Supplementary Information for

Cardiac injury of the newborn mammalian heart accelerates cardiomyocyte terminal differentiation

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Supplementary Figure Legends

Supplementary Figure 1: Effect of AR on nuclear localization of pericentrin. (**a**, **b**) Representative images of cryosections of P3 rat heart ventricles. red/cyan: cardiomyocytes (nuclei: Nkx2.5; cytoplasmic membrane: caveolin 3), green: pericentrin (pericentriolar matrix/centrosome), blue: nuclei (DAPI). Dotted circle: vessel. Arrows: examples of cardiomyocytes with pericentrin localized to the nucleus. Scale bars: a: 25 μm, b: 10 μm. (**a**) Note: 1: Non-myocyte with pericentrin localized to its centrosome (asterisk). 2: Cardiomoyocyte with pericentrin localized to its centrosome. 3: Cardiomyocyte with pericentrin localized to the nucleus (arrow). (**c**) Quantitative analysis of cardiomyocytes proximal (within 1 mm) to base or apex/resection with nuclear pericentrin signal from cryosections of P3 or P6 (SHAM and AR) rat hearts. Data are ± SD. p-values were calculated using two-tailed Student's t-test.

Supplementary Figure 2: Gross morphology of AR mouse hearts. Representative stereomicroscopic images of SHAM- and AR-operated mouse hearts at different postnatal time points. Arrow heads indicate myocardial scar. Scale bars: 1 mm.

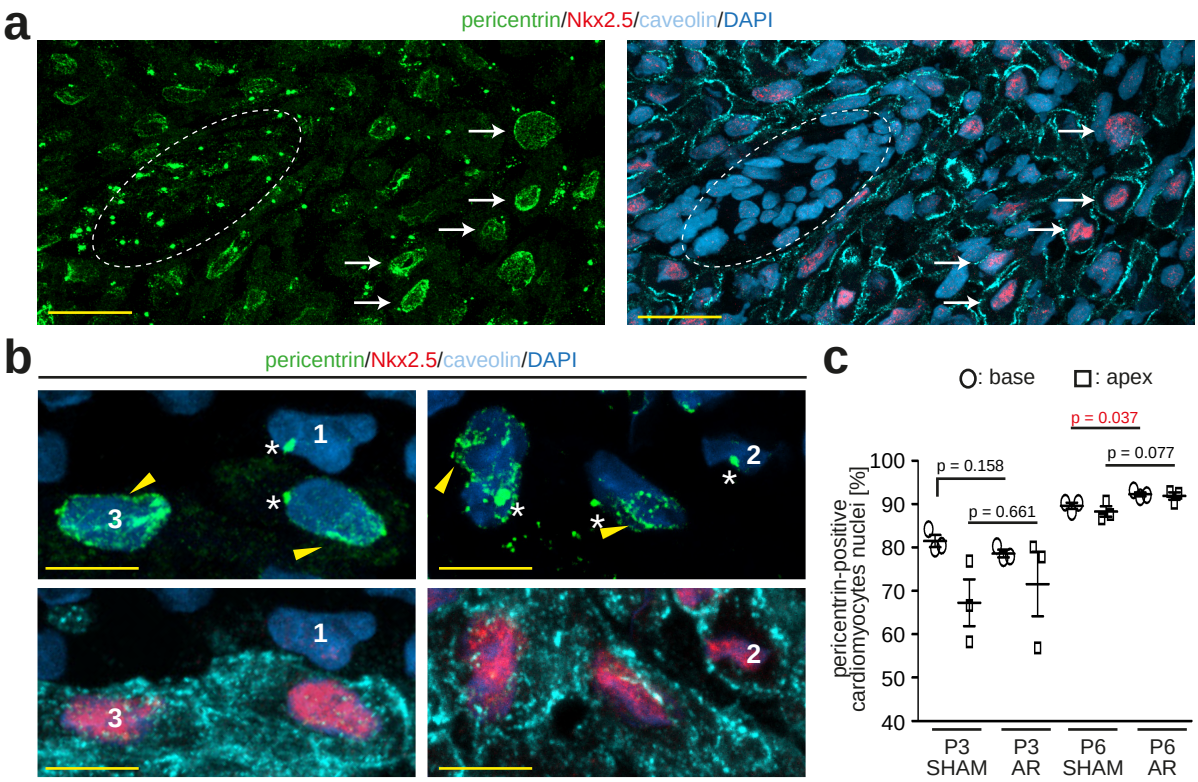
Supplementary Figure 3: Analysis of H3P-positive cardiomyocytes in AR rat hearts. Representative immunofluorescence images of sham- and AR-operated P6 rat hearts (scale bars: 1 mm). Red: cardiomyocytes (sarcomeric-α-actinin); green: mitotic cells (H3P); blue: nuclei (DAPI).

Supplementary Figure 4: Characterization of P1 cardiomyocyte cytokinesis *in vivo*. Representative images of rat heart sections stained for cytokinesis proteins Aurora B (purple) and Anillin (green) and cardiomyocytes (Troponin I, red). Nuclei were visualized with DAPI (blue). (**a**) Representative images of P1 SHAM rat cardiomyocytes exhibiting Anillin patterns

associated with "normal" cytokinesis. (**b**) Representative images of P1 SHAM rat cardiomyocytes exhibiting Anillin patterns associated with "abnormal" cytokinesis. Scale bars: 10 µm.

Supplementary Figure 5: Hypothetical model for the amount of H3P events to support proliferation along-side of binucleation after AR. (**a**) SHAM, binucleation with no proliferation. Experimental data: binucleation at P5 = 20%. The number of H3P events at P3 = 1-fold. (**b**) AR, binucleation with no proliferation. Experimental data: binucleation at P5 = 30%. The number of H3P events at P3 = 2-fold. (**c**, **d**) Assumption: all (c) or the additional (d) H3P events after AR indicate proliferation. This would result in a decrease in the binucleation rate which has not been observed. (**e**) Any significant increase in proliferation induced by AR requires a significant increase in binucleation and thus a higher fold-increase in H3P-positive cardiomyocytes than observed. P: postnatal day. Dashed arrows: no cell cycle activity from P3 to P5. Green arrows: mitosis (i.e. H3P event) from P3 to P5. Note, it is known that H3P events post-P3 are strictly followed by binucleation during normal (i.e. SHAM) neonatal heart development.

Zebrowski DC *et al.*, Figure S1



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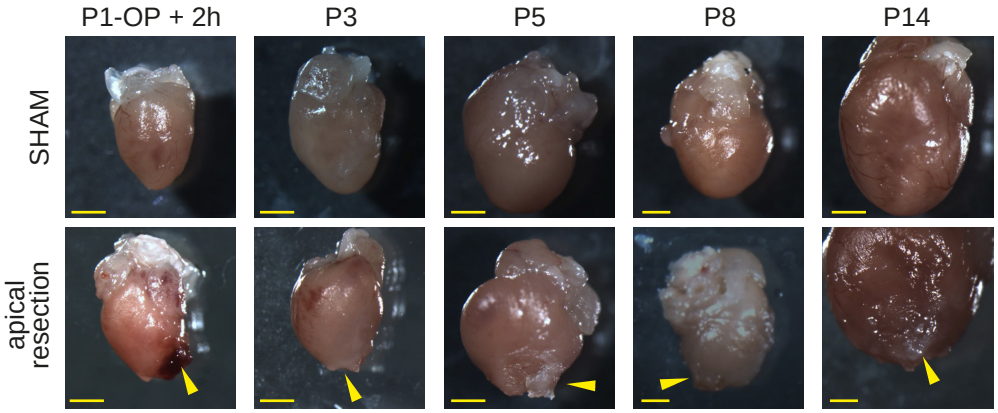

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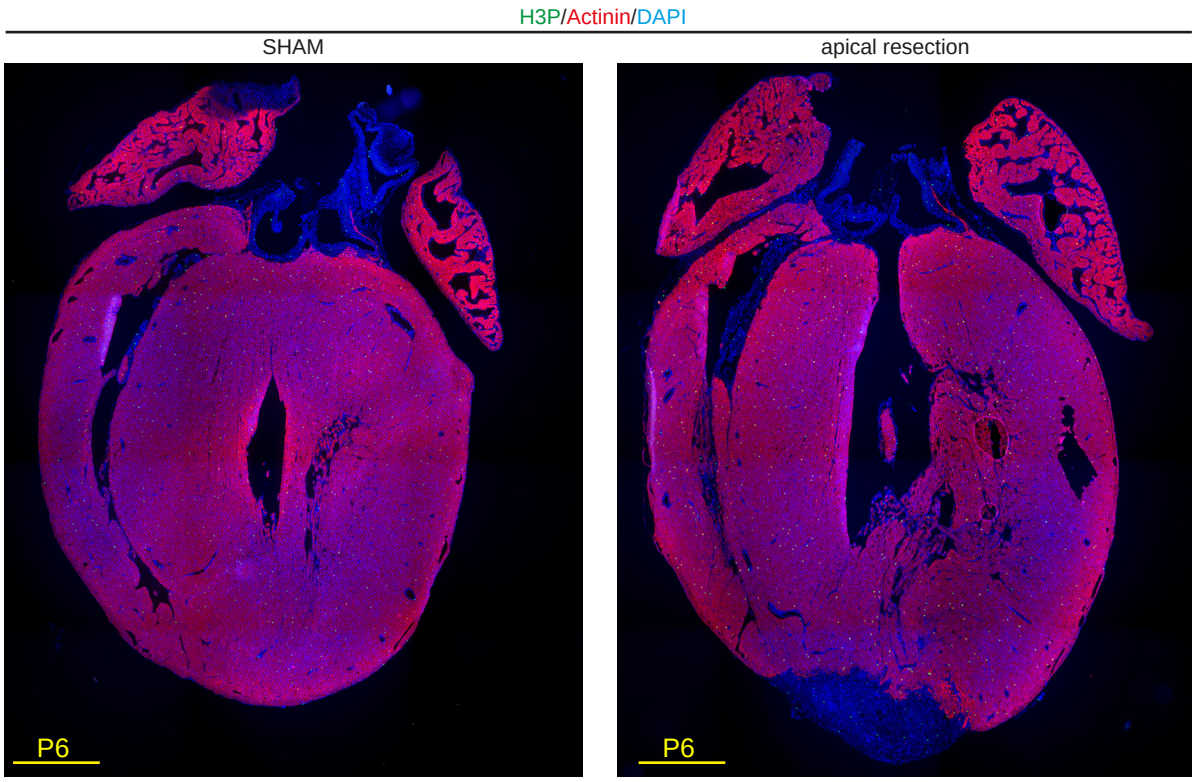

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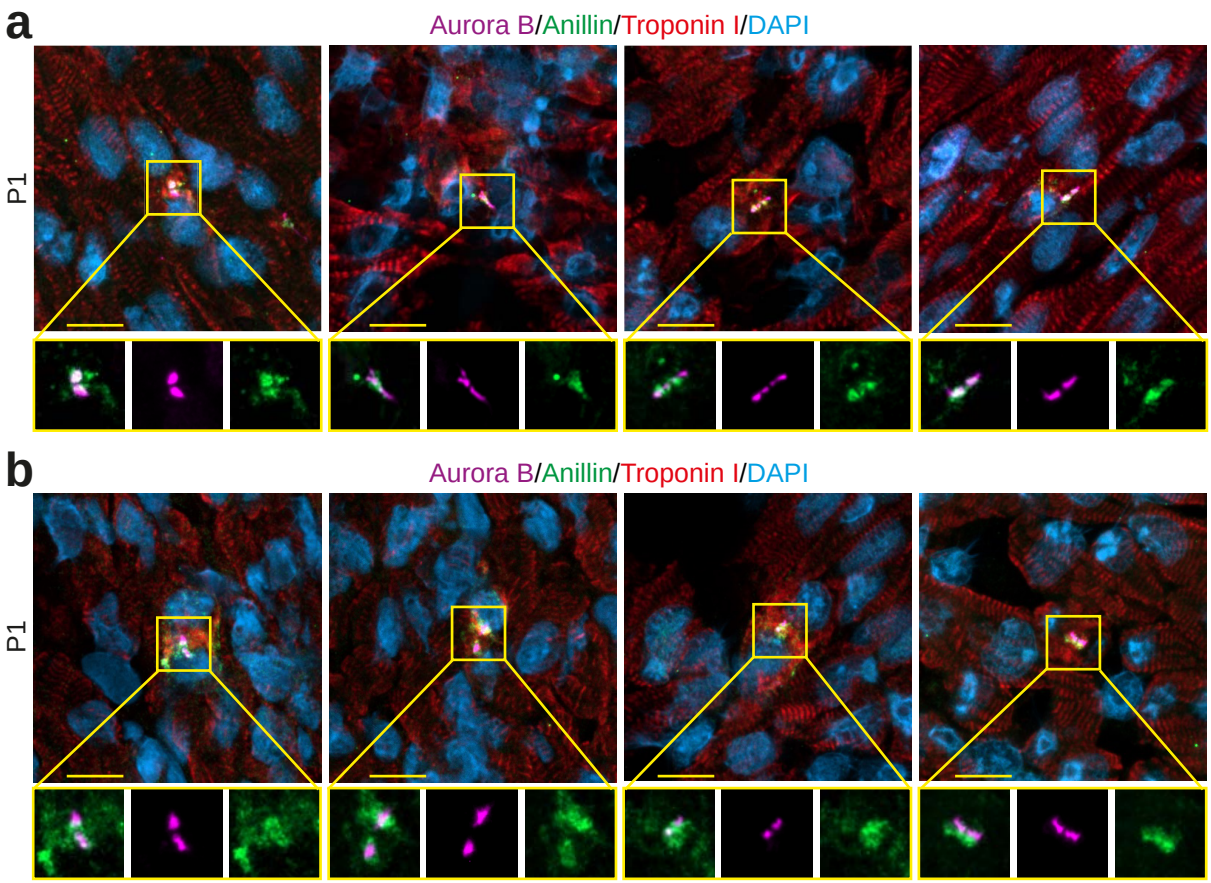
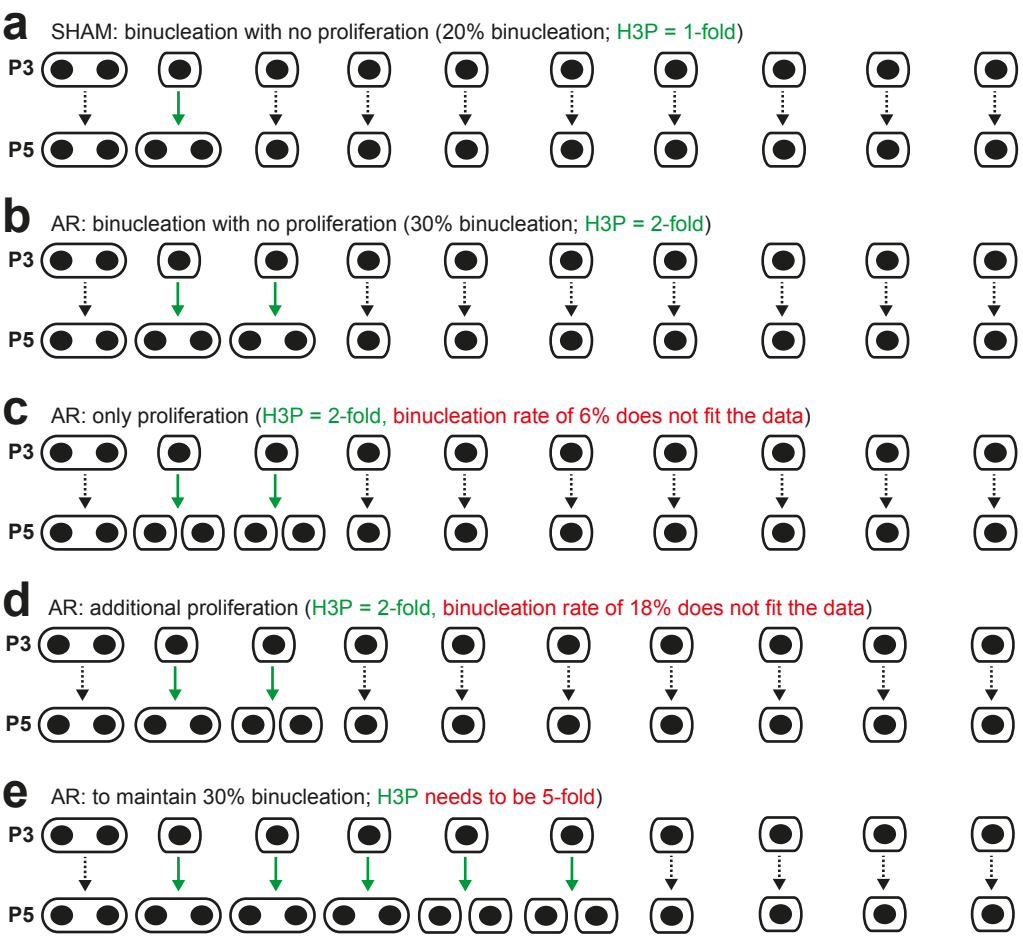
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