Supplementary Information:

Figure legends S1-S7

Tables S1-S3

Supplementary Experimental Procedures

Supplementary References

Supplementary Figure Legends

Supplementary Figure 1 (related to Figure 2) Antioxidant drugs reverse LTA4H-high mediated susceptibility

(A) Mean (±SEM) number of bacteria per infected macrophage in WT and LTA4H-high larvae in presence or absence of 20 μM GSH. ***$p < 0.001$ (one way ANOVA with Tukey's post-test)

(B) FPC in WT and LTA4H-high larvae in presence or absence of 20 μM GSH. *$p < 0.05$; ***$p < 0.001$ (one-way ANOVA with Tukey's post-test). Representative of 2 independent experiments.

(C) Mean (±SEM) number of bacteria per infected macrophage in WT larvae and LTA4H-high larvae in presence or absence of 40 μM amifostine. ***$p < 0.001$ (one way ANOVA with Tukey's post-test)

(D) FPC in WT and LTA4H-high larvae in presence or absence of 40 μM amifostine. *$p < 0.05$; **$p < 0.01$ (one-way ANOVA with Tukey's post-test). Representative of 3 independent experiments.

(E) Mean (±SEM) number of bacteria per infected macrophage in WT and LTA4H-high larvae in presence or absence of 20 μM TEMPOL. ***$p < 0.001$ (one way ANOVA with Tukey's post-test).

(F) FPC in WT and LTA4H-high larvae in presence or absence of 20 μM TEMPOL. *p < 0.05; ***p < 0.001 (one-way ANOVA with Tukey's post-test). Representative of 3 independent experiments.

Supplementary Figure 2 (related to Figure 2) C-PTIO does not reverse LTA4H-high susceptibility

FPC in WT and LTA4H-high siblings in presence or absence of 50 μM C-PTIO. (Student's t-test). Representative of 2 independent experiments.

Supplementary Figure 3 (related to Figure 2) TNF-high state promotes infected macrophage cell death.

(A) Number of neutral red positive cells in 3dpf uninfected fish. Difference not significant by Student's t-test. Representative of 2 independent experiments.

(B) Number of neutral red positive cells in 2dpi fish one day post-injection with TNF or vehicle and treated or not with 40 μM NAC. ***p < 0.001 (one-way ANOVA with Tukey's post-test).

Supplementary Figure 4 (related to Figure 3) Q-VD-OPH does not reverse LTA4H-high susceptibility

FPC in WT and LTA4H-high siblings in presence or absence of 25 μM Q-VD-OPh. ***p < 0.001 (one-way ANOVA with Tukey's post-test). Representative of 2 independent experiments.

Supplementary Figure 5 (related to Figure 3) Effects of chemical and genetic RIP1 and RIP3 inhibition on LTA4H-high, LTA4H-low and WT infection.

(A) Mean (±SEM) number of bacteria per infected macrophage in WT and LTA4H-high siblings in presence or absence of 10 μM Necrostatin-5. ***$p < 0.001$ (one way ANOVA with Tukey's post-test). Representative of 2 independent experiments.

(B) FPC in WT and LTA4H-high larvae in presence or absence of 10 μM Necrostatin-5. *$p < 0.05$; **$p < 0.01$ (one-way ANOVA with Tukey's post-test). Representative of 2 independent experiments.

(C) Percentage of animals in (B) with cording among WT and LTA4H-high larvae in presence or absence of 10 μM Necrostatin-5. *$p < 0.05$ (Fisher's exact test).

(D) FPC in WT and RIP1 morphant larvae. (one-way ANOVA with Tukey's post-test). Representative of 2 independent experiments.

(E) FPC in WT and RIP3 morphant larvae. (one-way ANOVA with Tukey's post-test). Representative of 2 independent experiments.

(F) FPC in WT larvae in presence of vehicle, 10 μM Necrostatin-1 or 10 μM Necrostatin-5. (one-way ANOVA with Tukey's post-test).

(G) FPC in WT and RIP1 morphants on LTA4H-low or WT background. ***$p < 0.001$ (one-way ANOVA with Tukey's post-test).

(H) FPC in WT and RIP3 morphant siblings on LTA4H-low or WT background. ***$p < 0.001$ (one-way ANOVA with Tukey's post-test).

Supplementary Figure 6 (related to Figure 4) Effect of gp91^{phox} knockdown on LTA4H-high fish.

(A) Mean (±SEM) number of bacteria per infected macrophage in WT and gp91^{phox} morphants. ***$p < 0.001$ (one way ANOVA with Tukey's post-test).

(B) FPC in WT and gp91^{phox} morphants. ***p < 0.001 (one way ANOVA with Tukey's post-test).

(C) Survival of WT and gp91^{phox} morphants (n=30 each) infected with *Pseudomonas aeruginosa* (p=0.013; log-rank test).

Supplementary Figure 7 (linked to Figure 5)

Cartoon of mPTPC formation showing proteins reported to be involved.

VDAC, voltage-dependent anion channel; PBR, peripheral type benzodiazepine receptor; ANT, adenine nucleotide transporter.

Table S1. Chemicals (in white rows) and drugs (in blue shaded rows) used in this work.

Chemical (supplier)	(Concentrations tested) (μM)	Rationale for concentration used
N-Acetyl cysteine (NAC) (Sigma)	40 (10, 20, 40, 50)	Minimum effective concentration. No concentration toxic
Reduced glutathione (GSH) (Sigma)	20 (10, 20, 40)	Minimum effective concentration. No concentration toxic
Amifostine (Ethyol) (Sigma)	40 (20, 40)	Minimum effective concentration. No concentration toxic
TEMPOL (Aldrich)	20 (20, 40)	Minimum effective concentration. Neither concentration toxic
C-PTIO (Sigma)	50 (25, 50)	Minimum effective concentration. No concentration toxic
Q-VD-OPh (R&D Systems)	25 (5, 25, 50)	Concentrations shown to reduce apoptosis in Mm-infected zebrafish. None effective and none toxic
Necrostatin-1 (Calbiochem)	10 (0.1, 1, 10, 100)	Minimum effective concentration. Toxic at 100 μM
Necrostatin-1, inactive control (Calbiochem)	10 (0.1, 1, 10, 100)	Concentrations matched to those of necrostatin-1. None effective and Toxic at 100 μM
Necrostatin-5 (Enzo Life Sciences)	10 (1, 10)	Minimum effective concentration. No concentration toxic
Necrox-5 (Enzo Life Sciences)	10 (0.1, 1, 10)	Minimum effective concentration. No concentration toxic
Necrosulfonamide (X. Wang)	10 (1, 10, 50)	Minimum effective concentration. Toxic at 50 μM
Alisporivir (Novartis)	7.5-10 (1, 5, 10)	10 μM minimum effective concentration when used singly. Insoluble starting at 11 μM
Desipramine (Sigma)	5-7.5 (1, 5, 7.5, 10, 50)	7.5 μM minimum effective concentration when used singly. Toxic at 10 μM

Actions in mammalian systems	Reference
ROS scavenger. Antioxidant. Mucolytic agent. Treatment of acetaminophen overdose. Intravenous, oral and inhaled administration. Approved drug since 1995.	(Zafarullah et al., 2003)
Endogenous ROS scavenger with potent effects against peroxynitrites, hydrogen radical and hydrogen peroxide. Antioxidant used as health supplement.	(Zunino et al., 1989)
Pro-drug administered to patients under radiotherapy for neck and head cancer as a cytoprotector in non-neoplastic tissues. Antioxidant. Hydroxyl radical scavenger. Intravenous administration. Approved drug since 1994.	(Kouvaris et al., 2007)
Superoxide dismutase activity. Antioxidant. Radioprotective during radiotherapy treatments. Pro-drug in phase I in humans.	(Metz et al., 2004)
NO scavenger.	(Soler et al., 2001)
Broad spectrum caspase inhibitor. Inhibitor of apoptosis mediated by the three major apoptotic pathways, caspase 9/3, caspase 8/10, and caspase 12.	(Caserta et al., 2003)
Specific inhibitor of RIP1. Inhibitor of necroptosis.	(Degterev et al., 2008)
Inactive molecule related to necrostatin-1.	(Degterev et al., 2008)
Specific inhibitor of RIP1 with different structure from Necrostatin-1. Inhibitor of necroptosis.	(Degterev et al., 2008)
Inhibitor of necrosis with antioxidant activity localizing mainly to the mitochondrion. Selectively blocks oxidative stress-induced necrotic cell death.	(Kim et al., 2010)
Inhibitor of necroptosis downstream of RIP3 activation by binding MLKL to cause steric hindrance of its binding to RIP3.	(Sun et al., 2012)
Specific cyclophilin D inhibitor. Desensitizes the mitochondrial permeability transition pore after cyclophilin D activation and transition pore formation. Oral drug in Phase III in clinical trials.	(Quarato et al., 2012)
Tricyclic antidepressant acting by inhibiting norepinephrine re-uptaking. It promotes aSMase proteolytic degradation and inactivation, avoiding ceramide-promoted cell death after TNF stimulation. Oral drug approved since 1964.	(Elojeimy et al., 2006)

Table S2 (linked to Figure 7) Effects of interventions used in LTA4H/TNF-high zebrafish

Intervention	Action	Effect in LTA4H-high genothype		
		Bactericidal activity	**Bacterial burden**	**Cell death/Cording**
TNF	MO knockdown	reduced to WT	reduced to WT	reduced to WT
Necrostatin-1	RIP1 inhibitor	reduced to WT	reduced to WT	reduced to WT
Necrostatin-5	RIP1 inhibitor	reduced to WT	reduced to WT	reduced to WT
RIP1	MO knockdown	reduced to WT	reduced to WT	reduced to WT
RIP3	MO knockdown	reduced to WT	reduced to WT	reduced to WT
NSA	MLKL/RIP3 interaction blocker	ND	reduced to WT	ND
PGAM5	MO knockdown	reduced to WT	reduced to WT	reduced to WT
Necrox-5	Antioxidant	reduced to WT	reduced to WT	reduced to WT
NAC	Antioxidant	reduced to WT	reduced to WT	reduced to WT
GSH	Antioxidant	reduced to WT	reduced to WT	reduced to WT
Amifostine	Antioxidant	reduced to WT	reduced to WT	reduced to WT
TEMPOL	Antioxidant	reduced to WT	reduced to WT	reduced to WT
CYPD	MO knockdown	remains high	reduced to WT	partial rescue
Alisporivir	CYPD inhibitor	remains high	reduced to WT	partial rescue
aSMase	MO knockdown	remains high	reduced to WT	partial rescue
Desipramine	aSMase degradation	remains high	reduced to WT	ND
Acid ceramidase	RNA overexpression	remains high	reduced to WT	partial rescue
CYPD/aSMase	MO knockdown	remains high	reduced below WT	reduced to WT
CYPD/acid	MO	remains high	reduced below WT	reduced to WT

ceramidase	knockdown/RNA overexression			
Alisporivir/ Desipramine	CYPD inhibitor/aSMase degradation	remains high	reduced below WT	reduced to WT

ND; not determined. MO; morpholino

Blue shaded rows represent interventions upstream of ROS that reduce both macrophage microbicidal activity and cell necrosis to WT, thus restoring overall bacterial burdens to WT levels; green shaded rows represent individual interventions downstream of ROS that retain increased macrophage microbicidal activity but also some degree of cell necrosis so that overall bacterial burdens are reduced to WT levels but not below; red shaded rows represent combinations of interventions downstream of ROS that retain increased macrophage microbicidal capacity while removing cell necrosis altogether, thus resulting in overall bacterial burdens below WT.

Table S3. Morpholinos used for gene knockdown in zebrafish embryos

Gene*	Used name**	Accession Number	Sequence (5'->3')	MO target	Reference	MO concentration (mM)
spi1b	PU.1	NM_198062	CCTCCATTCTGTACGGATGCAGCAT GGTCTTTCTCCTTACCATGCTCTCC	atg/5'UTR e4/i4	(Clay et al., 2007)	0.375 0.025
ripk1l	RIP1	NM_001043350.1	GGCTGACCGAAGTAGACATTGTTCC	atg/5'UTR	this work	0.2
rip3kl	RIP3	XM_001343791.1	TTTTAGAAATCACCTTGGCATCCAG	e2/i2	this work	0.15
lta4h	LTA4H	NM_213286	AGCTAGGGTCTGAAACTGGAGTCAT	atg/5'UTR	(Tobin et al., 2010)	0.2
pegam5	PGAM5	NM_001007323.2	AGCGCCCTCCGAAAAGACATGCTTC	atg/5'UTR	this work	0.15
ppid	CYPD	NM_001002065.1	TTGGGTTTGACATTTTCTTAGAT	atg/5'UTR	this work	0.15
smpdl3a	aSMase	NM_001014338	ACAGAACGAATAAAGCCATTCCTGA	atg/5'UTR	this work	0.2
cybb	gp91^{phox}	NM_200414.1	CATAATCCCGATAGCTTACGATAAC	e1/i1	(Yang et al., 2012)	0.8

*Gene abbreviations follow the "Zebrafish Nomenclature Guidelines" (https://wiki.zfin.org/display/general/ZFIN+Zebrafish+Nomenclature+Guidelines)

**Name, often simplified, used in the paper

Supplementary Experimental Procedures

Zebrafish lines and infection

The *Tg(mpeg1:YFP)*^{w200} line was created by cloning YFP into the Tol2 vector that contains the zebrafish *mpeg1* promoter (Ellett et al., 2011). Tol2 construct was injected together with transposase RNA into 1-4 cell stage embryos. Transgenic lines were identified by macrophage YFP expression in the next generation. Injections were carried out as detailed in (Tobin et al., 2010). Injection inocula were determined by injecting the same volume onto selective bacteriological plates and enumerating Mm colonies. Overall in vivo bacterial burden was determined using WT Mm by measuring fluorescence pixel counts as described in (Adams et al., 2011) at 3 dpi unless otherwise specified. The assay to determine intracellular bacterial burdens was performed by counting bacteria within individual macrophages 44 hours post-infection with 150-200 red fluorescent *erp* mutant Mm as described (Tobin et al., 2012). For quantification of percentage of animals with cording/extracellular bacteria, infected larvae were analyzed at 3 dpi.

ROS detection and quantification assay

ROS accumulation was assessed by the levels of the oxidized form of the cell-permeant ROS indicator acetyl ester of 5-(and 6-) chloromethyl-2',7'-dichlorodihydrofluorescein diacetate (CM-H_2DCFDA) (Invitrogen), as described (Anichtchik et al., 2008). Larvae were infected with 250-300 far-red fluorescent Mm, treated with TNF at 16 hpi, and then incubated at 29°C for 2 hours. The larvae were either viewed under the fluorescence microscope or assessed by fluorimetry. For the latter, they were transferred to the wells of an optical bottom 96-well plate (Nunc 265301), in groups of three larvae per well and

approximately 12 groups per condition. All water was then removed and replaced with 100 µl fish water supplemented with 1 µg/ml CM-H2DCFDA, and larvae were incubated for 2 hours. Fluorimetry was then performed using a Tecan GENios Pro microplate reader equipped with 485/20 nm and 535/25 nm excitation and emission filters, respectively (Takaki et al., 2012). Wells without fish were used as a negative control for spontaneous oxidation of CM-H2DCFDA, and H_2O_2 solution served as a positive control. Gain was optimized for individual experiments.

Mitochondrial ROS and cell death detection assays

Mitochondrial ROS production and cell death was assayed by the levels of the oxidized form of the cell permeant mitochondrion-targeted ROS indicator, MitoTracker Red CM-H_2Xros (Kweon et al., 2001) and the live cell impermeant nucleic acid stain SYTOX Green (both from Invitrogen). Larvae were infected with 200-250 blue fluorescent Mm, injected with TNF or vehicle at 16 hpi, and two hours later injected with 1 nl of a mixture of 4 mM MitoTracker Red CM-H_2Xros and 1 mM SYTOX Green in DMSO. Microscopical analysis was initiated 40 minutes after injection of the dyes and continued through 7 hours.

Microscopy

To enumerate macrophage and neutrophils and bacteria within macrophages, 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 using a 10x Plan Fluor 0.3 NA objective (Applied Scientific Instrumentation). Whole animal images were obtained on a motorized Nikon inverted Ti-E microscope using a 2x Apo 0.1 NA

objective for FPC and a 40x Plan Fluor 0.75 NA objective for bacterial cording. High-resolution confocal imaging was performed using a Nikon A1RSi confocal microscope system equipped with a 20x air Plan Apo 0.75 NA or 40x water Apo LWD 1.15 WI objective. It was used to detail granuloma structure and bacterial cording and for the assays examining mitochondrial ROS production and cell death using SYTOX. For confocal microscopy, larvae were mounted in 1.5 % low melting point agarose and a series of z-stack images with 1 μm step size were taken using the galvo scanner. Images were processed using Imaris (Bitplane Scientific Software). Widefield fluorescence images were captured on a CoolSnap HQ or CoolSnap CF CCD camera (Photometrics) using Elements AR software.

RIPK1L and RIPK3L domain analysis

Human RIP1 and RIP3 and their zebrafish counterpart amino acid residue sequences (Table S1) were analyzed with LALIGN for domain comparison (http://www.ch.embnet.org/software/LALIGN_form.html)(Myers and Miller, 1988). Domain predictions were made using Motif Scan (http://myhits.isb-sib.ch/cgi-bin/motif_scan#GRAPHIC).

Statistical Analysis

Statistical analysis was performed with Prism (Graphpad Software).

Supplementary References

Anichtchik, O., Diekmann, H., Fleming, A., Roach, A., Goldsmith, P., and Rubinsztein, D.C. (2008). Loss of PINK1 function affects development and results in neurodegeneration in zebrafish. J Neurosci *28*, 8199-8207.

Caserta, T.M., Smith, A.N., Gultice, A.D., Reedy, M.A., and Brown, T.L. (2003). Q-VD-OPh, a broad spectrum caspase inhibitor with potent antiapoptotic properties. Apoptosis *8*, 345-352.

Clay, H., Davis, J., Beery, D., Huttenlocher, A., Lyons, S., and Ramakrishnan, L. (2007). Dichotomous Role of the Macrophage in Early Mycobacterium marinum Infection of the Zebrafish. Cell Host and Microbe *2*, 29-39.

Degterev, A., Hitomi, J., Germscheid, M., Ch'en, I.L., Korkina, O., Teng, X., Abbott, D., Cuny, G.D., Yuan, C., Wagner, G., *et al.* (2008). Identification of RIP1 kinase as a specific cellular target of necrostatins. Nat Chem Biol *4*, 313-321.

Ellett, F., Pase, L., Hayman, J.W., Andrianopoulos, A., and Lieschke, G.J. (2011). mpeg1 promoter transgenes direct macrophage-lineage expression in zebrafish. Blood *117*, e49-56.

Elojeimy, S., Holman, D.H., Liu, X., El-Zawahry, A., Villani, M., Cheng, J.C., Mahdy, A., Zeidan, Y., Bielwaska, A., Hannun, Y.A., *et al.* (2006). New insights on the use of desipramine as an inhibitor for acid ceramidase. FEBS Lett *580*, 4751-4756.

Kim, H.J., Koo, S.Y., Ahn, B.H., Park, O., Park, D.H., Seo, D.O., Won, J.H., Yim, H.J., Kwak, H.S., Park, H.S., *et al.* (2010). NecroX as a novel class of mitochondrial reactive oxygen species and ONOO(-) scavenger. Arch Pharm Res *33*, 1813-1823.

Kouvaris, J.R., Kouloulias, V.E., and Vlahos, L.J. (2007). Amifostine: the first selective-target and broad-spectrum radioprotector. Oncologist *12*, 738-747.

Kweon, S.M., Kim, H.J., Lee, Z.W., Kim, S.J., Kim, S.I., Paik, S.G., and Ha, K.S. (2001). Real-time measurement of intracellular reactive oxygen species using Mito tracker orange (CMH2TMRos). Biosci Rep *21*, 341-352.

Metz, J.M., Smith, D., Mick, R., Lustig, R., Mitchell, J., Cherakuri, M., Glatstein, E., and Hahn, S.M. (2004). A phase I study of topical Tempol for the prevention of alopecia induced by whole brain radiotherapy. Clin Cancer Res *10*, 6411-6417.

Myers, E.W., and Miller, W. (1988). Optimal alignments in linear space. Comput Appl Biosci *4*, 11-17.

Quarato, G., D'Aprile, A., Gavillet, B., Vuagniaux, G., Moradpour, D., Capitanio, N., and Piccoli, C. (2012). The cyclophilin inhibitor alisporivir prevents hepatitis C virus-mediated mitochondrial dysfunction. Hepatology *55*, 1333-1343.

Soler, M., Camacho, M., Molins-Pujol, A.M., and Vila, L. (2001). Effect of an imidazolineoxyl nitric oxide on prostaglandin synthesis in experimental shock: possible role of nitrogen dioxide in prostacyclin synthase inactivation. J Infect Dis *183*, 105-112.

Sun, L., Wang, H., Wang, Z., He, S., Chen, S., Liao, D., Wang, L., Yan, J., Liu, W., Lei, X., *et al.* (2012). Mixed lineage kinase domain-like protein mediates necrosis signaling downstream of RIP3 kinase. Cell *148*, 213-227.

Takaki, K., Cosma, C.L., Troll, M.A., and Ramakrishnan, L. (2012). An in vivo platform for rapid high-throughput antitubercular drug discovery. Cell Rep *2*, 175-184.

Tobin, D.M., Roca, F.J., Oh, S.F., McFarland, R., Vickery, T.W., Ray, J.P., Ko, D.C., Zou, Y., Bang, N.D., Chau, T.T., *et al.* (2012). Host genotype-specific therapies can optimize the inflammatory response to mycobacterial infections. Cell *148*, 434-446.

Tobin, D.M., Vary, J.C., Jr., Ray, J.P., Walsh, G.S., Dunstan, S.J., Bang, N.D., Hagge, D.A., Khadge, S., King, M.C., Hawn, T.R., *et al.* (2010). The lta4h locus modulates susceptibility to mycobacterial infection in zebrafish and humans. Cell *140*, 717-730.

Yang, C.T., Cambier, C.J., Davis, J.M., Hall, C.J., Crosier, P.S., and Ramakrishnan, L. (2012). Neutrophils exert protection in the early tuberculous granuloma by oxidative killing of mycobacteria phagocytosed from infected macrophages. Cell Host Microbe *12*, 301-312.

Zafarullah, M., Li, W.Q., Sylvester, J., and Ahmad, M. (2003). Molecular mechanisms of N-acetylcysteine actions. Cell Mol Life Sci *60*, 6-20.

Zunino, F., Pratesi, G., Micheloni, A., Cavalletti, E., Sala, F., and Tofanetti, O. (1989). Protective effect of reduced glutathione against cisplatin-induced renal and systemic toxicity and its influence on the therapeutic activity of the antitumor drug. Chem Biol Interact *70*, 89-101.