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Table S1. Zebrafish transgenic and mutant lines

Genotype	Cell Lineage	Reference
was^{hu3280}	/	(Cvejic et al., 2008)
TgBAC(csf1ra:Gal4-VP16)i186	Macrophage	(Gray et al., 2011)
Tg(UAS-E1b:Eco.NfsB-mCherry)i149	/	(Gray et al., 2011)
Tg(mpx:GFP)i114	Neutrophil	(Renshaw et al., 2006)
Tg(lyz:DsRED2)nz50	Neutrophil	(Hall et al., 2007)
Tg(lyz:Gal4-VP16)i252b	Neutrophil	(Elks et al., 2012)
Tg(UAS:Kaede)rk8	/	(Hatta et al., 2006)
Tg(pU1:Gal4-UAS:eGFP)	Macrophage	(Hsu et al., 2004)

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Figure S1

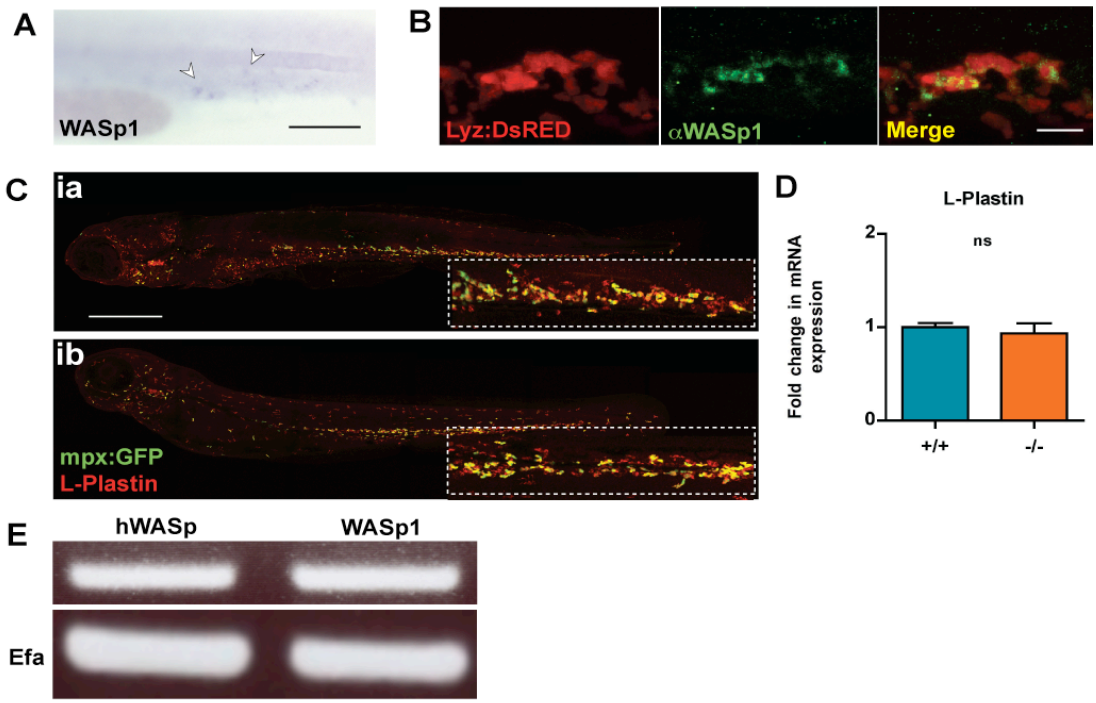

Figure S1. WASp1 is expressed in the hematopoietic lineages of Zebrafish larvae with WASp mutants showing no developmental defect in leukocyte numbers or distribution. (A) Whole-mount *in situ* hybridization for WASp1 in 1 dpf larvae, indicating expression in the early hematopoietic lineage (white arrowheads). (B) WASp1 antibody immunostaining depicting co-localisation with the transgenic neutrophil marker, lyz:DsRED in 3 dpf larvae. (C) WASp1 mutant has a normal distribution of leukocytes, as shown by L-Plastin immunostaining (red) in an mpx:GFP neutrophil transgenic line (green). (D) WASp1 mutant has a normal level of expression of the leukocyte marker L-plastin at 3 dpf, as shown by qPCR, indicating normal numbers of leukocytes. (E) RT-PCR showing transgenic expression of hWASp in "rescued" larvae is comparable to native levels of WASp1 transcript in WT larvae, with corresponding EFα control. Error bars (s.d). Scale bars: A, 100 µm; B, 10 µm; C, 200 µm.

Figure S2

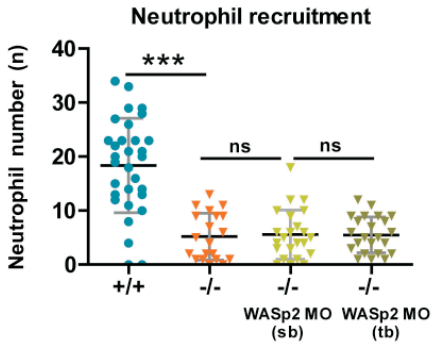

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Figure S2. WASp2 orthologue has no additional effect on WASp1 mutant neutrophil migration. Graph of neutrophil recruitment to a fin wound at 1.5 hrs post-wounding in WT, mutant, mutant with WASp2 splice block (sb) and translation block (tb) morpholino treatment. Knockdown of WASp2 does not increase the severity of the WASp1 mutant recruitment defect. Error bars (s.d); asterisks denote significance values of $p<0.001$ (***) via one way ANOVA.

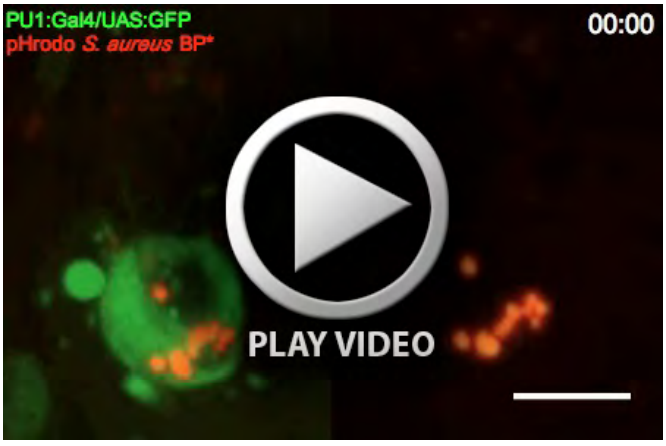
Movie 1. High-resolution protrusion analysis movies of neutrophils that are expressing various hWASp mutants as they migrate in response to wounds. Representative Protrusion Analysis of Spinning Disk Confocal movies of lyz:Gal4-VP16 UAS:Kaede neutrophils (green) from WT, mutant, $hWASp^{WT}$, $hWASp^{I294T}$, $hWASp^{H246D}$, $hWASp^{Y291F}$ "rescued" zebrafish lines. Approximate distance from, and orientation towards, the wound is the same for each cell, and the yellow arrow indicates the direction towards the wound. Newly formed protrusions (over a 10 sec period) are shown in magenta. New protrusions form almost entirely at the leading edge of wild type neutrophils (+/+), whereas in the null mutant, protrusions also frequently form at the rear of the cell (-/-), so that migration towards the wound is hindered. A similar lack of polarity is apparent in the $hWASp^{Y291F}$ expressing neutrophils which is also represented in quantification of protrusive area (Y291F). In contrast, $hWASp^{WT}$ and $hWASp^{H246D}$ protrusions appear normal (WT)(H246D), whilst $hWASp^{I294T}$ cells exhibit hyperprotrusive behaviour (I294T). Images were taken every 10 seconds; movie frame rate - 3 frames sec^{-1}. Scale bar represents 10 μm.

Movie 2. Phagocytosis of *S. aureus* by a macrophage in 2 dpf zebrafish larvae. Spinning Disk Confocal movie showing engulfment of eGFP expressing ***S. aureus*** by TgBAC(csf1ra:Gal4-VP16)^{i186}(UAS-E1b:Eco.NfsB-mCherry)^{i149} macrophage-labelled zebrafish line. In the first frame, several macrophages (red) can be seen attached to the wall of dorsal aorta; at increased magnification one of these macrophages (yellow boxed area) can be seen engulfing individual *S. aureus* (green), 10 min after injection of bacteria into the Duct of Culvier. A yellow arrow indicates when a membrane protrusion extends to engulf a microbe. Images were taken every 20 seconds, movie frame rate - 2 frames sec^{-1}. Scale bar represents 5 μm.

Movie 3. Acidification of a phagosome containing pHrodo *S. aureus* BioParticle in 2 dpf zebrafish larvae. Spinning Disk Confocal movie showing engulfment of pHrodo *S. aureus* BioParticles by Tg(pU1:Gal4-UAS:eGFP) macrophage-labelled zebrafish line. At the start of the movie a macrophage (green) phagocytoses a BioParticle (faint red), highlighted by yellow arrow, 10 minutes after injection of BioParticles into the circulation of 50 hpf zebrafish larvae. Subsequent acidification of the phagosome over the next 5 minutes is revealed by an increase in BioParticle fluorescence (brighter red). Images were taken every 10 seconds; movie frame rate - 3 frames sec^{-1}. Scale bar represents 5 μm.

Movie 1.

Movie 2.



Movie 3.