Tobin et al., Supplemental Material:

Figures, Tables, Movie Legend and Experimental Procedures.

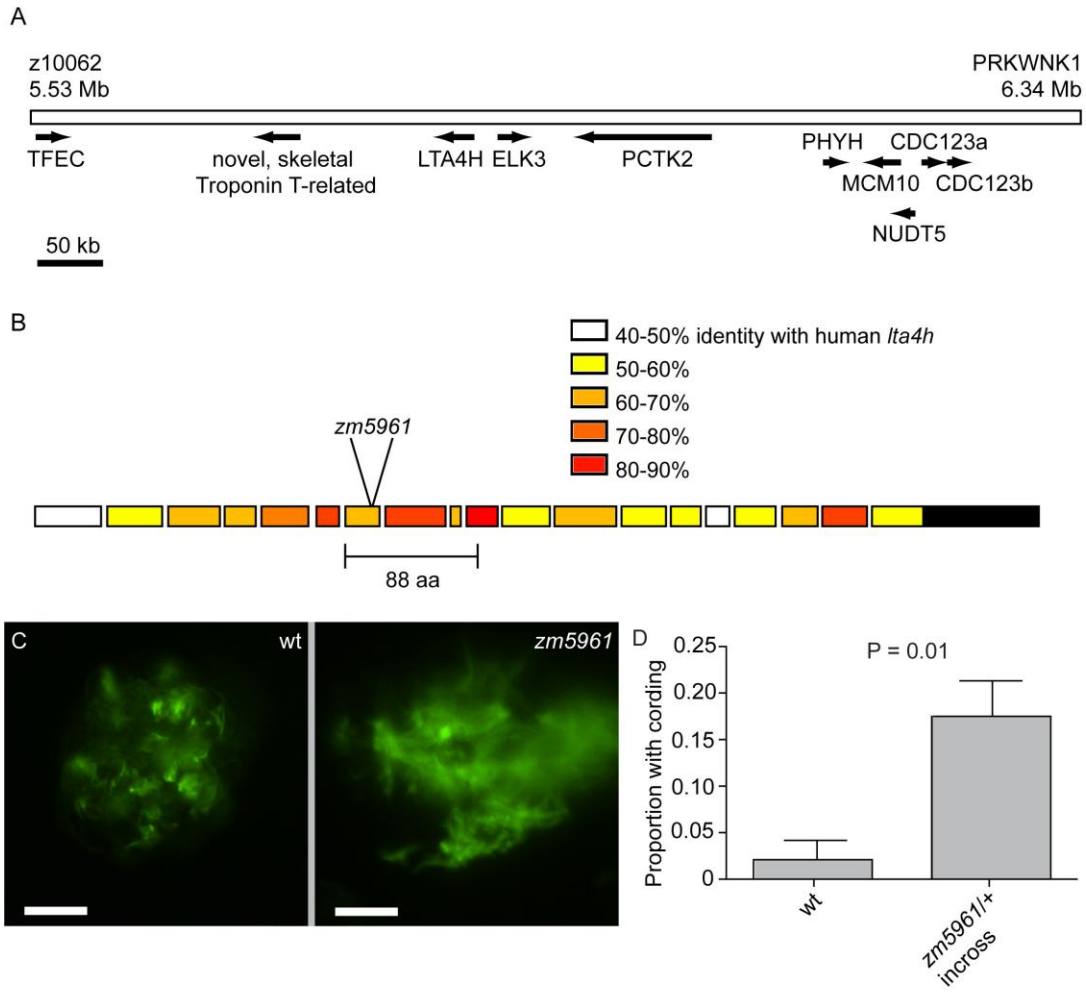

Figure S1, related to Figure 2. Mutations in the *lta4h* Locus Result in an Extracellular Cording Phenotype.

(A) Critical interval on zebrafish Chromosome 4 for *fh112*, bounded by closest polymorphic genetic markers. Predicted ORFs in the interval are indicated with arrows. Numbering and gene predictions based on Ensembl Zv6 assembly (www.ensembl.org).

(B) Gene structure of zebrafish *lta4h* locus with percent identity to human *lta4h* color-coded. Location of the retroviral insertion in the *zm5961*mutant and position of its in-frame 88 amino acid deletion is indicated. (C) Fluorescence image of non-cording bacteria within a granuloma in matched sibling control animals (left) and cording bacteria in *zm5961* (right) at five dpi with infection dose of 161 +/- 33 (SD) bacteria. Scale bars, 20 μm.

(D) Mean proportion of animals with cording in three independent groups of 15-25 animals five dpi with 161 +/- 33 (SD) bacteria. *P*=0.01 (Student's unpaired t-test). Error bars, SEM.

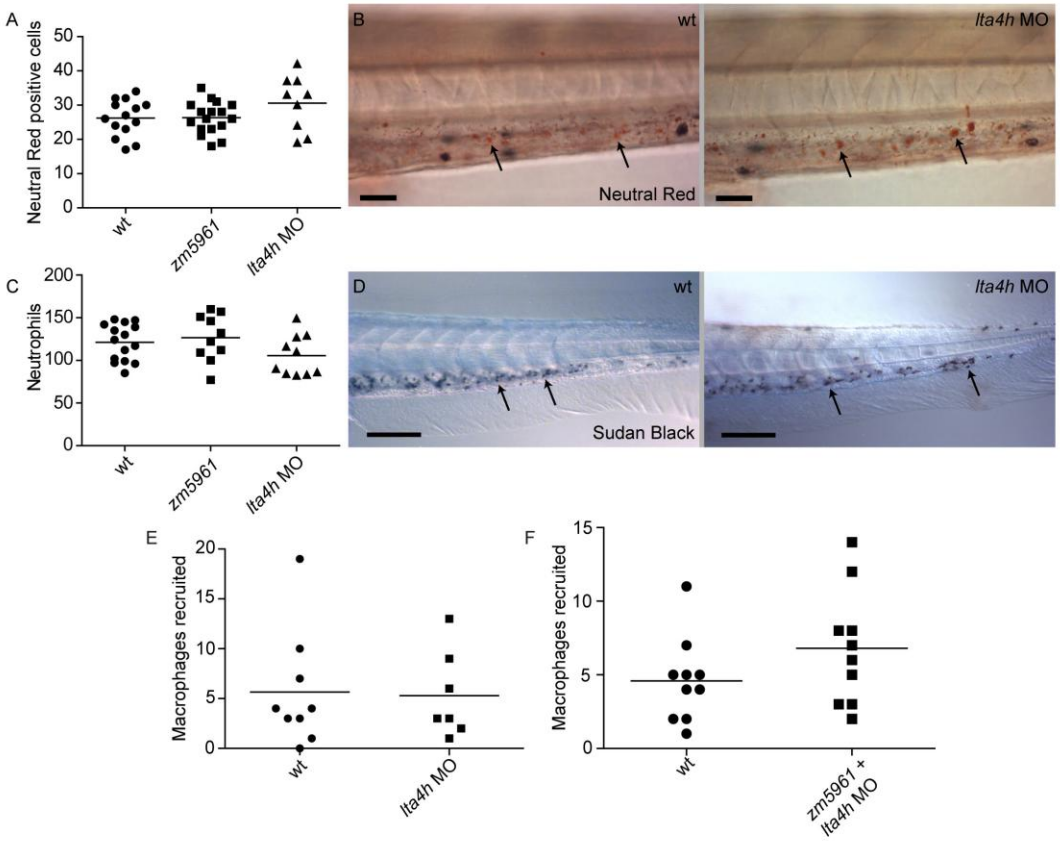

Figure S2, related to Figure 4. *lta4h* Deficiencies Do Not Affect Macrophage or Neutrophil Number or Recruitment.

(A,B) Live uninfected animals were stained with neutral red at three dpf to quantitate the number of macrophages in control, *zm5961* mutant, and *lta4h* morphant animals.

(A) The number of neutral red positive cells posterior to the yolk tube was determined. No significant differences in macrophage number were observed (one-way ANOVA).

(B) Example of neutral red positive cells (arrows) stained at three dpf in wildtype and *lta4h* morphant animals. Scale bar, 25 μm

(C, D) Uninfected fixed animals were stained with Sudan Black at three dpf to quantitate the number of neutrophils present.

(C) Quantitation of the number of neutrophils posterior to the yolk tube. No significant differences among control, *zm5961*, and *lta4h* morphant were found (one-way ANOVA).

(D) Example of Sudan Black neutrophil staining in the tail of wildtype and morphant animals. Scale bar, 100 μm

(E) LTA4H and control morphant animals were injected in the hindbrain ventricle with 150-200 bacteria at 24 hpf. The number of recruited macrophages was assessed six hours later by DIC microscopy. No significant differences between control and morphant were found using Student's unpaired t-test.

(F) WT animals compared to *zm5961* mutants that had also been injected with the *lta4h* morpholino. Animals were infected in the hindbrain ventricle and assayed for macrophage recruitment as described above.

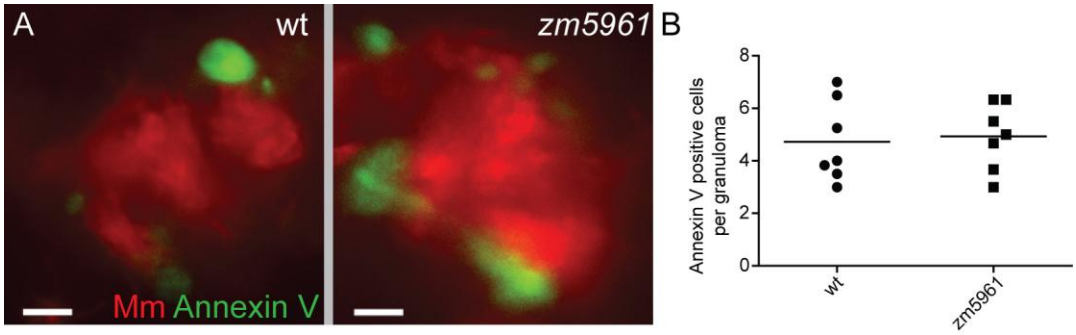

Figure S3, related to Figure 4. Mutation of *lta4h* Does Not Affect Annexin V Staining During Infection.

(A) Wildtype (left) and *zm5961* (right) granulomas after Annexin V staining (green). Bacteria are red. Staining was performed 4 dpi after infection with 250-300 CFU *M. marinum*. Scale bars, 5 µm.

(B) Quantitation of Annexin V positive cells per granuloma in wildtype and *zm5961* animals (n=7 animals for each class, total number of granulomas analyzed: 21 for wildtype, 25 for *zm5961*). No significant difference by Student's unpaired t-test.

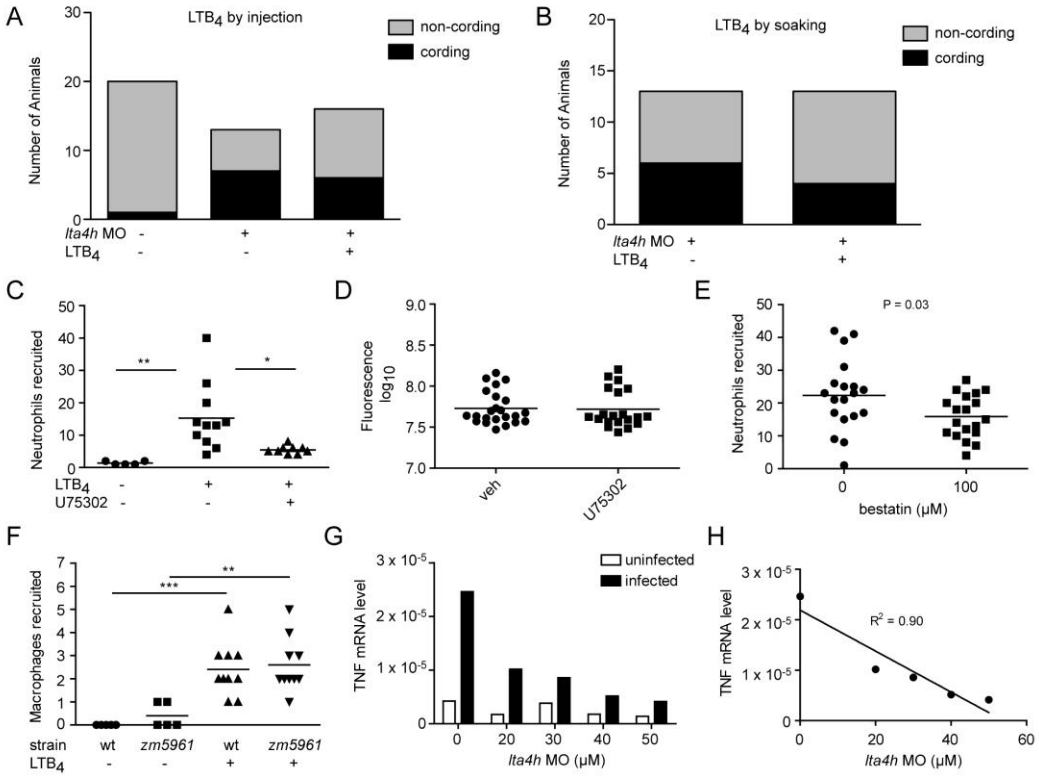

Figure S4, related to Figure 5. Exogenous LTB_4 Does Not Rescue the *lta4h* Hypersusceptibility Phenotype.

lta4h morphant embryos were infected, pooled, and then treated with either vehicle or exogenous LTB_4. Exogenous LTB_4 was either

(A) injected ($1.5x10^{-14}$ mol LTB_4 in 1% ethanol/PBS) into the caudal vein twice daily or (B) administered by soaking fish in 300 nM LTB_4 in 0.1% ethanol with daily water changes. Cording was scored at four dpi. No significant differences were found between the LTB4 and vehicle treated groups in (A) and (B).

(C) LTB_4-mediated recruitment of neutrophils to the right ear was abolished by soaking in 3 μM U75302, an inhibitor of the LTB_4 Receptor. *, $P<0.05$; **, $P<0.01$ (one-way ANOVA with Tukey's post test; other comparison not significant).

(D) Bacterial burden of vehicle-treated or U75302-treated animals assessed by FPC at 4 dpi. No significant difference by Student's unpaired t-test.

(E) Three dpf uninfected animals were injected in the right ear with $1.5x10^{-14}$ mol LTB_4 after overnight soaking in 100 μM bestatin or in vehicle. *P*=0.03 by Student's unpaired t-test.

(F) Wildtype or *zm5961* animals were injected with either vehicle or $1.5x10^{-14}$ mol LTB_4 into the hindbrain ventricle at 30 hpf. Recruited macrophages identified by DIC microscopy were scored at six hours post-injection. There was no significant difference between wildtype and *zm5961* in either the vehicle injected or LTB_4 injected groups (one-way ANOVA with Tukey's post-test). **, P<0.01; ***, P<0.001

(G) Levels of *tnf* mRNA relative to β-actin control measured by qRT-PCR for graduated doses of the *lta4h* morpholino in uninfected (white) and infected (black) animals at one dpi. Animals were infected at two dpf with 233 +/- 26 (SD) CFU *M. marinum*.

(H) Linear regression analysis of *tnf* mRNA levels for varying *lta4h* morpholino doses. R^2=0.90; *P*=0.01 (F test for a significantly non-zero slope).

Movie S1, related to Figure 4. Recruitment of active macrophages by exogenous LTB_4.

A 30-minute time lapse of macrophages recuited to the hindbrain ventricle, as shown in Figure 4A. Injection of $1.5x10^{-14}$ mol LTB_4 into this compartment at 24 hpf results in macrophage recruitment and dynamic membrane behavior typical of early zebrafish macrophages (Herbomel et al., 2001; Davis and Ramakrishnan, 2009). Macrophages were imaged by DIC microscopy as described.