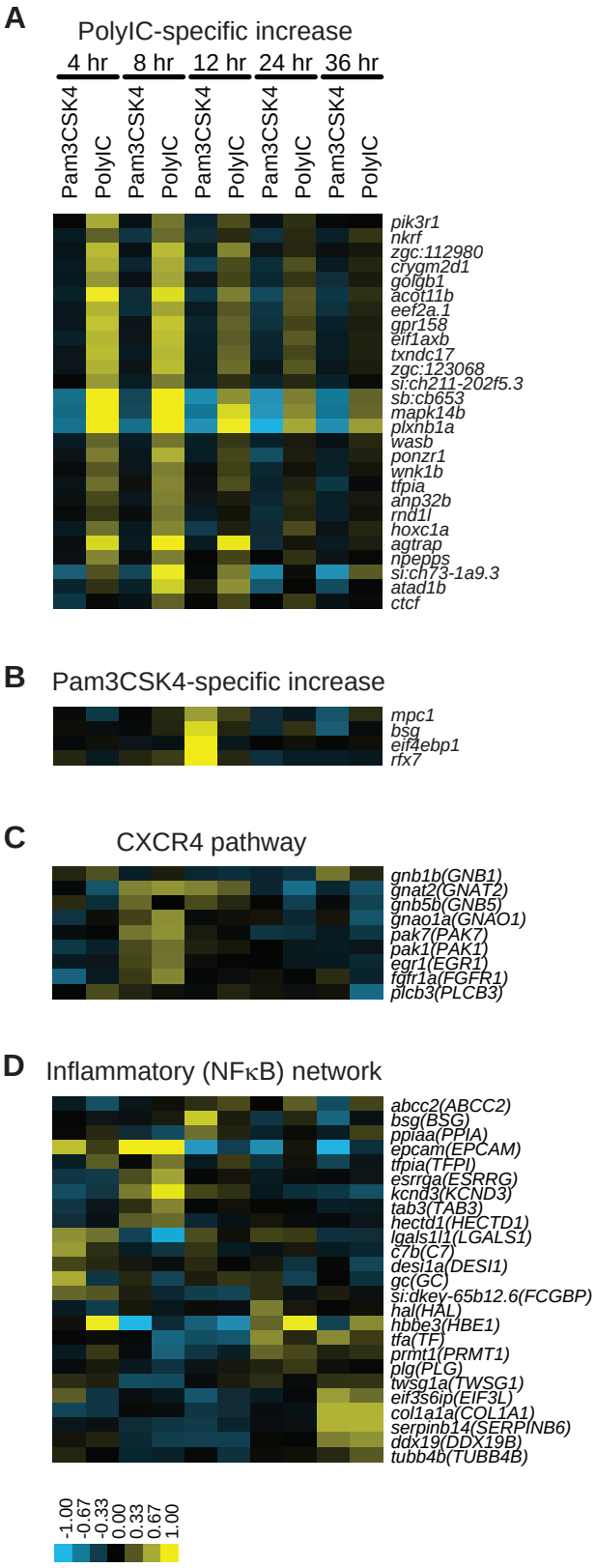

Figure S1. Heat maps of gene expression data. (A) Heat map of zebrafish genes that were primarily responsive to PolyIC. (B) Heat map of zebrafish genes that were primarily responsive to Pam3CSK4. (C) Heat map of zebrafish genes within the CXCR4 signaling pathway (see Fig. S2A). Corresponding human genes were defined by Ingenuity Pathway Analysis software (Qiagen) and are shown in parentheses. (D) Heat map of zebrafish genes within the inflammatory network (see Fig. S2B). Corresponding human genes were defined by Ingenuity Pathway Analysis software and are shown in parentheses.

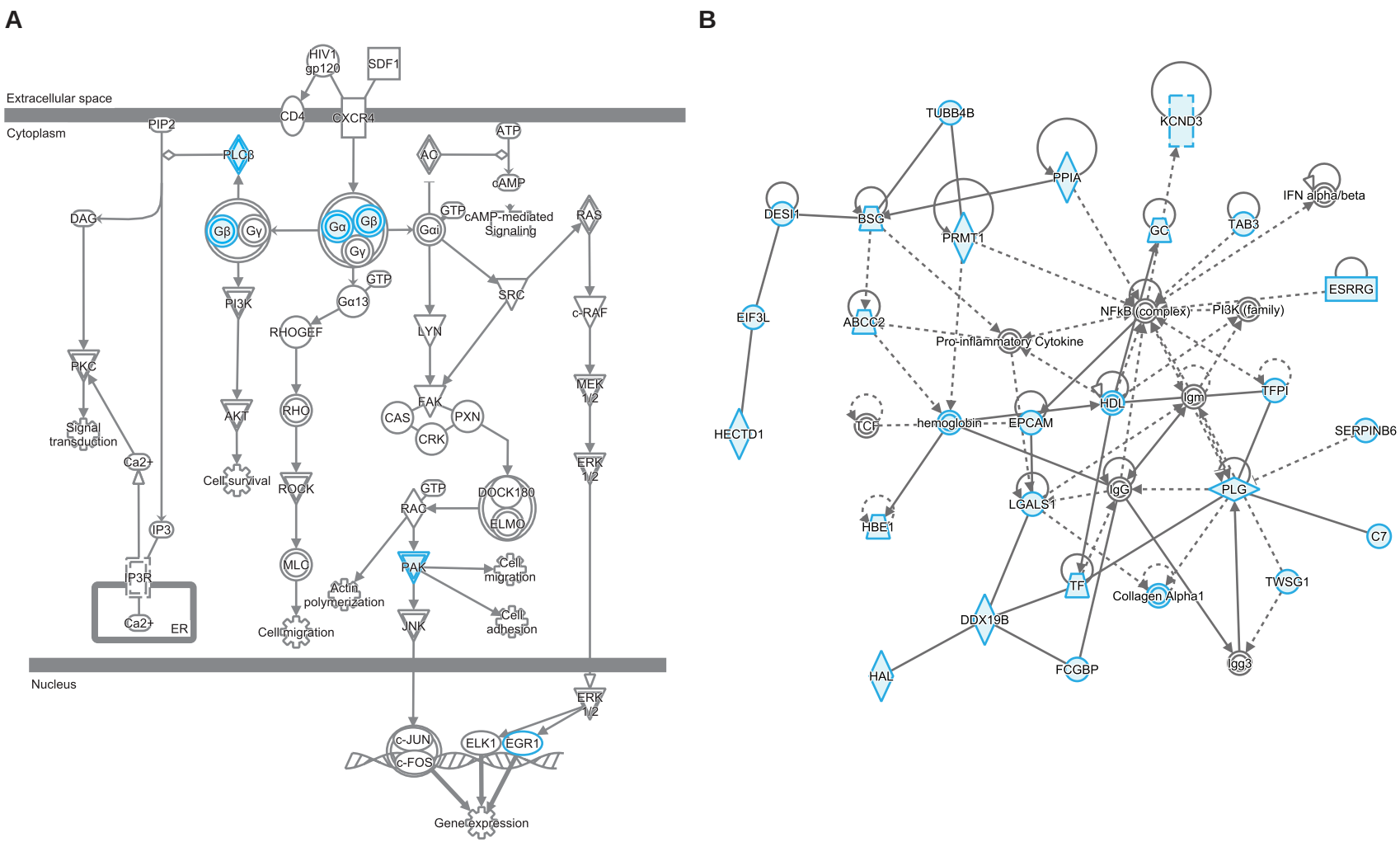

Figure S2. Human CXCR4 pathway and inflammatory network. (A) Components of the human CXCR4 pathway are indicated. Nodes which include genes identified with transcriptional changes in zebrafish larvae after exposure to Pam3CSK4 or PolyIC are outlined in blue and listed in Supplemental Fig. S1C. (B) Components of the human inflammatory (NF-κB) network are indicated. Genes identified with transcriptional changes in zebrafish larvae after exposure to Pam3CSK4 or PolyIC are outlined in blue and listed in Supplemental Fig. S1D. Images adapted from Ingenuity Pathway Analysis software (Qiagen).

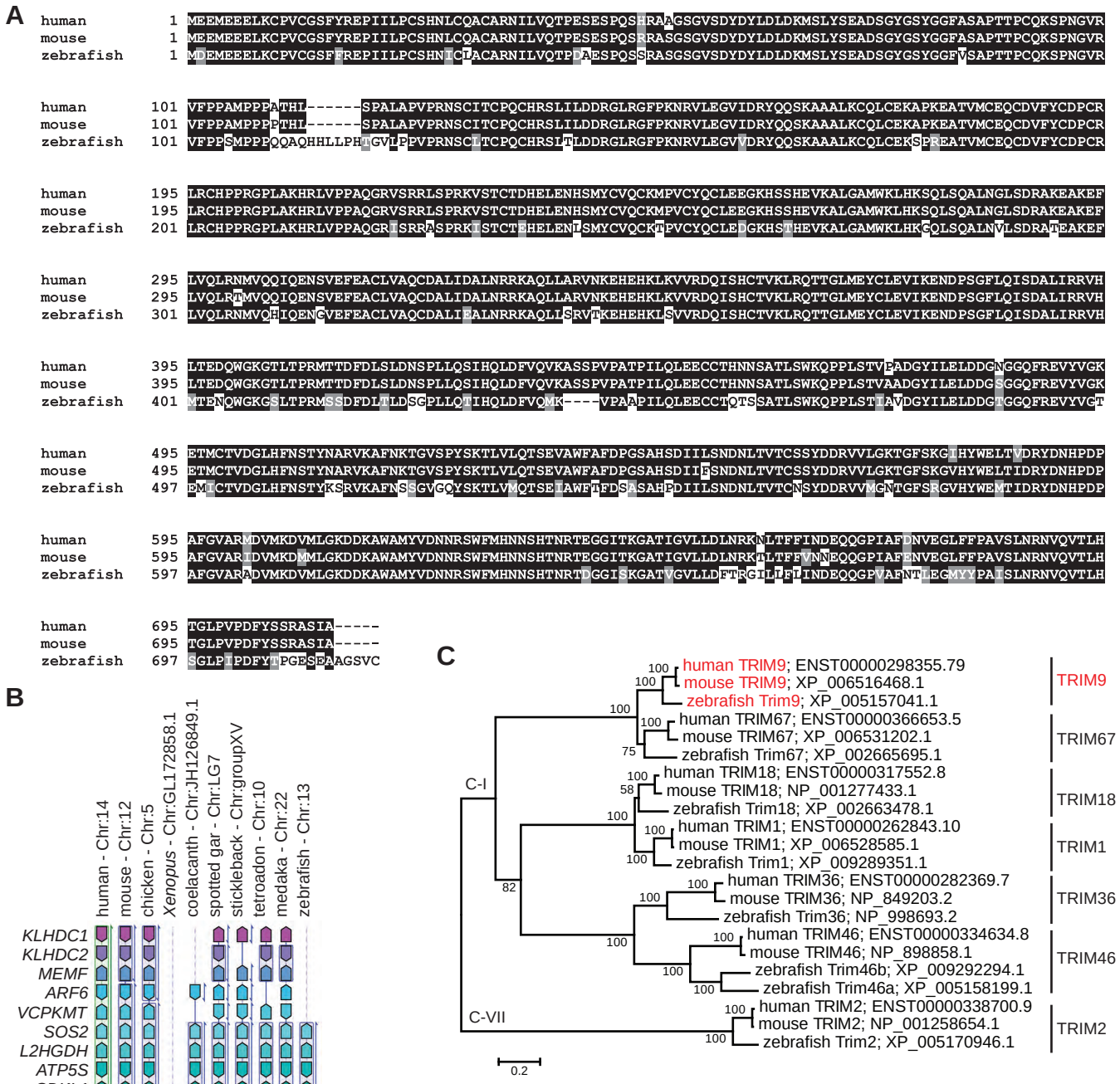

Figure S3. Conservation of Trim9 sequence. (A) TRIM9 proteins from human, mouse and zebrafish are aligned. Identical residues are shaded black and structurally similar residues are shaded gray. (B) The gene organization at the TRIM9 locus on human chromosome 14 is shown (left column) and compared to the TRIM9 locus in other vertebrate species using Genomicus (Louis et al. 2015. Nucleic Acids Res., 43, D682-D689) including the zebrafish trim9 locus on chromosome 13 (right column). (C) Phylogenetic comparison of TRIM C-I subfamily members from human, mouse and zebrafish using Neighbor-Joining (Saitou & Nei. 1987. Mol. Biol. Evol., 4, 406-425) and 2000 bootstrap replicates. Human TRIM protein sequences were acquired from ENSEMBL. Zebrafish and mouse TRIM protein sequences were identified by BLASTp searches using human sequences as queries. TRIM76, which has been classified as a C-1 family member, is excluded from this analyses because it encodes a partial RING domain, lacks a FN3 domain and is approximately twice as long as other C-I proteins. TRIM2, which is a C-VII subfamily member, is included as an outgroup. Sequence identification numbers are provided in panel C.

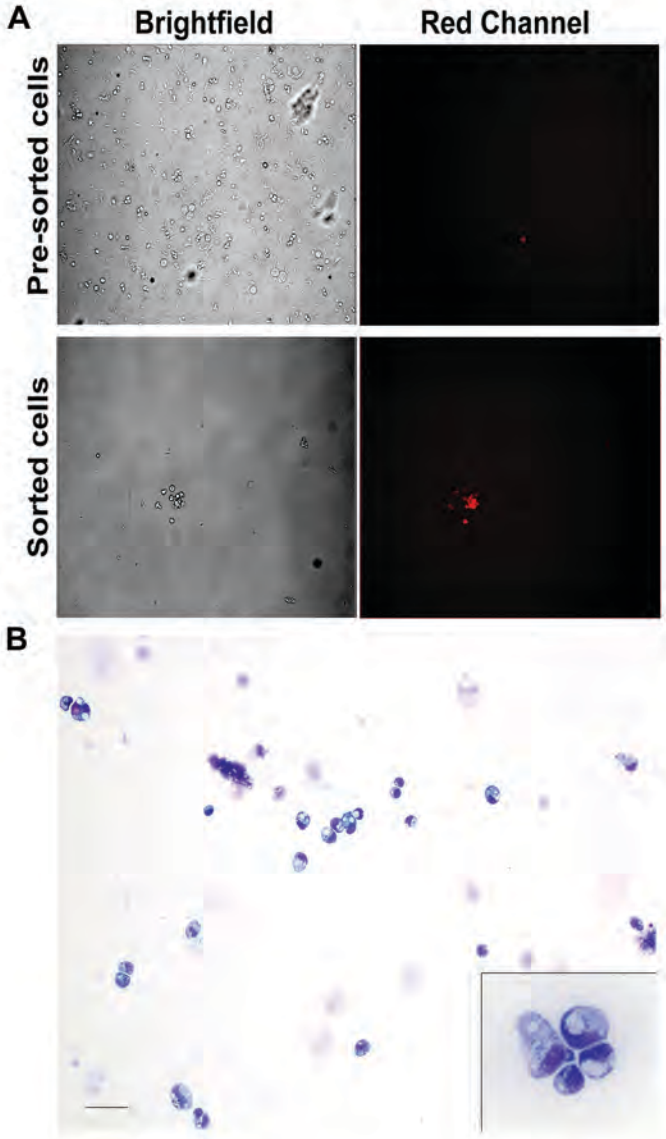

Figure S4. Macrophage isolation. (A) Cell suspensions made from larval *Tg(mpeg1:mCherry)* zebrafish at 120 hpf (top panels) were sorted for mCherry-positive cells (bottom panels). (B) Sorted cells were transferred onto slides by cytospin and stained with Diff-Quick (Dade Behring, Inc.). Approximately 95% of sorted cells possess an abundant, vacuolated cytoplasm and round to reniform nuclei, consistent with macrophages. Direct aliquots of cell suspensions pre- and post-sorting and cytospins were imaged on a Leica DM5000B microscope with Leica Application Suite software. Comparable results were obtained when sorting cells from *Tg(mpeg1.1:EGFP)* zebrafish. Scale bar = 50 μm.

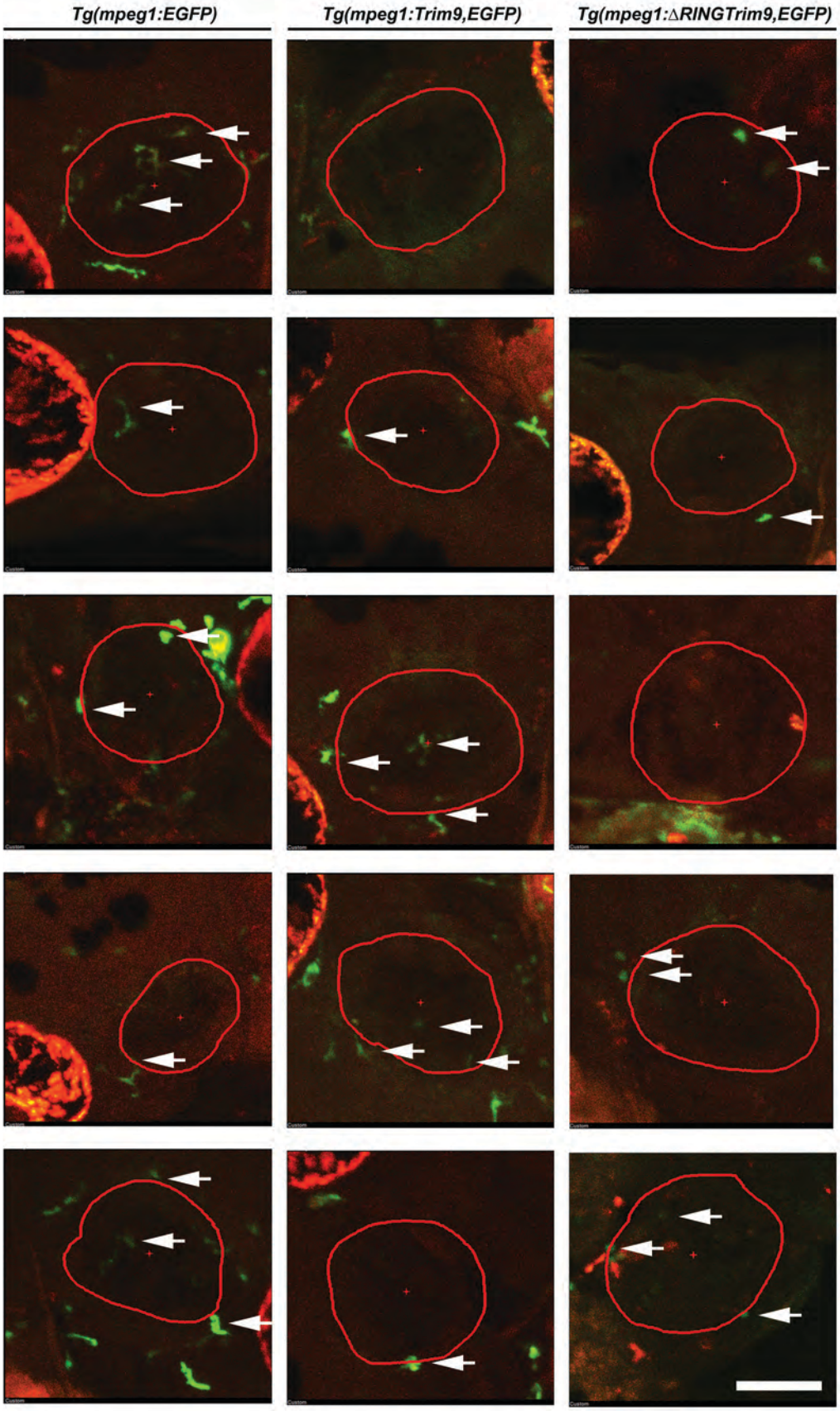

Figure S5. Macrophages are present in otic vesicle prior to chemotaxis assay. One cell *Tg(mpeg1.1:mCherry)* embryos were injected with the transgene indicated above and their otic vesicles (outlined with red) were imaged at 3 dpf. The observation of GFP+ cells (white arrows) demonstrate resident macrophage populations are in or around the otic vesicle in the absence of injection with PolyIC or Pam3CSK4. Scale bar = 100 μm.

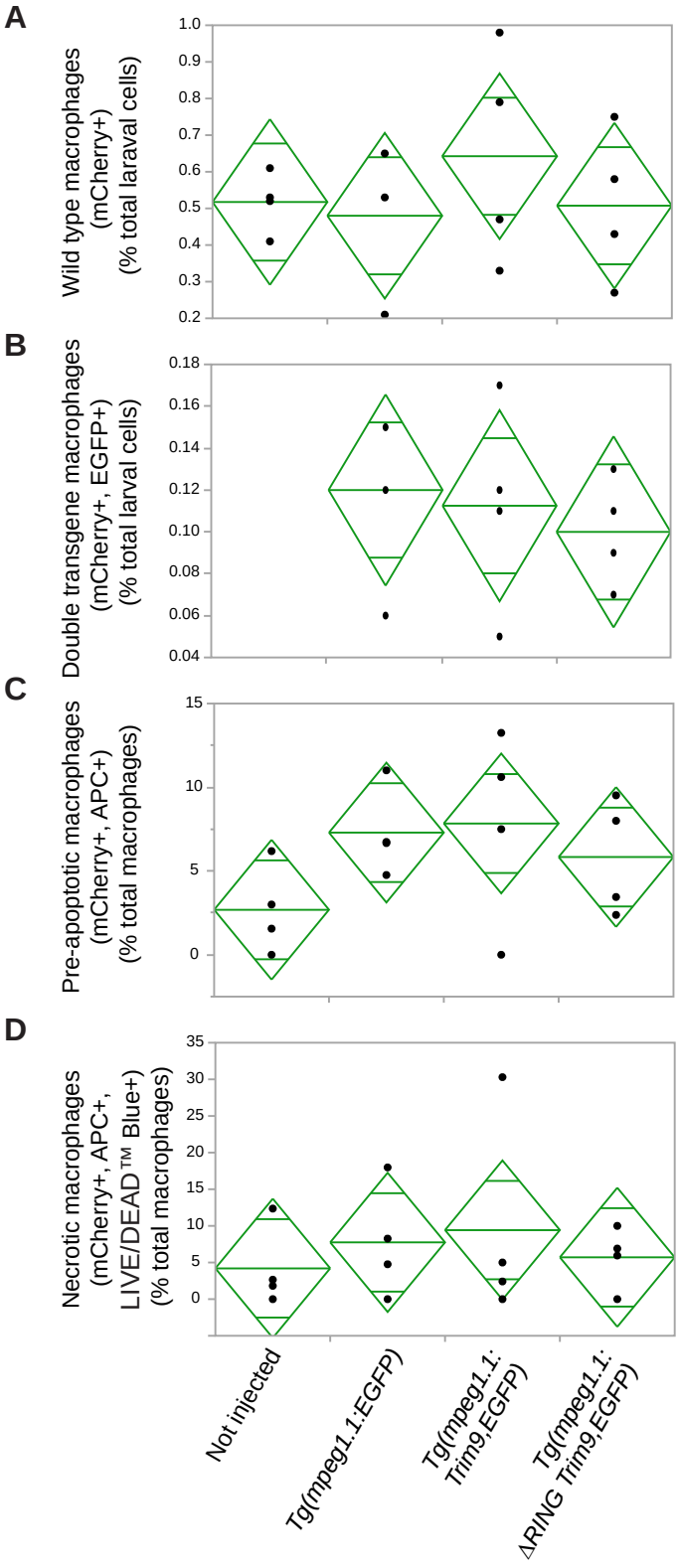

Figure S6. Trim9 transgenes do not induce cell death. One-cell transgenic *Tg(mpeg1.1:mCherry)* zebrafish embryos were either not injected or injected with 50 pg EGFP transgenes indicated at bottom of figure and 50 pg of Tol2 transposase mRNA. At 3 days post fertilization, larvae were euthanized and disaggregated into single cell suspensions for flow cytometry. Cells were incubated with APC Annexin V (BD Pharmingen™) to label apoptotic cells and with LIVE/DEAD™ Fixable Blue Dead Cell Stain Kit (ThermoFisher Scientific) to identify necrotic cells. Cells were analyzed using a Becton Dickinson LSRFortessa Flow cytometer (UNC-Chapel Hill Flow Cytometry Core Facility). Data from four biological replicates indicate that the expression of Trim9 or ΔRINGTrim9 do not alter significantly (A) the number of macrophages within a larvae, (B) the percentage of macrophages expressing the Trim9 transgene, (C) the percentage of pre-apoptotic macrophages and (D) the percentage of necrotic macrophages.