

Figure S1. Inhibition of hydrogen peroxide generation by DPI or in duox morphants.

Wild-type AB zebrafish larvae were injected with HyPer mRNA in combination with p53 MO alone or plus duox MO at 1 cell stage. Larvae at 3dpf were pretreated with DPI or DMSO for 1 hr before wounding as indicated. All images were taken 30 min post wounding using the same acquisition parameters and processed with identical post analysis. Note increased YFP488/YFP405 at the wound margin in the p53 morphant treated with DMSO, but not in the one treated with DPI or in the duox morphant treated with DMSO. Scale bar: 40 μ m.

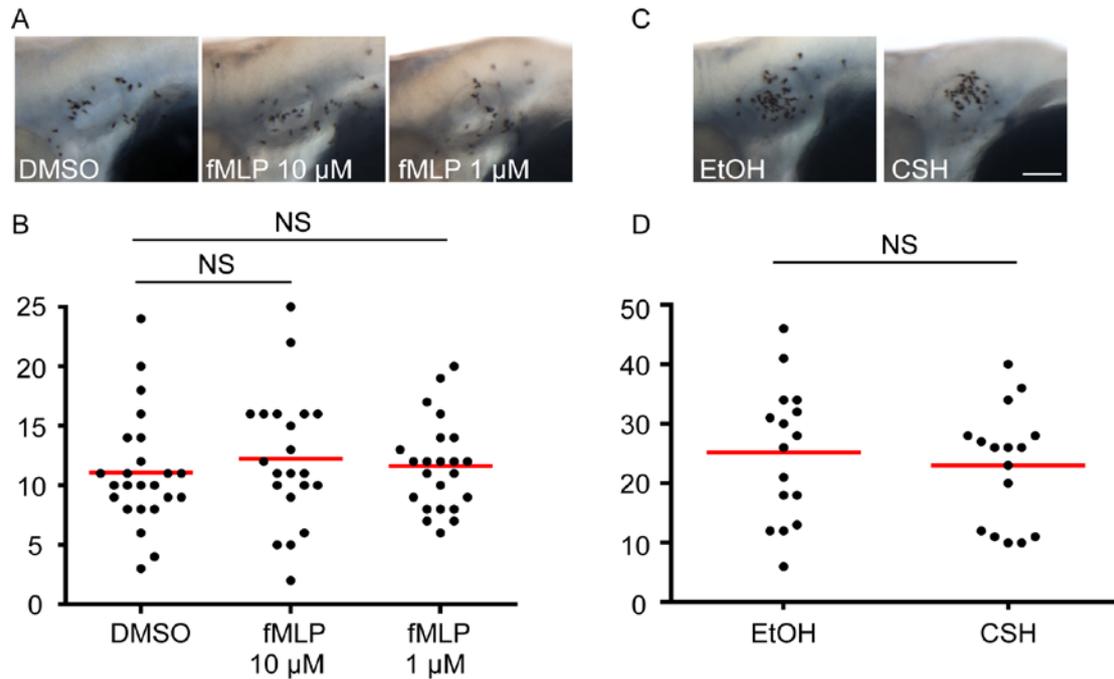


Figure S2. Formyl peptide receptor is not involved in neutrophil recruitment to *Pseudomonas aeruginosa* (PAK) infection.

A) Vehicle control, 10 μ M or 1 μ M fMLP were injected into the otic vesicle of 3 dpf wild-type zebrafish larvae. Representative images of neutrophils recruited to ear 1 hour post injection are shown.

B) Quantification of A. n=24 (DMSO), 21 (10 μ M) and 23 (1 μ M).

C) Wild-type zebrafish at 3 dpf were treated with vehicle control or 20 μ M cyclosporine H (CSH) for 1 hr and infected with ~1,200 cfu of PAK (pMKB1::mCherry). Representative images of neutrophils recruited to ear 1 hour post infection are shown.

D) Quantification of C. n=16 (EtOH) and 16 (CSH).

Results are representative of 3 independent experiments. NS, none significant, two-tailed Mann-Whitney test. Scale bar: 50 μ m.

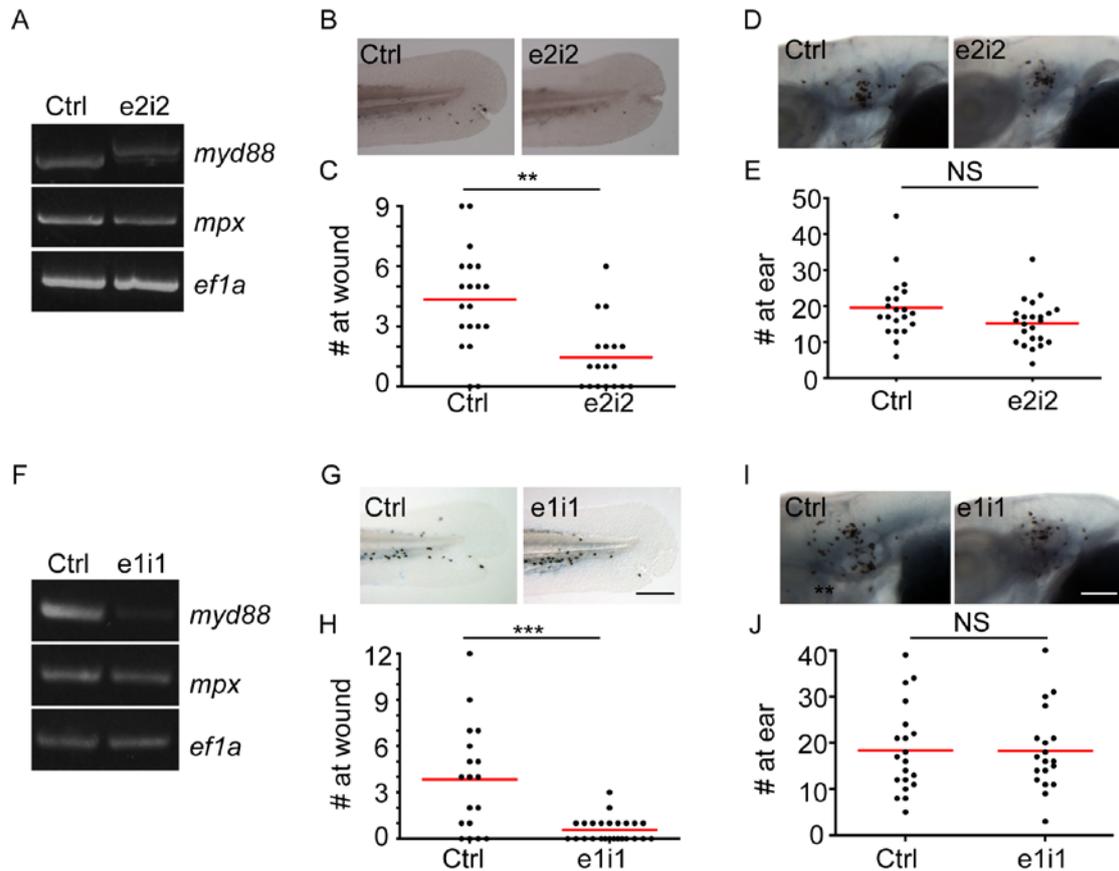


Figure S3. Myd88 is involved in neutrophil recruitment to wounding, but not to *Pseudomonas aeruginosa* (PAK) infection.

A-E) Wild-type AB zebrafish larvae were injected with standard control or myd88 (e2i2) morpholino oligonucleotide at 1 cell stage.

A) RT-PCR of myd88, mpx or ef1 α from DNA extracted from control or myd88 morphants at 3 dpf.

B) At 3 dpf, control or myd88 morphants were fixed 1 hpw. Representative images of neutrophils recruited to wounds are shown.

C) Quantification of B. n=21 (Ctrl), 23 (e2i2).

D) At 3 dpf, Control or myd88 morphants were fixed 1 hpi with ~1,200 cfu of PAK (pMKB1::mCherry). Representative images of neutrophils recruited to ear are shown.

E) Quantification of D. n=20 (Ctrl), 18 (e2i2).

D-F) Similar experiments were performed with another myd88 morpholino oligonucleotide e1i1. n=24 (Ctrl), 23 (e1i1) in H. n=16 (Ctrl), 16 (e1i1) in J.

Results are representative of 3 independent experiments. ***, p<0.001; **, p<0.01; NS, none significant, two-tailed Mann-Whitney test.

Supplemental Movie legend

Movie S1. Absence of H₂O₂ burst at localized PAK infection.

Wild-type AB zebrafish embryo were injected with HyPer mRNA at 1 cell stage. Embryos at 3 dpf were infected with ~ 1,400 cfu of PAK (pMKB1::mCherry) at the left ear and real-time ratiometric imaging was performed to measure relative amount of H₂O₂ generated in tissue. The right ear of the same fish was then wounded and generation of H₂O₂ was monitored under the same conditions. Scale bar: 50 μm.

Movie S2. Differential sensitivity to DPI during neutrophil response to simultaneous wounding and infection.

Tg(mpx:Dendra2) at 3 dpf were treated with DMSO, DPI or LY for 1 h, then infected with ~ 1,200 cfu of PAK (pMKB1::mCherry) immediately followed by tail transection. Movies are representative for 8 movies for each condition from 4 separate experiments.