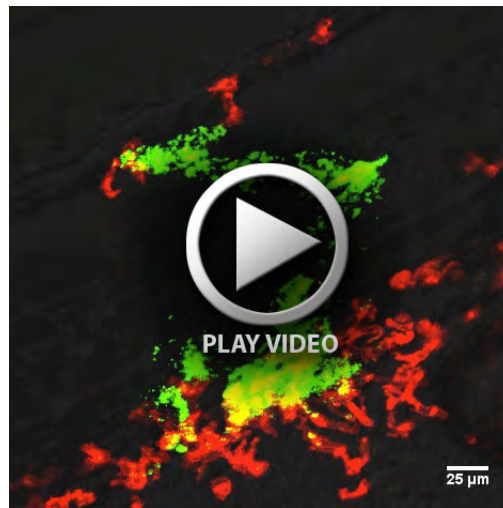
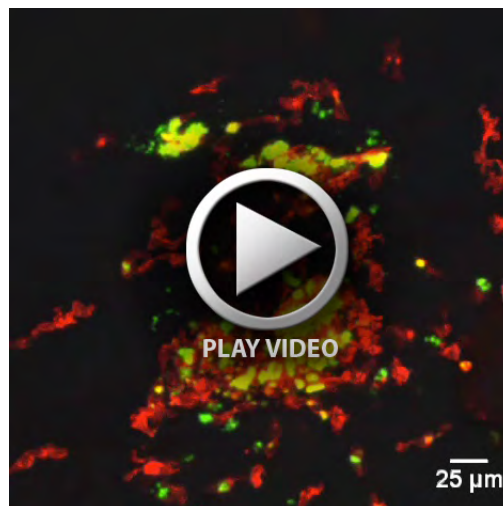


**Supplementary Movie 1.** Random patrolling of macrophages in a *Tg(mpeg1:Gal4-VP16/UAS-E1b:Kaede)* zebrafish larva. See Fig. 2A for details. Scale bar reported in the first movie stack (200  $\mu\text{m}$ ).



**Supplementary Movie 2.** Phagocytosis of GFP-labeled *M. marinum* in *Tg(mpeg1:mCherry-F)* macrophage reporter line. See Fig. 2B for details. Note that bacteria are also clustered in non-labeled cells, most likely neutrophils. Scale bar reported in the first movie stack (25  $\mu\text{m}$ ).



**Supplementary Movie 3.** Dissemination of GFP-labeled *M. marinum* by macrophages in *Tg(mpeg1:mCherry-F)* reporter line. See Fig. 2C for details. As in supplementary material Movie 1, note that bacteria are also clustered in non-labeled cells, most likely neutrophils. Scale bar reported in the first movie stack (25  $\mu\text{m}$ ).

## Supplementary material Table S1. Zebrafish fluorescent reporter lines for macrophages.

Marker	Transgenic line	Specificity	Promoter sequence	Reference
<b>apoeb</b> apolipoprotein Eb	<i>Tg(apoeb:lynEGFP)</i>	Microglial cells	Obtained from a BAC clone containing the <i>apoeb</i> locus, by BAC recombineering at the translation start	Peri and Nüsslein-Volhard, 2008
<b>coro1a</b> coronin, actin binding protein, 1A	<i>Tg(coro1a:EGFP)</i>	Macrophages, neutrophils and thymocytes <sup>(1)</sup>	-7.03-kb of <i>coro1a</i> upstream the translation start	Li et al., 2012
<b>csf1ra/fms</b> colony stimulating factor 1 receptor, a	<i>TgBAC(csf1ra:Gal4-VP16)/Tg(UAS-E1b:Eco.NfsB-mCherry)</i>	Macrophages (highly motile), and xanthophore cells (immobile)	Obtained from the BAC clone HUKGB735K06247Q containing the <i>csf1ra</i> locus, by BAC recombineering at the translation start	Gray et al., 2011
<b>fli1a</b> friend leukemia integration 1a	<i>Tg(fli1a:EGFP)</i>	Primitive macrophages (dull), endothelial cells (bright) and subsets of erythrocytes (dull)	5'UTR of <i>fli</i> was obtained from a PAC library	Lawson and Weinstein, 2002
<b>lyz/lysC</b> lysozyme	<i>Tg(lyz:EGFP)</i>	Neutrophils <sup>(2)</sup>	-11 kb of <i>lyz</i> upstream the translation start	Hall et al., 2007
	<i>Tg(lyz:DsRed2)</i>	Neutrophils <sup>(2)</sup>	-6.35 kb of <i>lyz</i> upstream the translation start	Hall et al., 2007
	<i>Tg(-4.1lyz:EGFP)</i>	Neutrophils <sup>(2)</sup>	-4.1 kb of <i>lyz</i> upstream the translation start	Zhang et al., 2008
	<i>Tg(-2.4lyz:EGFP)</i>	Neutrophils and primitive macrophages <sup>(3)</sup>	-2.4 kb of <i>lyz</i> upstream the translation start	Kitaguchi et al., 2009
	<i>Tg(lyz:Gal4-VP16)</i>	Neutrophils <sup>(2)</sup>	-11 kb of <i>lyz</i> construct as in Hall, 2007	Elks et al., 2011
<b>mhc2dab</b> major histocompatibility complex class II DAB gene	<i>Tg(mhc2dab:EGFP)</i> <i>Tg(mhc2dab:mCherry)</i>	Antigen presenting cells (APCs) <sup>(4)</sup>	-3.8 kb of <i>mhc2dab</i> upstream the transcription start	Wittamer et al., 2011
<b>mpeg1</b> macrophage expressed 1	<i>Tg(mpeg1:EGFP)</i> <i>Tg(mpeg1:mCherry)</i> <i>Tg(mpeg1:Gal4-VP16)</i> <i>Tg(mpeg1:mCherry-F)</i>	Macrophages <sup>(5)</sup>	-1.86 kb of <i>mpeg1</i> upstream the translation start	Ellett et al., 2011
	<i>Tg(mpeg1:mCherry-F)</i>	Macrophages	-1.86 kb of <i>mpeg1</i> upstream the translation start	Bernut et al., 2014
	<i>Tg(mpeg1:Dendra2)</i>	Macrophages <sup>(5)</sup>	As in Ellett et al., 2011	Harvie et al., 2013
	<i>Tg(mpeg1:YFP)</i>	Macrophages <sup>(5)</sup>	As in Ellett et al., 2011	Roca and Ramakrishnan, 2013
	<b>mpx/mpo</b> myeloid-specific peroxidase	<i>TgBAC(mpx:EGFP)</i>	Neutrophils <sup>(6)</sup>	Obtained from the BAC clone zC91B8 containing the <i>mpx</i> locus, by BAC recombineering at the translation start
	<i>Tg(mpx:GFP)</i>	Neutrophils (bright) and a subset of macrophages (dull)	-8 kb of <i>mpx</i> upstream the translation start	Mathias et al., 2006
	<i>Tg(-8mpx:mCherry)</i> <i>Tg(-8mpx:DsRed-F)</i> <i>Tg(-8 mpx:EGFP-F)</i>	Neutrophils (bright) and a subset of macrophages (dull)	As in Mathias, 2006	Yoo et al., 2010
	<i>Tg(-8mpx:Dendra2)</i>	Neutrophils (bright) and a subset of macrophages (dull)	As in Mathias, 2006	Yoo and Huttenlocher, 2011
<b>myd88</b> myeloid differentiation primary response gene (88)	<i>Tg(myd88:EGFP)</i> <i>Tg(myd88:DsRed2)</i>	Subsets of myeloid leukocytes, distal pronephric ducts and cloaca	-3.7 kb of <i>myd88</i> upstream the translation start	Hall et al., 2009
<b>ptprc/cd45</b> protein tyrosine phosphatase, receptor type, C	<i>Tg(ptprc:DsRed)</i>	Macrophages, granulocytes and T lymphocytes	-7.6 kb of <i>ptprc</i> upstream the transcription start	Bertrand et al., 2008
<b>spi1b/pu.1</b> spleen focus forming virus (SFFV) proviral integration oncogene spi1b	<i>Tg(-5.3spi1b:EGFP)</i>	Early myeloid cells <sup>(7)</sup>	-5.3 kb of <i>spi1b</i> upstream the translation start	Ward et al., 2003
	<i>Tg(-9.0spi1b:EGFP)</i>	Early myeloid cells (bright) and muscles (dull) <sup>(7)</sup>	-9 kb of <i>spi1b</i> upstream the translation start	Hsu et al., 2004
	<i>Tg(-4spi1b:Gal4/UAS:EGFP)</i>	Early myeloid cells <sup>(7)</sup>	-4 kb of <i>spi1b</i> upstream the translation start	Peri and Nüsslein-Volhard, 2008
	<i>Tg(-4spi1b:lynEGFP)</i>	Early myeloid cells <sup>(7)</sup>	As in Peri and Nüsslein-Volhard 2008	Zakrzewska et al., 2010

### Notes:

- <sup>(1)</sup> Expression in macrophages, neutrophils and thymocytes was shown for embryonic and young larval stages. Expression in myelomonocyte progenitors in head-kidneys of adults was also documented.
- <sup>(2)</sup> Discrepancies about expression of the construct in primitive macrophages reported.
- <sup>(3)</sup> Observations limited to embryonic and early larval stage. Labeling of primitive macrophages was determined based on the presence of fluorescent cells over the yolk at 26 hpf. Expression in macrophages from the second wave of primitive haemopoiesis and definitive haemopoiesis not demonstrated.
- <sup>(4)</sup> APCs are here indicated as macrophages, dendritic cells, B lymphocytes and eosinophils. Fluorescence visible from 5dpf and abundantly labeling APCs only from 12dpf; expression in keratinocytes reported in adults and juveniles.
- <sup>(5)</sup> Loss of expression after 6 dpf and in the adults reported; labeling of Langerhans dendritic cells in larvae suggested.
- <sup>(6)</sup> Existence of a set of GFP<sup>low</sup> macrophage-like cells distinguishable by confocal microscopy reported by some laboratories.
- <sup>(7)</sup> Expression documented from 12 to 30 hpf; Expression in early lymphoid cells reported for adult *Tg(-9.0spi1b:EGFP)*.

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## Supplementary material Table S2. Zebrafish reporters of subcellular compartments.

Marker	Reporter construct	Specificity	Notes	Reference
<b>rab5c</b> RAB5c, member RAS oncogene family	<i>Tg(h2afx:EGFP-rab5c)<sup>(1)</sup></i>	Early endosomes	-	Clark et al., 2011
	<i>Tg(UAS:mCherry-rab5c_S36N)<sup>(2)</sup></i>	Early endosomes	S36N substitution, dominant negative	Clark et al., 2011
	<i>Tg(UAS:mCherry-rab5c_Q81L)<sup>(2)</sup></i>	Early endosomes	Q81L substitution, constitutively active	Clark et al., 2011
<b>rab7a</b> RAB7, member RAS oncogene family a	<i>Tg(h2afx:EGFP-rab7)<sup>(1)</sup></i>	Late endosomes	-	Clark et al., 2011
	<i>Tg(UAS:mCherry-rab7_T22N)<sup>(2)</sup></i>	Late endosomes	T22N substitution, dominant negative	Clark et al., 2011
	<i>Tg(UAS:mCherry-rab7_Q67L)<sup>(2)</sup></i>	Late endosomes	Q67L substitution, constitutively active	Clark et al., 2011
<b>rab7b</b> RAB7, member RAS oncogene family b	<i>Plasmid(UAS:GFP-rab7b_T22N)<sup>(2)</sup></i>	Late endosomes	T22N substitution, dominant negative; the reporter consists of an UAS-expression plasmid; transgenic line not available	Ellis et al., 2013
<b>rab11a</b> RAB11a, member RAS oncogene family	<i>Tg(h2afx:EGFP-rab11a)<sup>(1)</sup></i>	Recycling endosomes	-	Clark et al., 2011
	<i>Tg(UAS:mCherry-rab11a_S25N)<sup>(2)</sup></i>	Recycling endosomes	S25N substitution, dominant negative	Clark et al., 2011
	<i>Tg(UAS:mCherry-rab11a_Q70L)<sup>(2)</sup></i>	Recycling endosomes	Q70L substitution, constitutively active	Clark et al., 2011
<b>lamp1</b> lysosomal-associated membrane protein 1	<i>Tg(hsp70l:lamp1-RFP)<sup>(3)</sup></i>	Lysosomes	-	Ellis et al., 2013
<b>lamp2</b> lysosomal membrane glycoprotein 2	<i>Tg(hsp70l:lamp2-EGFP)<sup>(3)</sup></i>	Lysosomes	-	Ellis et al., 2013
<b>cd63/lamp3</b> Cd63 antigen	<i>Plasmid(CMV/SP6:GFP-cd63)<sup>(4)</sup></i>	Lysosomes	The reporter consists of an expression plasmid for CMV-expression or mRNA preparation; transgenic line not available	Peri and Nüsslein-Volhard, 2008
<b>map1lc3b</b> microtubule-associated protein 1 light chain 3 beta	<i>Tg(CMV:EGFP-map1lc3b)<sup>(4)</sup></i>	Autophagosomes	-	He et al., 2009
	<i>Tg(hsp70l:RFP-Rno.Map1lc3b)<sup>(3)</sup></i>	Autophagosomes	Contains the Map1lc3b sequence from the rat ( <i>Rattus norvegicus</i> )	Ellis et al., 2013
<b>gabarapa</b> GABA(A) receptor-associated protein a	<i>Tg(CMV:EGFP-gabarapa)<sup>(4)</sup></i>	Autophagosomes	-	He et al., 2009
<b>rab32a</b> RAB32a, member RAS oncogene family	<i>Tg(4xUAS:EGFP-rab32a,myl7:EGFP)<sup>(2)</sup></i>	Vesicular trafficking	-	Ellis et al., 2013
	<i>Plasmid(UAS:GFP-rab32a_T27N)<sup>(2)</sup></i>	Vesicular trafficking	T27N substitution, dominant negative; the reporter consists of an UAS-expression plasmid; transgenic line not available	Ellis et al., 2013
<b>rab38b</b>	<i>Tg(UAS:GFP-rab38b-T23N, cmlc2:GFP)<sup>(2)</sup></i>	Vesicular trafficking	T23N substitution, dominant negative	Ellis et al., 2013

### Notes:

<sup>(1)</sup> *h2afx*: H2A histone family, member X constitutive promoter

<sup>(2)</sup> *UAS*: Upstream Activating Sequence for Gal4-dependent expression

<sup>(3)</sup> *hsp70l*: heat shock cognate 70-kd protein, like inducible promoter

<sup>(4)</sup> *CMV*: Cytomegalovirus constitutive promoter

## Supplementary table 2. References

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