

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 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).

Α	human	1	MEEMEEELKCPVCGSFY	REPIILPCSHN	ILCQACARNILVQT	PESES PQSHRA <mark>A</mark> GSG	VSDYDYLDLDKMSLYSI	EADSGYGSYGGFASAPTTPC	QKSPNGVR
	mouse	1	MEEMEEELKCPVCGSFY	REPIILPCSHN	ILCQACARNILVQT	PESES PQSRRASGSG	VSDYDYLDLDKMSLYSI	EADSGYGSYGGFASAPTTPC	QKSPNGVR
	zebrafish	1	MDEMEEELKCPVCGSFF	REPIILPCSHN	IC <mark>I</mark> ACARNILVQT	PD <mark>A</mark> ES PQS <mark>S</mark> RASGSG	VSDYDYLDLDKMSLYSI	EADSGYGSYGGF <mark>Y</mark> SAPTTPC	QKSPNGVR
	human	101	VFPPAMPPPATHL	SPALAPVPR	ENSCITCPQCHRSL	I LDDRGLRGFPKNRV	LEGVIDRYQQSKAAALI	(CQLCEKAPKEATVMCEQCD	VFYCDPCR
	mouse	101	VFPPAMPPP <mark>PTHL</mark>	SPALAPVPR	ENSCITCPQCHRSL	I LDDRGLRGFPKNRV	LEGVIDRYQQSKAAALI	(CQLCEKAPKEATVMCEQCD	VFYCDPCR
	zebrafish	101	VFPP <mark>S</mark> MPPPQQAQHHLI	PHTGVLPPVPR	ENSCITCPQCHRSL	<mark>T</mark> LDDRGLRGFPKNRV	LEGV <mark>Y</mark> DRYQQSKAAALI	(CQLCEK <mark>S</mark> PREATVMCEQCD	VFYCDPCR
	human	195	LRCHPPRGPLAKHRLVF	PPAQGRVSRRLS	PRKVSTCTDHELE	NHSMYCVQCKMPVCY	QCLEEGKHSSHEVKAL(SAMWKLHKSQLSQALNGLSD	RAKEAKEF
	mouse	195	LRCHPPRGPLAKHRLVF	PPAQGRVSRRLS	PRKVSTCTDHELE	NHSMYCVQCKMPVCY	QCLEEGKHSSHEVKAL(SAMWKLHKSQLSQALNGLSD	RAKEAKEF
	zebrafish	201	LRCHPPRGPLAKHRLVF	PPAQGRISRR <mark>A</mark> S	PRKISTCTEHELE	N <mark>I</mark> SMYCVQCK <mark>I</mark> PVCY	QCLEDGKHSTHEVKAL(SAMWKLHK <mark>E</mark> QLSQALN <mark>V</mark> LSD	RA <mark>T</mark> EAKEF
	human	295	LVQLRNMVQQIQENSVE	FEACLVAQCDA	LIDALNRRKAQLL	ARVNKEHEHKLKVVR	DQISHCTVKLRQTTGLI	ÆYCLEVIKENDPSGFLQIS	DALIRRVH
	mouse	295	LVQLR <mark>I</mark> MVQQIQENSVE	FEACLVAQCDA	LIDALNRRKAQLL	ARVNKEHEHKLKVVR	DQISHCTVKLRQTTGLI	ÆYCLEVIKENDPSGFLQIS	DALIRRVH
	zebrafish	301	LVQLRNMVQ <mark>H</mark> IQEN <mark>C</mark> VE	FEACLVAQCDA	LIEALNRRKAQLL	<mark>S</mark> RV <mark>T</mark> KEHEHKL <mark>S</mark> VVR	DQISHCTVKLRQTTGLI	ÆYCLEVIKENDPSGFLQIS	DALIRRVH
	human	395	LTEDQWGKGTLTPRMTT	DFDLSLDNSPI	LQSIHQLDFVQVK	ASSPVPATPILQLEE	CCTHNNSATLSWKQPP1	LSTV <mark>E</mark> ADGYILELDDGNGGQ	FREVYVGK
	mouse	395	LTEDQWGKGTLTPRMTT	DFDLSLDNSPI	LQSIHQLDFVQVK	ASSPVPATPILQLEE	CCTHNNSATLSWKQPP1	LSTVAADGYILELDDGSGGQ	FREVYVGK
	zebrafish	401	MTE <mark>N</mark> QWGKGSLTPRMSS	DFDL T LD <mark>SG</mark> PI	LQTIHQLDFVQMK	<mark>VPAA</mark> PILQLEE	CCT <mark>QTS</mark> SATLSWKQPP1	LSTIA <mark>V</mark> DGYILELDDGTGGQ	FREVYVG <mark>T</mark>
	human	495	ETMCTVDGLHFNSTYNA	RVKAFNKTGVS	PYSKTLVLQTSEV	AWFAFDPGSAHSDII	LSNDNLTVTCSSYDDR	/VLGKTGFSKGIHYWELTVD	RYDNHPDP
	mouse	495	ETMCTVDGLHFNSTYNA	RVKAFNKTGVS	PYSKTLVLQTSEV	AWFAFDPGSAHSDII	SNDNLTVTCSSYDDR	/VLGKTGFSKGVHYWELTID	RYDNHPDP
	zebrafish	497	E <mark>M</mark> ICTVDGLHFNSTY <mark>K</mark> S	RVKAFN <mark>S</mark> SGV <mark>C</mark>	OYSKTLVMQTSEI	AWF <mark>T</mark> FD <mark>SASAH</mark> EDII	LSNDNLTVTC <mark>N</mark> SYDDR	/VMG <mark>N</mark> TGFSRGVHYWEMTID	RYDNHPDP
	human	595	AFGVARMDVMKDVMLGF	DDKAWAMYVDN	INRSWFMHNNSHTN	RTEGGITKGATIGVL	LDLNRK <mark>N</mark> LTFFINDEQ	QGPIAFDNVEGLFFPAVSLN	RNVQVTLH
	mouse	595	AFGVARIDVMKDMLGF	DDKAWAMYVDN	INRSWFMHNNSHTN	RTEGGITKGATIGVL	LDLNRK <mark>TLTFFVNN</mark> EQ	QGPIAFENVEGLFFPAVSLN	RNVQVTLH
	zebrafish	597	AFGVAR <mark>A</mark> DVMKDVMLGF	DDKAWAMYVDN	INRSWFMHNNSHTN	RTDGGISKGATVGVL	LD <mark>FT</mark> R <mark>GI</mark> LTFTINDEQ	QGPVAF <mark>NTLEGMYY</mark> PAISLN	RNVQVTLH
В	human monse zepratish 4 fr:rd 2 fr: 2 fr:	icken - Chr:5 9 9 9 9000 - Chr:GL172858.1 2 5 5	elacanth - Chr:JH126849.1 otted gar - Chr:JF126849.1 ckleback - Chr:LG7 ckleback - Chr:groupXV roadon - Chr:10 sdaka - Chr:12 brafish - Chr:13 brafish - Chr:13			100 hu 100 c hu 100 hu 100 hu 75 zel 100 hu 58 mou 100 hu 58 mou 100 hu 100 hu 220 100 hu 100 hu 100 hu 28 mou 100 hu 100 hu 28 mou 100 hu 100 hu	man TRIM9; ENSTO buse TRIM9; XP_006 brafish Trim9; XP_00 nan TRIM67; ENSTO use TRIM67; XP_00 orafish Trim67; XP_00 nan TRIM18; ENSTO00 se TRIM18; NP_0012 rafish Trim18; XP_006 rafish Trim1; XP_006	0000298355.79 516468.1 05157041.1 0000366653.5 6531202.1 02665695.1 00317552.8 27743.1 02663478.1 000262843.10 528585.1 9289351.1 ENST00000282369.7 NB 940203.2	TRIM9 TRIM67 TRIM18 TRIM1
K K V(LHDC1	Ch C	co co co co co co co co co co co co co c		C-VII		zebrafish Trim36; 100 human TRIM4 mouse TRIM4 zebrafish Trir 200 zebrafish Trir 100 human TRIM 100 human TRIM 100 zebrafish Tri	use TRIM30, INP_049203.2 'afish Trim36; NP_998693.2 Juman TRIM46; ENST00000334634.8 nouse TRIM46; NP_898858.1 zebrafish Trim46b; XP_009292294.1 - zebrafish Trim46a; XP_005158199. human TRIM2; ENST00000338700.3 mouse TRIM2; NP_001258654.1 - zebrafish Trim2; XP_005170946.1	TRIM36
٨	ATP5S CDKL1 IAP4K5 ATL1 SAV1 NIN PYGL			Figure \$ human, r and struct	53. Conserv nouse and z turally simila	ration of Trin ebrafish are a r residues are	n9 sequence. igned. Identica shaded gray. ((A) TRIM9 prote I residues are shad B) The gene organ	eins from ded black ization a

m 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.

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HD12B U ABHD12B 🛡

FRMD6 🛑 🛄

GNG2 🔲

NID2

C14orf166 🗓

PTGDR Ń PTGER2

TXNDC16

GPR137C ERO1A PSMC6 STYX

GNPNAT1 📋

FERMT2 ERMT2 🗎 DDHD1 📄 Ū.

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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 postsorting 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 μ m.



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 μ m.



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 PharmingenTM) to label apoptotic cells and with LIVE/DEADTM 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 Δ RINGTrim9 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.