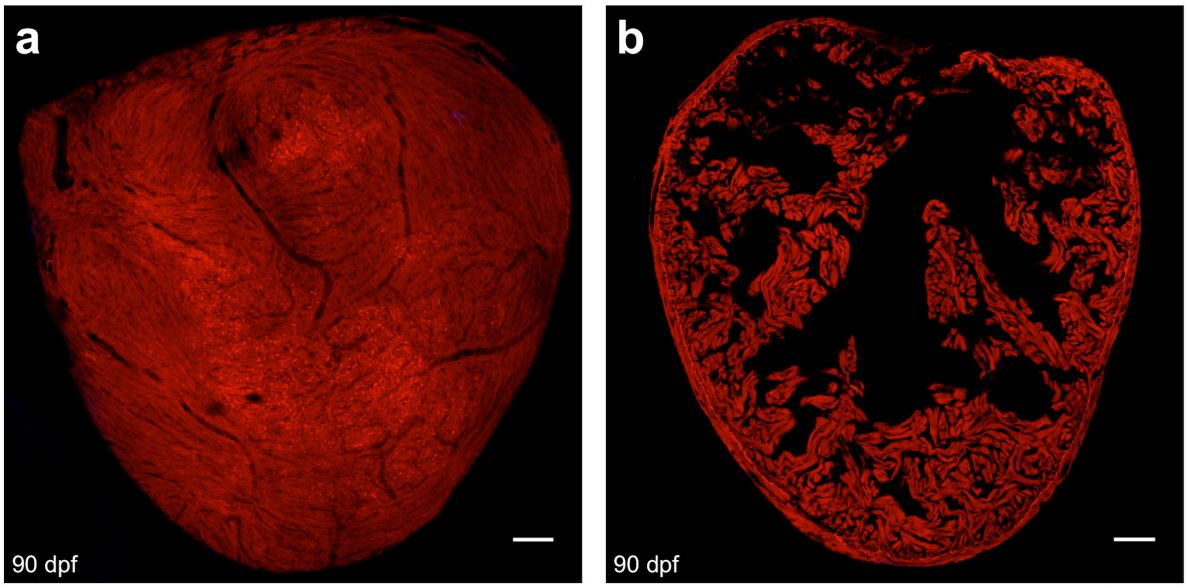
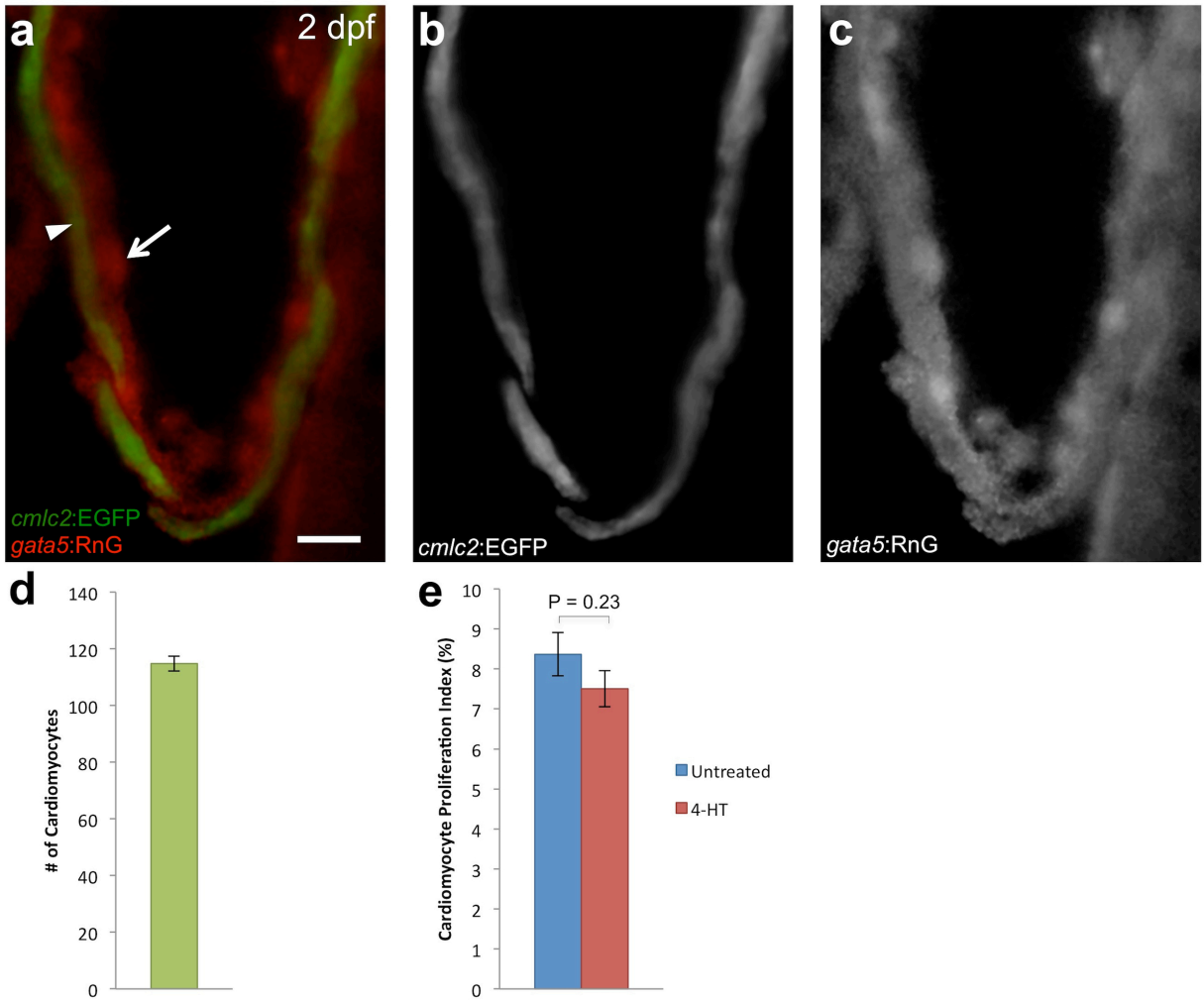
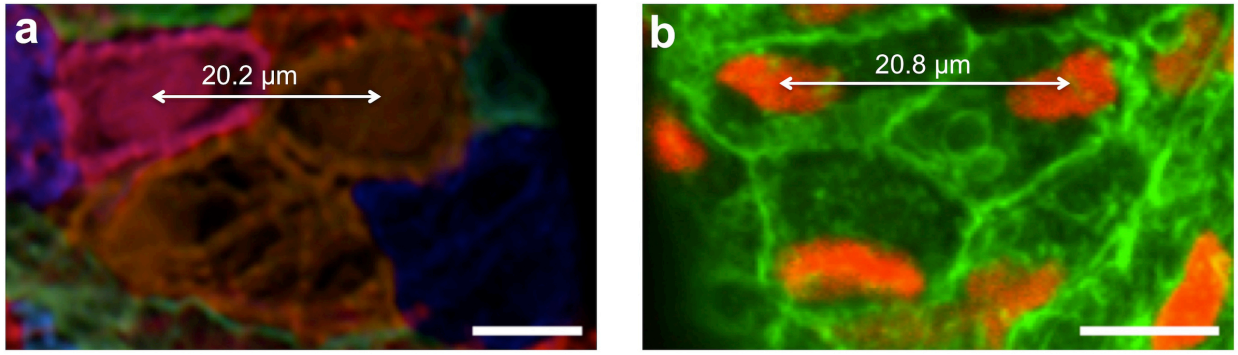


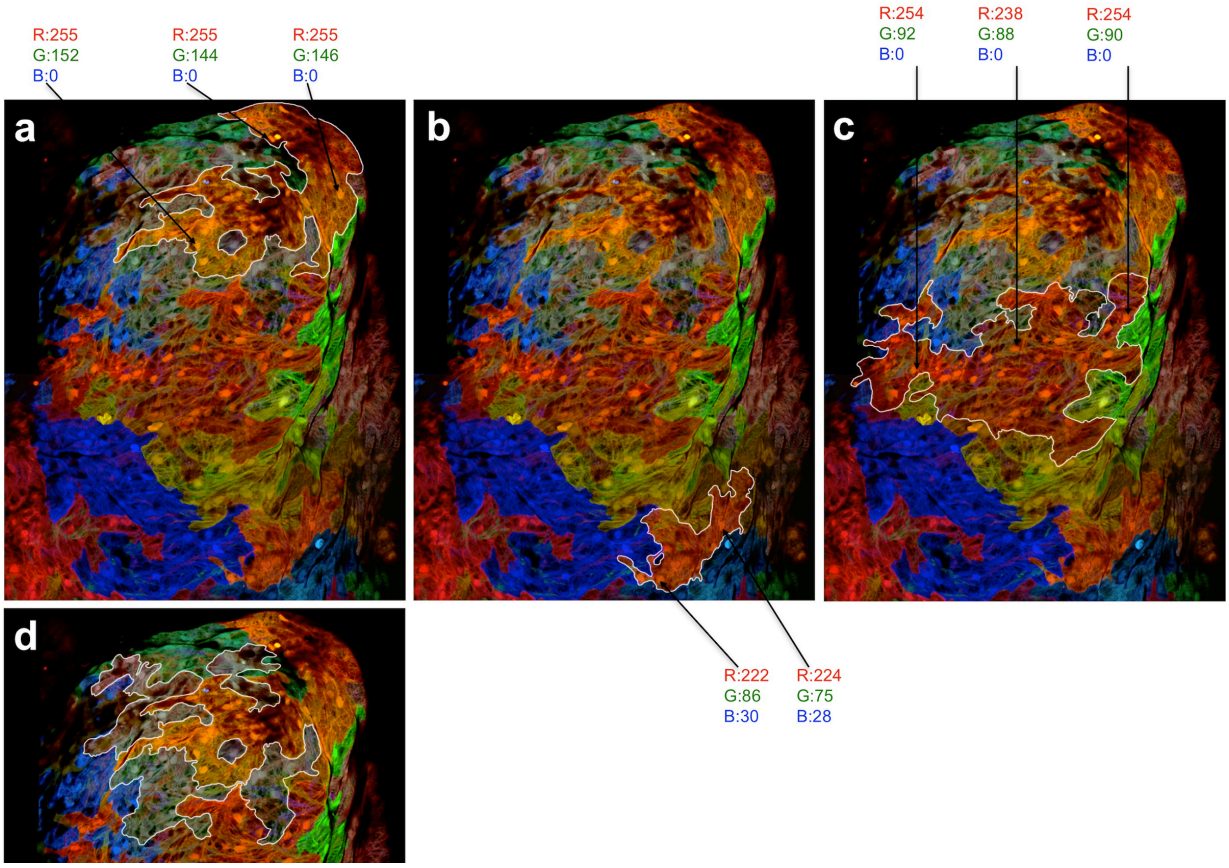
Supplementary Figure 1. Recombination and color generation require 4-HT treatment. a, b, Whole-mount surface image (**a**) and section image (**b**) of *cmlc2:CreER; priZm* ventricles at 90 dpf. Animals were treated with vehicle at 2 dpf and display no spurious recombination. Scale bars, 50 μm.



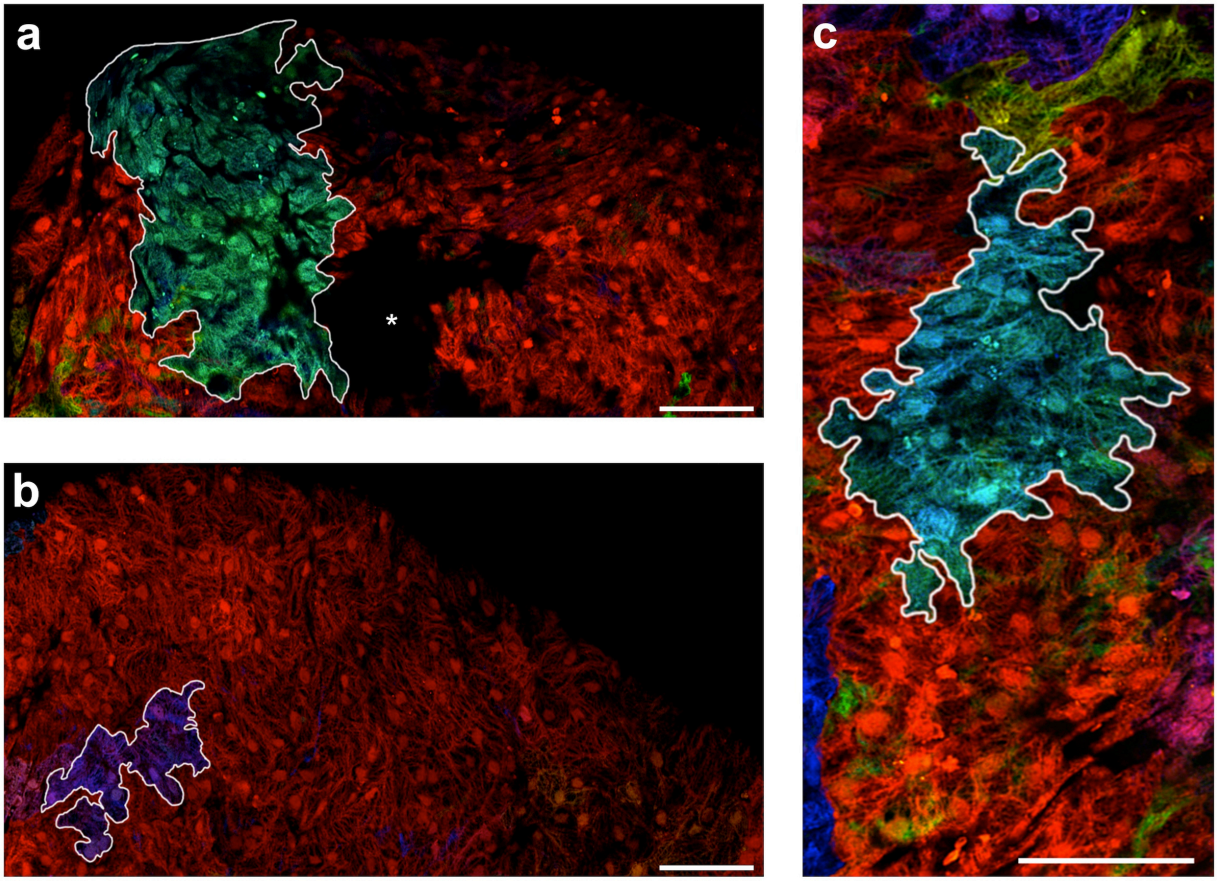
Supplementary Figure 2. Structure, cardiomyocyte number, and proliferation in the embryonic zebrafish ventricle. a-c, Section through a 2 dpf *cmlc2:EGFP; gata5:RnG* ventricle. At this stage, the myocardium (arrowhead; green in **a**) is of single-cell thickness. Cardiac muscle surrounds an inner layer of endocardium (arrow; red in **a**). **d.** Quantification of cardiomyocytes in the ventricular wall at 3 dpf. Mean ± s.e.m., n = 5 embryos. **e,** Standard 4-HT labeling treatment had no detectable effects on embryonic cardiomyocyte proliferation. Mean ± s.e.m., n = 5 (untreated) and 6 (4-HT) embryos. Student's t-test. Scale bar, 50 μm.



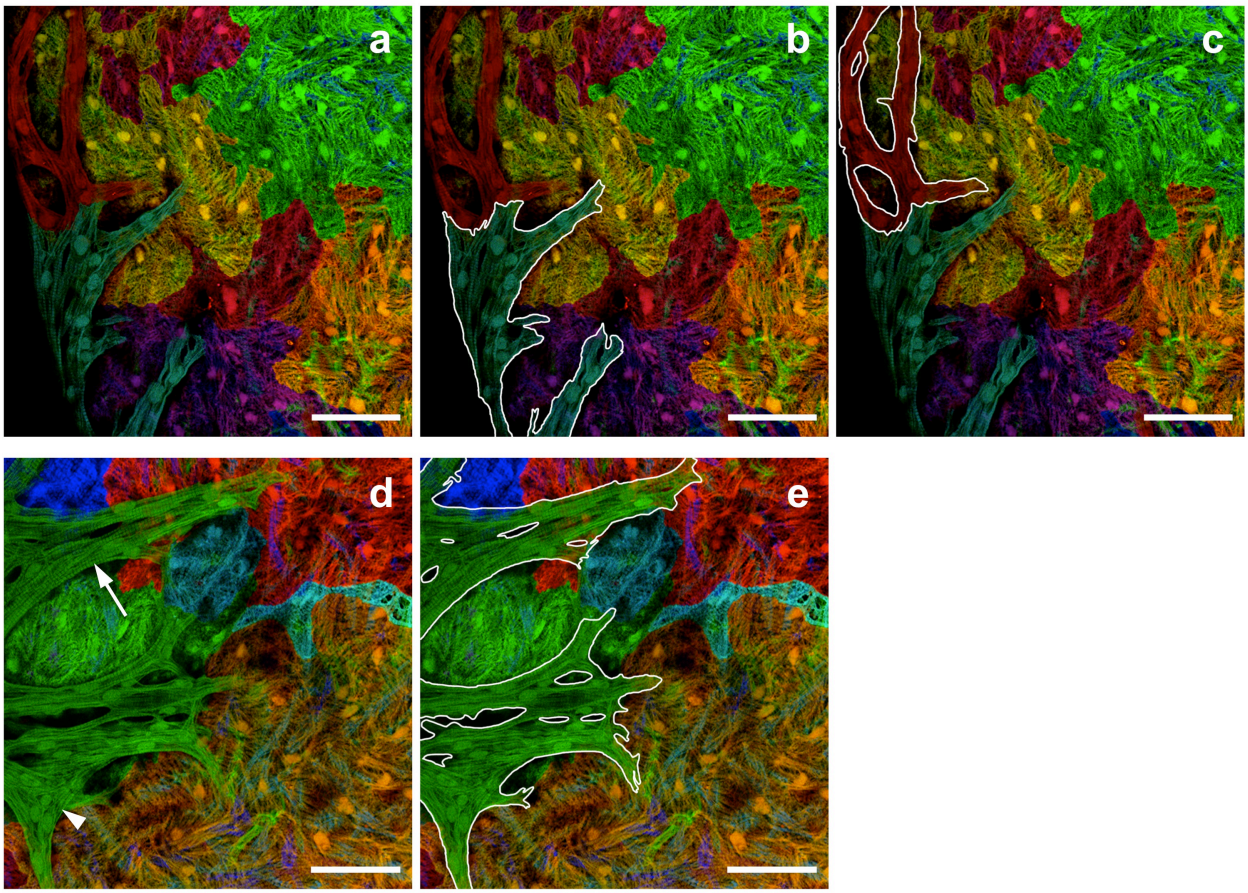
Supplementary Figure 3. Single cardiomyocytes comprise the majority of 10 dpf color clones. a, Surface myocardial image of a 10 dpf *cmlc2:CreER; priZm* ventricle, labeled at 2 dpf. Internuclear distance between adjacent myocardial cells is 20.2 µm. Because this image was taken 8 days after 4-HT labeling, it is likely that the presence of two adjacent brown cardiomyocytes reflects a division event. **b,** Surface myocardial image of a 10 dpf *cmlc2:nucDsRed2*; *β-actin:mGFP* embryo, with an internuclear distance of 20.8 µm. Scale bars, 10 µm.



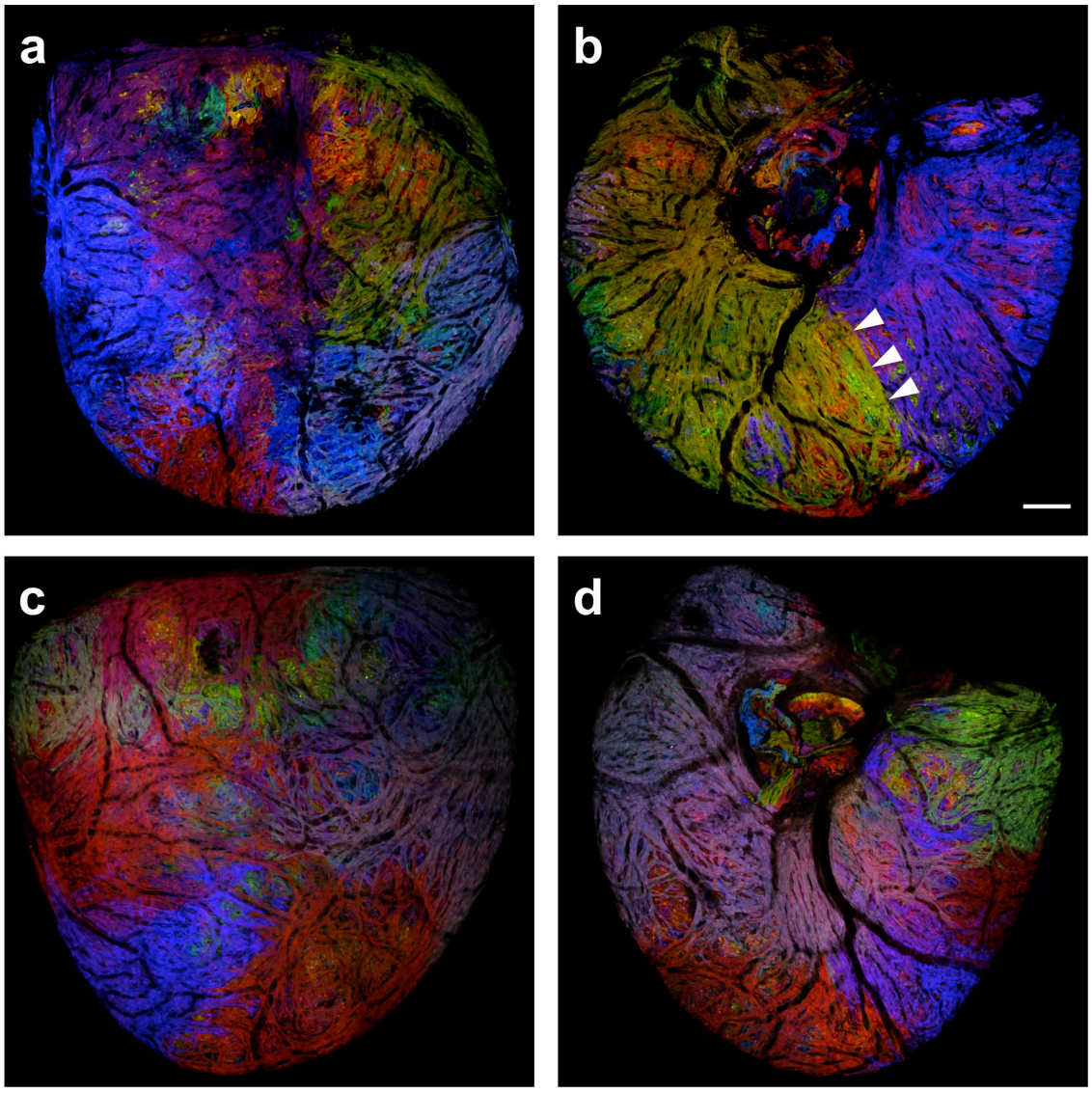
Supplementary Figure 4. Clonal color signatures and clone separation. a-c, Three orange clones of a 30 dpf ventricle were traced, and different regions throughout each clone were assigned Red/Green/Blue (RGB) values using Adobe Photoshop (black arrows). Note that the 3 different clones traced in **a**, **b**, and **c** have distinct RGB values, but the values are consistent from distant areas within each clone. Values were assessed from nuclei, which obscure fluorescence from underlying trabecular muscle better than cytosolic regions. **d**, The components of a gray clone that has been separated by a proliferating orange clone are traced, events that occurred in ~10% of clones.



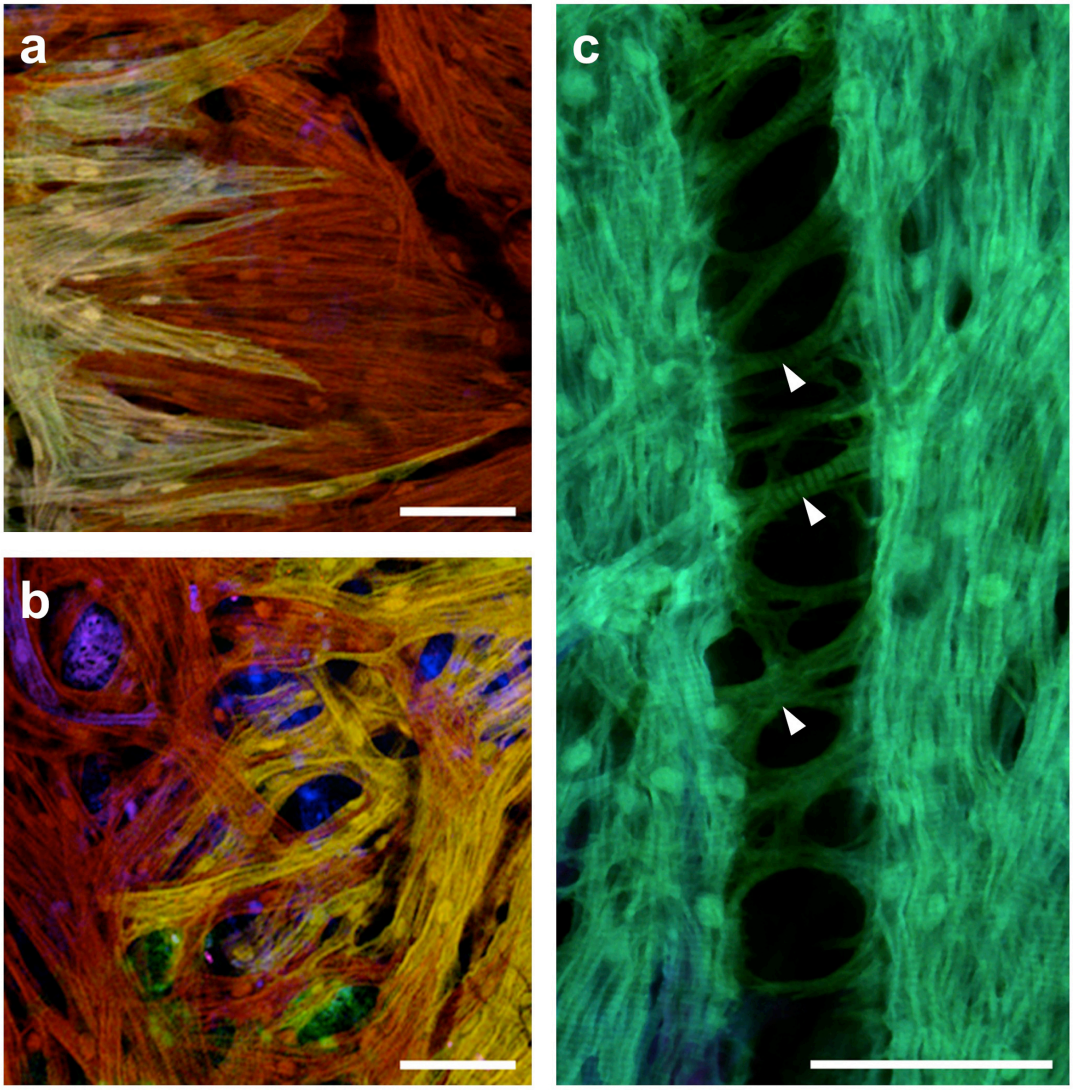
Supplementary Figure 5. Isolated clones also display diverse sizes. a-c, The likelihood that surface patches regularly formed from merging of independent clones of the same color is minute, given the high number of detected colors. Supporting this notion, we also observed diverse sizes and shapes of colored clones isolated within large areas of red, unrecombined myocardium. Here are shown three images of 30 dpf ventricles, in which a traced clone is surrounded by unrecombined myocardium. Large (**a**) and small (**b**) clones were observed. An asterisk indicates muscle tear that took place during tissue processing. Scale bars, 50 μm.



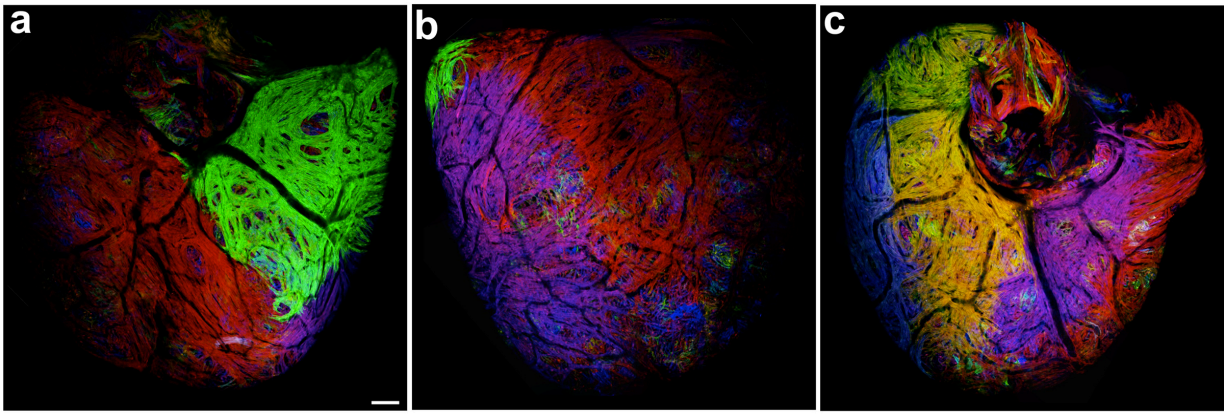
Supplementary Figure 6. Cortical cardiomyocyte clones at 10 weeks post-fertilization. a-c, High magnification view of 10 wpf surface myocardium, in which two distinct cortical clones (cyan and red) are in contact. Each clone is separately traced in **b** and **c**. **d, e,** High magnification view of a single green cortical clone that contains cardiomyocytes with clear striations (arrow) or with less organized structure (arrowhead). The clone is traced in **e**. Scale bars, 50 μm.



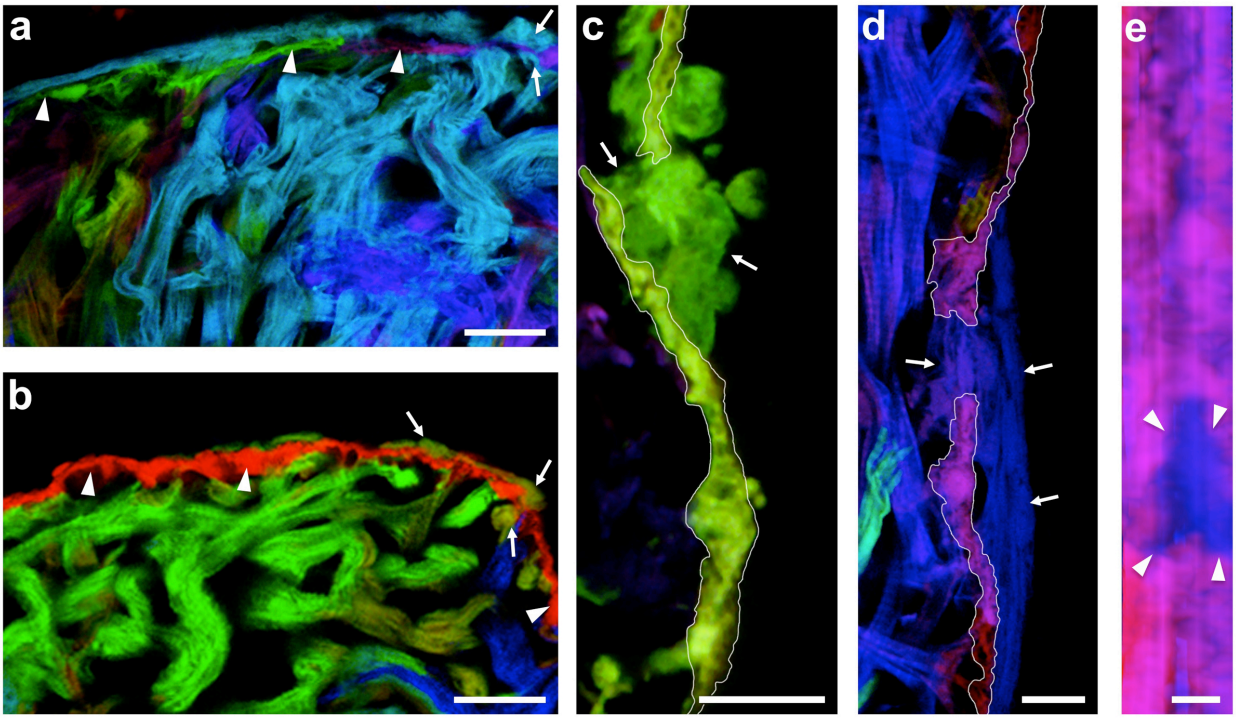
Supplementary Figure 7. Additional examples of adult ventricles, labeled at 2 dpf. **a-d,** Whole-mount images of 90 dpf *cmlc2:CreER; priZm* ventricles from animals that received 4-HT treatment at 2 dpf. Images in **a** and **b** are the front and back of the same ventricle, enabling visualization of full clonal extension. Arrowheads in **b** indicate a smooth boundary between clones running from base to apex. This was a rare event among our samples. Images in **c** and **d** are the front and back of a second ventricle. Scale bar, 100 μm.



Supplementary Figure 8. Surface interactions of cortical cardiomyocyte clones. **a,** High magnification view of a gray-tinted clone of cortical cardiomyocytes that appears to fit like a puzzle piece with a neighboring region of red cortical myocytes. **b,** A weaving interaction between a yellow clone of cortical myocytes and red myocytes. Indigo and green primordial layer cardiomyocytes are visible beneath. **c,** A vessel tract (arrowheads) is visible in a large green clone of cortical cardiomyocytes, with some myocytes extending over the structure. Scale bars, 50 μm.



Supplementary Figure 9. Additional examples of 90 dpf ventricles, labeled at 30 dpf. a-c, Whole-mount images of 90 dpf *cmlc2:CreER; priZm* ventricles from animals that underwent 4-HT labeling at 30 dpf, showing large cortical clones. Scale bar, 100 μm.



Supplementary Figure 10. Evidence that clones containing trabecular and cortical cardiomyocytes connect through a breach in the primordial layer. a-d, Four examples of 6-7 wpf *cmlc2:CreER; priZm* ventricles from animals that had been labeled at 2 dpf. Images were captured from areas near the base of the ventricle, where cortical muscle typically appears first. **a**, Primordial muscle is green and magenta from left to right (arrowheads). Cortical muscle is cyan, as is the adjacent trabecular muscle (arrows). **b**, The primordial layer is all red in this section (arrowheads). Cortical and trabecular muscle share the same two colors (green and hazel), and an area on the right of the image connects hazel muscle on each side (arrows). Scale bars, 20 μm (for **a**, **b**). **c**, Yellow primordial muscle is outlined in white, showing a gap breached by a green clone (arrows). **d**, The pink and red primordial layer is outlined in white, interrupted by a blue clone spanning trabecular and cortical muscle (arrows). **e**, 3D Imaris reconstruction of the confocal stack of images in **d**, rotated to

display a view from the periphery to the lumen to the heart. The primordial layer has a gap filled by the emergent blue clone (arrowheads). Scale bars, 10 μm (for **c**-**d**)