Supplementary Figure S1

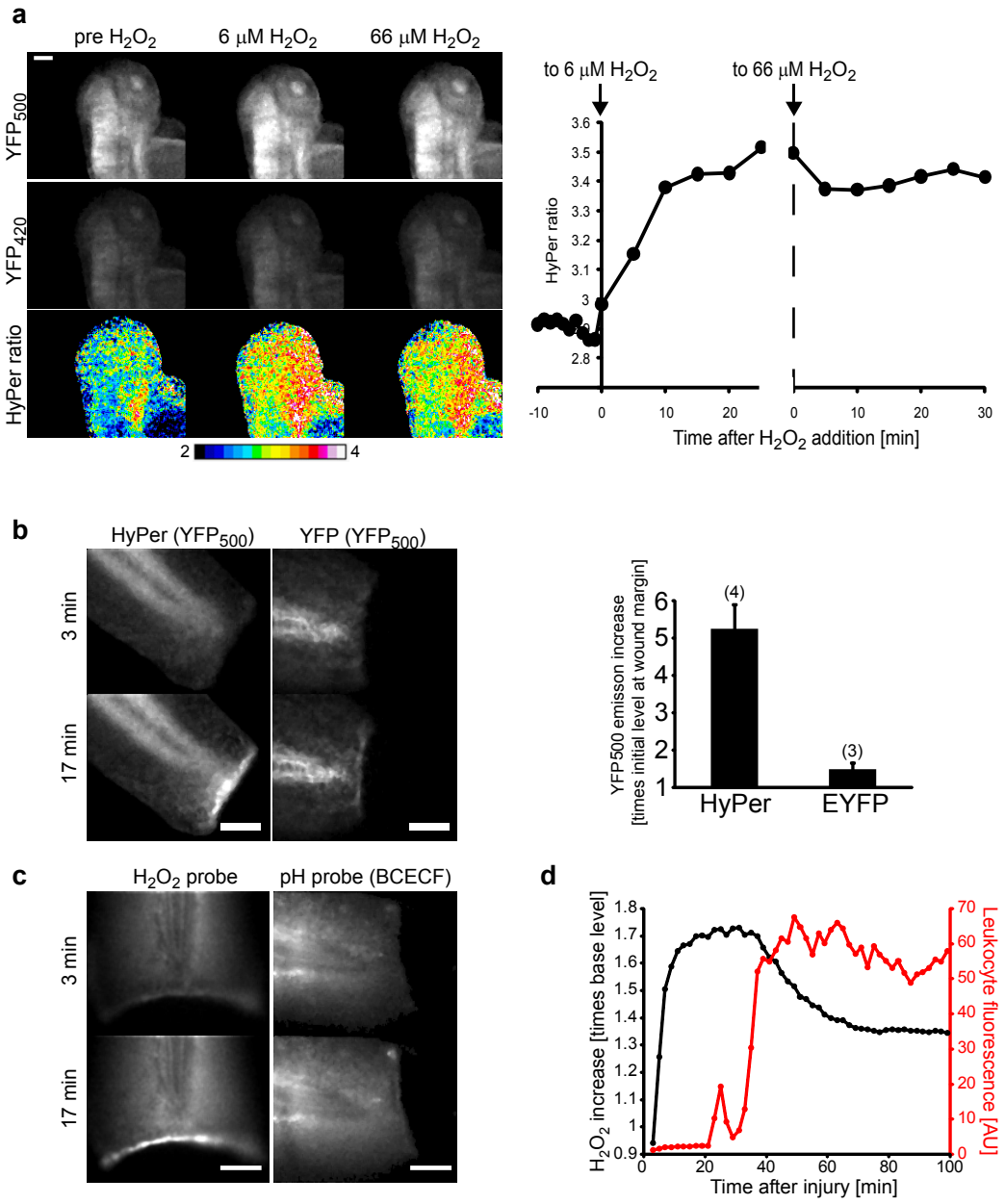

Figure S1. (a) Left panel: HyPer emission (YFP_{500}, YFP_{420}) and HyPer ratio of a 3 dpf zebrafish larva at indicated times before (-10 to -2 min) and after (0 - 25 min) subsequent addition of 6 μM and 60 μM H_2O_2 to the fish bathing water. Right panel: HyPer ratio (YFP_{500}/YFP_{420}) plotted vs. time after H_2O_2 addition. **(b)** Left panel: YFP_{500} of HyPer and EYFP expressed in 3 dpf larvae, imaged at indicated times after tail fin injury. Right panel: quantification of YFP_{500} emission of HyPer and EYFP (17 min pw). Up-regulation of emission is expressed as multiple of the initial emission at the wound margin (as measured 3 min pw). Error bars: SEM of indicated number of larvae (brackets). **(c)** Side by side comparison of an alternative fluorogenic H_2O_2 probe (Acetyl-pentafluorobenzenesulfonyl fluorescein) and pH probe (BCECF-AM) imaged at indicated times after tail fin injury. **(d)** Quantification of time-lapse sequence depicted in Figure 1e. H_2O_2 production (expressed at multiple of base value, f_{mult}; see Methods) and leukocyte fluorescence (*lysC::DsRED2*) at the wound margin plotted vs. time after injury. Note that scaling of individual fluorescence channels has been adjusted to improve grayscale contrast, but is the same between subsequent time-points of each channel. Scale bars: 100 μm.

Supplementary Figure S2

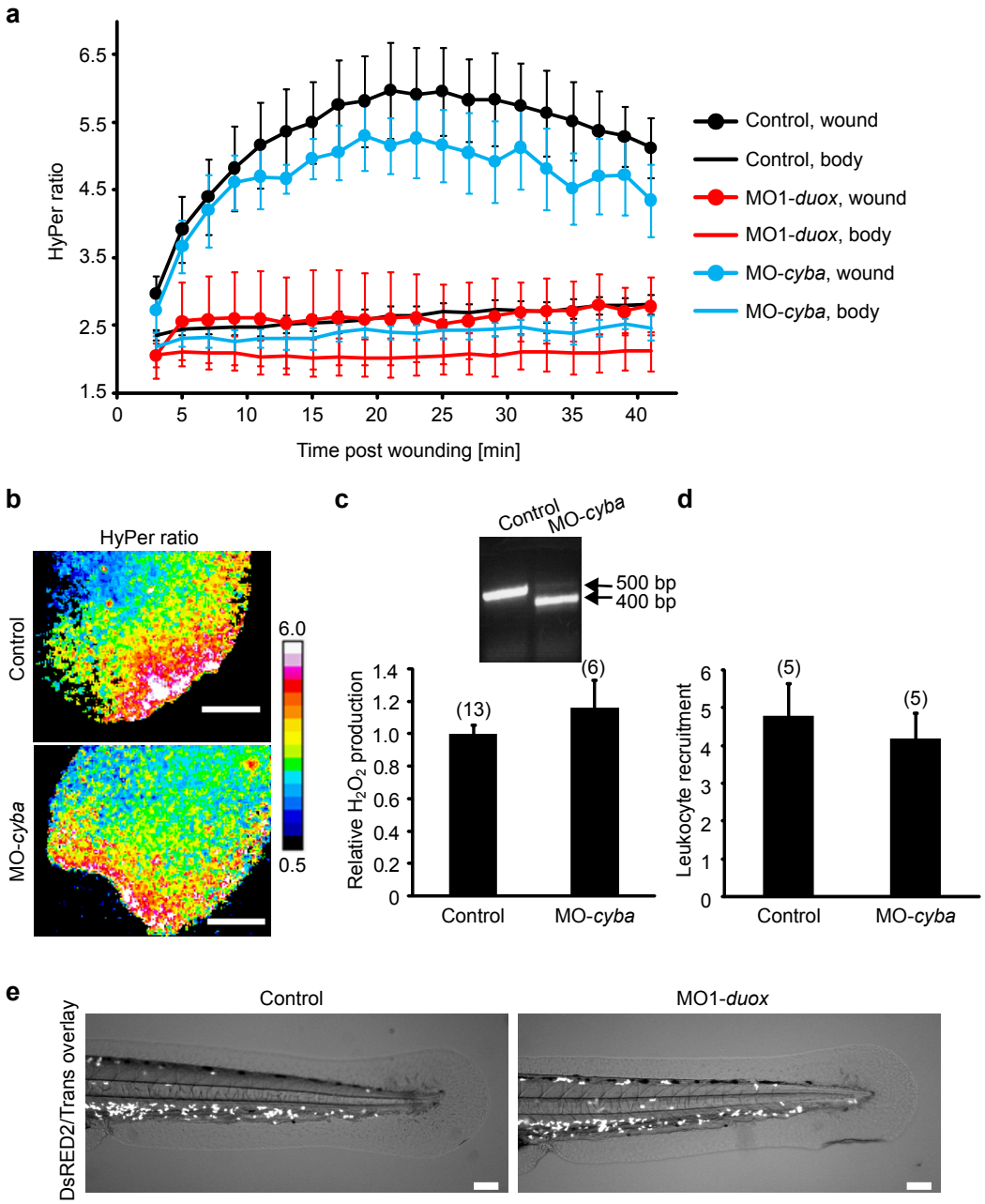

Figure S2. (a) Averaged kinetic profiles of wound margin (filled circles) and basal HyPer signals in response to *duox* and *cyba* morpholino knockdown (MO1-*duox*, MO-*cyba*) in 3 dpf HyPer expressing larvae. Error bars: SEM of n=3 larvae per sample. **(b)** Wound margin H_2O_2 production in response to *cyba* ($P22^{phox}$) morpholino knockdown in 3 dpf larvae compared to control, imaged 17 min pw. **(c)** Quantification of H_2O_2 production in *cyba* knockdown larvae (MO-*cyba*) compared to control, evaluated 17 min after wounding. Inset: RT-PCR of $P22^{phox}$ mRNA from MO-*cyba* injected and control larvae (3 dpf). Error bars: SEM of indicated number of larvae (brackets). **(d)** Quantification of leukocyte recruitment in *cyba* knockdown larvae (MO-*cyba*) compared to control. Error bars: SEM of indicated number of larvae (brackets). **(e)** Representative images of fluorescent leukocytes in wt (control), or *duox/p53* knockdown (MO1-*duox*) *lysC::DsRED2* larvae (3 dpf), superimposed with the corresponding transmission images. Scale bars: 100 μm.

Supplementary Figure S3

a

	Average Velocity [μm/min] (± s.e.m.)	Path Linearity (± s.e.m.)	Wound Directionality (± s.e.m.)	Number of cells moving* / Number of larvae inspected	Mean number of cells moving* in the tail fin (per larva)
No wound	4.68 ± 0.45	0.45 ± 0.06	n.a.	19 / 11	1.7
Wound, 100 μM DPI	4.99 ± 0.83	0.55 ± 0.10	0.53 ± 0.09	12 / 10	1.2
Wound, MO1-*duox*	5.79 ± 0.54	0.59 ± 0.05	0.48 ± 0.05	32 / 12	2.6
Wound, 5-MP	7.81 ± 0.27	0.78 ± 0.02	0.71 ± 0.02	100 / 12	8.3

* only cells describing a total pathlength of at least 50 μm were scored

T-test, p-values:

Average v	No wound	100 μM DPI	MO1-*duox*	5-MP
No wound	n.a.	0.75	0.12	1.35E-06
100 μM DPI		n.a.	0.42	6.32E-03
MO1-*duox*			n.a.	1.55E-03

Linearity	No wound	100 μM DPI	MO1-*duox*	5-MP
No wound	n.a.	0.42	0.08	8.50E-05
100 μM DPI		n.a.	0.67	0.04
MO1-*duox*			n.a.	5.25E-04

Wound dir	100 μM DPI	MO1-*duox*	5-MP
100 μM DPI	n.a.	0.66	0.09
MO1-*duox*		n.a.	2.01E-04

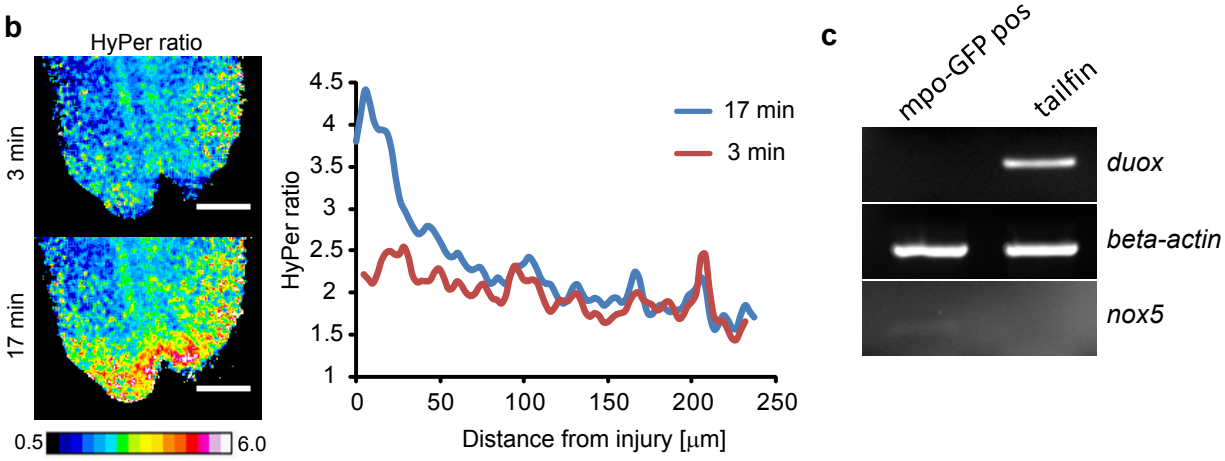

Figure S3. (a) Statistical evaluation of leukocyte tracks scored in non-injured, injured and 100 μM DPI treated, injured and MO1-*duox* or MO5-MP-*duox* (5-MP) injected larvae. Only adequately identifiable, at least 50 μm long tracks outside a 50 μm radius around the center of mass of the wound, within the ventral tail fin tissue were scored for analysis (see Methods). Lower panels: summary of p-values (ttest). P values < 0.05 are highlighted. **(b)** Wound margin H_2O_2 production as detected by HyPer in response to a small tail fin incision at indicated times after injury. Right panel: spatial [H_2O_2] profile at the indicated times after wounding, measured along a line approximately normal to the wound margin. Scale bar: 100 μm. **(c)** *Duox*, *nox5* and *beta-actin* mRNA expression in green fluorescent leukocytes (isolated by FACS from disaggregated 3 dpf *mpo::GFP* larvae) and dissected tail fin tip tissue of 3 dpf AB larvae as evaluated by semi-quantitative RT-PCR.