Supporting Information

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SI Materials and Methods

Mouse Genotyping. The following primers and PCR conditions were used for genotyping: Cre; 5′-GTCCAATTTACTGACCG-TACACC-3′ and 5′-GTTATTCGGATCATCAGCTACACC-3′; 94 °C for 5 min, 94 °C for 30 s, 58 °C for 70 s, 72 °C for 60 s, repeat 35 cycles, 4 °C for 20 min.

Tamoxifen Injections. Tamoxifen (TM) (Sigma) was prepared by dissolving in corn oil (Sigma) to a concentration of 20 mg/mL. TM was injected intraperitoneally using a tuberculin syringe and a 25-gauge needle. Pups younger than postnatal day (P) 10 were given 0.5 mg per injection (typically on an alternating day regimen), and older mice were given 2 mg per injection (daily injections were tolerated). For the clonal analysis described in Fig. 1*J*, 1 mg TM was given at P4 and analyzed at P5.

Histology and Tissue Analysis. For fixation, tissues, and organs were placed in 4% (vol/vol) paraformaldehyde for 18–24 h at 4 °C. Samples were prepared for embedding by soaking in 30% (wt/vol) sucrose in PBS at 4 °C for 24 h. The samples were removed from the sucrose solution and tissue blocks were prepared by embedding in Tissue Tek OCT (Sakura Finetek) under dry ice to freeze the samples within the compound. Frozen blocks were mounted on a MicroM HM550 cryostat (MICROM International) and 7- to 20-μm-thick sections were transferred to Superfrost/Plus adhesive slides (Fisher brand). Slides were incubated at 37 °C in the dark for 20 min before storage or immunostaining.

Antibodies. Immunostaining was performed using the following primary antibodies: PECAM-1/CD31 (Abcam ab28364; 1:50), α-smooth muscle actin (Sigma A2547; 1:500), α-actinin (Sigma A7811; 1:200), c-kit (Santa Cruz sc-5535; 1:200), c-kit (R&D Systems AF1356; 1:100), troponin I (Millipore mab1691; 1:250), Gata4 (Santa Cruz sc-5535; 1:100), Mesp1 (Aviva Systems Biology ARP39374; 1:100), Connexin43 (Millipore mab3068; 1:50), Isl1 (Abcam ab20670; 1:100), wheat germ agglutinin-Alexa Fluor 647 conjugate (Invitrogen W32466; 1:500).

Immunohistochemistry. Sections were washed in PBS and incubated with primary antibodies diluted in blocking solution (5% goat serum, 0.5% Triton X-100 in PBS) at room temperature for 1–3 h or overnight at 4 °C. Sections were then washed in PBS and incubated with fluorescent-conjugated secondary antibodies for 1 h at room temperature. Fluorescent and bright-field images were taken with a Leica DM5500B microscope and Leica CTR5500 camera (Leica Microsystems) using Metamorph 7.7.3.0 software, on a Leica M205FA dissection microscope and Leica DFC310FX camera (Leica Microsystems) using Leica Application Suite 4.1.0 software, and on a Leica Sp2 confocal microscope using LCS software. Adobe Photoshop was used to adjust image levels and process image overlays.

Myocardial Infarction. Animal protocols were approved by the Stanford University Animal Care and Use Committee guidelines. All surgical procedures were performed on 8- to 10-wk-old male and female experimental mosaic analysis with double markers (MADM) mice (as described above) by a single, experienced microsurgeon. After induction with inhaled 2–3% isoflurane, mice were intubated and ventilated, and anesthesia was maintained with inhaled 1–2.5% isoflurane. After a left thoracotomy was performed, the middle portion of left anterior descending (LAD) artery was ligated for 30 min, followed by reperfusion. Infarction was visually confirmed by blanching of the anterolateral region of the left ventricle along with dyskinesis. For sham-operated animals, the left thoracotomy was performed only, without ligation of the LAD artery.

Statistical Analysis. Statistical testing was performed with Microsoft Excel v12.2.8 (Microsoft). Results are presented as mean ± SEM and these values were compared using the unpaired Student *t* test (significance was assigned for $P < 0.05$).

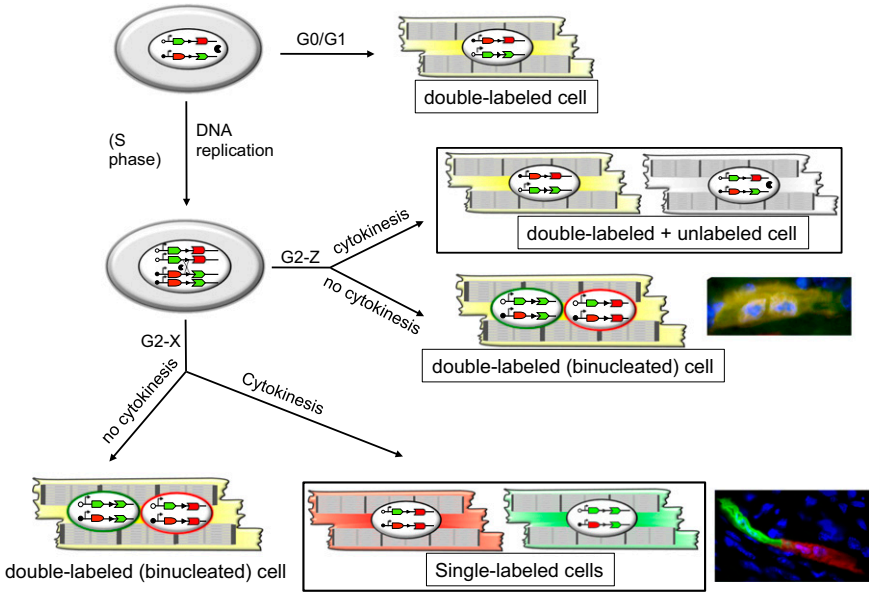

Fig. S1. Model for MADM recombination. Before S phase, G_0/G_1 recombination leads to double-labeled cells; G_0/G_1 recombination could also occur in binucleated cells, which would also yield double-labeled cells (not shown). After S phase, a G2-Z segregation event would lead to two mononucleated daughter cells or a single binucleate daughter cell, depending on whether or not cytokinesis occurs. If there is cytokinesis, one daughter cell would be double-labeled and its sibling cell would be unlabeled. A binucleated daughter cell resulting from G2-Z segregation without cytokinesis would be double-labeled. After S phase, a G2-X segregation event would lead to a double-labeled daughter cell if cytokinesis does not occur (although the nuclei would be "single-labeled"; that is, either GFP^+ or RFP^+ at the DNA level) or to two uniquely labeled daughter cells ("single-labeled") if there is abscission. Note that these single-labeled cells are the only cells that are a result of division that occurred during the presence of Cre (and any subsequent chase period), and hence are the only labeled cells that are used to study clonal cell division giving rise to cardiomyocytes; although G2-Z segregation can also result in daughter cells, because double-labeled cells can arise through binucleation or by G_0/G_1 recombination, the inability to discriminate among these various origins of double-labeled cells we only refer to single-labeled cells (arising from G2-X recombination) as definitively the result of mitosis. Single-labeled cardiomyocytes from the Myh6CreERT2;MADM-$11^{GT/TG}$ model as an example of G2-X–derived single-labeled newborn daughter cell clones.

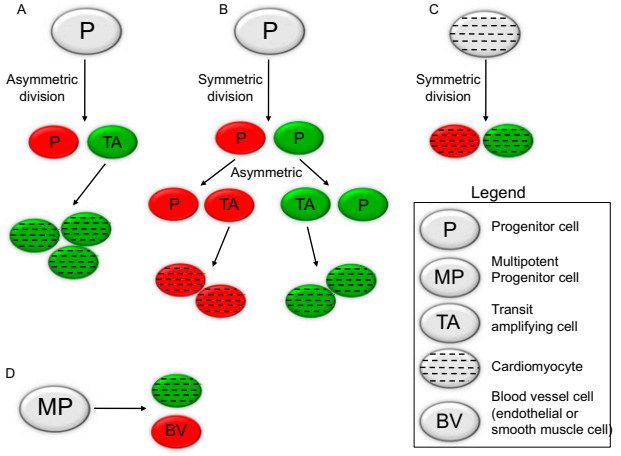

Fig. S2. Possible outcomes of MADM clonal analysis. MADM G2-X recombination results in asymmetric labeling of the two daughter cells and, thus, is an ideal platform to study the parity of a precursor/parent cell and dissect the precursor–progeny relationship and evaluate for self-renewal, multipotentiality, and so forth. [In this figure, the GFP^+ and RFP^+ cell are interchangeable (only one possibility is shown for the sake of clarity)]. (*A*) Asymmetric division would lead to self-renewal of the precursor cell and the generation of a daughter transit-amplifying cell that would further proliferate, leading to a single RFP^+ cell and multiple GFP^+ cells (depending on the proliferative capacity of the TA cell). (*B*) Symmetric division could lead to two progenitor cells, which could subsequently undergo asymmetric division to yield two alternate-single-label clones. (*C*) Symmetric division could also occur in existing cardiomyocytes and form two alternate-label sister cardiomyocytes. (*D*) A multipotent progenitor could also lead to alternate-labeled blood vessel cells (endothelial cells and smooth muscle cells) and cardiomyocytes.

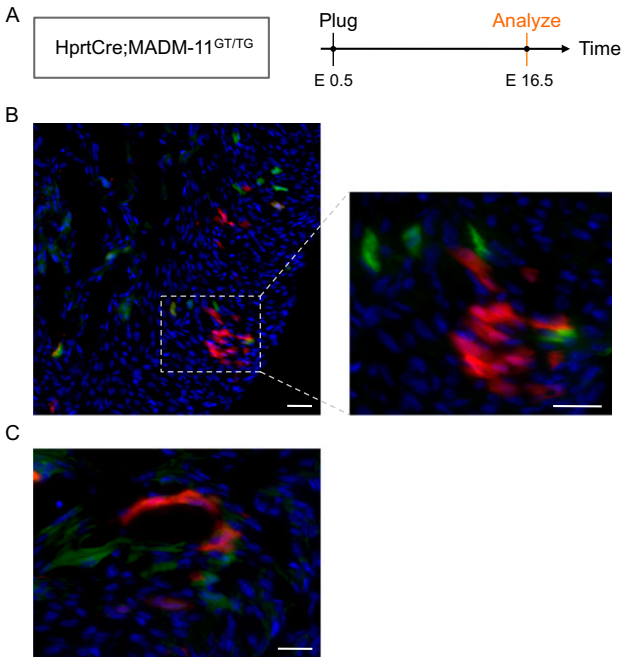

Fig. S3. Proliferation in the embryonic heart. (*A*) HprtCre;MADM model and time line for analysis. (*B*) Section of developing left ventricle (LV) demonstrates a RFP$^+$ cluster. (Scale bar, 20 μm.) (*Inset* scale bar, 5 μm.). (*C*) Section of LV demonstrates a GFP$^+$ and RFP$^+$ clones (n = 3 embryos). (Scale bar, 5 μm.)

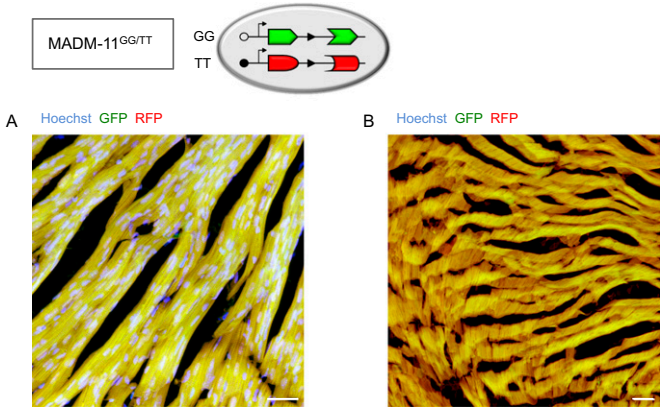

Fig. S4. Lack of silencing at the Hipp11 locus. In a MADM-11$^{GG/TT}$ mouse, only double-labeled cells are present. (*A* and *B*) Section from the LV. (Scale bars, 10 μm.)

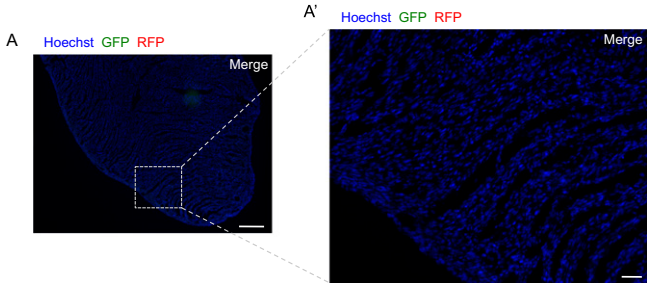

Fig. S5. MADM recombination does not occur in the absence of TM administration. (*A*) A 1-mo-old mouse heart 7-μm section stained with Hoechst, and the merged image of the GFP, tdTomato, and Hoechst channels is displayed. (Scale bar, 100 μm.) (*A′*) Boxed region in *A* zoomed to further demonstrate absence of MADM recombination labeling without TM injection. (Scale bar, 30 μm.) These sections are representative of all sections of the heart analyzed for n = 4 mice each for both the Myh6CreERT2;MADM and β-actinCreER;MADM models.

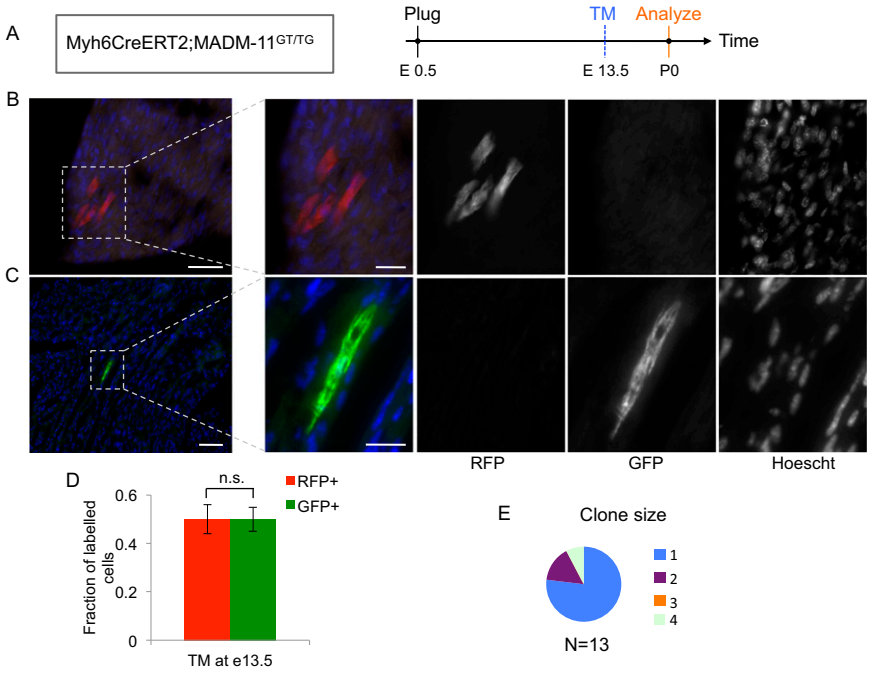

Fig. S6. Myh6-expressing cardiomyocytes undergo division during embryonic development. (*A*) Myh6CreER; MADM$^{GT/TG}$ model and timeline for TM delivery and analysis. (*B*) A single-label clone of RFP^{+} cardiomyocytes born from a cardiomyocyte during development. (Scale bars, 20 μm and 5 μm in the zoomed-in region, *Right*.) (*C*) A single-labeled GFP^{+} cardiomyocyte indicates cardiomyocyte division during development using the Myh6CreERT2;MADM$^{GT/TG}$ model. (Scale bars, 20 μm and 5 μm in the zoomed-in region, *Right*.) (*D*) Percent of single-labeled cells born between embryonic day (E)13.5 and P0. (*E*) Clone size analysis of cardiomyocyte division between E13.5 and P0 (n = total number of single-labeled cells, based on n = 3 mice; P values calculated with Student t test. Error bars indicate SEM).

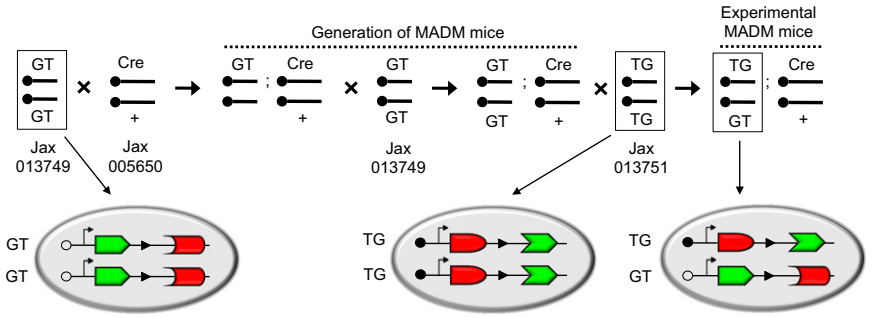

Fig. S7. Genetic breeding strategy to generate experimental MADM mice. In the first cross, the Cre transgenic mouse is crossed with the GT/GT mouse (or the TG/TG mouse), and its progeny back-crossed with the GT/GT strain (or TG/TG strain). The progeny of this second cross are homozygous for the GT/GT (or TG/TG) transgenes and thus can be used to generate the experimental MADM mice, which are formed when second-progeny mice are crossed with TG/TG mice (or GT/GT mice). The MADM-homozygous and the Myh6CreERT2 mouse strains are available at Jackson Laboratories. The β-actinCreER strain has been published previously (1).

1. Zong H, Espinosa JS, Su HH, Muzumdar MD, Luo L (2005) Mosaic analysis with double markers in mice. *Cell* 121:479–492.