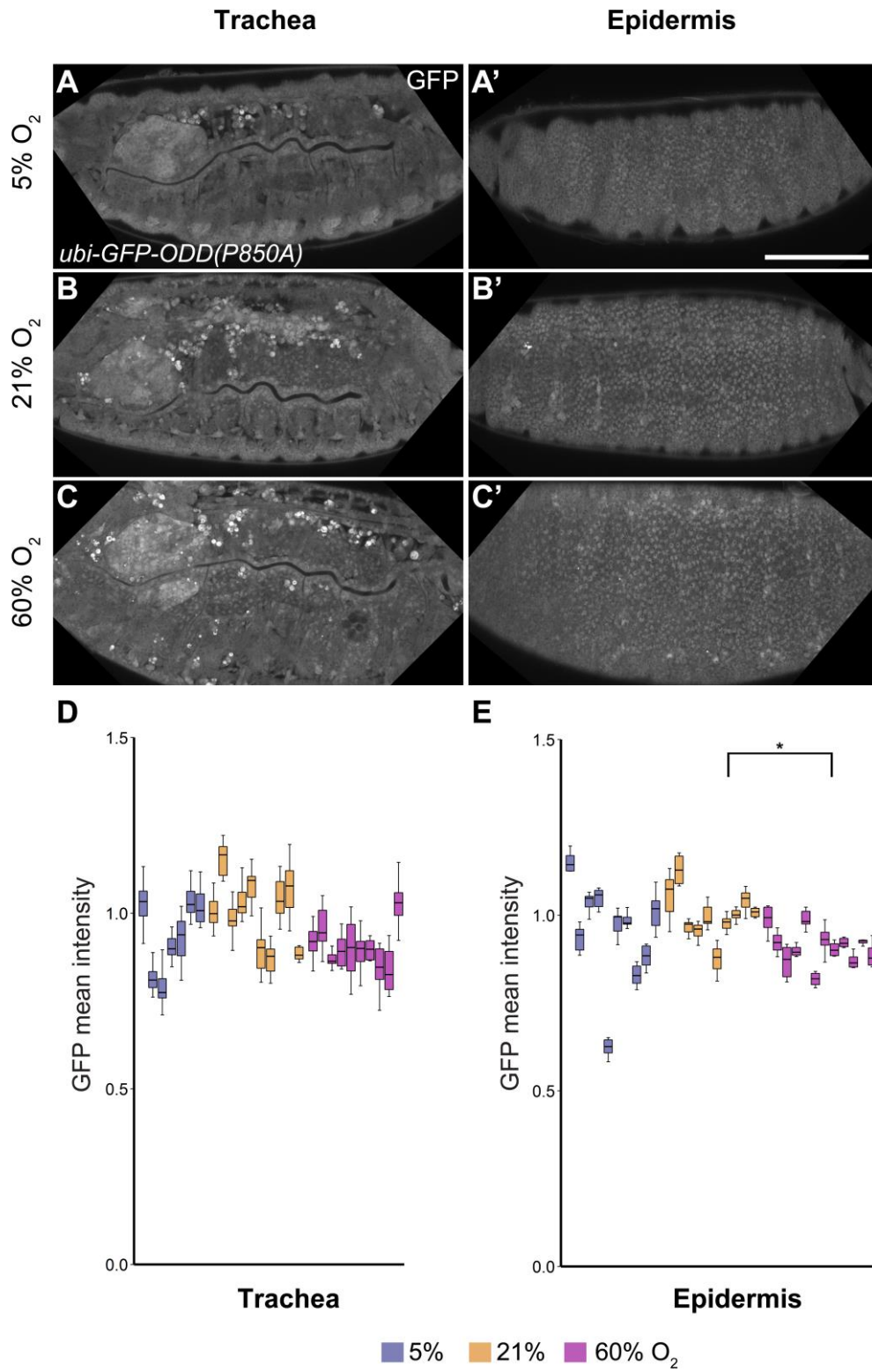


Supplemental Figures



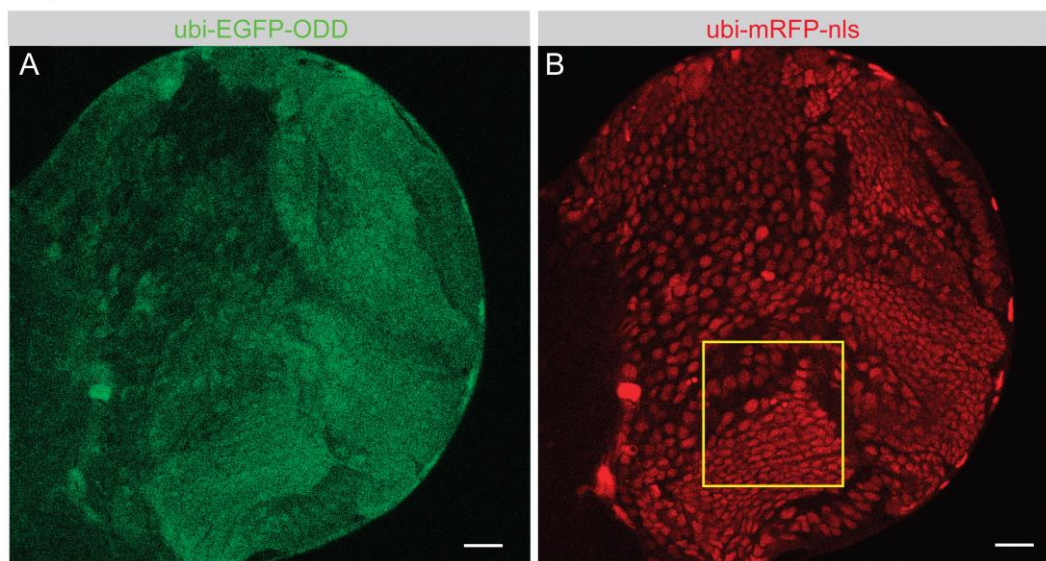
## Supplemental Figure 1

Proline 850 is required for O<sub>2</sub>-dependent changes in GFP-ODD accumulation.

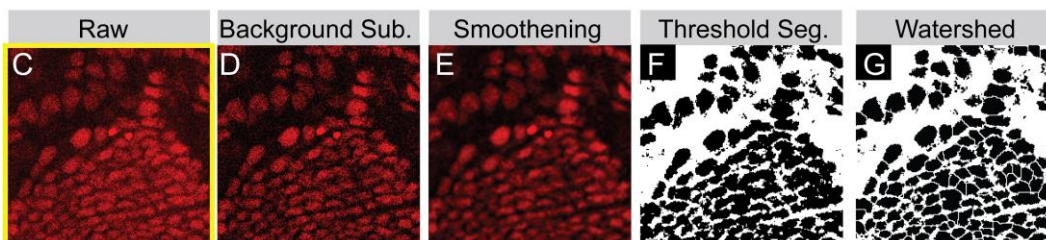
Embryos expressing *ubi-GFP-ODD(P850A)* were incubated at (A) 5% O<sub>2</sub>, (B) 21% O<sub>2</sub>, or (C) 60% O<sub>2</sub>. Optical sections of the same embryos taken at two different depths show the tracheal dorsal trunk (A-C) and the epidermis (A'-C'). Quantification of GFP-ODD(P850A) signals in tracheal (D) and epidermal (E) cells indicates that GFP-ODD(P850A) does not increase after incubation in 5% O<sub>2</sub> relative to 21%, in contrast to the changes observed for GFP-ODD(wild-type) (compare Fig. 1B). No significant differences were observed in the levels of GFP-ODD(P850A) between 5% and 21% O<sub>2</sub> in the epidermis (5% decrease; student's t-test, p=0.3) and trachea (4% decrease; student's t-test, p=0.1). The changes in GFP-ODD(P850A) levels between 21% and 60% O<sub>2</sub> (epidermis: 8% decrease, student's t-test, p=0.005; trachea: 7% decrease, p=0.01) are smaller than the changes observed for GFP-ODD(wild-type) under the same conditions. (F) Bar plots of the individual embryos used for quantification of the mean intensities shown in (D) and (E). Each box represents data from a single embryo in which fluorescence was measured in at least 8 cells.

Scale bar, 100 μm. \*\*\*, p≤0.001; \*\*, p≤0.01; \*, p≤0.05.

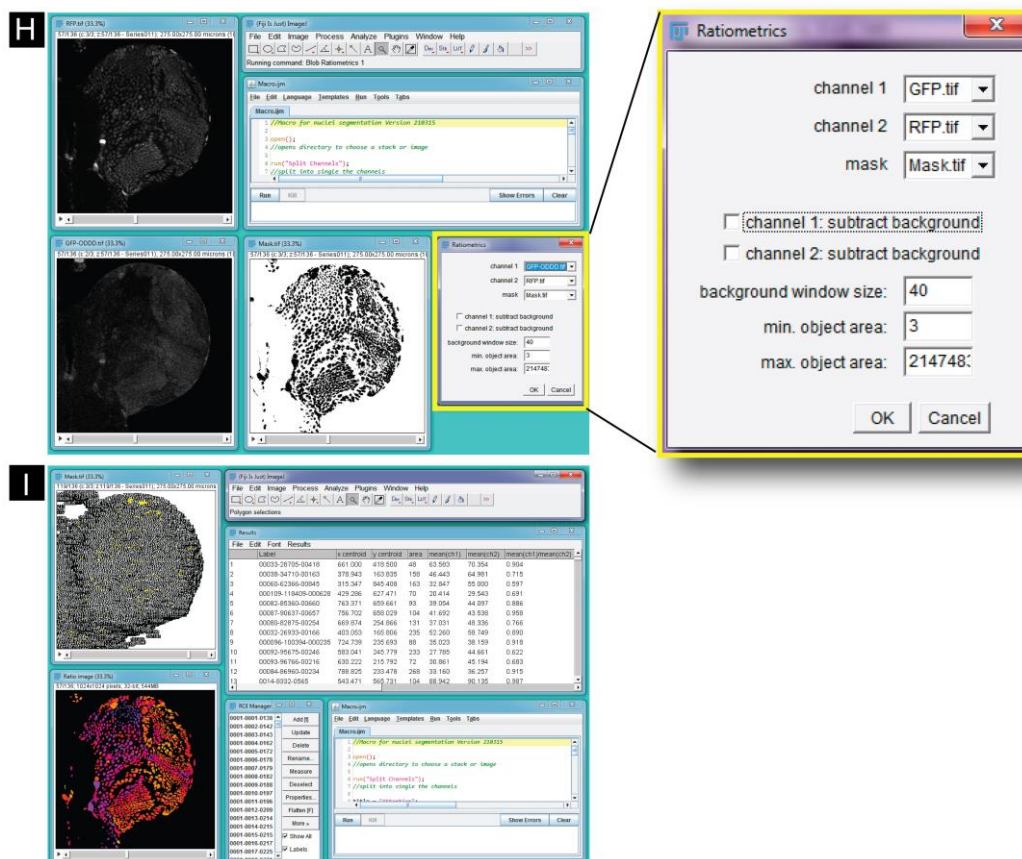
### Acquisition of confocal stacks



### Segmentation of nuclei with Macro Language in Fiji



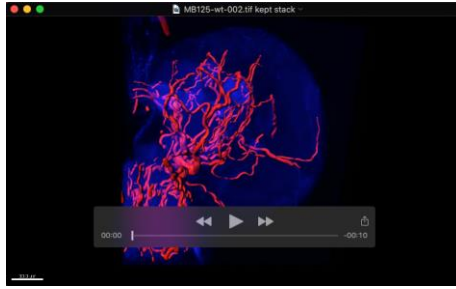
### Analysis with Ratiometric Plugin in Fiji



## Supplemental Figure S2

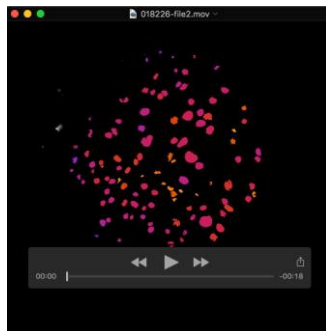
Overview of image segmentation and ratiometric analysis.

A single confocal section of the larval brain at 96 hALH shows GFP-ODD (A, green) and mRFP-nls (B, red). Prior to segmentation of nuclei, raw confocal image stacks of the mRFP channel (C) were subjected to background subtraction (D) and smoothing (E). Nuclei were segmented using Autothreshold (F) and Watershed (G) algorithms. A plugin was developed in Fiji to perform the ratiometric analysis. A screenshot of Fiji is shown before (H) and after (I) running the ratiometric analysis. In the ratiometric plugin interface GFP, RFP and segmented mask image stacks are selected. The plugin allows the user to specify parameters for background subtraction, as well as for minimum and maximum size of segmented objects.



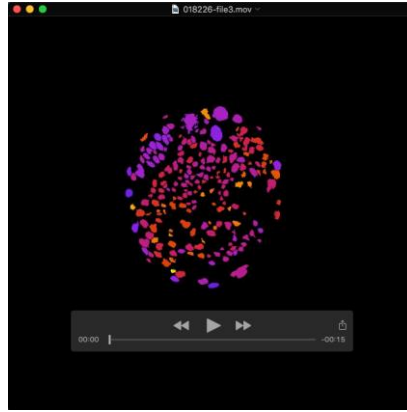
### Supplemental Movie 1

3-D animation of a larval brain hemisphere labelled with anti-Dlg (blue, to show the outline of the brain) and with Calcofluor (red, to show surface of the tracheal lumen). The tracheal lumen was rendered using Imaris 7.6 for measurement of surface area. The animation was made from the specimen shown in Fig. 4A.



### Supplemental Movie 2

Animated z-stack through the ratiometric color map of the *ubi-GFP-ODD*, *ubi-mRFP-nls* expressing brain shown in Fig. 4C. Overlaid is the tracheal system stained with calcofluor.



### Supplemental Movie 3

Animated z-stack through the ratiometric color map of the *ubi-GFP-ODD (P850A)*, *ubi-mRFP-nls* expressing control brain shown in Fig. 4D.