Supporting Online Material:

Materials and Methods

Bacterial Strains

WT strain M (ATCC #BAA-535) and the ΔRD1 strain derived from it (*1*) were transformed with plasmids containing transcriptional fusions of genes encoding green-fluorescent protein (GFP) to a constitutive Mm promoter as described (*1-3*). Bacteria were grown in 7H9 media supplemented with oleic acid-albumin-dextrose-complex and 0.05% Tween-80 unless otherwise stated.

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Embryo Infection and Husbandry

All zebrafish experiments were conducted in conformity with the Public Health Service Policy on Humane Care and Use of Laboratory Animals using protocols approved by the Institutional Animal Care and Use Committee of the University of Washington. Zebrafish embryos of the AB line were injected with Mm or PBS (mock-injected) using phenol red as a visual marker at 30 hours post fertilization (hpf) via caudal vein as described (*4*). Innocula at injection were determined by injecting the same volume onto a hemocytometer and counting bacteria by fluorescence microscopy and/or by injecting the same volume onto selective bacteriologic plates and enumerating bacteria when colonies formed in 5-7 days. Bacterial burdens in embryos were determined by plating lysed embryos or where noted by fluorescence pixel counts (FPC). After images have been taken with standardized exposure times, FPC integrates the number of pixels in each image with values above a background threshold, as determined by matched images of uninfected animals. For the microarray experiments, ~150-200 CFU of WT Mm and 200-

300 ΔRD1 Mm were injected per embryo. 30-50 embryos were maintained throughout the experiment in 100x15 mM Petri dishes in water with 0.003% PTU to prevent melanization. Each day, any dead embryos were removed and 50% of the water was replaced. At 5 dpi, all embryos were examined by low-power fluorescence microscopy and the outliers of infection (low or extremely high levels of infection) as assessed visually were removed from the final pool. Approximately 40% of each total pool was removed, leaving pools of 30 to 90 embryos for further analysis.

Whole genome transcriptional profiling – Affymetrix platform

Total RNA was extracted from groups of zebrafish larvae (31-82 larvae/group, 3 biological replicate groups/condition) using established methods (*5*). RNA quality was confirmed using an Agilent 2100 Bioanalyzer. Total RNA was used to prepare cRNA targets that were hybridized (30 μg/sample) to Affymetrix GeneChip Zebrafish Genome Arrays using established protocols (*6*). GeneChip data were analyzed using dChip software (build date Dec. 11, 2005) (*7*). CEL files were normalized (Invariant set method), and model-based expression values were generated (PM-MM model). Expression values were then exported into MeV software (*8*) and probesets differentially expressed between any two different conditions (i.e., WT-infected vs. mock-infected at 5 dpi; mock-infected vs. uninjected at 5 dpi) were identified in MeV as those that exhibited (i) a minimal fold-change of 1.5, (ii) a paired *t* test p value of ≤ 0.05, and (iii) an average intensity value of ≥ 10 in at least one condition (table S1 and S2). Zebrafish genes represented by Affymetrix probesets were identified using NetAffx (July 14, 2006 release; http://www.affymetrix.com/analysis/), the Zebrafish Affy Chip Annotation

Project (http://134.174.23.160/zfaca/hash/master020106public.aspx), and through manual annotation using the zebrafish genome assembly at Ensembl (http://www.ensembl.org/Danio_rerio/).

Whole genome transcriptional profiling – Compugen platform

Total RNA was extracted from groups of zebrafish larvae (154-180 larvae/group, 2 biological replicate groups/condition) using established methods (*5*). RNA quality was confirmed using an Agilent 2100 Bioanalyzer. Total RNA was used to prepare cDNA targets that were labeled and hybridized (12 μg/sample) to glass slides printed with the Zebrafish Oligonucleotide Library (oligonucleotides designed by Compugen and synthesized by Sigma-Genosys) using established protocols (*5*). Slides were scanned and expression values measured and normalized as described using ScanArray software (Perkin Elmer) (*5*). Each experiment consisted of pairwise competitive hybridizations from two treatment groups (i.e., WT-infected vs. mock-infected at 5 dpi), plus reciprocal dye-swap replicates. Since biological duplicates were generated for each treatment group, a total of four DNA microarrays were utilized per comparison of the two treatment groups. Oligonucleotide elements that (i) received "present" flags in all four microarrays and (ii) displayed a >6 mean signal-to-noise ratio across both dye channels in all four microarray replicates were identified and all others were excluded. The normalized $\log_2$ ratio of median dye intensities for each remaining element were exported into MeV software (*8*) and probesets differentially expressed between WT-infected and mock-infected larvae were identified as those that exhibited (i) a minimum fold-change of 1.5 and (ii) a paired t-test p-value of ≤ 0.05 (table S1 and S3). Zebrafish genes represented by

Compugen oligonucleotides were identified using the following methods: (i) annotation provided by Compugen, (ii) the Zebrafish Chip Annotation Database (http://giscompute.gis.a-star.edu.sg/~govind/zebrafish/version2/), and/or (iii) through manual annotation using the zebrafish genome assembly at Ensembl (http://www.ensembl.org/Danio_rerio/). Mammalian homologs of zebrafish genes identified using either the Affymetrix or Compugen platforms were identified using BioMart (http://www.biomart.org/) in addition to the databases described above. Raw and processed data from the Affymetrix and Compugen platforms were submitted to Gene Expression Omnibus under accession numbers GSE8327 and GSE8846 (http://www.ncbi.nlm.nih.gov/geo/).

Quantitative RT-PCR

Total RNA from infected or mock-injected embryos used for DNA microarray analysis were also used as templates for generating cDNA (Superscript II reverse transcriptase; oligo dT primers; Invitrogen) for quantitative real-time RT-PCR analysis. For SYBR Green: qRT-PCR assays were performed such that each 20μL reaction contained either 250 nM of gene-specific primers or B-actin specific control primers (see table S4 for a list of gene-specific primers). SYBR green PCR Master Mix (Applied Biosystems) was used at 1X. TAQMAN: Assays were performed with 900nM of each unlabeled primer and 250nM of 5'FAM-DHQ probes (Applied Biosystems). Taqman Universal Master Mix was used at 1X. All qRT-PCR assays were performed in triplicate with an ABI Prism 7300 Real Time PCR System (Applied Biosystems). Data were normalized to B-actin (ΔΔCT analysis). qRT-PCR was used to verify changes in gene expression

observed in DNA microarray assays of WT vs. mock, ΔRD1 vs. mock, and mock vs. untouched samples at 6dpf/5dpi.

Fluorescence In Situ Hybridization and TUNEL analysis

RNA transcripts were localized in situ, singly or in combination (multiplex), as described in (*9*). Briefly, digoxigenin or fluorescein-labeled antisense RNA probes were generated and hybridized to zebrafish embryos that were fixed in 4% paraformaldehyde and permeabilized with proteinase K. Primary sheep anti-hapten antibodies were detected with HRP-conjugated secondary antibodies, and TSA visualization with Molecular Probes Alexa 555 (red) or Alexa 488 (green) was performed. TUNEL labeling was performed as described (*9*). Granuloma diameter was determined as described (*10*) and only granulomas between 20 and 50 μm were analyzed. Mean granuloma diameter (μm±SEM) was 29±9, 27±7, and 30±11 for control infected with WT Mm, control infected with ΔRD1 Mm, and *mmp9* morphant (e2) injected with WT Mm, respectively.

Gelatinase Zymography

Embryos were collected and rinsed once with lysis buffer (150mM NaCl,10mM HEPES, 2 mM DTT, 0.1% Triton X-100, pH 8.0), then resuspended and ground in lysis buffer supplemented with EDTA-free protease inhibitor (Calbiochem #539137). Gelatinases were purified from lysates using gelatin-sepharose beads (Amersham 17-0956-01). Briefly, gelatin-sepharose beads were washed three times with equilibration buffer (50mM Tris-HCl pH 7.5, 0.5M NaCl, 10mM $CaCl_2$, 0.01% Tween 20, 5mM O-phenanthroline) and resuspended in equilibration buffer. An equal volume of beads and

lysate was added to a Micro Biospin Column (Biorad) and allowed to bind overnight at 4ºC with gentle rocking. Beads were then washed with buffer 1 (50mM Tris-HCl pH7.5, 0.5M NaCl, 10mM $CaCl_2$, 0.05% Tween 20, 5mM O-phenanthroline) and twice with buffer 2 (50mM Tris-HCl pH7.5, 10mM $CaCl_2$, 0.05% Tween 20, 5mM O-phenanthroline). Next gelatinases were eluted with elution buffer (100 mM Tris-HCl pH6.8, 4% SDS, 20% Glycerol, 200ug/ml bromophenol blue) and loaded and run on a 10% Zymogram gel (Invitrogen), which was developed per the manufacturer's instructions.

Gelatinase Fluorimetry

Embryos were homogenized in lysis buffer (as described above) and centrifuged to remove cellular debris. DQ gelatin (Molecular Probes D12054) was added to embryo lysates per the manufacturer's instructions. Samples were read on a microplate reader (Tecan GENiosPro) after two to four hours of incubation at room temperature.

Morpholino injections

Morpholinos were obtained from Genetools. Control, *pu.1, myd88, and tr1* morpholinos were injected as previously described (*10-12*). *mmp9* morpholinos were directed to the exon 2-3 boundary (5'-GAATAATGTCCCACCTGTATGTGAC-3'), the exon 6-7 boundary (5'-GTAAGTTTACCTCTGTTAGGGCAGA-3'), and the translations start (5'-CGCCAGGACTCCAAGTCTCATTTTG-3') and used at 0.5 mM, 0.5 mM, and 0.1mM respectively. 5nL of this mixture was injected per embryo into the yolk at the one- to four-cell stage. Each of the *mmp9* morpholinos diminished gelatinase activity when used

singly or in combination, and gave similar infection phenotypes, consistent with specific knock down of Mmp9 activity.

Bacterial Supernatant Injections

Bacterial supernatants were prepared from either WT or ΔRD1 grown in an albumin-free minimal medium (Sauton's) supplemented with 0.05% Tween 80 to mid log phase, filtered through a 0.2 μm filter (Millipore), and then concentrated ~ 16-fold by centrifugation in an Amicon Ultra-15 Ultracel-3K (Millipore #UFC900324). Protein concentrations were assessed using Coomassie Plus (Pierce #232236). Similar amounts of either WT or ΔRD1 supernatant (approximately 10 nL of supernatant containing $22x10^{-15}$g of total protein) were injected at two days post fertilization. RNA was harvested at four hours post injection.

ESAT-6 and CFP-10 injections

Endotoxin free *M. tuberculosis* rESAT-6-$His_{(6)}$ and rESAT-6-$His_{(6)}$ proteins were obtained from Colorado State University under the NIH TB Research Materials Contract, and solubilized in 10mM ammonium bicarbonate. ~ 10 nL of a 500 μg/ ml solution of protein was injected into embryos at two days post fertilization.

Microscopy

Widefield microscopy was performed on a Nikon E600 equipped with DIC optics, a Nikon D-FL-E fluorescence unit with 100W Mercury lamp and MFC-1000 z-step controller (Applied Scientific Instrumentation). Objectives used included 10x Plan Fluor,

0.3 NA and 60x Oil Plan Apo, 1.4 NA. Widefield fluorescence and DIC images were captured on a CoolSnap HQ or CoolSnap CF CCD camera (Photometrics) using MetaMorph 7.1 (Molecular Devices). Confocal microscopy was performed on an Olympus Fluoview FV1000 laser scanning confocal microscope equipped with lasers capable of 405nm, 637nm, 488nm, and 561nm excitation. Objectives used were 10x UplanApo air, 0.4 NA and 20x UplanApo air, 0.75 NA.

Image Processing

Where indicated, z-stacks were deconvolved using AutoDeblur Gold CWF, Version X1.4.1 (Media Cybernetics), with default settings for blind deconvolution. Additional dataset analysis and visualization was performed using Imaris x64 6.0 (Bitplane) and MetaMorph 7.1 (Molecular Devices). Movies were produced either directly from Imaris or from stacks compiled in MetaMorph with some graphics added using ImageJ. Volumetric rendering of bacteria discerned by DIC microscopy (Fig. 3C; movies S2 and S3) were performed as follows. Stacks of DIC images taken concurrently with fluorescence stacks were scanned manually for the location of bacteria. By tracing these locations a new stack was constructed with locations of bacteria represented as maximum intensity. This stack was combined with the fluorescence channels in Imaris to render the locations of bacteria in three dimensions.

Statistical Analysis

Statistical analysis was performed with Prism (Graphpad Software) for all comparisons.

Supplemental Figures and Movie Legends

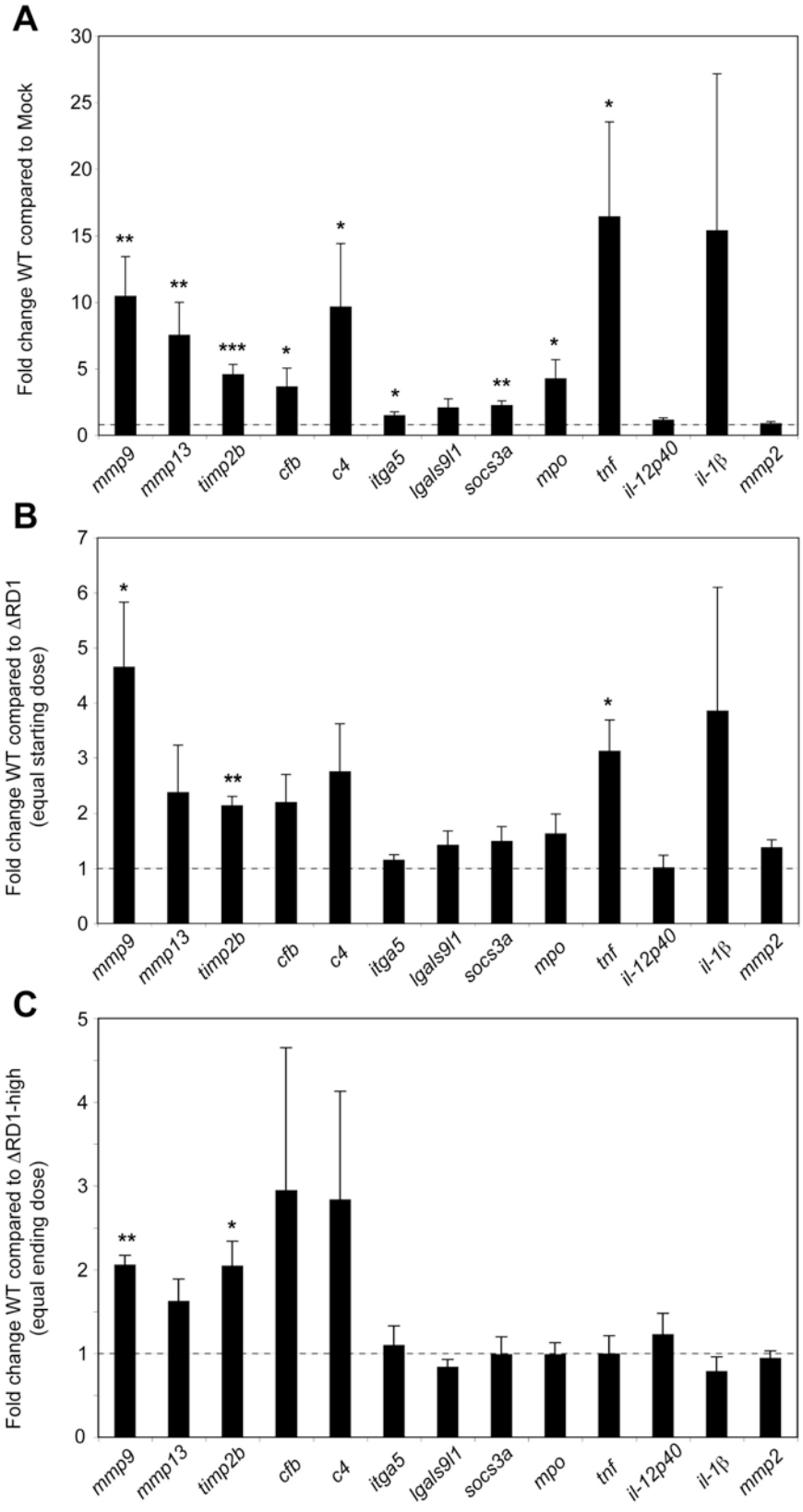

fig. S1. Immune response genes regulated by infection. (**A**) Relative gene expression levels by qRT-PCR of WT- and mock-infected embryos at 5 dpi. Experiments were performed on at least 5 biological replicates for each gene. The induction of each gene was normalized to β-actin. Bars represent means of fold changes over mock ± SEM. Infecting dose183±37. (**B**) Relative gene expression levels at 5 dpi of WT- and ΔRD1-infected embryos. Bars represent fold changes of WT-infected over ΔRD1-infected ± SEM. Similar doses of bacteria were injected (WT dose 193±36 and ΔRD1 dose 217±63) for each experiment, and data represent at least 3 biological replicates for each gene. (**C**) Relative gene expression levels of 5 dpi WT- and ΔRD1-high-infected embryos. Bars represent fold changes of WT-infected over ΔRD1-high-infected ± SEM. ΔRD1 starting bacterial doses were adjusted to achieve equal ending counts at 5 dpi as WT (WT ending count 1601±1071 and ΔRD-high ending count 1531±1011). Induction of *tumor necrosis factor* (*tnf*) and *interleukin 1b* (*il1b*) was RD1-independent, consistent with mouse studies (*13, 14*). Data represent 3 biological replicates for each gene. * $p < 0.05$, ** $p < 0.01$, ***$p < 0.005$ (Student's t-test).

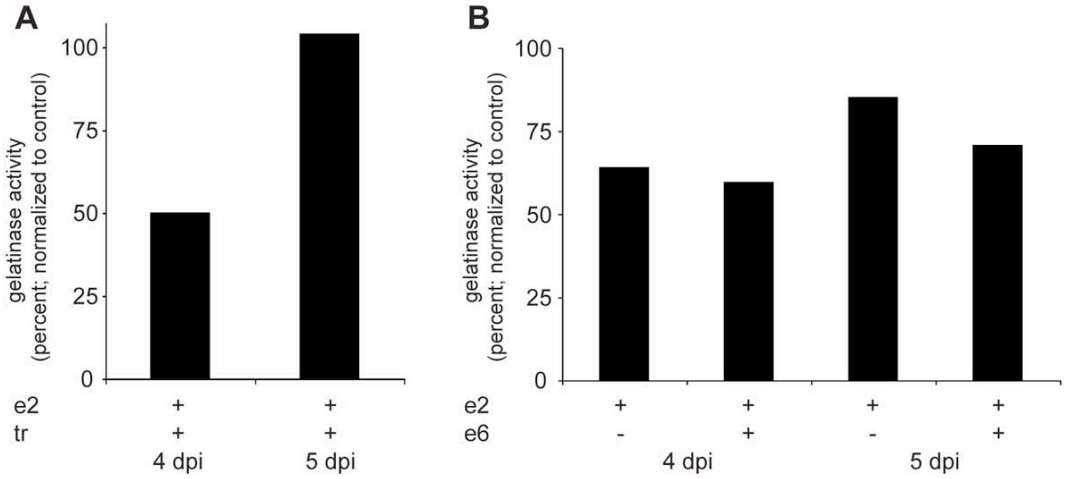

fig. S2. *mmp9* morpholinos decrease Mmp9 activity during infection. Embryos injected with *mmp9* gene specific or control morpholinos at the 2-4 cell stage were infected with WT at 30 hpf. Total gelatinase activity in whole embryos was assayed at 4 and 5 dpi. (**A**) A combination of the translation blocking (tr) morpholino, and the splice blocking morpholino directed against the second exon/intron boundary (e2) was injected prior to infection with 107±38 WT. (**B**) e2 was injected singly or in combination with another splice blocking morpholino e6, directed against the sixth exon/intron boundary prior to infection with 64±17 WT. Gelatinase activity was normalized to control morpholino injected WT-infected embryos.

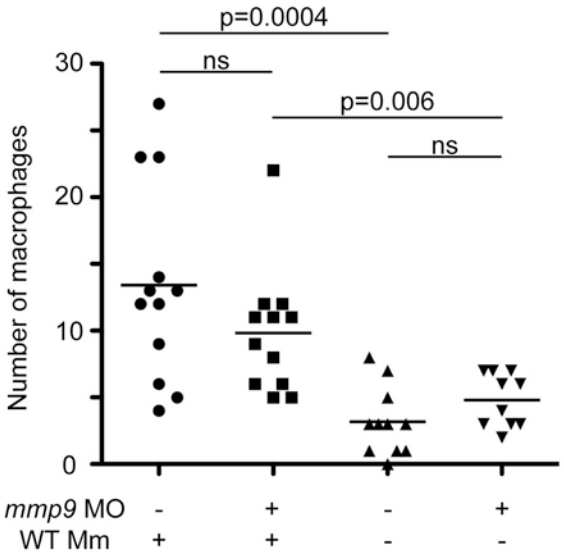

fig. S3. Mmp9 is not required for initial macrophage recruitment to bacteria. Macrophage recruitment to hindbrain ventricle assessed 4 hours after injection of WT into this cavity at 32 hpf. Control (con), and *mmp9* morphants (tr in combination with e2) (MO) showed no difference in macrophage recruitment to Mm. Bars represent means.

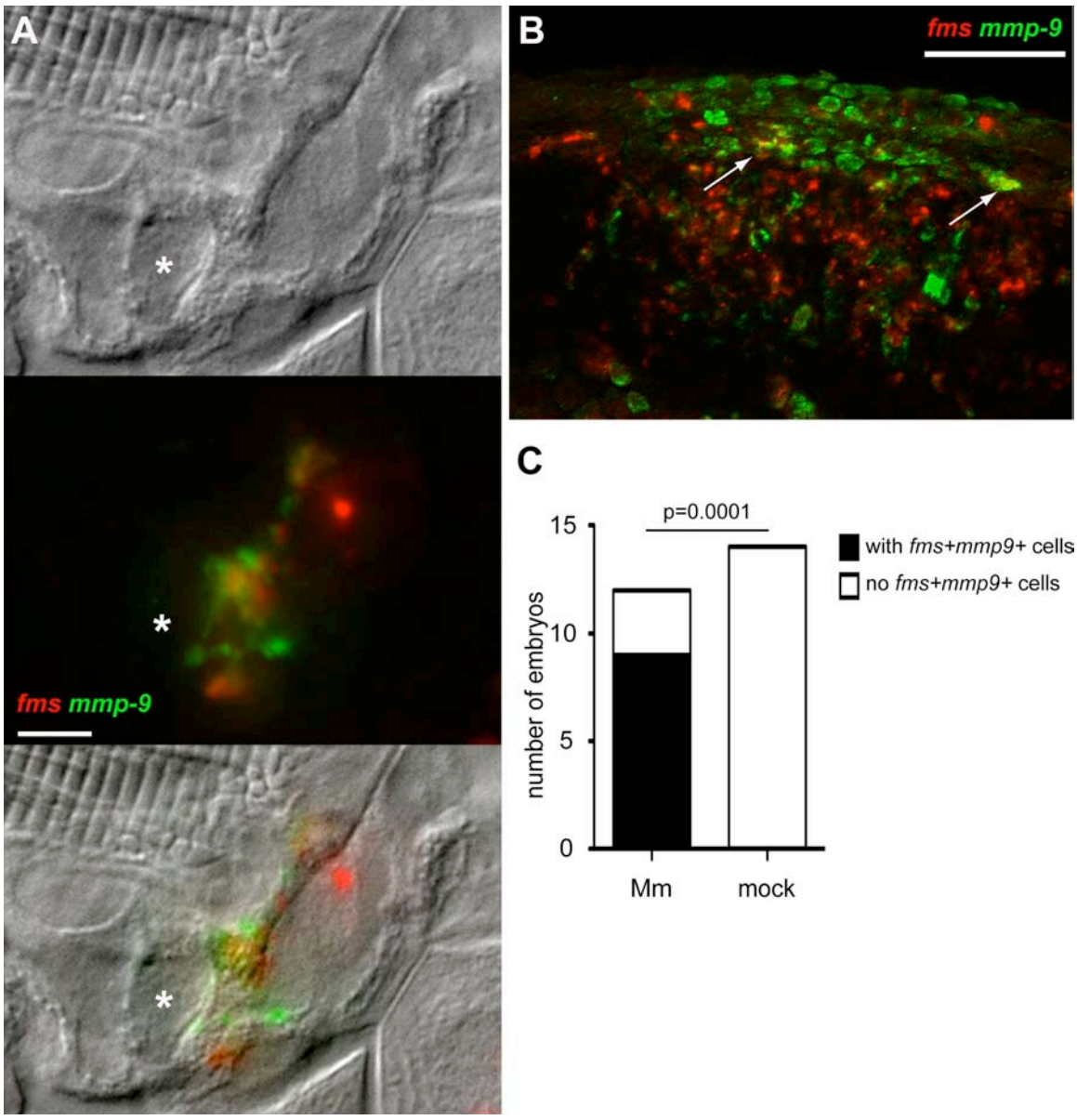

fig. S4. Infection-dependent *mmp9* induction in macrophages. *mmp9*-expressing macrophages in 5 dpi WT-and mock infected embryos were identified by dual FISH for *fms* and *mmp9* co-expression. (**A** and **B**) The majority of WT-infected embryos had some *mmp9*-expressing single macrophages. (**A**) DIC, deconvolved fluorescence, and overlay images of a single macrophage expressing *mmp9*. *, nucleus of the macrophage. Scale bar, 5μm; (**B**) Some granuloma macrophages also expressed both markers (arrows). Scale bar, 50μm. (**C**) Number of embryos with any *mmp9*-positive macrophages (*fms*+*mmp9*+ cells) in infected and uninfected embryos. p=0.0001 (Fisher's exact test of a contingency table).

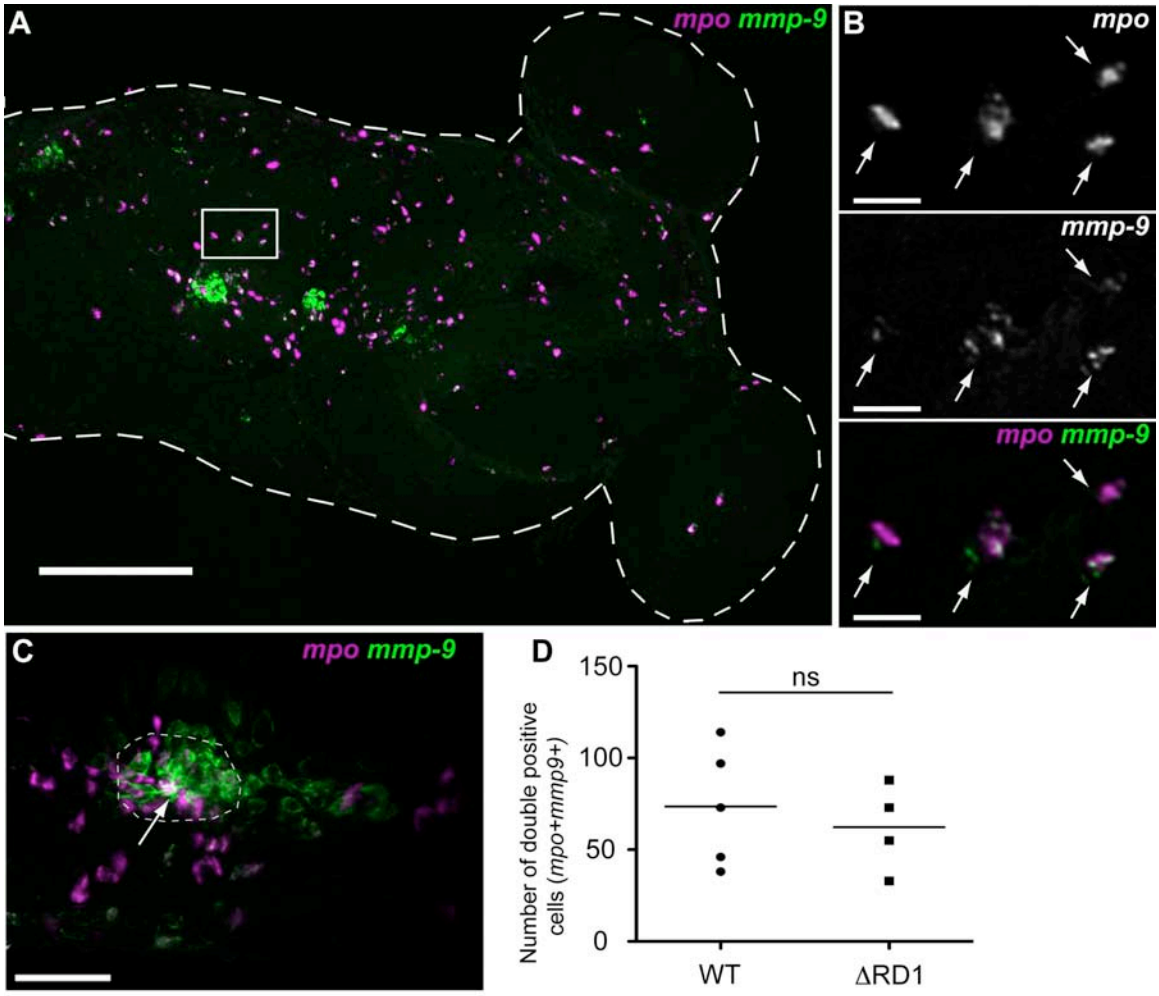

fig. S5. Infection-dependent and RD1-independent induction of *mmp9* in neutrophils. (**A**) Image of of the head region of 5 dpi-embryos after FISH with probes directed against *mmp9* (green) and the neutrophil-specific marker *mpo* (magenta). White dotted line demarcates embryo outline. Deconvolved confocal image. Scale bar, 200μm. (**B**) Higher magnification of boxed inset in (A) showing cells expressing both *mpo* and *mmp9* (arrows) Scale bar, 20μm. (**C**) area of a granuloma (demarcated by dotted white outline) showing lack of co-localization of *mmp9* and *mpo*. Arrow points to the only cell expressing both markers. Also deconvolved. Scale bar, 50μm. (**D**) Enumeration of *mpo* and *mmp9* double-positive cells (*mpo*+*mmp9*+) in the head region of WT- and ΔRD1-infected embryos at 5dpi. Bars represent means. ns, not significant (Student's t-test).

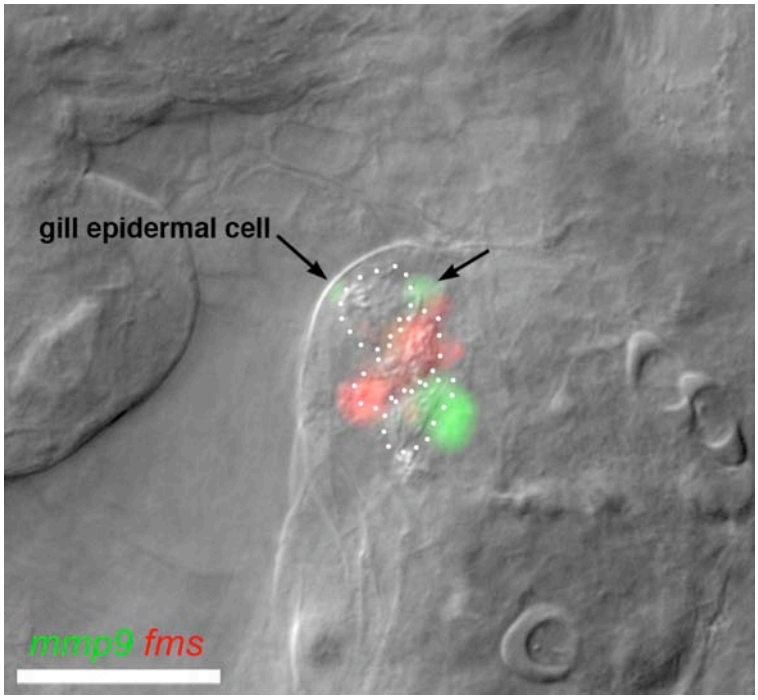

Fig. S6. Epithelial cell *mmp9* expression is associated with the earliest discernible macrophage aggregates. Fluorescence and DIC overlay image following FISH with *mmp9* (green) and *fms* (red) probes of nascent granuloma in gill at 5 dpi WT-infected embryo. Dotted white lines represent infected macrophages in which bacteria can be discerned by DIC imaging. Arrows point to proximate gill epidermal cells that have begun to express *mmp9*. Widefield fluorescence data deconvolved. Scale bar, 20µm. Also see movie S3.

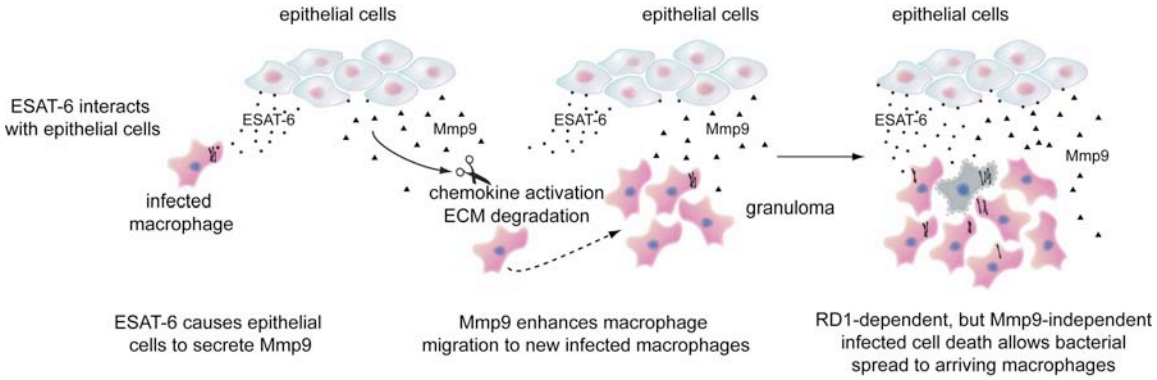

fig. S7 Model of steps of Mmp9-mediated granuloma formation depicting release of ESAT-6 (a virulence protein secreted from the RD1 locus) from infected macrophages, induction of Mmp9 in neighboring epithelial cells, which causes activation of chemokine gradients and/or extracellular matrix (ECM) degradation, resulting in migration of uninfected macrophages to the infected macrophage and their continued movement within the forming granuloma (*15*). ESAT-6 may be released from infected cells via its pore-forming activity (*16, 17*). RD1 is also required for infected cell death (*1, 15, 18*) that produces a second signal directing nearby macrophages to phagocytose dying infected macrophages (*15*), resulting in bacterial spread within the granuloma. This work shows that RD1-dependent cell death proceeds through an Mmp9-independent pathway. However, RD1-induced cell death may enhance ESAT-6 release allowing its interaction with epithelial cells.

Movie S1 3-D reconstruction of *mmp9* expression by epidermal cells adjacent to a WT Mm grauloma

Focus-through stack of multiplex fluorescence in situ hybridization (FISH) of *mmp9* (green) and *fms* (red) localization with corresponding DIC images of a WT Mm granuloma in an embryo at 5 days post infection. Non-expressing periderm cells (white outlines) overlay *mmp9*-expressing epidermal cells (black outlines). This movie is an enlargement of the same granuloma shown in Figure 3B. Relative location in the z-plane is represented by the graph in the lower left panel. Upper left, fluorescence; Upper right, DIC with cell outlines; lower left, overlay. Widefield fluorescence data deconvolved. Scale bar, 10µm.

Movie S2 3-D reconstruction of WT Mm granuloma in muscle shows *mmp9* expressed only by distant overlying epidermal cells.

Focus through and 3-D reconstruction of multiplex FISH of *mmp9* (green) and *fms* (red) localization with corresponding DIC images of a WT Mm granuloma in an embryo at 5 days post infection. This movie is an enlargement of the same granuloma shown in Figure 3C. Bacterial locations have been inferred from DIC images and pseudo-colored in blue (see Supplemental Materials and Methods). Widefield fluorescence data deconvolved. Grid lines represent 10µm.

Movie S3 3-D reconstruction of a nascent WT Mm granuloma shows *fms* negative cells already expressing *mmp9*.
Focus through and 3-D reconstruction of multiplex FISH of *mmp9* (green) and *fms* (red) localization with DIC images of a WT Mm granuloma in an embryo at 5 days post infection. This movie is an enlargement of the same granuloma shown in Figure S6. Bacterial locations have been inferred from DIC images and pseudo-colored in blue (see Supplemental Materials and Methods). Widefield fluorescence data deconvolved. Grid lines represent 10μm.

References

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Table S1: Immune genes that are differentially expressed in Mm-infected compared to mock-infected zebrafish

		Affymetrix		Compugen		qRT-PCR	Gene Induction and/or
Gene name	**Ensembl Gene ID**	**Probe ID**	**FC**	**Probe ID**	**FC**	**validation**	**Functional Role in Mammalian TB**
ECM and MMP							
matrix metalloproteinase 9 (mmp9) *	ENSDARG00000042816	Dr.967.1.S1_at	4.9	--	--	+	(*1-5*)
matrix metalloproteinase 13 (mmp13) *	ENSDARG00000012395	--	--	CGENXEB_456014688_0	2.81	+	(*1, 5-8*)
tissue inhibitor of metalloproteinase 2b (timp2b) *	ENSDARG00000075261	Dr.15281.1.A1_at	2.7	--	--	+	(*4, 9*)
Innate immune cell markers							
coronin, actin binding protein, 1A (coro1a) *	ENSDARG00000054610	--	--	CGENXEB_456001924_0	2.3	ND	(*10-12*)
myeloid-specific peroxidase (mpx/mpo) *	ENSDARG00000019521	--	--	CGENXEB_456000173_0	2.03	+	(*13-15*)
Complement							
complement C4 (c4) *	ENSDARG00000038424	Dr.12491.1.A1_at	4.3	CGENXEB_456008645_0	2.66	+	(*5, 8, 16-21*)
complement factor B (cfb) *	ENSDARG00000055278	Dr.190.1.S1_at	3.8	CGENXEB_456000406_0	2.33	+	
complement component 3a (c3a) *	ENSDARG00000012694	--	--	CGENXEB_456004596_0	2.36	ND	
complement component 3c (c3c) *	ENSDARG00000052207	--	--	CGENXEB_456000237_0	2.33	ND	
complement component 3c (c3c) *	ENSDARG00000052207	--	--	CGENXEB_456015311_0	2.27	ND	
Adhesion							
integrin alpha 5 (itga5) *	ENSDARG00000006353	Dr.22498.1.A1_at	2.7	--	--	+	(*5, 22*)
galectin 9, like 1 (lgals9l1) *	ENSDARG00000025903	Dr.25862.1.A1_at	2.2	--	--	NS	(*5, 23, 24*)
galectin 1, like 2 (lgals1l2) *	ENSDARG00000054942	Dr.13015.1.S1_at	2.1	--	--	ND	
CD82 antigen (cd82)	ENSDARG00000026070	Dr.10301.1.A1_at	2	--	--	ND	
Cytokines							
suppressor of cytokine signaling 3a (socs3a) *	ENSDARG00000025428	Dr.6431.1.S1_at	2.4	--	--	+	(25)
Coagulation/Acute Phase							
coagulation factor V (f5) *	ENSDARG00000055705	Dr.18429.1.A1_at	2.6	--	--	ND	(*26, 27*)
serum amyloid A (saa) *	ENSDARG00000045999	--	--	CGENXEB_456005085_0	2.68	ND	(*5, 8, 21, 28*)
novel pentraxin-related gene	ENSDARG00000056462	--	--	CGENXEB_456015990_0	2.48	ND	

* genes upregulated on Affymetrix or Compugen arrays during late stage tuberculosis of adult zebrafish (29)

Table S2: Differentially expressed genes identified using the Affymetrix platform

				WT/Mock comparison[a]			WT/Un comparison[b]			Mock/Un comparison[c]		
Probe ID	**Gene annotation**	**Gene symbol**	**Ensembl ID**	**Fold-change**	**P-value**	**Significant**	**Fold-change**	**P-value**	**Significant**	**Fold-change**	**P-value**	**Significant**
Dr.8591.1.A1_at	similar to hemopexin	zgc:152945	ENSDARG00000051912	**7.14**	0.0185	*	4.83	0.0164	*	0.68	0.1542	
DrAffx.2.1.S1_at	collagen 1 alpha 1	col1a1	ENSDARG00000012405	**5.16**	0.0261	*	2.11	0.0285	*	0.41	0.0274	
Dr.967.1.S1_at	matrix metalloproteinase 9	mmp9	ENSDARG00000042816	**4.88**	0.0245	*	10.68	0.0384	*	2.19	0.3314	
Dr.12491.1.A1_at	complement C4	c4	ENSDARG00000038424	**4.26**	0.0313	*	5.56	0.0304	*	1.31	0.298	
Dr.3073.1.A1_at	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 7	serpina7	ENSDARG00000035492	**3.92**	0.0405	*	2.76	0.1046		0.70	0.5082	
Dr.190.1.S1_at	complement component factor B	cfb	ENSDARG00000055278	**3.83**	0.0254	*	4.43	0.0229	*	1.16	0.3335	
Dr.22401.1.A1_at	DBH-like monooxygenase protein 1 homolog	moxd1	ENSDARG00000031136	**3.57**	0.0144	*	2.83	0.0015	*	0.79	0.3898	
Dr.22923.1.A1_at	similar to synaptophysin-like protein 2 (SYPL2)	zgc:110355	ENSDARG00000000690	**3.32**	0.0225	*	1.48	0.2297		0.45	0.0481	
Dr.6347.1.A1_at	de-etiolated homolog 1 (Arabidopsis)	det1	ENSDARG00000006145	**3.08**	0.0426	*	1.05	0.8686		0.34	0.0344	*
Dr.17591.1.S1_at	similar to complement component 1, q subcomponent-like 4	LOC100149559	---	**2.97**	0.0366	*	3.82	0.018	*	1.28	0.2229	
Dr.22498.1.A1_at	integrin, alpha 5 (fibronectin receptor, alpha polypeptide)	itga5	ENSDARG00000006353	**2.73**	0.0257	*	1.38	0.2097		0.51	0.1798	
Dr.15281.1.A1_at	tissue inhibitor of metalloproteinase 2b	timp2b	ENSDARG00000075261	**2.73**	0.0452	*	2.89	0.0195	*	1.06	0.8471	
Dr.18429.1.A1_at	coagulation factor V	f5	ENSDARG00000055705	**2.60**	0.0235	*	2.94	0.0028	*	1.13	0.672	
Dr.17437.1.S1_at	similar to C1q-like protein	zgc:136272	ENSDARG00000023157	**2.54**	0.037	*	2.25	0.1793		0.89	0.7754	
Dr.14949.1.A1_at	similar to T28C6.9	LOC798149	---	**2.49**	0.0049	*	2.34	0.0805		0.94	0.8601	
Dr.17438.1.S1_at	similar to TRAF2 binding protein (TIFA)	LOC560548	ENSDARG00000022134	**2.46**	0.0033	*	3.11	0.0144	*	1.26	0.2223	
Dr.6431.1.S1_at	suppressor of cytokine signaling 3a	socs3a	ENSDARG00000025428	**2.36**	0.0224	*	2.74	0.0156	*	1.16	0.5108	
Dr.18445.1.S1_at	coiled-coil domain containing 58	ccdc58	ENSDARG00000045351	**2.35**	0.027	*	1.20	0.3654		0.51	0.2194	
Dr.14272.1.A1_at	similar to tripartite motif-containing protein 16 (TRIM16)	zgc:153258	ENSDARG00000076839	**2.34**	0.0246	*	1.25	0.3869		0.54	0.0617	
Dr.1077.1.S1_at	similar to cystatin/stefin	zgc:153129	ENSDARG00000045980	**2.26**	0.0081	*	1.51	0.28		0.67	0.3581	
Dr.422.1.A1_at	unknown	wu:fb74b10	---	**2.26**	0.0494	*	1.40	0.2041		0.62	0.0345	
Dr.25862.1.A1_at	lectin, galactoside-binding, soluble, 9 (galectin 9)-like 1	lgals9l1	ENSDARG00000025903	**2.24**	0.0341	*	2.05	0.0353	*	0.92	0.0208	
Dr.5656.1.A1_at	thioredoxin-related transmembrane protein 2	tmx2	ENSDARG00000007786	**2.20**	0.0123	*	2.07	0.0179	*	0.94	0.1358	
Dr.25858.1.A1_at	unknown	wu:fc11a05	---	**2.15**	0.0317	*	1.27	0.2193		0.59	0.0561	
Dr.25719.1.A1_at	similar to serologically defined colon cancer antigen 8 (SDCCAG8)	B8JK76_DANRE	ENSDARG00000078947	**2.14**	0.0214	*	1.20	0.5371		0.56	0.2236	
Dr.20067.2.A1_at	calreticulin like	calrl	ENSDARG00000020103	**2.14**	0.0444	*	2.00	0.0489	*	0.94	0.1784	
Dr.18825.1.S1_at	chemokine CXL-C24a	LOC796252	ENSDARG00000074870	**2.14**	0.0258	*	2.53	0.003	*	1.18	0.4116	
Dr.5816.1.A1_at	inhibitor of growth family, member 2	ing2	ENSDARG00000013042	**2.12**	0.0382	*	1.27	0.2354		0.60	0.0834	
Dr.1259.1.A1_at	similar to peptidyl-glycine alpha-amidating monooxygenase (PAM) precursor	LOC100148119	ENSDARG00000042071	**2.11**	0.0407	*	2.20	0.042	*	1.04	0.6499	
Dr.4306.1.A1_at	similar to caseinolytic protease X	zgc:92303	ENSDARG00000029063	**2.11**	0.0485	*	2.03	0.0513		0.96	0.8099	
Dr.18470.1.A1_at	poliovirus receptor-related protein 3 precursor (nectin-3)	zgc:113035	ENSDARG00000006604	**2.08**	0.039	*	1.37	0.0117		0.66	0.2039	
Dr.13015.1.S1_at	lectin, galactoside-binding, soluble, 1 (galectin 1)-like 2	lgals1l2	ENSDARG00000054942	**2.06**	0.003	*	1.93	0.0342		0.94	0.6881	
Dr.10301.1.A1_at	CD82 antigen	cd82	ENSDARG00000026070	**2.03**	0.0431	*	1.73	0.0697		0.85	0.0359	
Dr.1368.4.A1_at	unknown	--	---	**2.02**	0.0345	*	1.96	0.0672		0.97	0.7213	
Dr.6259.1.S1_at	nucleobindin 2b	nucb2b	ENSDARG00000036291	**2.02**	0.0447	*	1.50	0.0583		0.74	0.2123	
Dr.10242.1.S1_at	protein inhibitor of activated STAT, 4 -like	pias4l	ENSDARG00000042215	**0.49**	0.0425	*	0.64	0.0234		1.31	0.1098	
Dr.7530.1.A1_at	similar to SAC3 domain-containing protein 1 (SAC3D1)	wu:fd60e07	ENSDARG00000071271	**0.48**	0.0133	*	0.55	0.0673		1.13	0.4209	
Dr.23670.1.A1_at	unknown	--	---	**0.48**	0.0144	*	0.52	0.1113		1.08	0.7694	
Dr.21021.1.S1_at	dopamine beta hydroxylase	dbh	ENSDARG00000069446	**0.48**	0.0465	*	0.64	0.2552		1.35	0.1979	
Dr.4485.3.A1_x_at	unknown	--	---	**0.45**	0.0428	*	0.49	0.1528		1.10	0.4937	
Dr.437.1.A1_x_at	unknown	wu:fb97g08	---	**0.42**	0.0336	*	0.60	0.158		1.42	0.2786	

[a] Comparison of 5dpi zebrafish infected with wild-type Mm versus mock infected zebrafish.
[b] Comparison of 5dpi zebrafish infected with wild-type Mm versus uninfected zebrafish.
[c] Comparison of 5dpi mock-infected zebrafish versus uninfected zebrafish.

Table S3: Differentially expressed genes identified using the Compugen platform

					WT/Mock comparison[b]	
Probe ID	**GenBank ID[a]**	**Gene annotation**	**Gene symbol**	**Ensembl ID**	**Fold-change**	**P-value**
CGENXEB_456014688_0	AW420822	matrix metalloproteinase 13	mmp13	ENSDARG00000012395	**2.81**	0.03305
CGENXEB_456005475_0	BE557057	similar to cornifelin	--	---	**2.76**	0.00526
CGENXEB_456005085_0	BI883516	serum amyloid A	saa	ENSDARG00000045999	**2.68**	0.03946
CGENXEB_456008645_0	BI672168	complement C4	c4	ENSDARG00000038424	**2.66**	0.02899
CGENXEB_456008142_0	AI943154	proteasome 26S subunit, ATPase, 6	psmc6	ENSDARG00000037038	**2.54**	0.03437
CGENXEB_456001154_0	AL591172	unknown	si:busm1-48c11.5	---	**2.51**	0.01677
CGENXEB_456015990_0	BM103840	novel pentraxin-related gene	si:ch211-234p6.13	ENSDARG00000056462	**2.48**	0.00003
CGENXEB_456004596_0	AW116558	complement component 3a	c3a	ENSDARG00000012694	**2.36**	0.00650
CGENXEB_456000237_0	AF047415	complement component 3c	c3c	ENSDARG00000052207	**2.33**	0.00032
CGENXEB_456000406_0	U34662	complement component factor B	cfb	ENSDARG00000055278	**2.33**	0.00041
CGENXEB_456001924_0	BM095940	coronin, actin binding protein, 1A	coro1a	ENSDARG00000054610	**2.30**	0.00554
CGENXEB_456015311_0	AI722510	complement component 3c	c3c	ENSDARG00000052207	**2.27**	0.00278
CGENXEB_456013868_0	AW076614	unknown	--	---	**2.09**	0.00801
CGENXEB_456004722_0	AW117001	scinderin like b	scinlb	ENSDARG00000058348	**2.09**	0.02431
CGENXEB_456013886_0	AW232713	unknown	wu:fj24h11	---	**2.05**	0.00603
CGENXEB_456000173_0	AF349034	myeloid-specific peroxidase	mpx	ENSDARG00000019521	**2.03**	0.00038
CGENXEB_456000113_0	U31079	heat shock protein 47	hsp47	ENSDARG00000019949	**0.50**	0.00334

[a] GenBank IDs selected by Compugen for probe design.
[b] Comparison of 5dpi zebrafish infected with wild-type Mm versus mock infected.

Table S4: qRT-PCR primers used in this study

Gene	Forward Primer 5' to 3'	Reverse Primer 5' to 3'	Probe (if applicable)
b-actin	CTGAATCCCAAAGCCAACAGAGA	GCCTGGATGGCCACATACAT	CATGATCTGTGTCATCTTC
b-actin	ACCTGACAGACTACCTGATG	TGAAGGTGGTCTCATGGATAC	
c4	GGAGGTGAAGCCTGTATTGC	TGACACACGTCATCTGAGCA	
c9	CGACCGATGAGTCAGATGAA	GCTGCTCTCTTCCACCTTTG	
cfb	TTTGCCAACCTAATGGGAAG	CACTGTGGCTCTGTTCCTGA	
il-12ba (p40)	AACATCTGAGAGCTTCTGGAAACTC	GGCACTTCTACCCTATTTACACCTT	CACCACAAGACAATTTG
il-1beta	TGGACTTCGCAGCACAAAATG	GTTCACTTCACGCTCTTGGATG	
itga5	CGAGTCGCATCAGTCACACT	CCACACTGGAGCAAGACAGA	
lgals9l1	AACCCACGCTATGAGGACAC	GGATTCTGGAAGGCAACAGA	
mmp2	CCTTCCACAAGACCAAGAAGAC	AGTGCACCATCTAGGTCATCG	
mmp13	CTGGAATGACCGGGAAGGT	TGGAGCCAAACTCAAGCATCTTT	CCAGTACAGAGGTTTAAAC
mmp9	CATCACTGAAATCCAGAAGGAGCTT	GTTCACCATTGCCTGAGATCTTC	AAGGACGGGCGCTACT
mpx/mpo	TTGCCTTCACATCCCACATA	TGTTCATCACCACAGCCAAT	
socs3a	CTGAGACTGGTCCAGCACTACATG	TTTTCTCCCCTCCTGTGTAGATG	
timp2b	GAGCAATGGCAAGCAGGAATAC	GCGCAGTGTGCCATTTGAATT	CTGATCACAGGTAATTTG
tnf	TTCCAAGGCTGCCATCCATTTA	GGTCATCTCTCCAGTCTAAGGTCTT	ACAGGTGGATACAACTCT