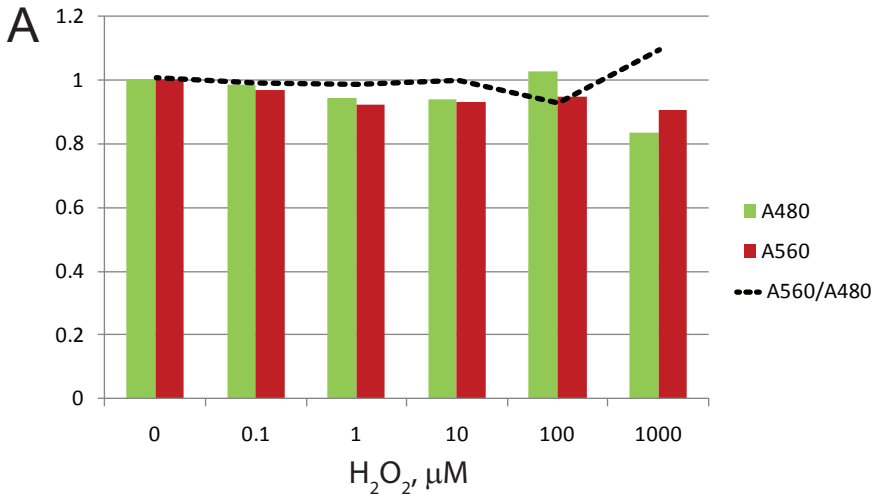


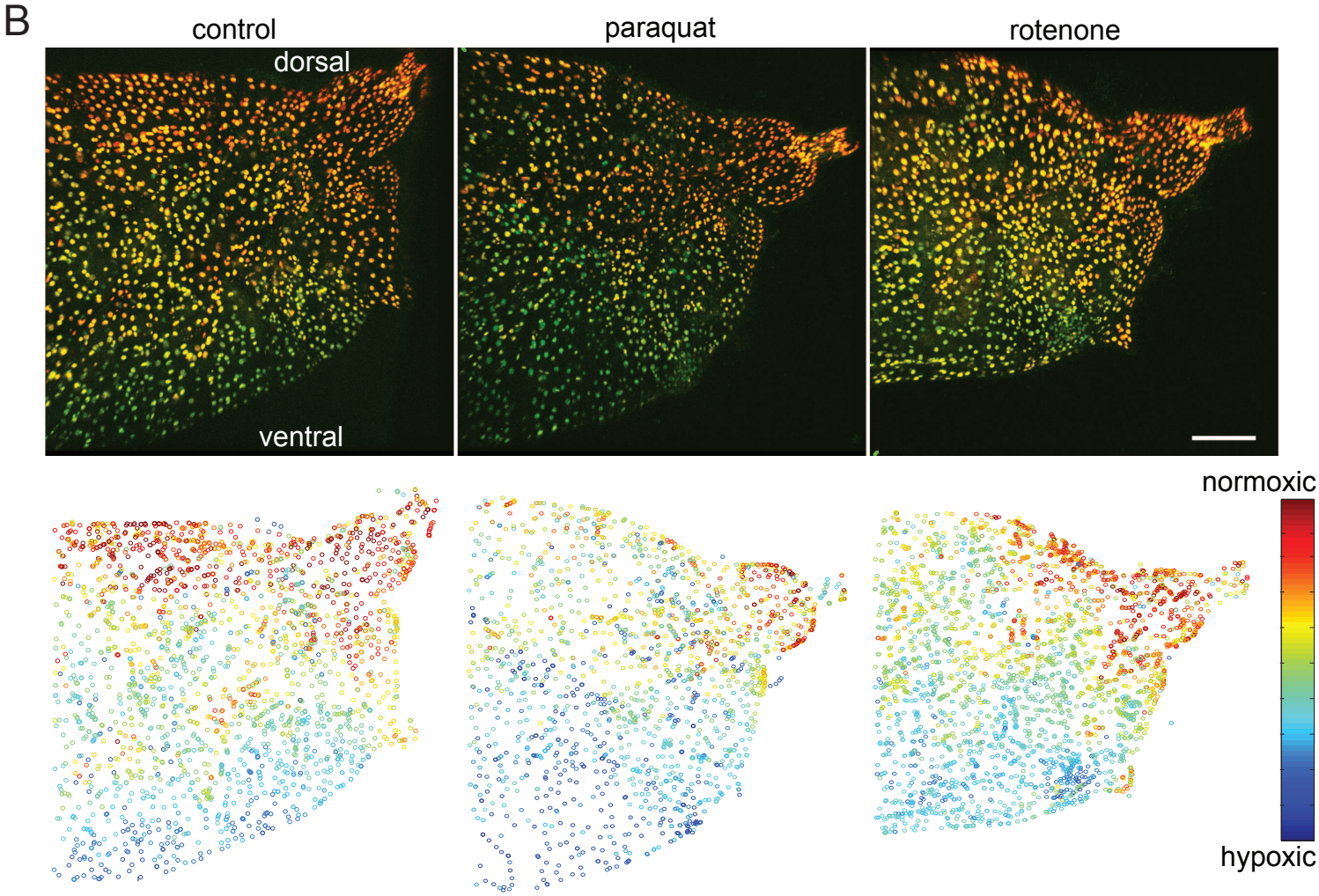


Fig S1. Timer maturation is not affected by ROS significantly. A. H_2O_2 does not have a strong effect on Timer maturation. Timer samples were obtained as in Fig 1 and let to mature for 48 h at room temperature in the presence of designated concentrations of H2O2. Graph shows absorbance at 480 nm (green) and 560 nm (red), and their ratio (black line); all values were normalized to the corresponding value at zero H_2O_2. B. Larvae raised as in Fig 1 and exposed to ROS-inducing agents paraquat or rotenone for 3 days do not display any significant change in the color of nlsTimer maturation. Lateral images of abdominal regions are shows to display the most hypoxic and most normoxic regions of the larvae. Dorsal and ventral sides are marked. n=5. Bar is 200 μm.

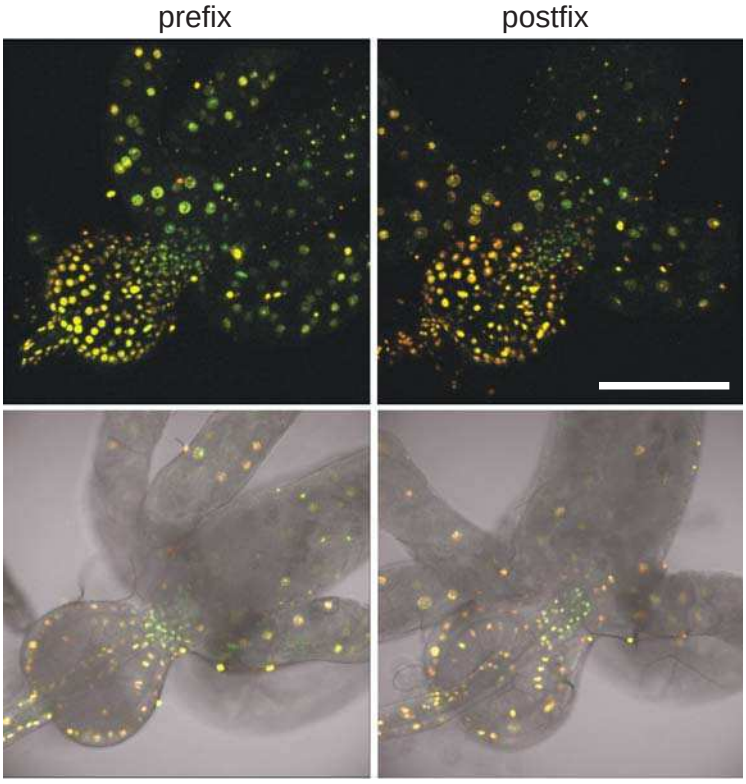


Fig S2. PFA fixation retains the nlsTimer red/green gradient in a slightly red-shifted dynamic range. Larvae prepared as in Fig 2 were dissected, organs were immobilized on poly-L-lysine coated slide and imaged prior and after PFA fixation. Bar is 100 μm.

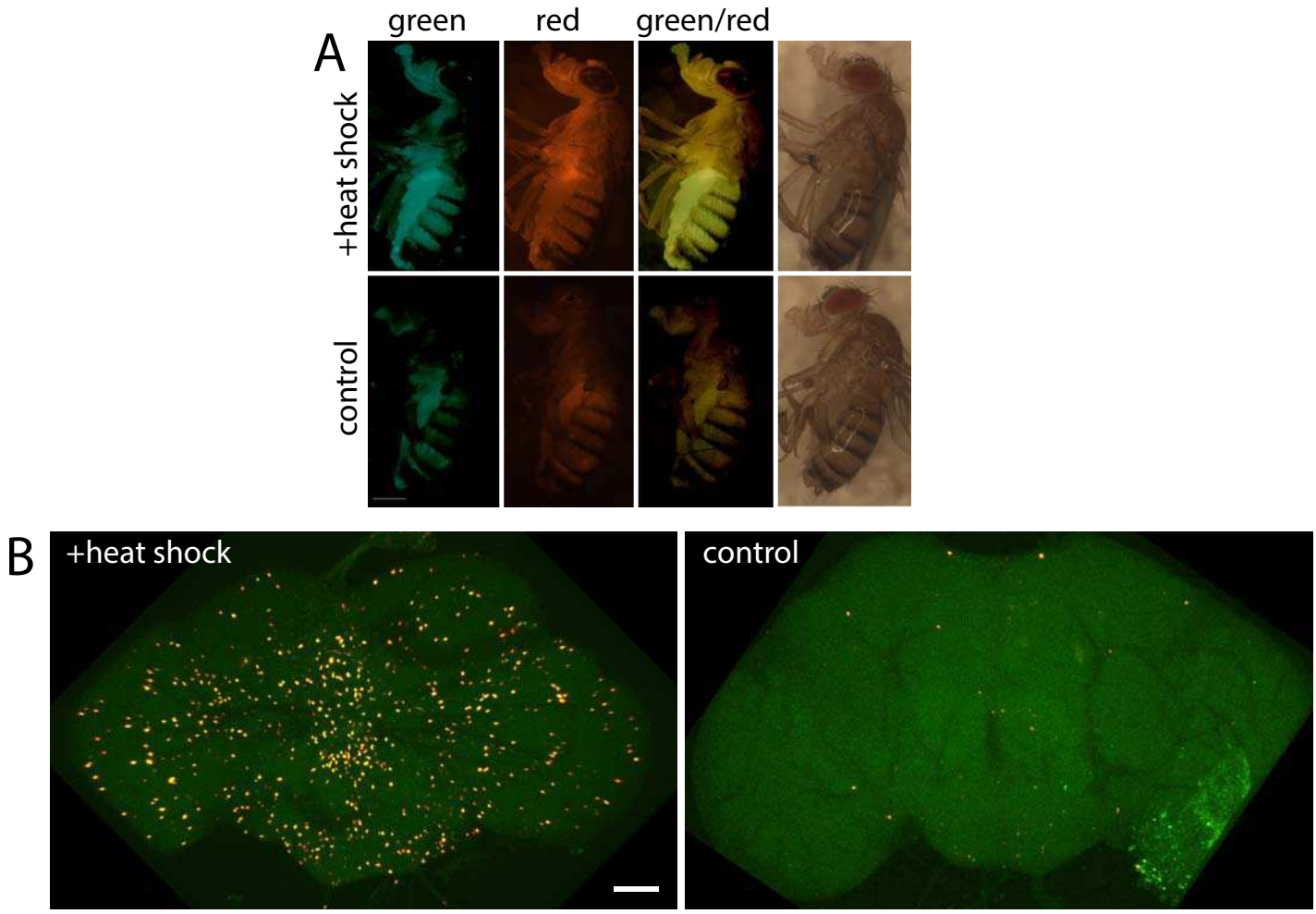
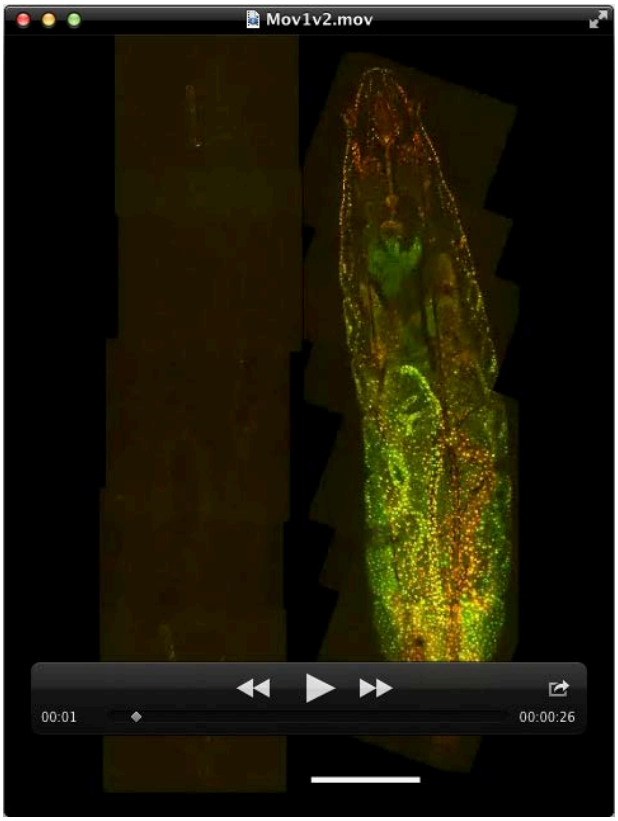


Fig S3. nlsTimer probe can be used in adult animals. A. 3 day-old flies were exposed to heat shock for 1h at 37°C and let to recover for 3 days at 25°C. While the head and the thorax express more reddish fluorescence, the abdomen displays green coloration. Bar is 1 mm. n>10. B. nlsTimer induction in adult brains, 3 days after the heat shock. Slight differences between individual cells can be observed. n=5. Bar is 50 µm.



Movie S1. Detailed view of the larvae shown in Fig 1A-H. Individual z-planes are shown as animation. Automated nuclei recognition and ratiometric analysis of fluorescence signals was performed with Imaris (Bitmap).