

humanSHIP1a	--MVCWNHGNITRSKAEELLSRTGKDGSFLVRASESISRAYALCVLYRNCVYTYRILPN	58
humanSHIP1b	--MVCWNHGNITRSKAEELLSRTGKDGSFLVRASESISRAYALCVLYRNCVYTYRILPN	58
zebrafishShip1	MFSPOWPWHGNITRSKAEDLSSLKAGKDGSFLLRDSESIQGAYALCVLYQNCVYTYRILPN	60
	* :*****:*****:*****:*****:*****:*****:*****:*****:*****:	
	SH2	
humanSHIP1a	EDDKFTVQASEGVSMRFFTAKLDQLIEFYKKENMGLVTHLQYPVPLEEDTGDDPEEDTVE	118
humanSHIP1b	EDDKFTVQASEGVSMRFFTAKLDQLIEFYKKENMGLVTHLQYPVPLEEDTGDDPEED-TE	117
zebrafishShip1	DDKKLSVQASEGVPIRFFSALPDVLDAYYKENMGLITHLQYAVQKEEPPEEEPEDDIIFS	120
	:*.*:*****:*****:*****:*****:*****:*****:*****:*****:*****:	
	SH2	
humanSHIP1a	SVVS-PPELPPRNIPLTASSCEAKEVPFSNENPRATETSRPSLSETLFQRLQSMDTSGLP	177
humanSHIP1b	SVVS-PPELPPRNIPLTASSCEAKEVPFSNENPRATETSRPSLSETLFQRLQSMDTSGLP	176
zebrafishShip1	SPLSLPQLPPRNIFNSDKESHNDP--SRGTDRGTEPSRPSISDTYFQRLQQIDISNLP	178
	* :* **:*****:*****:*****:*****:*****:*****:*****:*****:*****:	
humanSHIP1a	EEHLKAIQDYLSQLAQDSEFVKTGSSSLPHLKKLTTLLCKELYGEVIRTLPSLESQRL	237
humanSHIP1b	EEHLKAIQDYLSQLAQDSEFVKTGSSSLPHLKKLTTLLCKELYGEVIRTLPSLESQRL	236
zebrafishShip1	EDHQKS1QEYFRASVCLDAEQVQNGNQTLPHFKKLTONICKNLNSEARTLPSFEALQKV	238
	*: * :*:*****:*****:*****:*****:*****:*****:*****:*****:*****:	
humanSHIP1a	FDQQLSPGL-RPRPQVPGEANPINMVSKLSQLTSLLSIEDKVKALLHE--GPESPHRPS	294
humanSHIP1b	FDQQLSPGL-RPRPQVPGEANPINMVSKLSQLTSLLSIEDKVKALLHE--GPESPHRPS	293
zebrafishShip1	LDQQLSPGAGRLRTQLSADANPS-VAFRLEQLTKLQISIEDDKTKNAVFEVGYDGHRNS	297
	:*** *** * *.***:*****: .: :*.***.*:*****: .: * * .: *** *	
humanSHIP1a	LIPPVTFEVKAESLGIPQKMQLKVDVESGKLIKKSKDGSEDKFYSHKKILQLIKSQKFL	354
humanSHIP1b	LIPPVTFEVKAESLGIPQKMQLKVDVESGKLIKKSKDGSEDKFYSHKKILQLIKSQKFL	353
zebrafishShip1	LIPPVTFEVKSDSLGSNKMYLKVDEGGKVYFKKSQDGDPEDKYFVHNKILQLVKSQKMH	357
	*****:*****:*****:*****:*****:*****:*****:*****:*****:*****:	
humanSHIP1a	NKLVLVETEKEKILRKEYVFADSKKREGFCOLLOQMKNKHSEQEPDMITIFIGTWNMG	414
humanSHIP1b	NKLVLVETEKEKILRKEYVFADSKKREGFCOLLOQMKNKHSEQEPDMITIFIGTWNMG	413
zebrafishShip1	NRLVLVETEKGTQSKDFVDDTKKREGFCOLLOQMKNKHSGKPEPDMITIFVGWNMG	417
	*: * :***** * :*****:*****:*****:*****:*****:*****:*****:	
	IPPC	
humanSHIP1a	NAPPKKITSWFLSKGQGKTRDDSADYIPHDIYVIGTQEDPLSEKEWLEILKHSLOEITS	474
humanSHIP1b	NAPPKKITSWFLSKGQGKTRDDSADYIPHDIYVIGTQEDPLSEKEWLEILKHSLOEITS	473
zebrafishShip1	NANPPQNITSWFQSKGQGKTHDDTANQIPHDIYVIGTQEDPLGEKEWIETIRGALDITN	477
	** :* :*****:*****:*****:*****:*****:*****:*****:*****:*****:	
	IPPC	
humanSHIP1a	VTFKTVAIHTLWNIRIVVLAKPEHENRISHICTDNVKTGIANTLGNKGAVGVSMFNGTS	534
humanSHIP1b	VTFKTVAIHTLWNIRIVVLAKPEHENRISHICTDNVKTGIANTLGNKGAVGVSMFNGTS	533
zebrafishShip1	ISFKQIATQTLWSIRIVVLAKPEHENRFSHIFSDSVKTGIANALGNKGAVGVSMFNRTS	537
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	IPPC	
humanSHIP1a	LGFVNSHLTSGSEKKLRRNQNYMNILRFLALGDKKLSPFNITHRFTLFWFGDLNRYRVDL	594
humanSHIP1b	LGFVNSHLTSGSEKKLRRNQNYMNILRFLALGDKKLSPFNITHRFTLFWFGDLNRYRVDL	593
zebrafishShip1	FGFVNSHLTSGSEKKLRRNQNYVSLRFLNLGDKKVNPFDITHRFTLFWLGDLNRYRVDL	597
	:*****:*****:*****:*****:*****:*****:*****:*****:*****:	
	IPPC	
humanSHIP1a	PTWEAETIIQKIKQQYADLSSHDQLLTERREQKVFLHFEEEEITFAPTYRFERLTRDKY	654
humanSHIP1b	PTWEAETIIQKIKQQYADLSSHDQLLTERREQKVFLHFEEEEITFAPTYRFERLTRDKY	653
zebrafishShip1	PSHEAENIVMKIKQQYKELLAQDQLKIEKDEEKVFLVEEEITFAPTYRFERDTRERY	657
	*: ***.*:*****:*****:*****:*****:*****:*****:*****:*****:*****:	
	IPPC	
humanSHIP1a	AYTKQKATGMKYNLPSWCDRVWLWKSYPYLHVVCQSYGSTSDIMTSDHSPVFATFEAGVTS	714
humanSHIP1b	AYTKQKATGMKYNLPSWCDRVWLWKSYPYLHVVCQSYGSTSDIMTSDHSPVFATFEAGVTS	713
zebrafishShip1	AYTKAKATGTKNLPSWCDRVLRKSYPYLHVVCNSYGCNTDIMTSDHSPVFATFDVGVTS	717
	****:*****:*****:*****:*****:*****:*****:*****:*****:*****:	
	IPPC	
humanSHIP1a	QFVSKNGPGTVDSQGQIEFLRCYATLTKTSQTKFYLEFHSSCLESFVKSQEGENESEG	774
humanSHIP1b	QFVSKNGPGTVDSQGQIEFLRCYATLTKTSQTKFYLEFHSSCLESFVKSQEGENESEG	773
zebrafishShip1	QFVSKND-LSNDARGAIKILNCVAVLCTSKTKFFIEYHSSCLEKFVRSPDGENQE-MEG	775
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	IPPC	
humanSHIP1a	ELVVKFGETPLKLPKPIISDPYEYLLDQHILISIKSSDSDESYGEGCIALR-LEATETQLPI	833
humanSHIP1b	ELVVKFGETPLKLPKPIISDPYEYLLDQHILISIKSSDSDESYGEGCIALR-LEATETQLPI	832
zebrafishShip1	SIKVRGEMTQLTPPIADPEYLLDQHILICIKSTDSDESYGEVGVALRSAESSYTEFSI	835
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	IPPC	
humanSHIP1a	YTPLTHHGE LTGHFQGEIKLQTSQGK TREKLYDFVKTERDESSGP KTLKSLTSHDPMKQW	893
humanSHIP1b	YTPLTHHGE LTGHFQGEIKLQTSQGK TREKLYDFVKTERDESSGP KTLKSLTSHDPMKQW	892
zebrafishShip1	K--LTHHGERTGWLTAQOLPKSEGKQTEKLYDFIKVGDDPGAG---KGKTCM-----	884
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Fig. S1. Amino acid alignment of human SHIP1a, SHIP1b and zebrafish SHIP1. Zebrafish SHIP1 shows 61% overall identity to human SHIP1a and SHIP1b. The highest level of homology is seen within the conserved domains for SH2 (src homology 2) and IPPc (inositol polyphosphate 5-phosphatase catalytic domain).

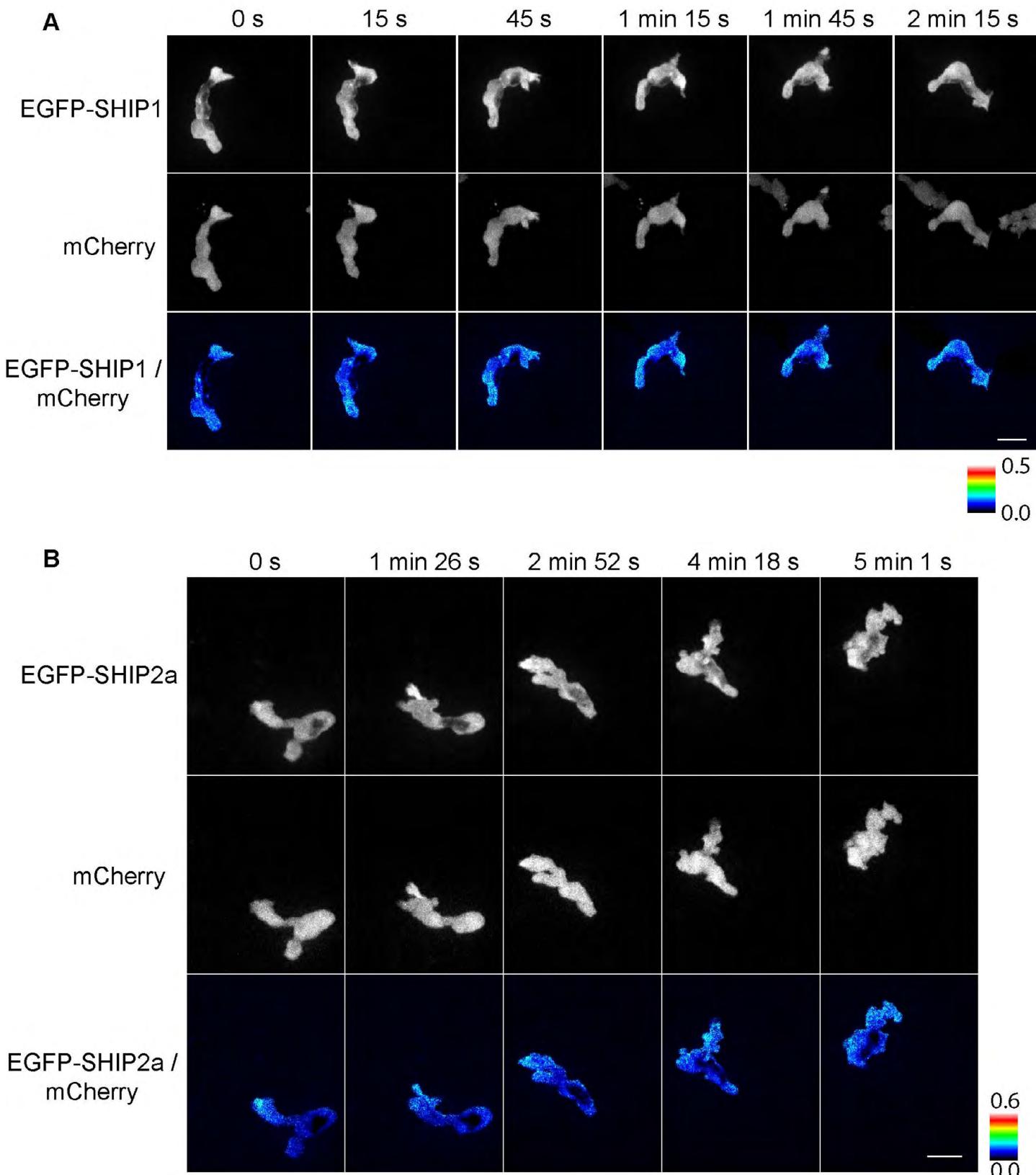


Fig. S2. Localization of zebrafish SHIP1 and SHIP2a in vivo. Time-lapse ratiometric imaging of zebrafish EGFP-SHIP1/mCherry (A) and zebrafish EGFP-SHIP2a/mCherry (B) using *lyz:EGFP-SHIP1* or *lyz:EGFP-SHIP2a*, respectively, injected into *Tg(mpox:mCherry)* embryos. Both zebrafish SHIP1 and SHIP2a show intermittent localization at the front of motile neutrophils. Images are acquired using a NA1.3 /60x water immersion objective on a spinning disk confocal microscope equipped with a Photometrics Evolve EMCCD camera (detail in Material and Methods). Representative images are presented as maximum intensity projections. The numerical value of ratiometric analysis is shown in the scale. Scale bar, 10 μ m.

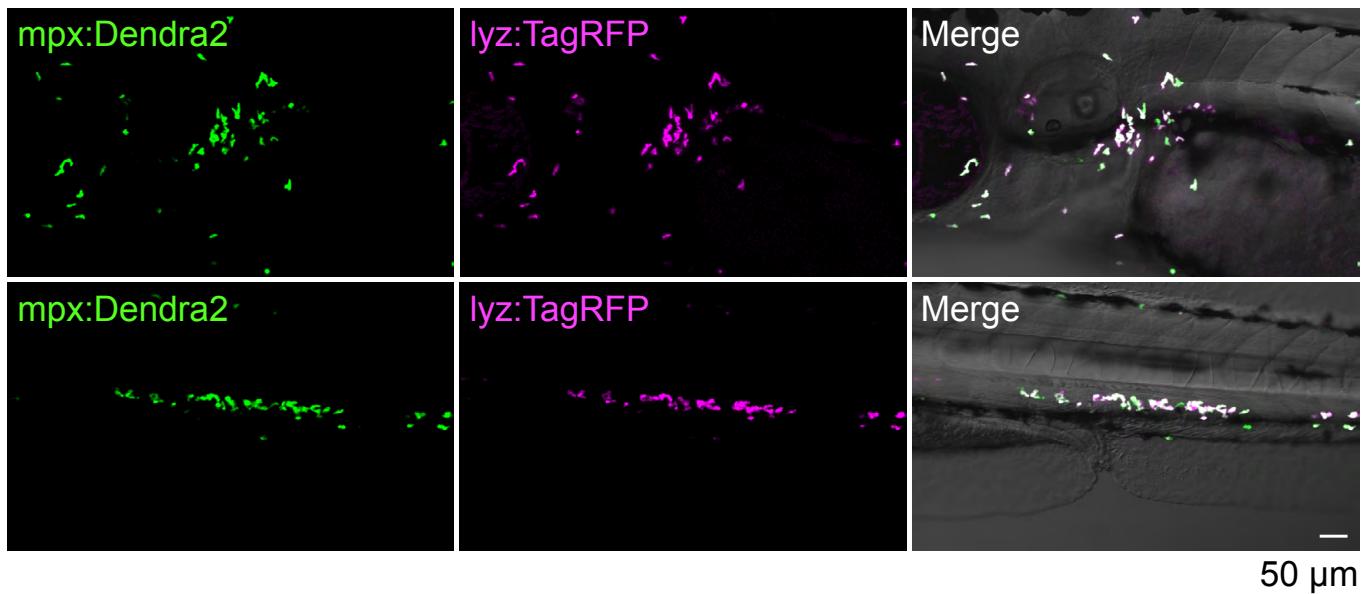


Fig. S3. Overlapping expression of mpx:Dendra2 and lyz:TagRFP expression in zebrafish neutrophils. Confocal images showing Dendra2 expressing neutrophils (left panel –green expressing cells), TagRFP expressing neutrophil (middle panel –magenta expressing cells) and overlaid image of dendra2, TagRFP signal along with the corresponding DIC image. The *mpx⁺/lyz⁺* positive cells appear white in the merged panel. Two representative embryos were selected to show neutrophils in the head (top panel) and in the caudal hematopoietic tissue (CHT) (bottom panel) at 3 dpf.

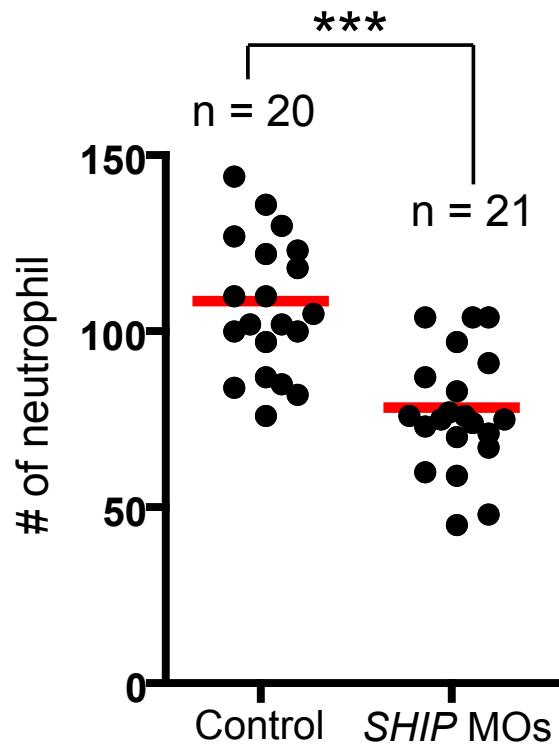


Fig. S4. Reduced neutrophils in *SHIP* morphants. Zebrafish embryos were injected with either control or *SHIP1* (MO1) and *SHIP2a* (MO2) MO. Embryos were fixed at 2.5 dpf and Sudan Black staining was performed to facilitate counting of total neutrophil numbers. n = number of embryo counted. ***p<0.001 (two-tailed, unpaired t test).

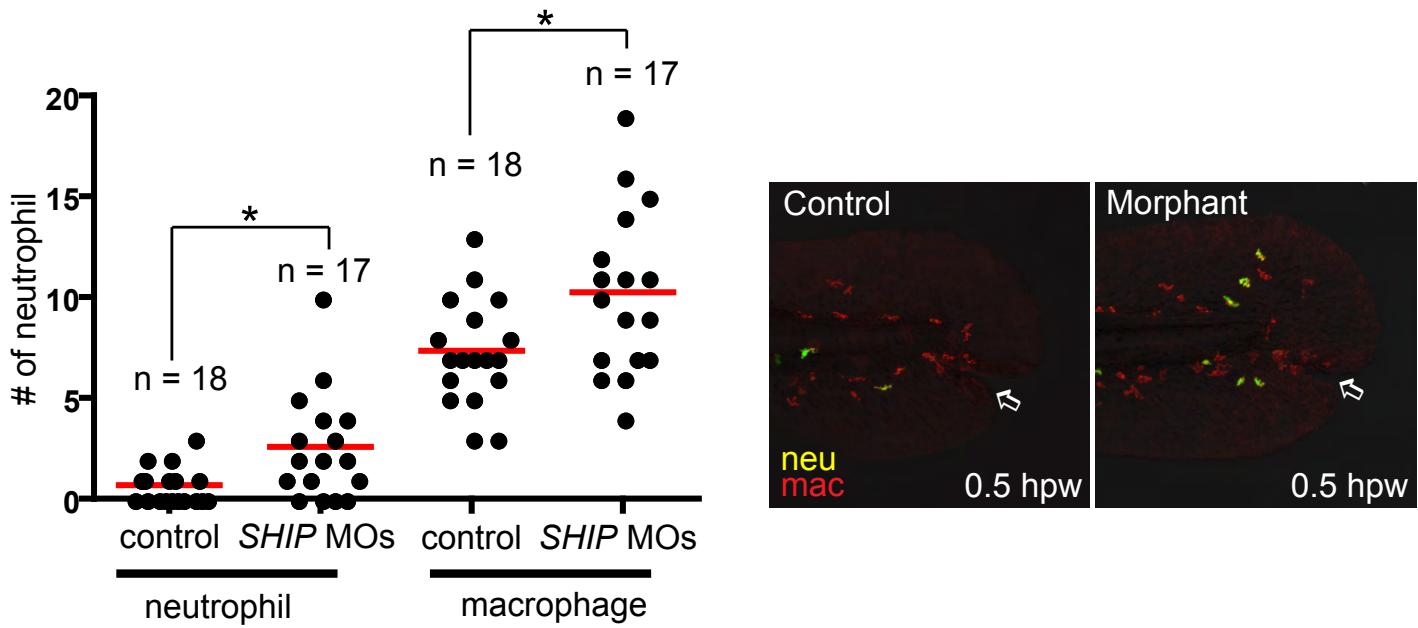


Fig. S5. Increased macrophage wound recruitment in *SHIP* morphants. Quantification of macrophage numbers recruited to tail fin wounds in combined morpholino knockdown of *SHIP1* (MO1) and *SHIP2a* (MO2) at 0.5 hpw. Confocal imaging at wounds in control or *SHIP* MOs injected *Tg(mpz: dendra2; green)* embryos at 2.5 dpf immunolabeled with antibody to L-plastin (red) that labels all leukocytes. Overlapping signals are yellow indicating MPX⁺, L-plastin⁺ neutrophils while red indicates MPX⁻, L-plastin⁺ macrophages. Open arrow indicates location of the wound. n = number of embryo wounded and counted. * <0.05 (two-tailed, unpaired t test).