# Supplementary Information for

# Long-term *in vivo* single cell lineage tracing of deep structures using three-photon activation

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**Table S1. Survival rates of control zebrafish embryos**

| Experiment | Control fish | | |
|---|---|---|---|
| | # of fish | Survival 24 h | Survival to adulthood |
| cyclofen | 100 | 85 | 24/30 |
| three-photon | 100 | 90 | 23/30 |
| DMNB-cyclofen | 100 | 83 | 27/30 |

For the control conditions shown in Fig. 2d-i, including incubation with cyclofen, three-photon illumination alone, and incubation with DMNB-cyclofen alone, 100 zebrafish embryos per condition were used and survival scored after 24 h. Thirty treated embryos per condition were grown for 3 months to score survival to adulthood.

**Table S2. Survival rates of photoactivated zebrafish embryos**

| Experiment | Irradiated fish | | Control fish | |
|---|---|---|---|---|
| | # of positive fish after irradiation | Survival to adulthood | # of fish | Survival to adulthood |
| #5 | 12 | 12 | 15 | 13 |
| #6 | 4 | 2 | 15 | 11 |
| #7 | 1 | 1 | 15 | 12 |

In three independent photoactivation experiments (numbering of experiments as in Table S3), the surviving embryos that had undergone successful cardiomyocyte labeling (positive fish after irradiation) were grown for 3 months to score survival to adulthood. In each experiment, 15 embryos were incubated with DMNB-cyclofen but not irradiated, and grown in parallel to score survival of control fish.

**Table S3. Efficiency of three-photon activation**

| Experiment | # of irradiated fish | Survival 24 hpi | # of positive fish 24 hpi | Survival to adulthood |
|---|---|---|---|---|
| #1 | 48 | 33 | 15 (30%) | n.a |
| #2 | 74 | 63 | 11 (15%) | n.a |
| #3 | 72 | 60 | 12 (17%) | n.a |
| #4 | 22 | 18 | 4 (18%) | n.a |
| #5 | 72 | 61 | 12 (17%) | 12 |
| #6 | 22 | 21 | 4 (18%) | 2 |
| #7 | 15 | 15 | 1 (7%) | 1 |

Summary of the results from 7 independent experiments using 3-photon activation of DMNB-cyclofen to label zebrafish cardiomyocytes. The survival of the treated embryos was scored 24 h post irradiation (hpi), when the number of embryos that had undergone successful cardiomyocyte labeling (positive fish) was also scored. In 3 experiments, successfully labeled embryos were grown for 3 months to score survival to adulthood (see more details in Table S2). Factors that may underestimate the overall efficiency of 3-photon-mediated cardiomyocyte labeling include: 1) initially fish were unlabeled and their heart was beating, thus making it hard to maintain the focus on the heart wall; 2) fish were immersed in medium containing the activating molecule (DMNB-cyclofen), but it is possible that the targeted cardiomyocyte had no DMNB-cyclofen available in its cytoplasm; 3) the expression of GFP could be below the limit of fluorescence detection; and 4) the activated cardiomyocyte(s) could not survive irradiation. Despite all these limitations, the efficiency of activation was kept constant at ~18%, most likely reflecting a high degree of optimization of the overall procedure.

Cardiomyocytes in the zebrafish embryo at the stages that we used for photoactivation are ~ 100 $\mu m^2$ in area and have rectangular shape. Because fish were initially unlabeled, it was difficult to target *a priori* an individual cell to be activated. We decided to irradiate patches of 50 $\mu m^2$ or 100 $\mu m^2$, areas smaller and larger, respectively, than the one of an individual cell, and relate the labeled patch with the irradiated one, in 74 zebrafish embryos (experiment #2 in Table S3). The results are summarized in Table S4.

**Table S4. Single-cell labeling**

| **Illuminated patch size** | **Survival 24 hpi** | **# of positive fish 24 hpi** | **Labeled patch size 24 hours post irradiation** | **# of labeled cells** |
|---|---|---|---|---|
| 50 $\mu m^2$ | 45 | 7 | ~90 $\mu m^2$ | 1 |
| 100 $\mu m^2$ | 18 | 4 | ~200 $\mu m^2$ | 2-3 |

Number of zebrafish embryos irradiated in patches of 50 $\mu m^2$ or 100 $\mu m^2$ that survived 24 h post irradiation (hpi), indicating the number of embryos that had undergone successful cardiomyocyte labeling (positive fish), the size of the labeled patch at 24 hpi as measured using spinning disc microscopy, and the putative number of labeled according to the labeled patch size. The labeled area corresponded to a single cardiomyocyte when we irradiated a patch of 50 $\mu m^2$, and we did not observe any 2-cell size activated area in these experiments. In the case of irradiating a patch of 100 $\mu m^2$, the result was a labeled area corresponding to 2-3 cells. Similar results were obtained using the Tg(actb2:ERT2-Cre-ERT2) / Tg(Xla.Eef1a1-actb2:LOXP-LOX5171-FRT-F3-EGFP,mCherry) zebrafish line, in which we could activate an individual cell in the tail of the zebrafish embryo (Fig. S1).

**Exploring activation depth**

To explore how deep we could activate cells through the zebrafish embryo, we decided to use a transgenic zebrafish line [Tg(actb2:ERT2-Cre-ERT2)/Tg(Xla.Eef1a1-actb2:LOXP-LOX5171-FRT-F3-EGFP,mCherry)] with ubiquitous expression of GFP that switches to mCherry upon activation. This fish line allowed us to image the fish before irradiation for activation, to determine the depth of the area being activated.

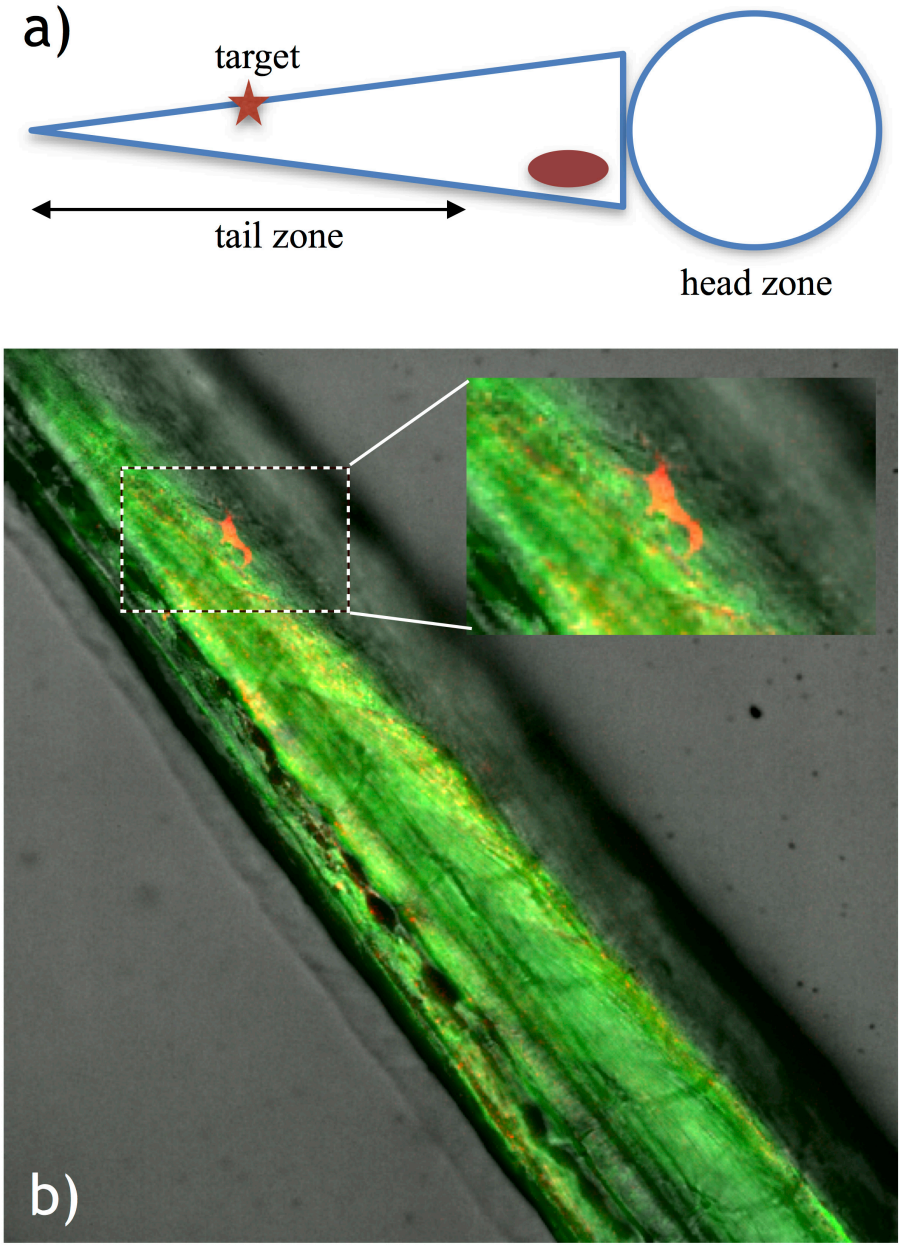


**Figure S1. Single cell labeling in the zebrafish tail. a)** Diagram depicting the embryo positioning for the experiment and the target area in the embryo tail. **b)** Labeled cell identified by red fluorescence (mCherry expression), demonstrating successful photoactivation in the zebrafish tail. The inset shows a magnification of the boxed area.

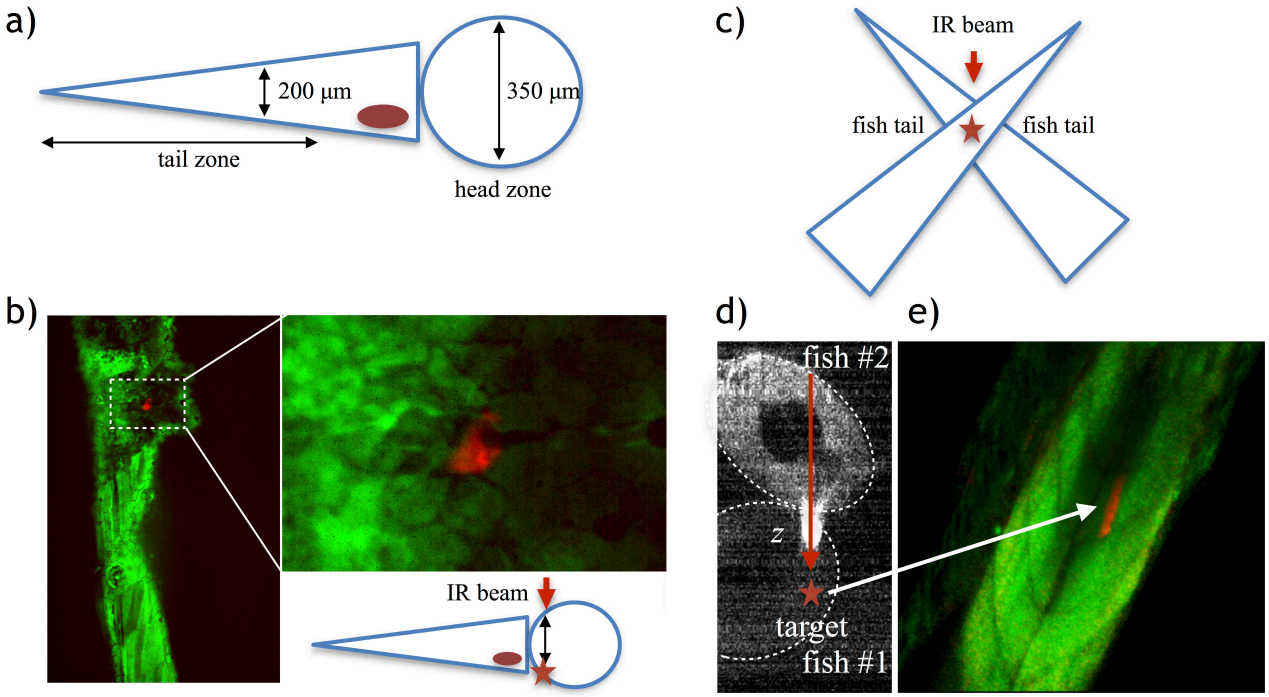


**Figure S2 Exploring activation depth. a)** Schematic of a 5-7 dpf zebrafish showing relative dimensions. **b)** Successful labeling (red fluorescence) in the head area after irradiating a patch of 50 µm$^2$, which resulted in an activation area of 150 µm$^2$ corresponding to a single cell and depth *z* ~ 200 µm (the boxed area is magnified in the right image, scale bar = 20 µm). **c-d)** We also designed an experiment where we could irradiate through fish tissue in the tail zone, but deeper than 200 µm. We superposed two fish in a crossed position, as shown in the schematic (**c**), and irradiated the crossed area using this configuration. We could activate areas of 1600 µm$^2$ passing through tissue length z ~ 360 µm (**d**) (Scale bar = 80 µm).