000098700	TGGACTTCGCAGCACAAAATG	GTTCACTTCACGCTCTTGGATG
il4	ENSDARG00000087909	GCAGGAATGGCTTTGAAGGG	GCAGTTTCCAGTCCCGGTAT
jupa	ENSDARG00000070787	GCACATGGGAGAGGCTCC	GTTTAACTGAGACTCCACCTCTGCAG
mmp2	ENSDARG00000017676	ACTGCTGTTCCCGATGACCTAG	TTGACCTCGCCGACTTTGAT
mmp9	ENSDARG00000042816	TCATGATCTCTGCGAAGTGG	TTGCCTTTTCCTCTCTGCAT
mmp13a	ENSDARG00000114451	ATGGTGCAAGGCTATCCCAAGAGT	GCCTGTTGTTGGAGCCAAACTCAA
nfkbiaa	ENSDARG00000005481	GCCGGACAGCCCTTAAATTC	TCATAAATACAATCGTCCTCAGACATG
nfkbiab	ENSDARG00000007693	CTCACCGAGGACGGAGACA	CTCTTCGGGATAACGCAATCA
pck1	ENSDARG00000013522	AACTCACTGCTGGGGAAGAA	GTCTCCCACACACTCCACCT
pglyrp2	ENSDARG00000062998	TGCAGGAGGATTTCACCATTC	CTGGGCAGCTGGTGGTTACT
pglyrp5	ENSDARG00000068382	GACACACAAACACCGTGGACAT	CCCCATCCTCTGCCTTCATA
rnasel2	ENSDARG00000043196	CGCATTGTCAATACCGTGGTA	GCCTTTATCACAACGCAAAACA
rnasel3	ENSDARG00000036171	TTGTGCTGCTGCTCCTTTGT	CGCCTTATTTCTGCTGGTTGA
rnasel4	ENSDARG00000076983	TCACTCGGAGATCGTACATCTTTC	CGGGTCTTCATGATCAATTGC
saa	ENSDARG00000045999	CGCAGAGGCAATTCAGAT	CAGGCCTTTAAGTCTGTATTTGTTG
scara3	ENSDARG00000008060	CAATTCTTAAAGGTTTGCCTGGTCC	CCAGAAACTCCTAGCAAGCCTTG
socs3a	ENSDARG00000025428	CACTAACTTCTCTAAAGCAGGGAAGAC	CTGAAGGTCTTGAAGTGGTAAAACG
socs3b	ENSDARG00000026611	GAAAACTCCCAAGATTGAGTCGGATAAC	CAAGCCTACTATGCGTTACCATGG
stat3	ENSDARG00000022712	ACAGCAGGATGGCCAGGTTGC	TCTGTCTGGTGGCTGCTGCCT
tjp1a	ENSDARG00000077506	GAGTACAACAATGGAGGAAACTGTC	CATCACCACTCTATCGTTCTCTTGTAGAAG
tjp1b	ENSDARG00000079374	CATATGTGGGTCTTCCCCAAACACGAC	GTCTCATCCATTGCTGCACTGTTGGAGTC
tnfa	ENSDARG00000009511	GCGCTTTTCTGAATCCTACG	TGCCCAGTCTGTCTCCTTCT
xbp1-s	ENSDARG00000035622	TGTTGCGAGACAAGACGA	CCTGCACCTGCTGCGGACT
xbp1-u	ENSDARG00000035622	GAGGAGCCCACAAAGTCCTC	CGAAGTGCTTTTTCCTCTGG
xbp1-RT	ENSDARG00000035622	GGGTTGGATACCTTGGAAA	AGGGCCAGGGCTGTGAGTA

Table S3: Antibodies, dyes and fluorescent probes

Primary Antibodies

Target	Supplier	Catalog Number	Clone	Application	Dilution
4E8	Abcam	ab73643	FES 4E8/1	Immunofluorescence / Dot Blot	1:500 / 1:250
DsRed	Clontech	632496	1510004	Immunofluorescence	1:500
GFP	Aves	GFP-1010	polyclonal	Immunofluorescence	1:500
ZO-1	Invitrogen	33-9100	ZO1-1A12	Immunofluorescence	1:500

Secondary Antibodies, Dyes, and Fluorescent Probes

Reagent	Supplier	Catalog Number	Stock Concentration	Application	Final Concentration	Delivery / route of administration
7-AAD	Sigma	A9400	1 mg/mL (5% methanol / 95% PBS w/ Ca2+/Mg2+)	FACS / flow cytometry	5 µg/mL	cell suspension immersion
Acridine Orange	Sigma	A8097	10 mg/mL (in H2O)	Live imaging	1 µg/mL	immersion
CellROX Green	ThermoFisher	C10444	2.5 mM (in DMSO)	FACS / flow cytometry	5 µM	cell suspension immersion
Donkey anti Mouse IRDye 680	Li-Cor	926-68072	1.0 mg/mL	western blotting	100 ng/mL	N/A
Donkey anti Rabbit 568	ThermoFisher	A10042	2 mg/mL	Staining vibratome sections	10 µg/mL	N/A
Goat anti Chicken 488	ThermoFisher	A11039	2 mg/mL	Staining vibratome sections	10 µg/mL	N/A
Goat anti mouse 568	ThermoFisher	A11004	2 mg/mL	Staining vibratome sections	10 µg/mL	N/A
Goat anti Mouse 647	ThermoFisher	A32728	2 mg/mL	Staining vibratome sections	10 µg/mL	N/A
Hoechst 33258	ThermoFisher	H3569	10 mg/mL	Staining vibratome sections	5 µg/mL	N/A
Texas Red 10kDa Dextran	ThermoFisher	D1828	1% w/v (in 1x DPBS)	Intestinal permeability	0.10%	oral gavage

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