Supplemental Experimental Procedures

Transgenic line generation

ifabp:tdtomato:p2a:dn-rbpj (*ifabp:dn-rbpj*): the *dn-rbpj* sequence was amplified from *pDONR221-su[H]dn1*. Using *pDestTol2pA2* as the backbone, a *tol2* destination vector was generated by using an *ifabp* 5' entry vector (Table S1), with a pME entry vector to introduce *tdTomato* and a *p2A:notch* 3' entry vector. The *p2A:notch* fusions were accomplished using SOE PCR using primers *attB2/5'p2a*, *3'p2A/5'suHdn_R*, *5' suHdn_F*, and *attB3/3' suHdn_R* (Table S2) resulted in a sequence containing *attB2:P2A-dnRBPj:attB3*. The PCR fragment was then recombined into the gateway Donor vector *pDONR P2r-P3* following manufacturer's instructions (Invitrogen, Carlsbad, CA). The 3' entry plasmid was used in multi-site gateway reactions with the *p5E:ifabp* and *pMEtdTomato* plasmids and recombined into *pDestTol2pA2* according to manufacturer's instructions (Invitrogen, Carlsbad, CA); the resulting construct carried *ifabp:tdtomato:p2a:dn-rbpj*.

The *ifabp:dn-rbpj* construct was microinjected into AB/Tu embryos at the 1-cell stage for tol2-mediated transgenesis as previously described (Kwan et al., 2007) and allowed to develop normally. Larvae were screened for GFP expression in the heart and tdTomato expression in the intestinal epithelium at 7-8 dpf. Individuals expressing both markers were reared to adulthood and kept as stable transgenic lines.

Morpholino injections

myd88 and *tnfR* MO-injected animals were generated as previously described (Bates et al., 2007). Splice blocking morpholinos (Gene Tools, Philomath, OR) were

injected into embryos at the one cell stage. The *myd88*e212 morpholino was injected at 5 pmol per embryo. To knockdown *tnfR,* the *TR1v1/TR1v2* morpholinos were injected at 1.2 pmol and 6 pmol respectively (Bates et al., 2007). Mock-injected controls where injected with only phenol red and water. Morpholino sequences are presented in Table S3. Splice-blocking was verified by RT-PCR using the primers in Table S4. For both morpholinos, efficacy of target knock down was also assessed by assaying for reduction of intestinal myeloid peroxidase activity positive neutrophils to GF levels, as described (Bates et al., 2007).

Histological analysis

To determine goblet cell numbers, fixed larvae were washed 3x 10 min in Phosphate Buffered Saline (PBS) and equilibrated in 100 mM Tris pH7.5, 10 mM $MgCl_2$ for 10 min. Goblet cells were labeled with 0.04% Alcian Blue (Sigma Aldrich, St. Louis, MO), 10 mM $MgCl_2$ in Ethanol for 4 d at room temperature. Labeled larvae were then rehydrated and destained with successive 5 min washes of 100 mM Tris pH7.5, 10 mM $MgCl_2$ in 80%, 50% and 25% Ethanol. Paraffin-embedded larvae were sectioned in 7 μm thick slices and goblet cells were counted in 30 sections retrograde from the vent.

2F11 antibody (ab71286; 1:500; ABCAM, Cambridge, MA) staining in histological sections was performed as previously described (Crosnier et al., 2005). The 2F11 antibody recognizes the pan-secretory cell antigen Annexin 4A (Zhang et al., 2014). 7 dpf larvae were fixed in 1% formaldehyde for 3 h at room temperature. Larvae were then washed 3x for 10 min with PBS, blocked in Saponin Block Buffer: PBS, 1% BSA (Sigma Aldrich, St. Louis, MO), 1% DMSO (Sigma Aldrich, St. Louis, MO), 0.5%

Saponin (Sigma Aldrich, St. Louis, MO), 2% Goat Serum (Jackson Immunoresearch, West Grove, PA). Larvae were then incubated with the 2F11 antibody diluted 1:1000 in Saponin Block Buffer at 4°C overnight, followed by 4 1h washes with PBS + 1% Triton-X-100. AlexaFluor488-conjugated goat anti-Rabbit secondary antibodies (Life Technologies, Grand Island, NY) were diluted into Saponin Block buffer and incubated with the larvae overnight at 4°C followed by 3x 30 min washes with PBS + 1% Triton-X-100 and 3x 5 min washes in PBS. 2F11 stained secretory cells in whole mount larvae were visualized on a Leica (DFC360FX) fluorescence stereo microscope. All 2F11 stained secretory cells within a 50 μm length of the intestinal bulb were counted.

EECs were directly enumerated on a Leica fluorescence stereo dissection scope (Leica DFC360FX). 7 dpf *nkx2.2a:egfp;ifabp:dn-rbp-j* larvae were anaesthetized and mounted in 4% methyl cellulose on glass slides. All GFP positive cells within the region of intestinal tdTomato expression were counted and compared against WT sibling controls.

Identification of the vdrb:GAL4 *genomic insertion site*

The *vdrb:GAL4* insertion site was identified using a method similar to that developed to identify transposon insertion sites in bacteria (Stephens et al., 2015). Briefly, 1.5 μg of genomic DNA was pooled from 14 individual larvae and sheared to a median fragment size of 300 bp, end-repaired with NEB Quick-blunting kit (New England Biolabs, Ipswich, MA), then cleaned up and A-tailed using Klenow Fragment (3'->5' exo-) (New England Biolabs, Ipswich, MA). Standard paired-end Illumina adapters with T-overhangs were then ligated to the DNA fragments with T4 ligase (New

England Biolabs, Ipswich, MA) overnight at room temperature. The ligation reaction was then cleaned up with a Qiagen (Germantown, MD) MinElute column to remove excess adapter sequences. 28 ng of the cleaned ligation was used as template in a 50 ul PCR using Phusion high-fidelity polymerase (cycling conditions: 1:00 initial denature at 98 C followed by 30 cycles of 98 C for 10 seconds, 56 C for 30 seconds, 72 C for 20 seconds, followed by final extension at 72 C for 2 minutes). The forward primer (*tol2_addP5*; Table S5) was designed to add a 5 nucleotide barcode and full-length Illumina adapter sequence while targeting a 22 bp sequence of the tol2 construct (CCCTAAGTACTTGTACTTTCAC) located 34 bp from the transposon end. The reverse primer (*P7_amp*; Table S5) targeted the asymmetric ends of the ligated Illumina adapters that would not contain a priming site for the reverse primer unless the forward primer had extended its template. The PCR product was run on a 1.5% agarose gel and a fragment in the range of 150-300 bp was extracted. The final Illumina library was spiked in as a small portion of another Illumina library and run on a single lane of the Illumina GAII. Raw sequences were first identified by their 5 nucleotide barcode and the presence of the primer sequence used to target the tol2 construct, then end-trimmed and quality filtered to remove low quality base calls at the end of sequences as well as low-quality sequences overall. The remaining 34 bp of the tol2 construct end sequence that was not directly targeted by the primer was used to identify reads that came from amplified products that correctly targeted the tol2 construct integrated into the genome. As expected, the accuracy of the targeting primer (forward) was low, with only 3.2% of the quality reads that contained the target primer sequence also containing the 34 bp of the untargeted tol2 construct end sequence. Finally, after filtering out Illumina adapter

sequences, the potential genomic insertion site was identified in each read as the sequence after the tol2 inverted repeat (TIR) sequence at the transposon end. These 1608 potential genomic insertion site sequences were aligned to the Zv9 build of the zebrafish genome with Bowtie2 (Langmead and Salzberg, 2012). Of the 1596 quality alignments, all but 1 aligned to the same zebrafish genomic sequence (Zv9 build chromosome 6:38936157) located in the first intron of the *vdrb* gene (ENSEMBL Gene ID ENSDARG00000070721) suggesting there was only a single tol2 insertion in this transgenic line. To independently verify the insertion site, we designed primers that anneal to the *vdrb* gene and the tol2 construct (vdrbGal4_check and tol2_out, Table S5) so that we would amplify a 794 bp fragment only if the identified insertion site was correct. All of the original 14 individuals were tested and showed the expected amplification. Sanger sequencing of the amplified products confirmed the predicted insertion site, identifying the tol2 construct ends adjacent to the predicted zebrafish genomic sequence (*vdrb:GAL4* insertion site sequence).

References

Bates, J.M., Akerlund, J., Mittge, E., and Guillemin, K. (2007). Intestinal alkaline phosphatase detoxifies lipopolysaccharide and prevents inflammation in zebrafish in response to the gut microbiota. Cell Host Microbe *2*, 371-382.

Crosnier, C., Vargesson, N., Gschmeissner, S., Ariza-McNaughton, L., Morrison, A., and Lewis, J. (2005). Delta-Notch signalling controls commitment to a secretory fate in the zebrafish intestine. Development *132*, 1093-1104.

Kwan, K.M., Fujimoto, E., Grabher, C., Mangum, B.D., Hardy, M.E., Campbell, D.S., Parant, J.M., Yost, H.J., Kanki, J.P., and Chien, C.B. (2007). The Tol2kit: a multisite gateway-based construction kit for Tol2 transposon transgenesis constructs. Dev Dyn *236*, 3088-3099.

Langmead, B., and Salzberg, S.L. (2012). Fast gapped-read alignment with Bowtie 2. Nat Methods *9*, 357-359.

Stephens, W.Z., Wiles, T.J., Martinez, E.S., Jemielita, M., Burns, A.R., Parthasarathy, R., Bohannan, B.J., and Guillemin, K. (2015). Identification of Population Bottlenecks and Colonization Factors during Assembly of Bacterial Communities within the Zebrafish Intestine. MBio *6*, e01163-01115.

Zhang, D., Golubkov, V.S., Han, W., Correa, R.G., Zhou, Y., Lee, S., Strongin, A.Y., and Dong, P.D. (2014). Identification of Annexin A4 as a hepatopancreas factor involved in liver cell survival. Dev Biol *395*, 96-110.

Table S1. *ifabp:dn-rbpj* promoter sequence

atcactgttacgcagattctgcaaattctacaaaatgtttgtagtaaatatagtataaaagatgtttttagcttacttttttacattattt
tatacatttaaacatgcatgaaatcaattattatcagcttggacctttttcttgtccctactcttagtgggacgatgccaaaggtctt
acaatgggacgataccaaaggtcttcagaggtcttatttaattgcactgttaagagattaaggataaggtaatgatgagataa
accatcatcagagattatcatgtctcagtttgatatctcgtctctttatcttgtcatggagcctccagataaaacttccacactgc
atgttgcttaatttgaatcatttgcaggctttgtacactattcatcattacagataatgctgaagctgttctttcacagccttgcatc
aatatttacaacattttcaaacaatacaaggggataattcagtaatacaatttaaaataaggttccaataactatattactaataa
ctaacaatgagcaaaatacataatgctgttatttttagtataactggatgagtactgcagattttaaataaaatattcatggaatta
ttgtcgttactgtataatgttggtgaatgtatggtctgtttttgacatttgtgtgcatttatttttatatctaaagtgttttatagatttgca
ttttaatattagtagtaaatgcaaatgataaatactttatctttaaattaaaaatattattatgcattacttatctgtgataaatatatc
gtatgtgttatgttactgataaaatgttacaataataatggttcggttagggttaaggttttaggacaaaacacaactgttcatta
gtattcagatcagatctatgaaataatattggcaggtgcaaattttcattttaataatgtactagtactgtaaatatttgagttagct
ttagaactggctttgaagtatattcactggtagctgtctgttaagtagaactgcacaaaaaaaaggtacgaggaagaacgaa
gcattgccatatactgtattctcaattttagctgcaatagctgcacttttgtagtaaaaagttcttcaaagacccttttttatatagtg
tgaagaacttttcaataatcttaaaacatttttctacataaataaaatttttgtgcaatgaaatatttcaaaaagtactttataaaac
caaccattcatgtcaacacagagccttaaaataattgttaccagtatttctcaatagccaatgaggcatgcagcaagattgta
aactaatttaactgtaaccattaaatctaaagttttgacctttgacctttcaccacaattgtttaaaaatcaatacatgtggctaga
attcatagctttcaaacactcttcatagtccctcagtagttactgcaacacaaggcacttactagtataacagacacaccctac
acagtttaaacagcaaacaattcaaacaaaagcaggactttgttatgagaacaacagagcgtgtatgtttgaccatcgcagt
acaagtgagtgcttgttctcaggacttgaaatctccgctctttgcacaatcaatgaataagcaaggcatgctgggatgtgtgt
aacatatagcctgttgggcgggtgagatttatacttggtgagctttactcggccacatcagcatgaagataatactcagataa
ggcaacgcttcgccactcgcacaggtataaaagagtggctcggggtaaagttaggccactgtcaggatcacacaacag

Table S2. Primer sequences used for *ifabp* line

Name	Sequence
attB2/5'p2a	GGG GAC AGC TTT CTT GTA CAA AGT GG
3'p2A/5'suHdn_R	GGA ATG CCA GGT TGT GCC ATA GGA C
5' suHdn_F	ATG GCA CAA CCT GGC ATT CC
attB3/3' suHdn_R	GGG GAC AAC TTT GTA TAA TAA AGT TG

Table S3. Morpholino sequences

Name	Sequence
*myD88*e2i2	5′-GTTAAACACTGACCCTGTGGATCAT-3′
TRlv1	5′-TACGTCCTTGTGCATTGCTGGCATC-3′
TRlv2	5′-CTGCATTGTGACTTACTTATCGCAC-3′

Table S4. Splice-blocking primers

Name	Sequence
MyD88elF	5′-TCTTGACGGACTGGGAAACTCG-3′
MyD88e5R	5′-GATTTGTAGACGACAGGGATTAGCC-3′
TR1F	5′-GCATGGATCCATATCAGGACTTGGTGGA-3′
TR1R	5′-TCGAGAATTCTTACGAAACGCTTGTGTT-3′

Table S5. Primer sequences used for tol2 insertion site identification

Name	Sequence
tol2_addP5	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTA CACGACGCTCTTCCGATCTXXXXX**CCCTAAGTACTTGTA CTTTCA*C**
P7_amp	CAAGCAGAAGACGGCATACGA
vdrbGal4_check	TAAACACCGACCTTGGCGAG
tol2_out	ACTTTGAGTAGCGTGTACTGGC

XXXXX: 5 nucleotide barcode
*: Phosphorothioate bond
BOLD: Sequence targeting inside tol2 transposon

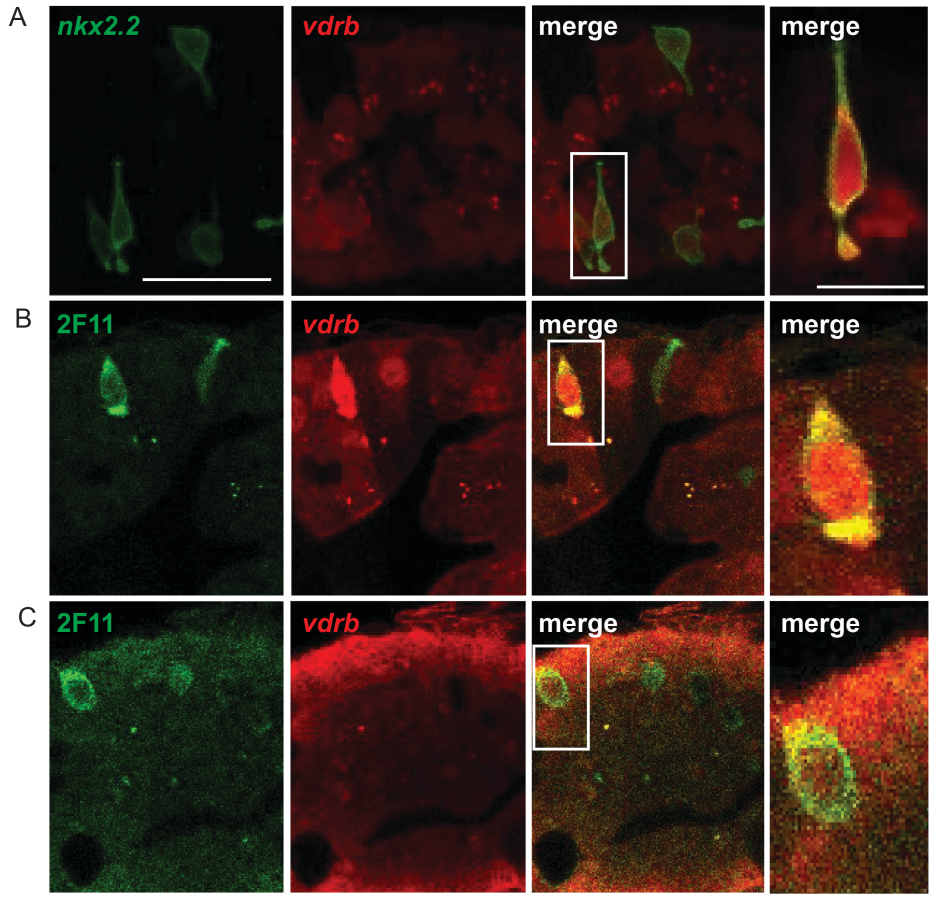

Figure S1. *Tg*(*vdrb:GAL4); Tg(UAS:mCherry*) expression throughout the larval intestinal epithelium, including in secretory cells. Co-expression of the enteroendocrine specific *Tg*(*nkx2.2a:mEGFP)* transgene with *UAS:mCherry* driven by the *vdrb:GAL4* transgene [*Tg(vdrb:mCherry)*] (A). *Tg*(*vdrb:mCherry*) stained with secretory cell marker 2F11 showing expression in both enteroendocrine cells (B) and goblet cells (C). Each image is a single confocal plane. Scale bar = 25 μm in all panels except the three on the far right. Scale bar for the three panels on the far right = 10μm.

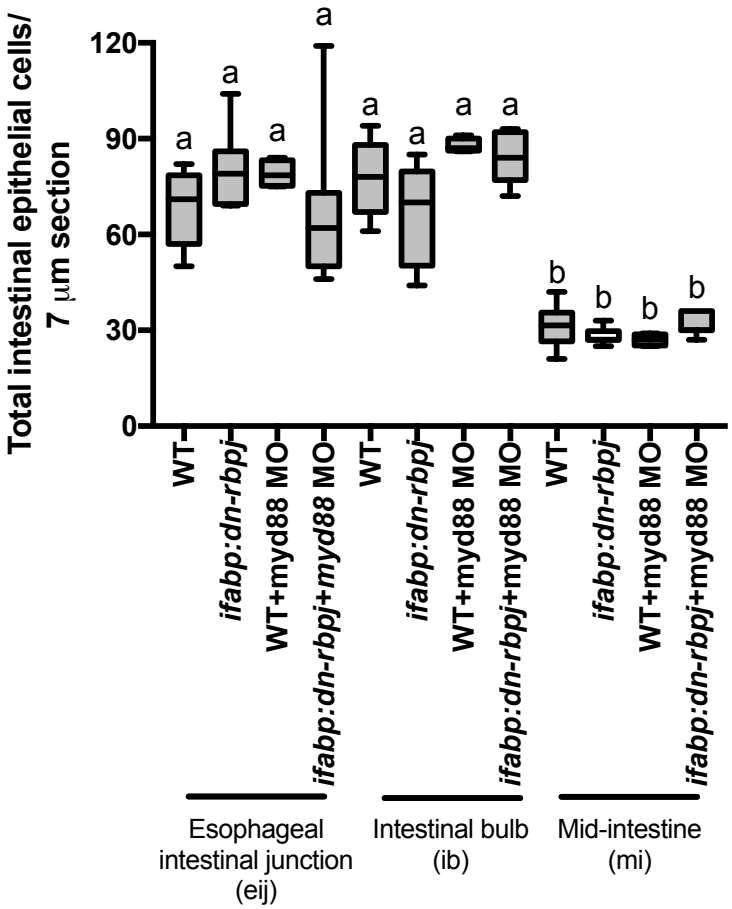

Figure S2. The number of total epithelial cells differs due to larval intestinal region, but not fish or treatment type.