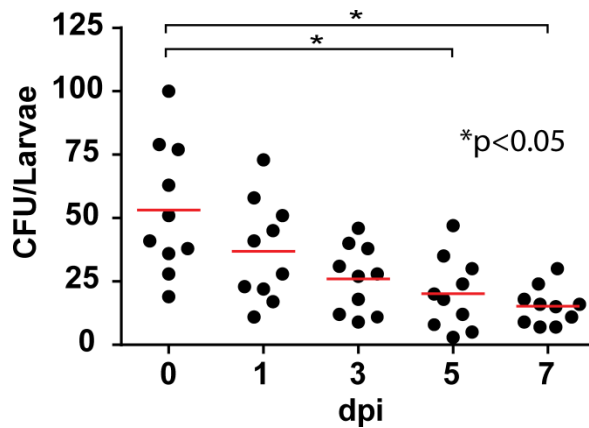


## Supplemental Information

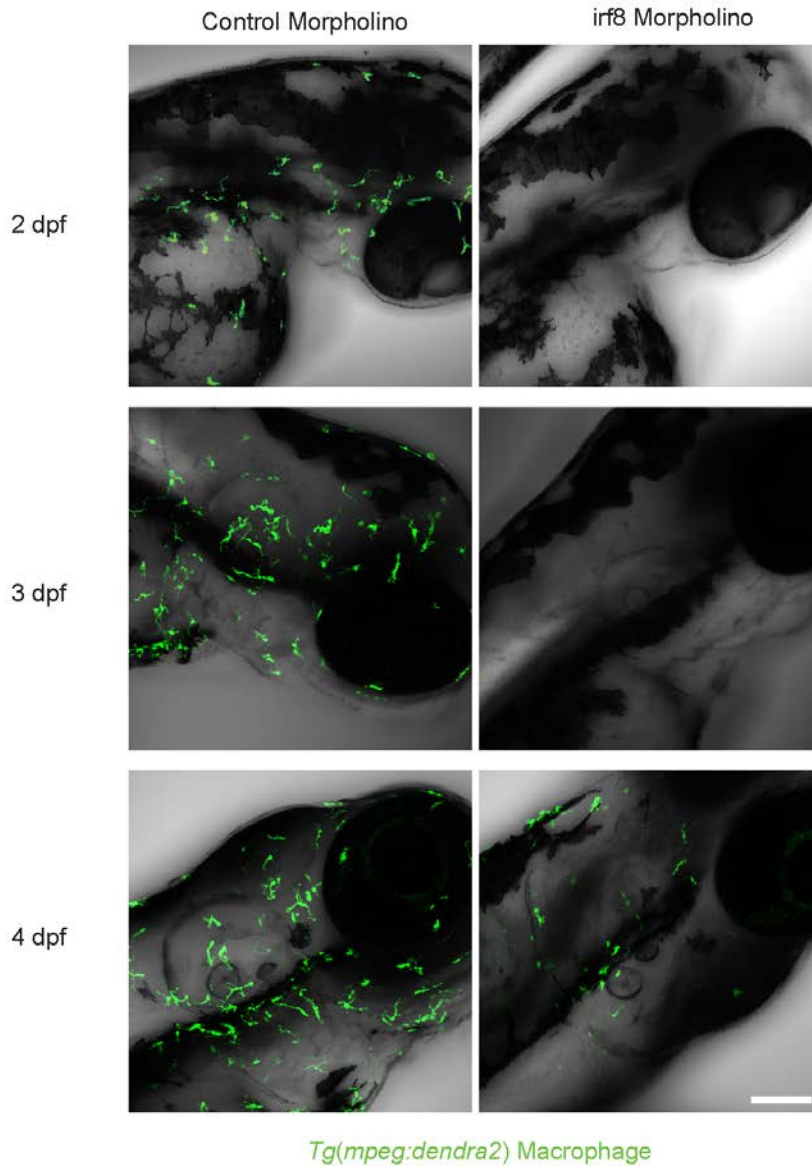
### Distinct Innate Immune Phagocyte Responses to *Aspergillus fumigatus* Conidia and Hyphae in Zebrafish Larvae

Benjamin P. Knox, Qing Deng, Mary Rood, Jens C. Eickhoff, Nancy P. Keller, and Anna Huttenlocher



**Figure S1. Decrease in *A. fumigatus* CFU's over time from single immunocompetent larvae.** Immunocompetent larvae infected with *A. fumigatus* were individually homogenized and plated at the time points indicated showing the spread of original fungal inoculum per larvae and decrease over time. Significant reduction in fungal CFU obtained by days 5 and 7 post infection. Graph representative of data from two independent experimental replicates. \*,  $p < 0.05$  as determined by Kruskal-Wallis test with Dunn's multiple comparison posttest.

## Supplemental Information



**Figure S2. Irf8 morpholino knockdown efficacy over time.** Macrophage development in *Tg(mpeg:dendra2)* larvae is completely inhibited with the irf8 morpholino for 3 days post fertilization (dpf). Starting at 4 dpf, macrophages begin to return, albeit in diminished numbers. Scale bar, 100  $\mu$ m.

## Supplemental Information

**Table S1. *Aspergillus fumigatus* strains used in this study.**

Strain	Genotype	Source
AF293.1	<i>pyrG</i> -	Xue et al., 2004
TJW54.2	<i>pyrG</i> -; $\Delta$ <i>laeA</i> :: <i>A. parasiticus pyrG</i>	Bok and Keller, 2004
TJW55.2	<i>pyrG</i> -; <i>A. parasiticus pyrG</i>	Bok and Keller, 2004
Afs82	<i>gpdA</i> :: <i>mCherry</i> :: <i>his2A ptrA</i>	Szewczyk and Krappmann, 2010
$\Delta$ <i>sidA</i>	<i>pyrG</i> -; $\Delta$ <i>sidA</i> :: <i>pyrG</i>	Dr. Wen-Bing Yin
TJMP131.5	<i>gpdA</i> :: <i>GFP</i> :: <i>his2A pyrG</i>	Dr. Jonathan M. Palmer
TJMP132.4	$\Delta$ <i>laeA</i> :: <i>A. fumigatus argB</i> ; <i>Af-pyrG</i> :: <i>gpdA(p)</i> :: <i>GFP2-5</i> :: <i>Af h2A</i> :: <i>h2A(t)</i>	Dr. Jonathan M. Palmer
TBK1.1	<i>pyrG</i> -; <i>A. fumigatus gpdA</i> :: <i>YFP</i> :: <i>A. fumigatus pyrG</i>	This Study

### Reference

**Xue T, Nguyen CK, Romans A, Kontoyiannis DP, May GS. 2004.** Isogenic auxotrophic mutant strains in the *Aspergillus fumigatus* genome reference strain AF293. Arch Microbiol **182**: 346–353.

## Supplemental Information

**Table S2. Primers used in this study.**

<b>Primer Name</b>	<b>Sequence</b>
QC-pFNO3- pSK495-F	GGAGCTGGTGCAGGCGCTGGAGCCGGTGCCATGGTCAGCAAG GGCGAGGAACTGTTCCACC
QC-pFNO3- pSK495-R	GGGTGAAGAGCATTGTTTGAGGCGACCGGTTCACTTGTACAGC TCGTCCATGCCAGGG
AfgpdA-YFP- pyrG-5'F	TCCACTATGCTGTACGTGCTCCAAGC
AfgpdA-YFP- pyrG-5'R	GGCACCGGCTCCAGCGCCTGCACCAGCTCCCTGGGAATCGAC CTTGCGGATGTAGGC
AfpBPK1-F	GGAGCTGGTGCAGGCGCTGG
AfpBPK1-R	CTGTCTGAGAGGAGGCACTGATGC
AfgpdA-YFP- pyrG-3'F	GCATCAGTGCCTCCTCTCAGACAGATTCCGAAGTCGGATACAA CATGCCCTGCGG
AfgpdA-YFP- pyrG-3'R	ACCTCACAATGGTAGCGCATCTGG
AfgpdA-YFP- DIGN-F	TGATCAGGTCGTTGG

## Supplemental Information

**Movie S1.** Time lapse microscopy of invasive hyphal growth in the hindbrain ventricle of highly immunosuppressed pu.1 morphant larvae. Scale bar, 100µm.

**Movie S2.** Time lapse microscopy of macrophages rapidly phagocytosing *A. fumigatus* conidia. Scale bar, 100µm.

**Movie S3.** Time lapse microscopy of macrophage inflammation around *A. fumigatus* hyphae. Scale bar, 100µm.

**Movie S4.** Time lapse microscopy of neutrophils and *A. fumigatus* conidia. Scale bar, 100µm.

**Movie S5.** Time lapse microscopy of neutrophils in macrophage-deficient irf8 morphant larvae with *A. fumigatus* conidia. Scale bar, 100µm.

**Movie S6.** Time lapse microscopy of neutrophils co-localizing with, migrating towards, and adhering to *A. fumigatus* hyphae. Scale bar, 100µm.

**Movie S7.** Time lapse microscopy of neutrophils in macrophage-deficient irf8 morphant larvae with *A. fumigatus*  $\Delta laeA$  conidia. Scale bar, 100µm.