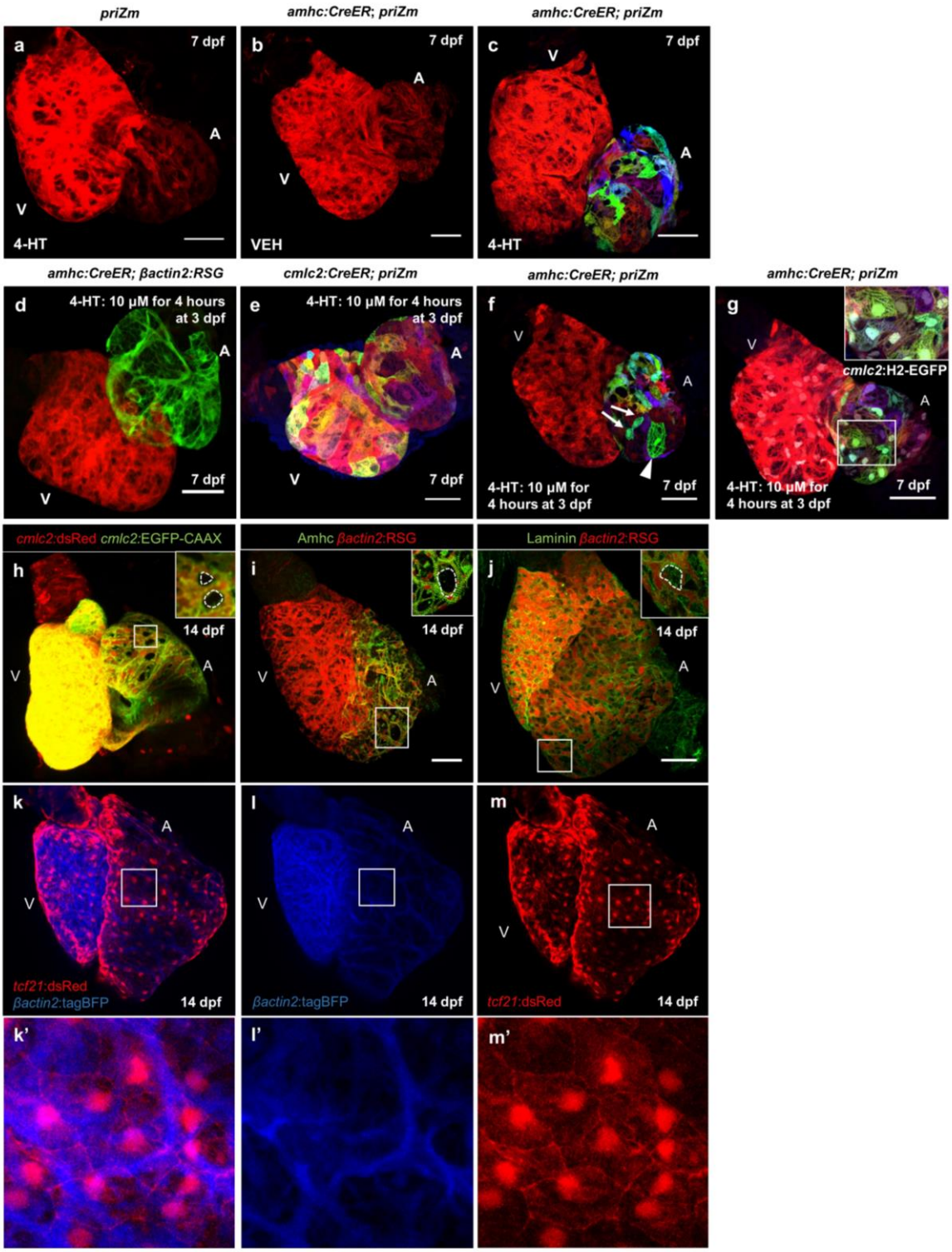



**Fig. S1. *amhc:*CreER-mediated recombination of the *priZm* cassette fluorescently labels individual zebrafish atrial cardiomyocytes.** (**a-c**) Surface myocardium images of 7 dpf *priZm* hearts with and without the *amhc:CreER* transgene and after 4-HT or vehicle (VEH) treatment. (**d**) Surface myocardium of a 7 dpf *amhc:CreER; βactin2:RSG* heart after treatment with 4-HT at 3 dpf. (**e**) Surface myocardium of a 7 dpf *cmlc2:CreER; priZm* heart after treatment with 4-HT at 3 dpf. Scale bars are 50 μm. (**f**) Surface myocardium of a 7 dpf *amhc:CreER; priZm* heart from a larva treated with

4-HT at 3 dpf. Labeled cardiomyocytes are present in singlet (arrowhead) and doublet (arrows) clones. (**g**) Atrial cardiomyocytes from a 7 dpf *amhc:CreER; priZm* heart with nuclei marked by *cmlc2:*H2-GFP. Scale bars are 50 µm. (**h**) 3D reconstruction of a 14 dpf heart from an animal expressing the *cmlc2:dsRed* and *cmlc2:EGFP-CAAX* transgenes. Non-myocardial regions of the atrial wall are indicated (dotted lines). (**i, j**) Surface myocardium of 14 dpf hearts expressing *βactin2:RSG* and immunostained for Atrial myosin heavy chain (Amhc) or Laminin, respectively. A non-myocardial region of the atrial wall is indicated with dotted lines. Laminin immunofluorescence covers the entire atrial surface. Scale bars are 50 µm. (**k-m**) Surface of a 14 dpf heart from an animal expressing the epicardial reporter *tcf21:dsRed* and the myocardial reporter *βactin2:tagBFP.* Epicardial cells coat the entire atrial surface, whereas there are myocardial gaps. White boxes indicate magnified area.

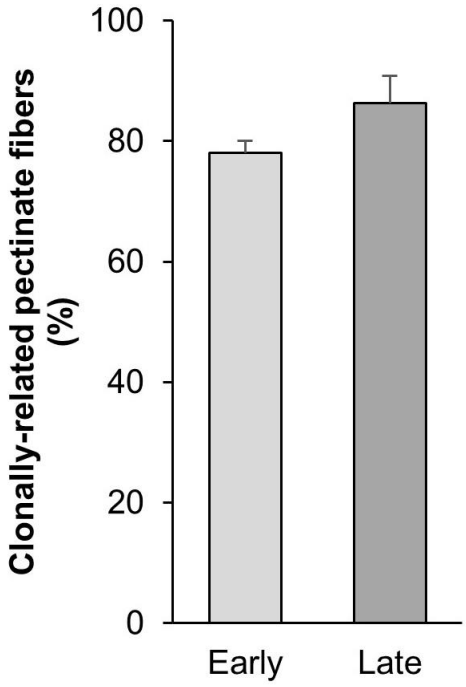



**Fig. S2. The clonal relationship of pectinate fibers with atrial wall cardiomyocytes is consistent after early and late *priZm* recombination.** (**a**) Percentage of pectinate fibers that are clonally related to adjacent atrial wall cardiomyocytes as determined by shared fluorescent reporter expression after recombination of the *priZm* cassette at 3 dpf (Early; n = 11 hearts) or 12 dpf (Late; n = 6 hearts) in larval development. Hearts labeled at 3 dpf were imaged at 42 dpf and hearts labeled at 12 dpf were imaged at 28 dpf.

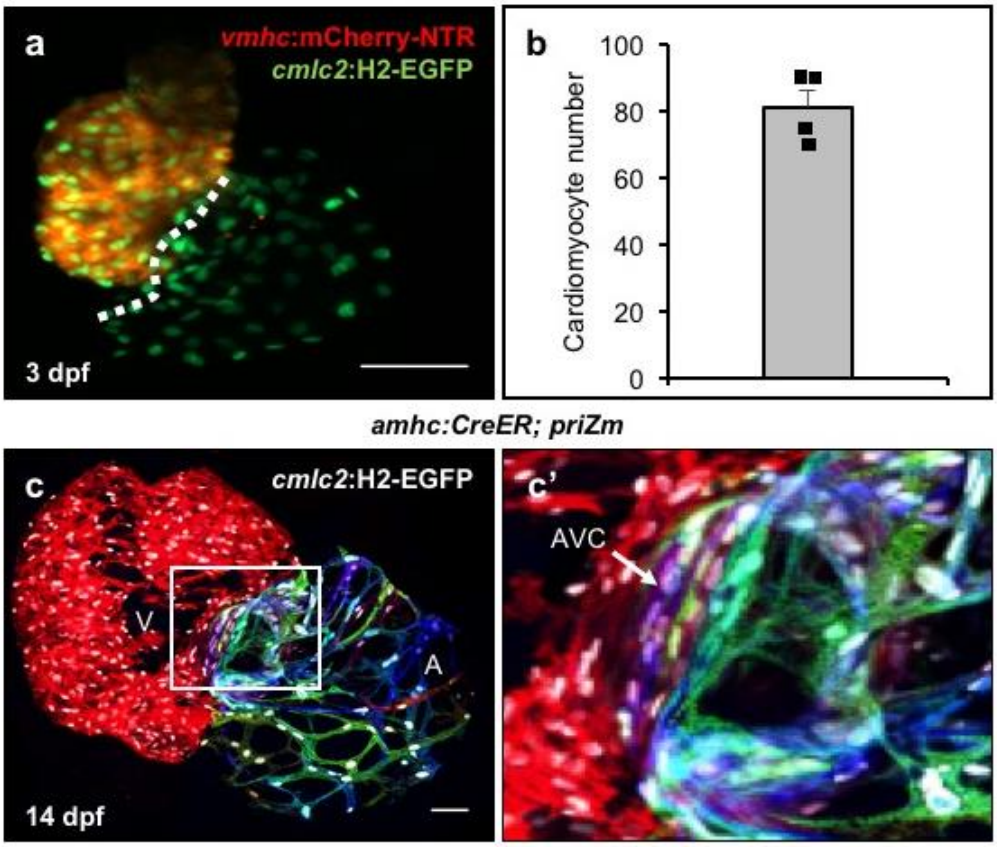



**Fig. S3. Embryonic atrial cardiomyocytes contribute to the atrial wall and atrio-ventricular canal.** (**a**) Maximum intensity projection of a z-stack from a 3 dpf heart expressing *vmhc:mCherry-NTR* and *cmlc2:H2-EGFP*. (**b**) Number of atrial cardiomyocytes in 3 dpf zebrafish hearts (mean +/- SEM, n = 4, with individual data points shown). (**c**) Surface myocardium from a 14 dpf *amhc:CreER; priZm*; *cmlc2:H2-EGFP* heart. An expanded view of the AV canal (AVC), dense with cardiomyocyte nuclei (white), is shown. Scale bars are 50 µm.

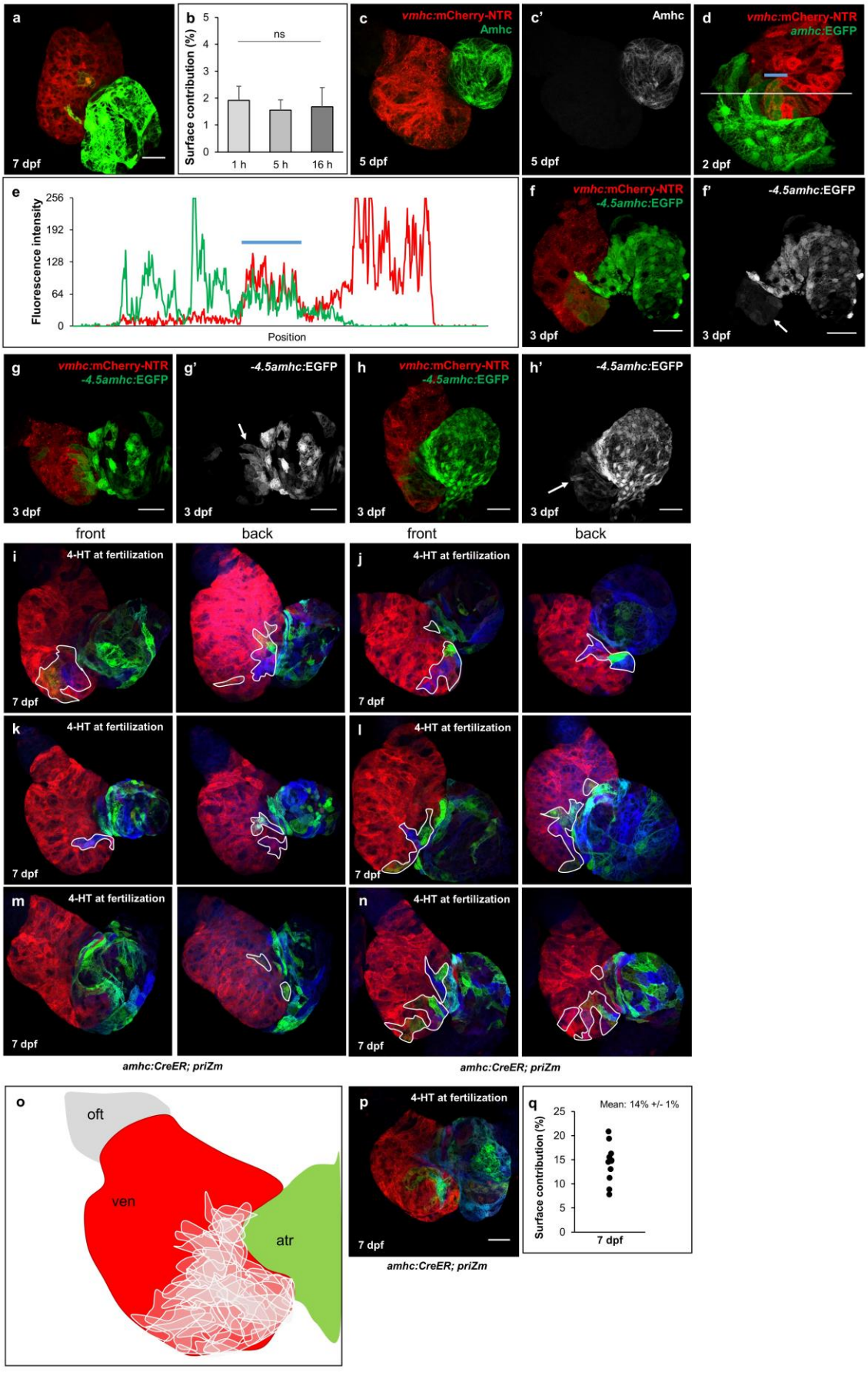
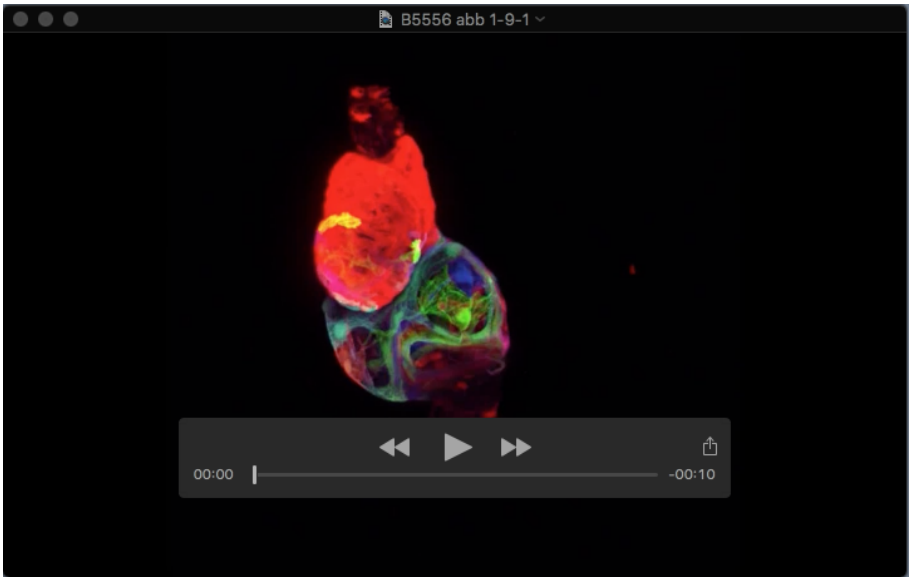
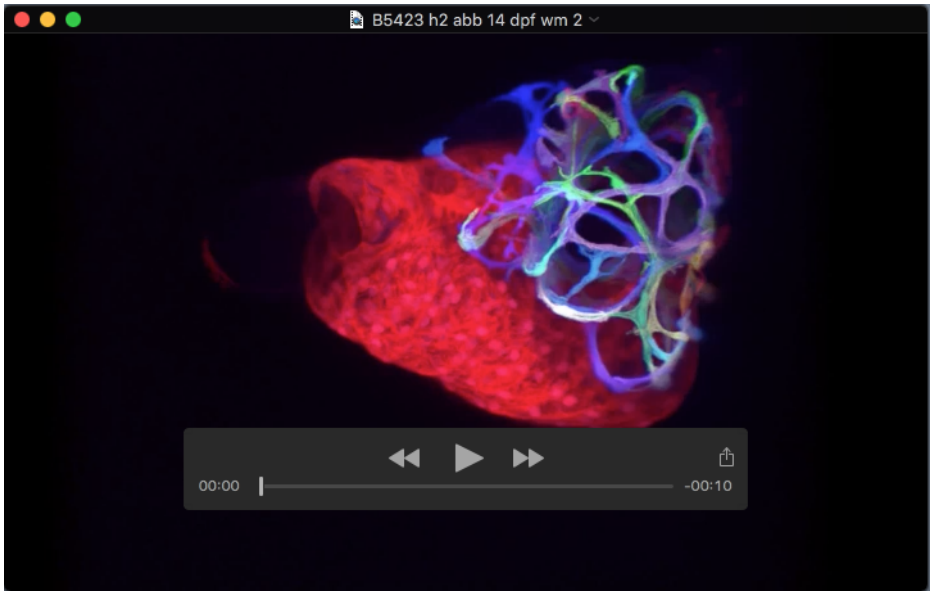


**Fig. S4. *amhc:*CreER-expressing cardiomyocytes are transient and localized to the ventricular apex and atrio-ventricular junction.** (**a**) Surface myocardium of a 7 dpf *amhc:CreER; β-actin2:RSG* heart from an animal treated with 4-HT for 1 hour at 3 dpf, indicating areas of EGFP fluorescence. (**b**) Percentage of the ventricular surface area with EGFP fluorescence in 7 dpf *amhc:CreER; β-actin2:RSG* hearts, after treatment with 4-HT at 3 dpf for 1 hour (n = 11 hearts), 5 hours (n = 14 hearts) or 16 hours (n = 9 hearts) (mean +/- SEM). Differences in the means are not significant (ns) by one-way ANOVA ($p > 0.05$) after arcsine transformation. (**c, c'**) Surface myocardium of a 5 dpf *vmhc:mCherry-NTR* heart stained for Amhc, immunofluorescence, indicating no detectable ventricular Amhc. (**d, e**) Surface myocardium of a 2 dpf *amhc:EGFP; vmhc:mCherry-NTR* heart indicating some *amhc*:EGFP fluorescence in the ventricle. White bar indicates measured region for fluorescent intensity profile. Graph plots mCherry and EGFP fluorescent intensity in arbitrary units from 0-256 with position on x-axis indicating distance along the white line in (D). Blue bar indicates a region of EGFP and mCherry co-expression. Scale bars are 50 μm. (**f-h**) Images of 3 dpf hearts from *vmhc:mCherry-NTR* animals injected immediately after fertilization with a linearized plasmid containing an *EGFP* sequence preceded by a 4.5 kb region of the *amhc* promoter. Arrows indicate regions of EGFP fluorescence in the ventricle. Scale bars are 50 μm. (**i-n**) Surface myocardium of 7 dpf hearts (front and back) from *amhc:CreER; priZm* animals treated with 4-HT for 24 hours immediately after fertilization. Non-red cardiomyocytes in the ventricle are outlined in white. (**o**) Cartoon of generic 7 dpf ventricle overlaid with outlines from (C-H) to demonstrate localization of labeled ventricular cardiomyocytes. (**p**) Surface myocardium of a 7 dpf heart from an *amhc:CreER; priZm* animal treated with 4-HT for 24 hours immediately after fertilization. Scale bar is 50 μm. (**q**) Percentage of the ventricular surface area expressing non-red fluorescent proteins at 7 dpf when treated with 4-HT after fertilization (mean +/- SEM, n = 11 hearts).

## SUPPLEMENTAL VIDEOS




**Movie 1. 3D reconstruction of a heart from an *amhc:CreER; priZm* animal treated with 4-HT at 3 dpf and collected at 7 dpf.**




**Movie 2. 3D reconstruction of a heart from an *amhc:CreER; priZm* animal treated with 4-HT at 3 dpf and collected at 14 dpf.**