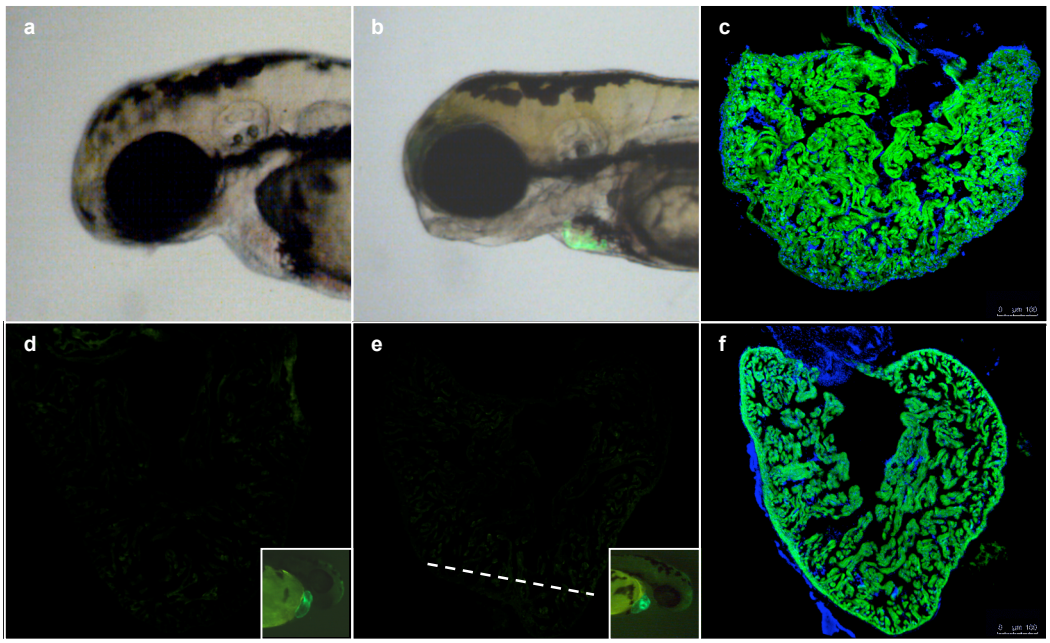
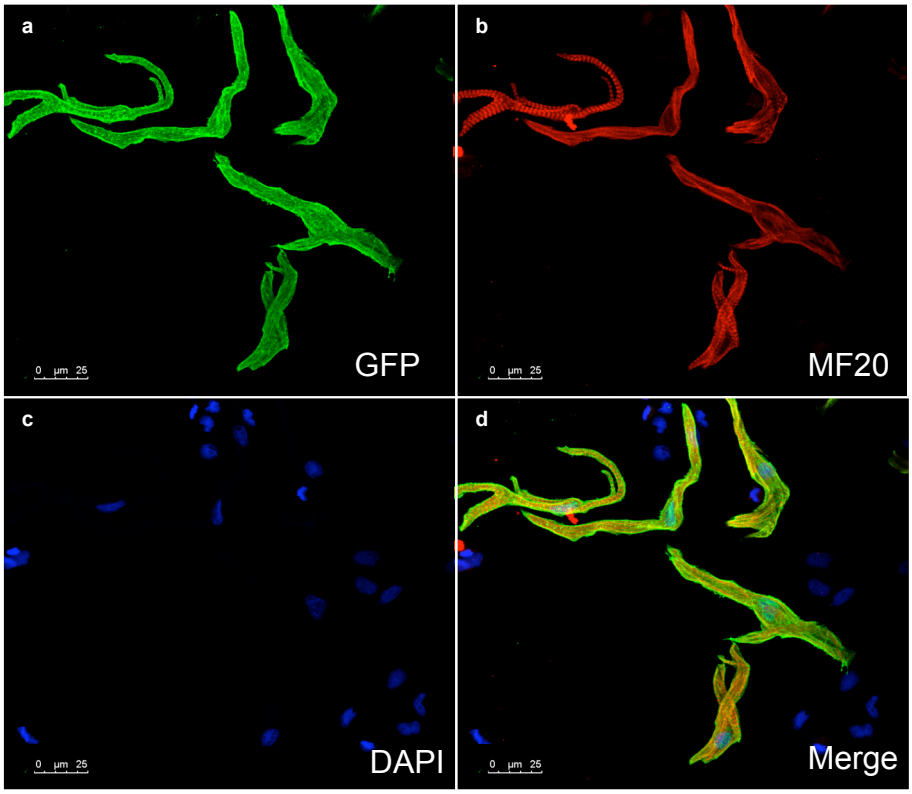
Supp fig.1



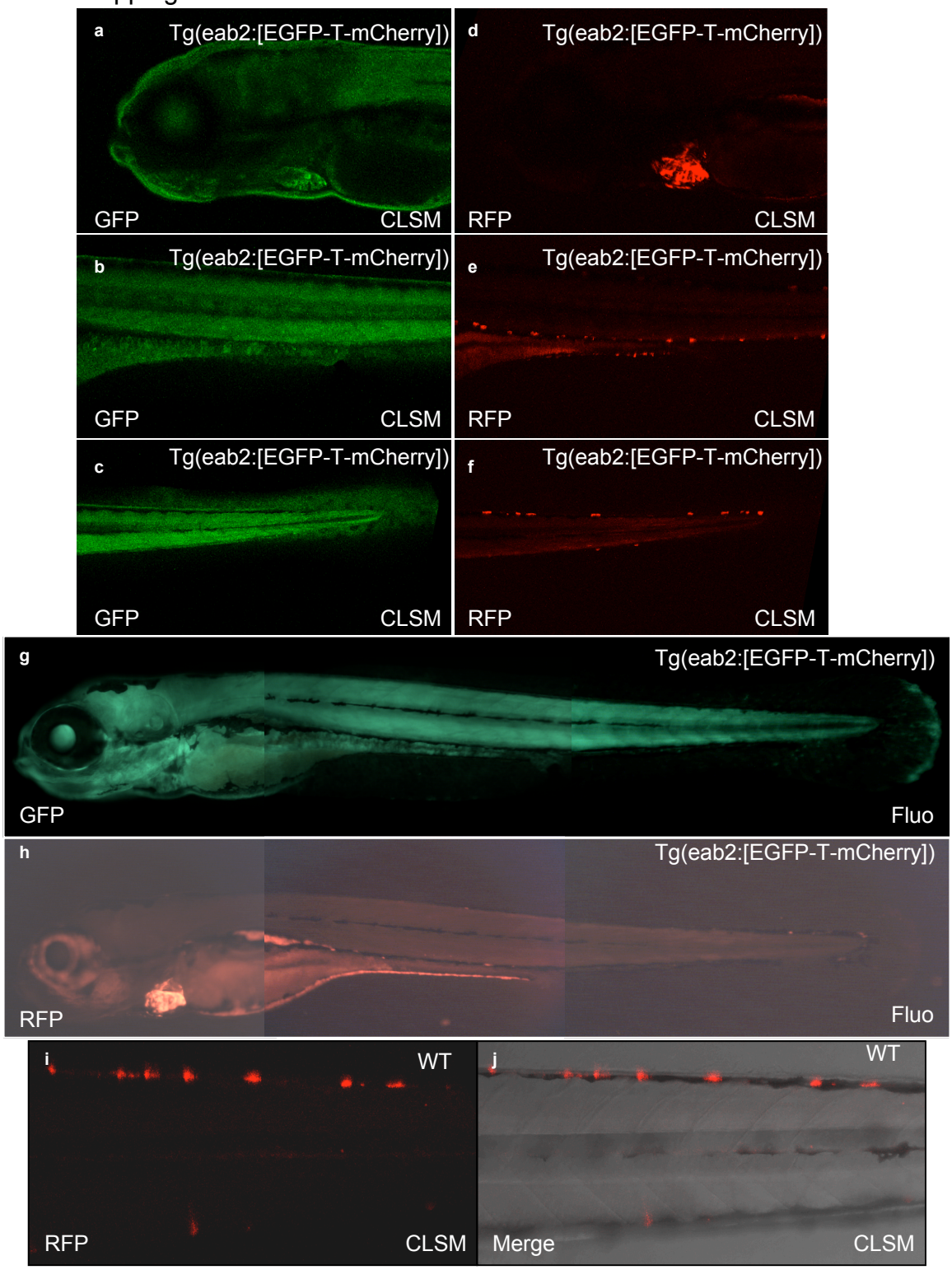
Supplementary fig.1. Cre/tamoxifen in zebrafish. (A) 48hpf embryo before 4-OHT treatment. (B) The same embryo after 24hrs 4-OHT. (C) Heart taken from a 3mnth tg-cmlc2a-Cre-Ert2: tg-cmlc2a-LnL-GFP zebrafish treated with 4-OHT at 48hpf. (D) An untreated 3mnth tg-cmlc2a-Cre-Ert2: tg-cmlc2a-LnL-GFP zebrafish heart, inset shows a treated embryo from an outcross of the adult fish. (E) An untreated 3mnth tg-cmlc2a-Cre-Ert2: tg-cmlc2a-LnL-GFP zebrafish heart 7dpa, inset shows a treated embryo from an outcross of the adult fish. (F) A 3mnth tg-cmlc2a-Cre-Ert2: tg-cmlc2a-LnL-GFP zebrafish heart taken from an adult treated with 4-OHT.

Supp fig.2



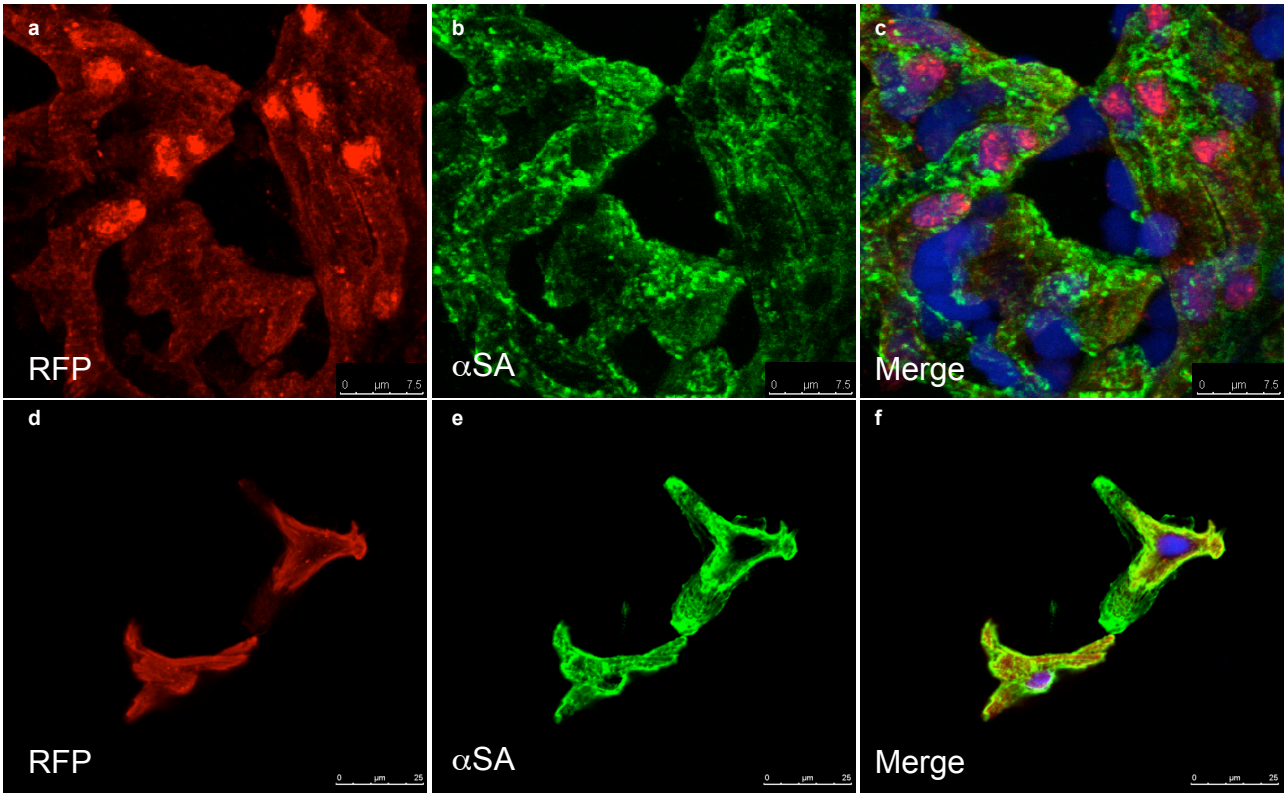
Supplementary fig.2. GFP expression is restricted to cardiomyocytes.(A-D) Dissociated GFPpos cardiomyocytes (from tg-cmlc2a-Cre-Ert2: tg-cmlc2a-LnL-GFP) in culture immunolabelled for GFP(A) and MF20 (B).

Supp fig.3



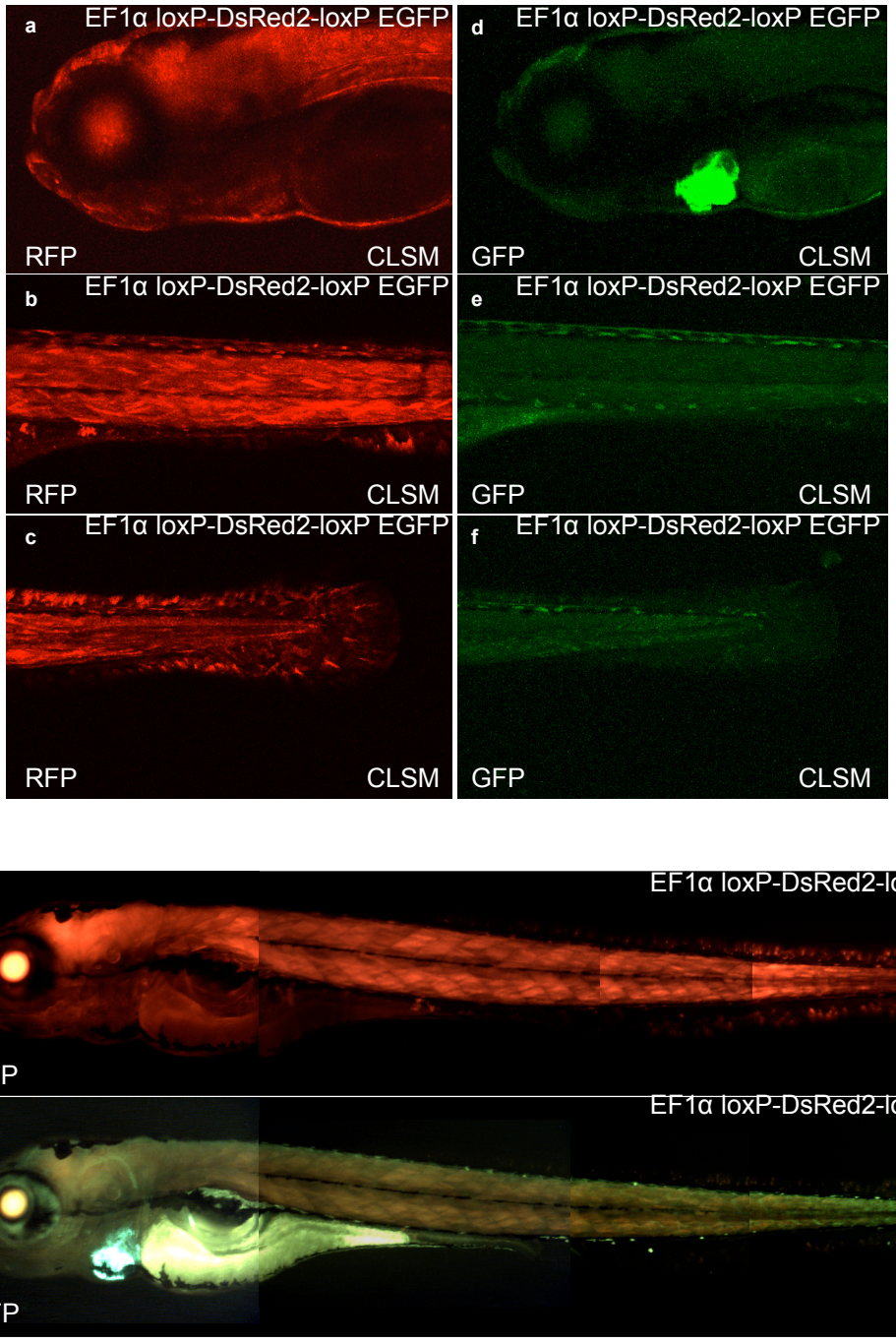
Supplementary fig.3. Cre expression is restricted to the heart.(A-F) Confocal images (maximum projection z-stack) of a 4-OHT treated embryo taken from a cross between tg-cmlc2a-Cre-Ert2: tg-cmlc2a-LnL-GFP and Tg(eab2:[EGFP-T-mCherry]) (red cells along the top and bottom of the tail in E,F and H are due to xanthophore/iridophore autofluorescence. Similar autofluoresence is detected in non transgenic wildtype embryos using confocal microscopy I,J). (G,H) Fluorescent images of a 4-OHT treated embryo taken from a cross between tg-cmlc2a-Cre-Ert2: tg-cmlc2a-LnL-GFP and Tg(eab2:[EGFP-T-mCherry]). (H) has been overexposed.

Supp fig.4



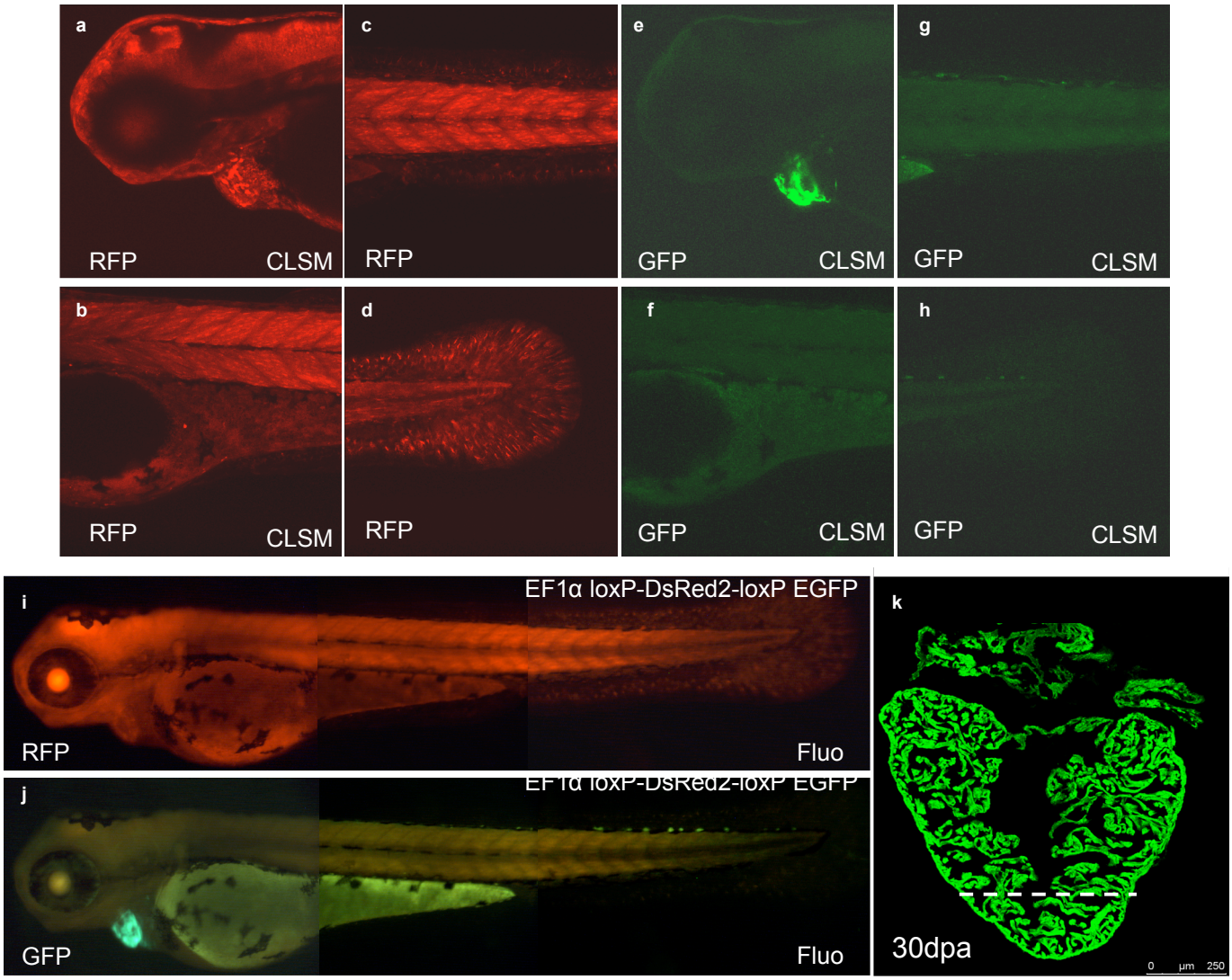
Supplementary fig.4. Cre expression is restricted to cardiomyocytes.(A-C) Confocal images of a heart taken from a tg-cmlc2a-Cre-Ert2/Tg(eab2:[EGFP-T-mCherry]) 4-OHT treated 2 month old zebrafish labelled with (A) anti-RFP (RFP) (red) (to label mCherry expressing cells) and (B) anti-a sarcomeric actin (aSA) (green) (to label cardiomyocytes). (C) Merged image shows mCherry^{pos} cells are also a sarcomeric actin positive (n=1564 cells from 4 different hearts). (D-F) Dissociated cardiomyocytes from tg-cmlc2a-Cre-Ert2/Tg(eab2:[EGFP-T-mCherry]) 4-OHT treated 2 month old zebrafish in culture, immunolabelled for (D) anti-RFP (RFP) (red) and (E) a sarcomeric actin (aSA) (green).(F) Merged image shows mCherrypos cells are also a sarcomeric actin positive (n=843).

Supp fig.5



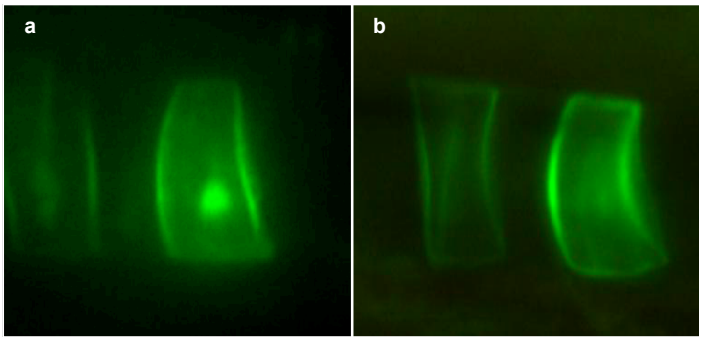
Supplementary fig.5. Cre expression is restricted to the heart.(A-E) Confocal images (maximum projection z-stack) of a 4-OHT treated embryo taken from a cross between tg-cmlc2a-Cre-Ert2: tg-cmlc2a-LnL-GFP and EF1α loxP-DsRed2-loxP EGFP (green cells along the top and bottom of the tail in E,F and H are due to xanthophore/iridophore autofluorescence).(G,H) Fluorescent images of a 4-OHT treated embryo taken from a cross between tg-cmlc2a-Cre-Ert2: tg-cmlc2a-LnL-GFP and EF1α loxP-DsRed2-loxP EGFP. (H) has been overexposed.

Supp fig.6



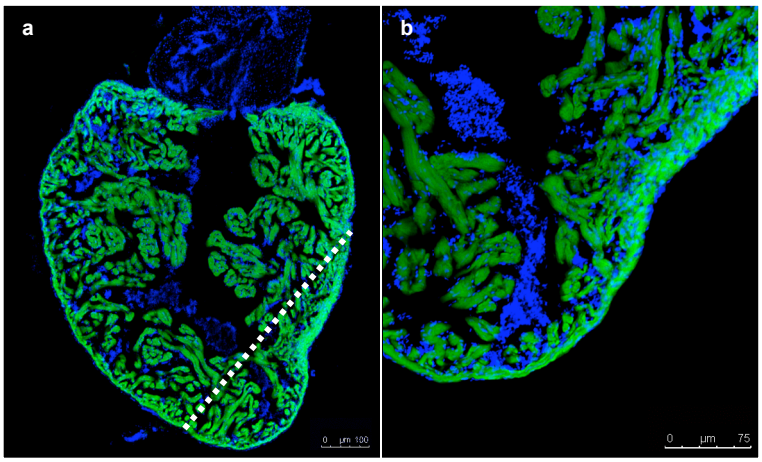
Supplementary fig.6. Cre expression is restricted to the heart in a 2nd cmlc2a-Cre-Ert2 line .(A-H) Confocal images (maximum projection z-stack) of a 4-OHT treated embryo taken from a cross between tg-cmlc2a-Cre-Ert2: tg-cmlc2a-LnL-GFP and EF1α loxP-DsRed2-loxP EGFP (green cells along the top and bottom of the tail in E,F and H are due to xanthophore/iridophore autofluorescence).(I,J) Fluorescent images of a 4-OHT treated embryo taken from a cross between tg-cmlc2a-Cre-Ert2: tg-cmlc2a-LnL-GFP and EF1α loxP-DsRed2-loxP EGFP. (H) has been overexposed.(K) A 30 dpa regenerating heart taken from a 2nd cmlc2a-Cre-Ert2: tg-cmlc2a-LnL-GFP line, the dashed white line indicates the plane of amputation.

Supp.fig.7



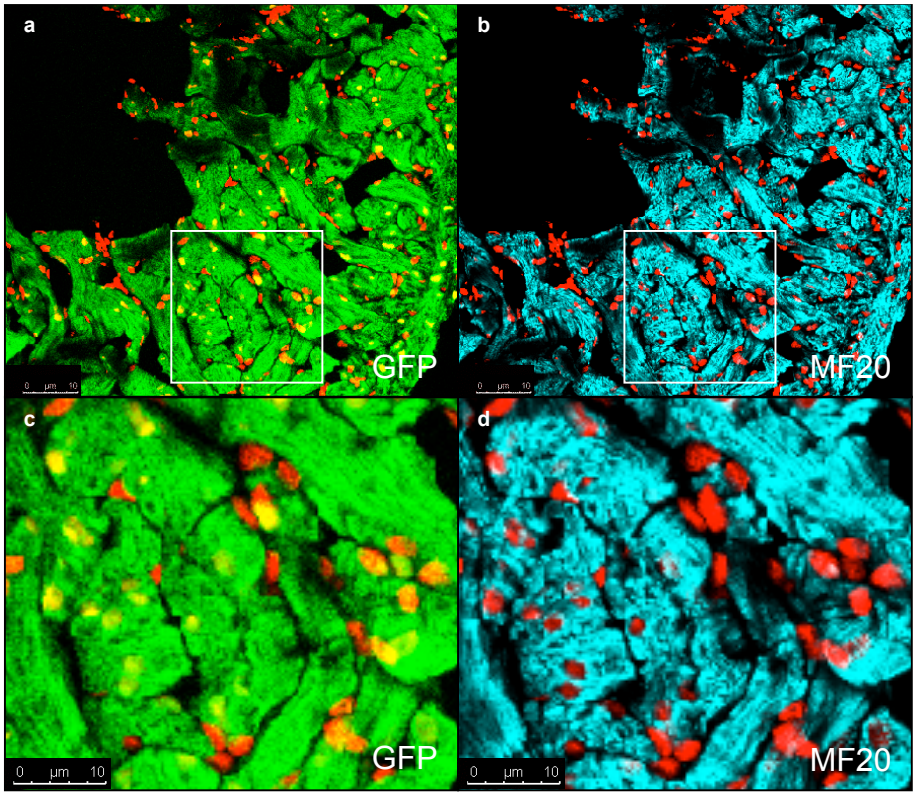
Supplementary fig.7.Ert2-Cre-Ert2 is not retained in the nucleus following removal of 4-OHT. (A) A columnar cell in the notochord of a 48hpf Ert-GFP expressing embryos treated with 4-OHT for 24hrs. (B) The same cell after 3days wash, note that GFP is no longer concentrated in the nucleus.

Supp fig.8



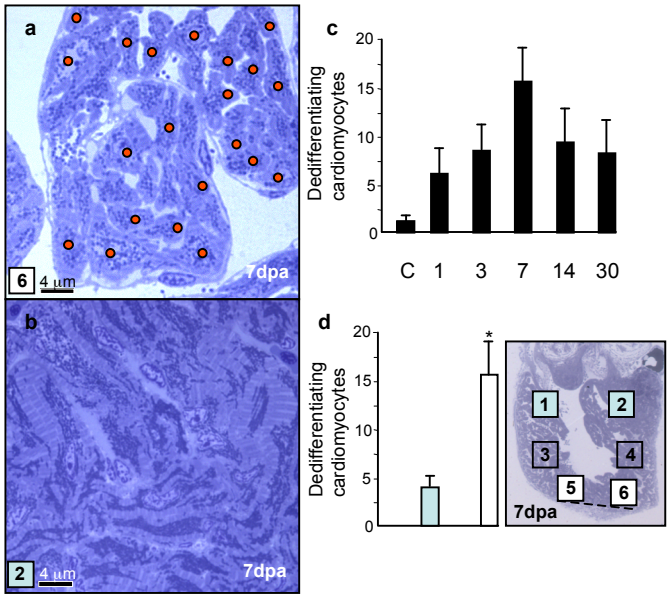
Supplementary fig.8. No difference in cardiomyocyte labelling when inducing Cre activity at embryonic or adult stages. Non-recombined adults were treated with 4-OHT to induce Cre activity. Heart amputations were carried out after a one week wash period. Subsequently, regeneration was allowed to proceed for 30 days before collection. (A) 30dpa heart, the dashed line indicates the plane of amputation, all the regenerated tissue below is clearly GFP^{pos} . (B) Higher magnification.

Supp fig.9



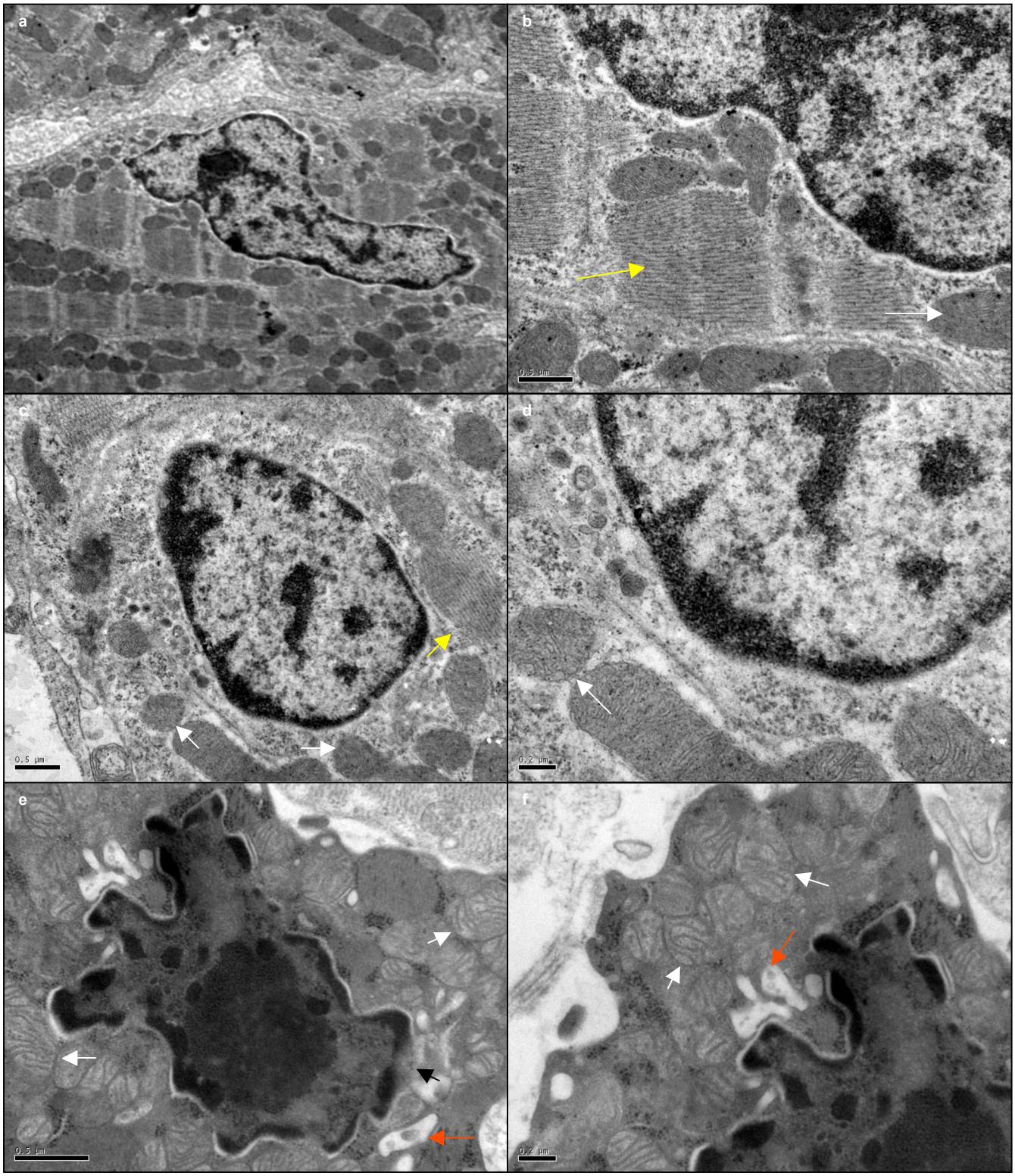
Supplementary fig.9. All regenerated cardiomyocytes derive from differentiated GFP^{pos} cardiomyocytes. (A,C) A 30dpa heart from a tg-cmlc2a-Cre-Ert2: tg-cmlc2a-LnL-GFP zebrafish immuno labelled with anti-GFP. (B,D) The same heart immuno labelled with anti-MF20. Total cardiomyocytes counted=4836 (3 time points were analysed 7,14 and 30 dpa. For each time point 2 different hearts where analysed. For each heart 2 sections were analysed).

Supp fig.10



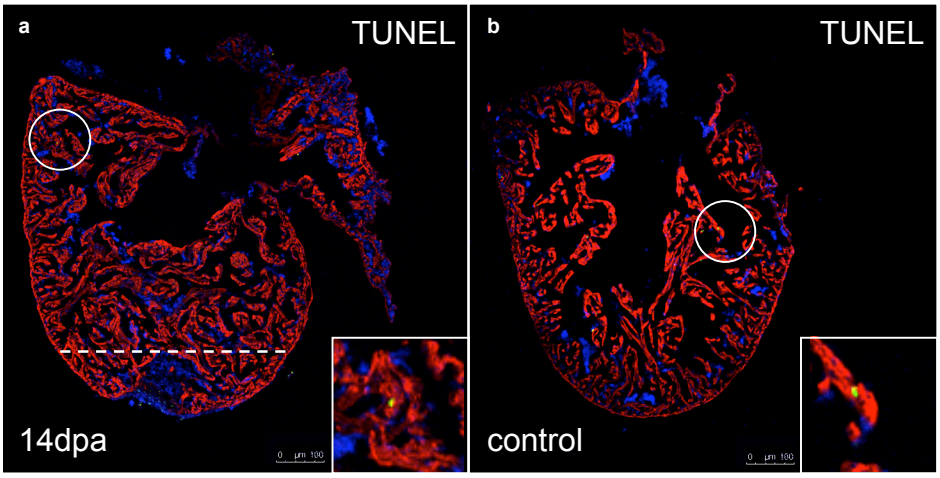
Supplementary fig.10. Structurally/morphologically altered cardiomyoctes during regeneration. (A and D inset) Structurally/morphologically altered cardiomyoctes adjacent to the wound at 7dpa. Red dots indicate counted cells. (B and D inset) Structurally/morphologically normal cardiomyoctes not adjacent to the wound. (C) Indicates the average number of structurally/morphologically altered cardiomyoctes at the given time points (the average was calculated from cell counts taken from positions 1-6 (D.inset). (D and inset) Indicates the position of structurally/morphologically altered cardiomyoctes (the average was calculated from cell counts taken from positions 1+2 (blue) and 5+6 (white))

Supp fig.11



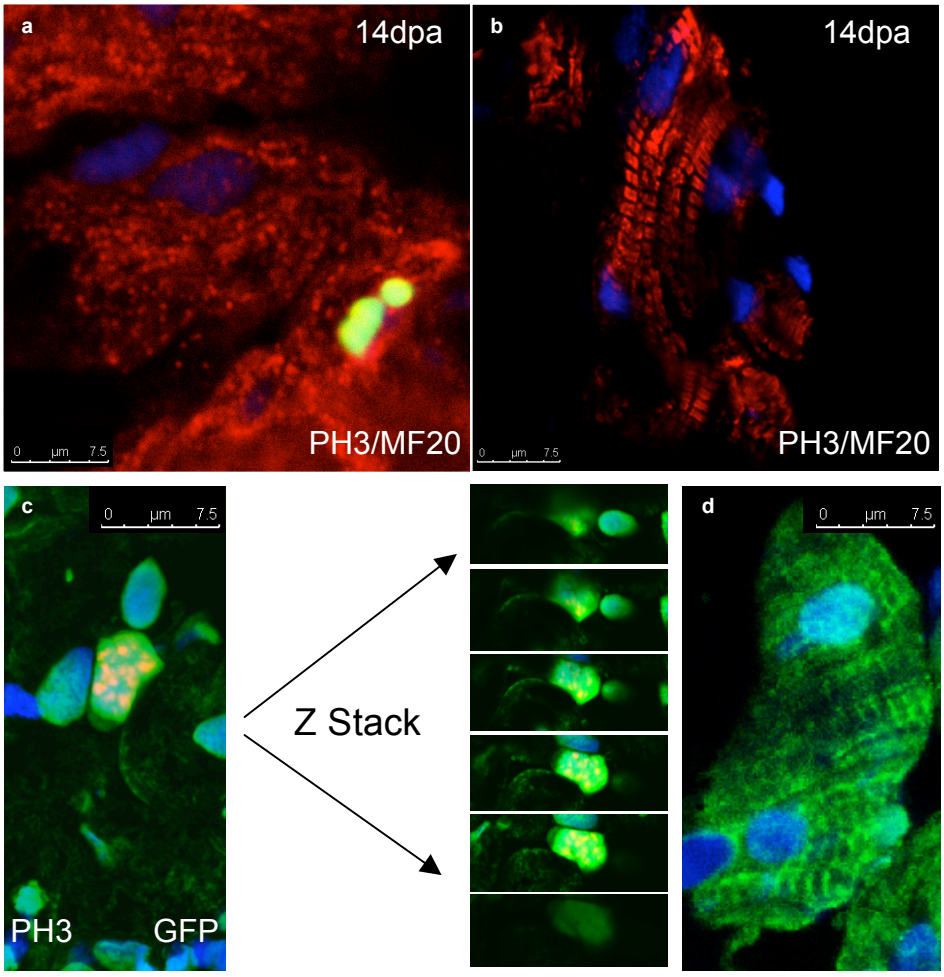
Supplementary fig.11. Ultrastructure of structurally/morphologically altered cardiomyoctes. (A,B) A normal cardiomyocyte. White arrow indicates mitochondria, yellow arrow indicates sarcomere. (C,D) A structurally/morphologically altered cardiomyocte. White arrow indicates mitochondria, yellow arrow indicates sarcomere. (E,F) A dying cardiomyocyte. White arrow indicates mitochondria, red arrow indicates autophagic vacuoles, black arrow indicates disrupted nuclear membrane.

Supp.fig.12



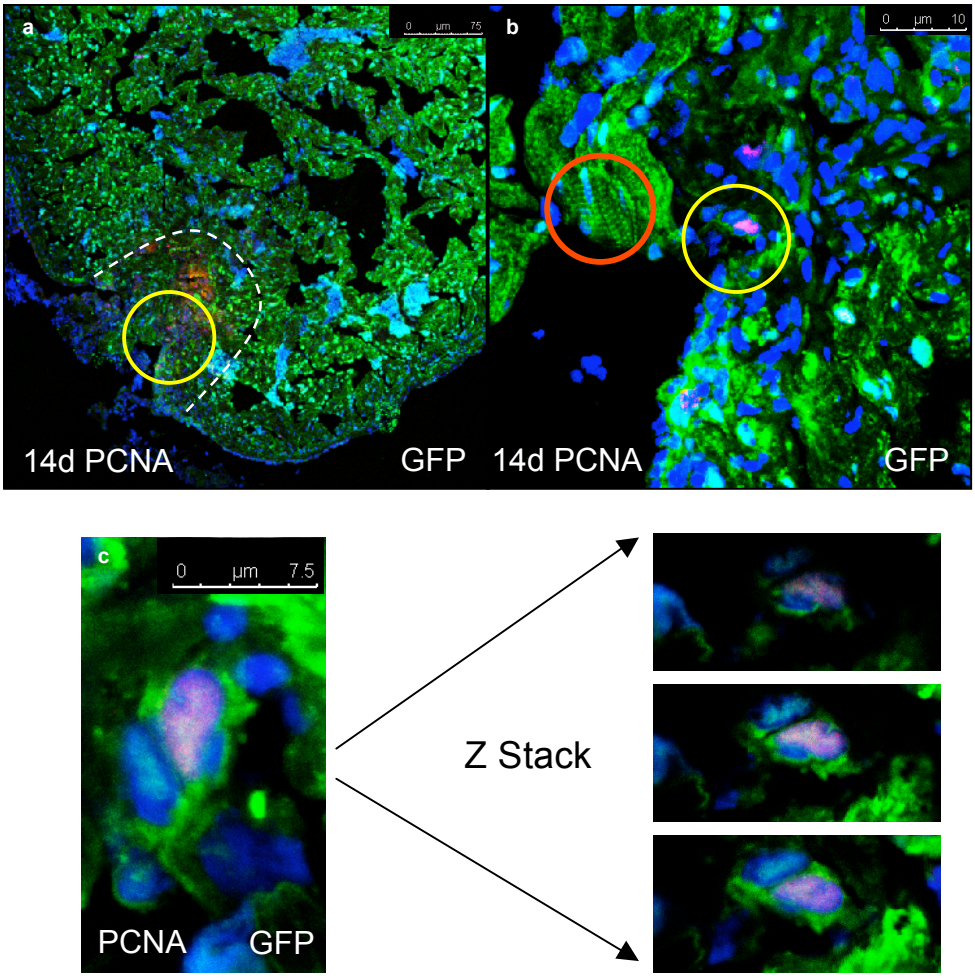
Supplementary fig.12. No increase in apoptosis during regeneration. (A) A 14dpa regenerating TUNEL (green)/MF20 (red) labelled heart. The white dashed line indicates the plane of amputation. The white circle/inset highlights a TUNEL positive cardiomyocyte. We found on average one apoptotic cardiomyocyte per section (n=9 sections from 3 hearts). (B) A non amputated TUNEL/MF20 labelled heart. The white circle/inset highlights a TUNEL positive cardiomyocyte. We found no increase in the amount of cardiomyocytes undergoing apoptosis (n=14 sections from 5 hearts)

Supp.fig.13



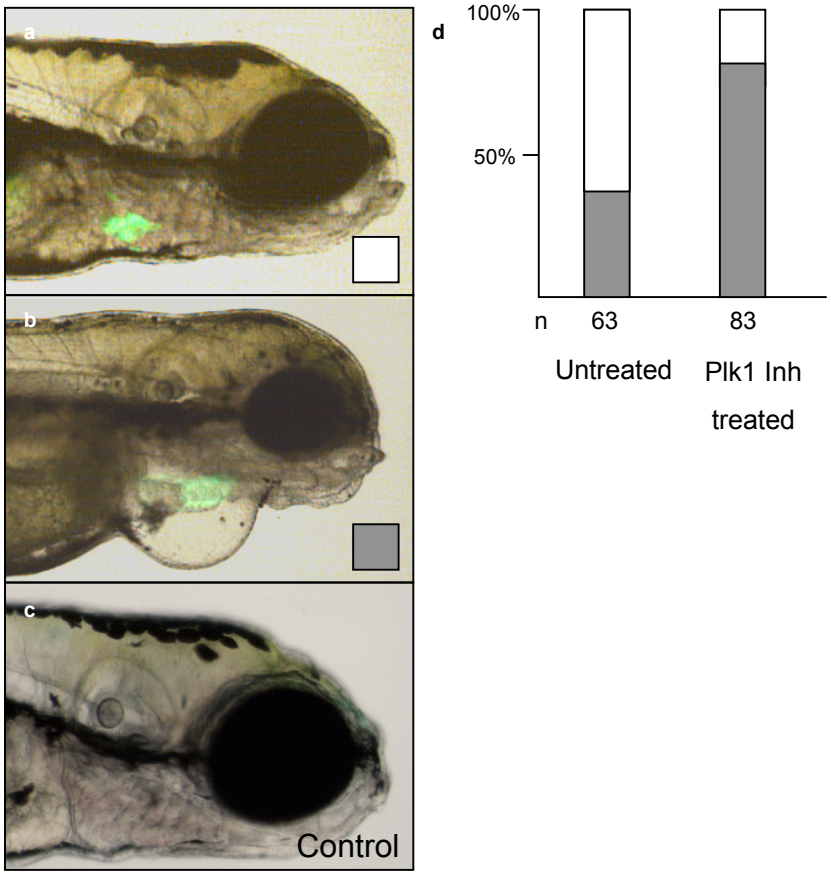
Supplementary fig.13. Cardiomyocytes undergoing cell division do not display any organised sarcomeric structure. (A) A 14dpa regenerating heart labelled with anti-P3H (green) and anti-MF20(red). (B) A control area from the same heart labelled with anti-P3H (green) and anti-MF20 (red), note the organised sarcomeric structure.(C) A 14dpa regenerating heart labelled with anti-P3H (red) and anti-GFP(green).(D) A control area from the same heart, note the organised sarcomeric structure.

Supp.fig.14



Supplementary fig.14. Cardiomyocytes undergoing cell division do not display any organised sarcomeric structure. (A-C) A 14dpa regenerating heart labelled with anti-PCNA (red) and anti-GFP(green). White dashed line indicates the regenerating area, yellow circle indicates the region of interest and red circle indicates a cardiomyocyte with organised sarcomeric structure.

Supp fig.15



Supplementary fig.15. *Plk1* is necessary for cardiac regeneration.
Heart regeneration following MTZ induced cardiomyocyte ablation was scored as, regenerated (indistinguishable from an untreated embryo)(A) or no regeneration (any visible cardiac defect and associated oedema) (B) at 96hpf.(C) Shows a wildtype embryo treated with MTZ and cyclapolin9.
(D) Embryos in which cardiomyocytes had been ablated were either allowed to recover in the absence (untreated) or presence of a Plk1 inhibitor (Plk1 Inh treated). Grey bar indicates the percentage of embryos with a defective/non-regenerated heart and the white bar indicates the percentage of embryos with a normal heart.