Supplementary Figure Legends

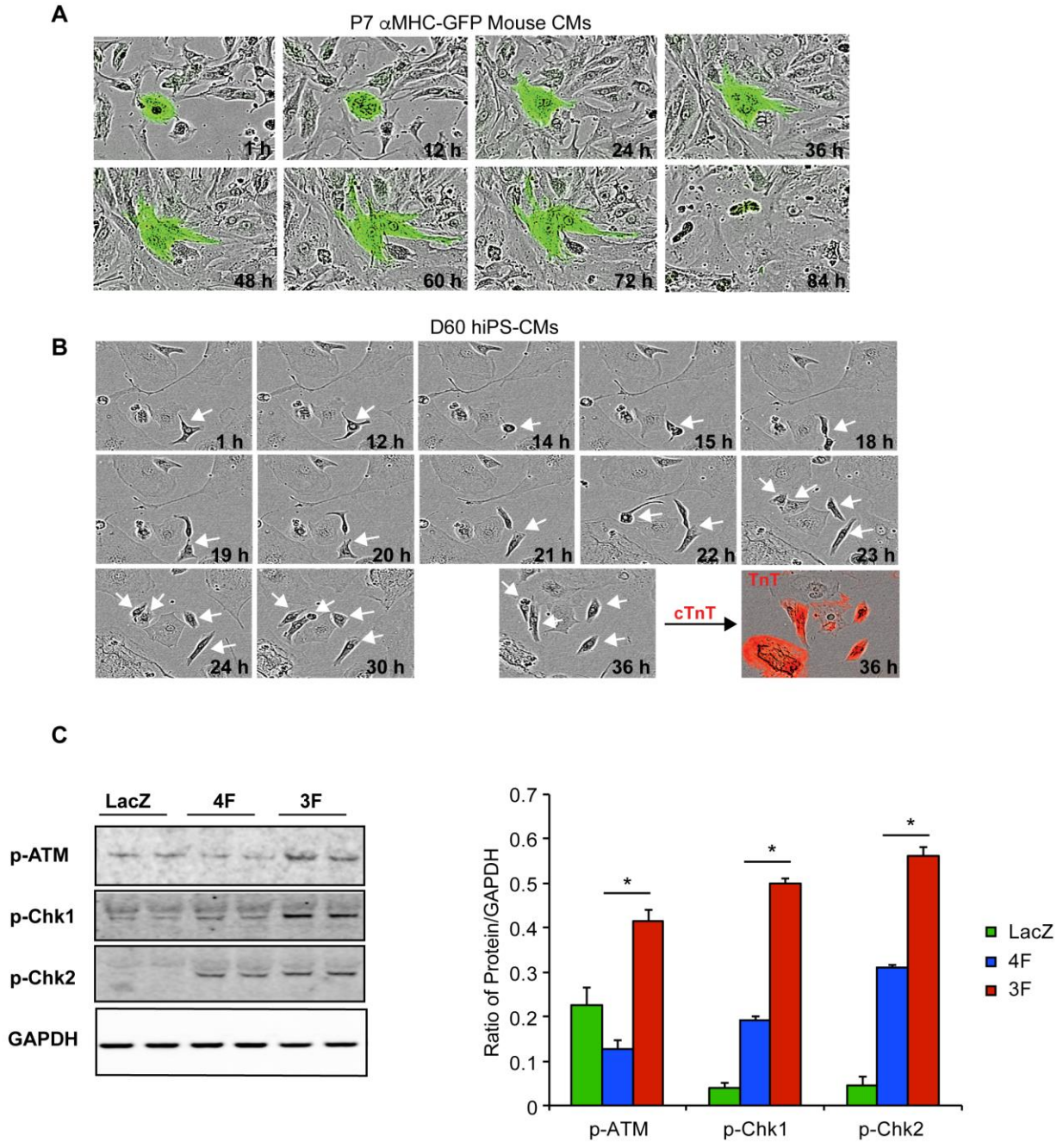

Figure S1. CDK1/CCNB/AURKB (3F)-Induced Cell Division in Mouse and Human Cardiomyocytes and DNA Damage Response, Related to Figures 1-2 and Supplementary Videos 1-2)

(A) Time lapse imaging of cell division in P7 mouse cardiomyocytes isolated from α-MHC-GFP transgenic mice overexpressing CDK1, CCNB and AURKB (3F). Panels are representative of images recorded every hour for 4 days and demonstrate cell division of a cardiomyocyte, followed by rapid cell death seen in last panel (see Supplementary Video 1 and 2).

(B) Time lapse imaging of cell division in 60-day-old hiPS-derived cardiomyocytes overexpressing 3F. Panels are representative of images collected every hour for 2 days. Last panel represents immunocytochemistry for cardiac Troponin T (cTnT) in the 36-hour cells. Arrows denote dividing cells and their progeny.

(C) Representative western blots and quantification for the indicated DNA damage response markers (p-ATM, p-Chk1 and p-Chk2) in response to virus encoding 4F, 3F or LacZ (control) in human iPS-CMs (n=3 independent experiments with two replicates in each; *$p<0.05$, bars indicate means with SEM).

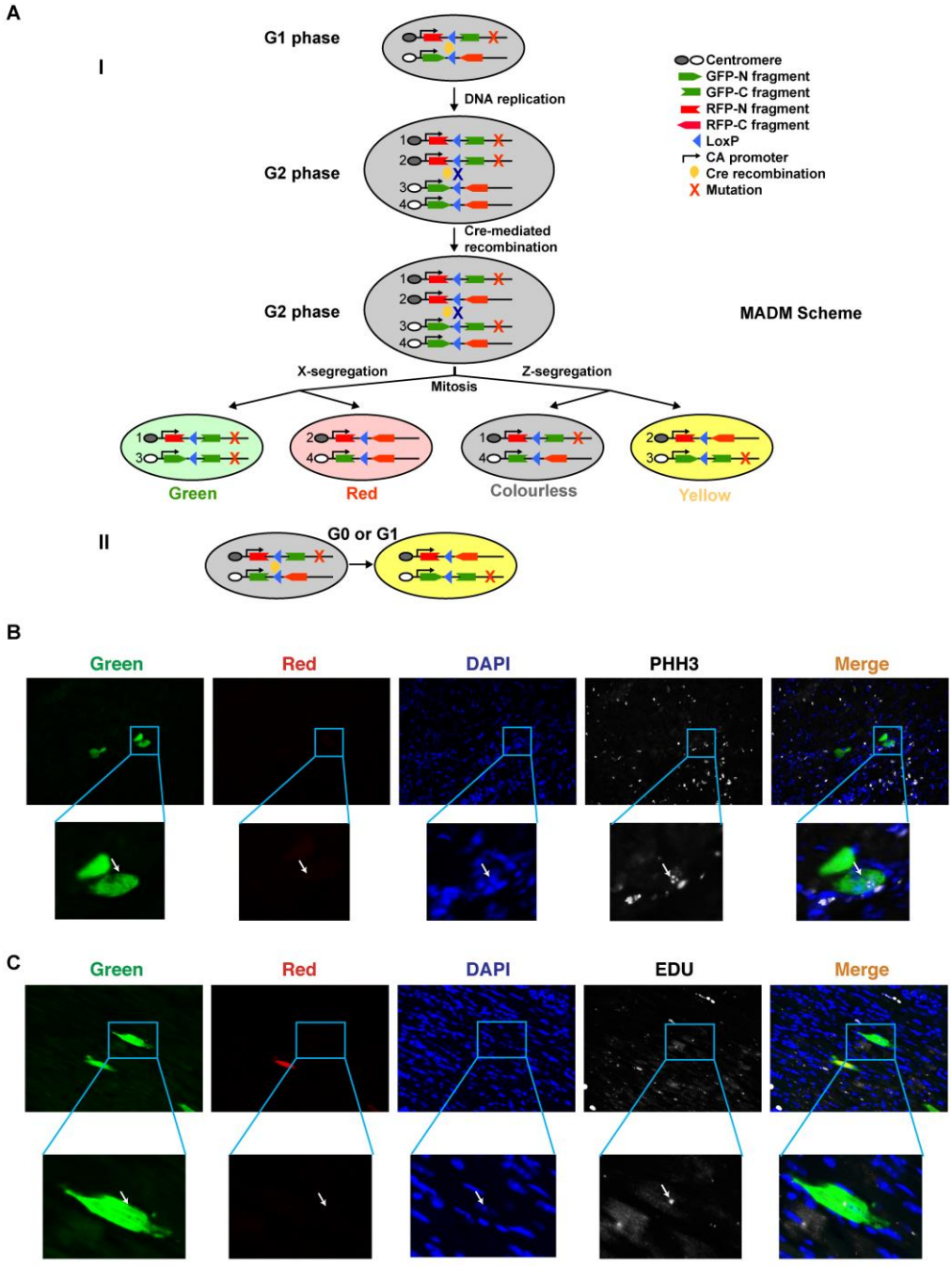

Figure S2. Validation of the Mosaic Analysis with Double Markers (MADM) System to Detect 4F-Induced Cardiomyocyte Proliferation *In Vivo,* Related to Figure 3

(A) Schematic diagram showing the principle behind the lineage tracing of proliferating cells in MADM mice (adapted from (Gitig, 2010)).

(B, C) Representative histologic images of cardiomyocyte-specific α-MHC-Cre MADM hearts infected with 4F at the time of infarct and sectioned 4 days later. Single-colored cardiomyocytes stained positive for PHH3 (B) and EDU incorporation (C). Low and high magnification of indicated areas are shown,

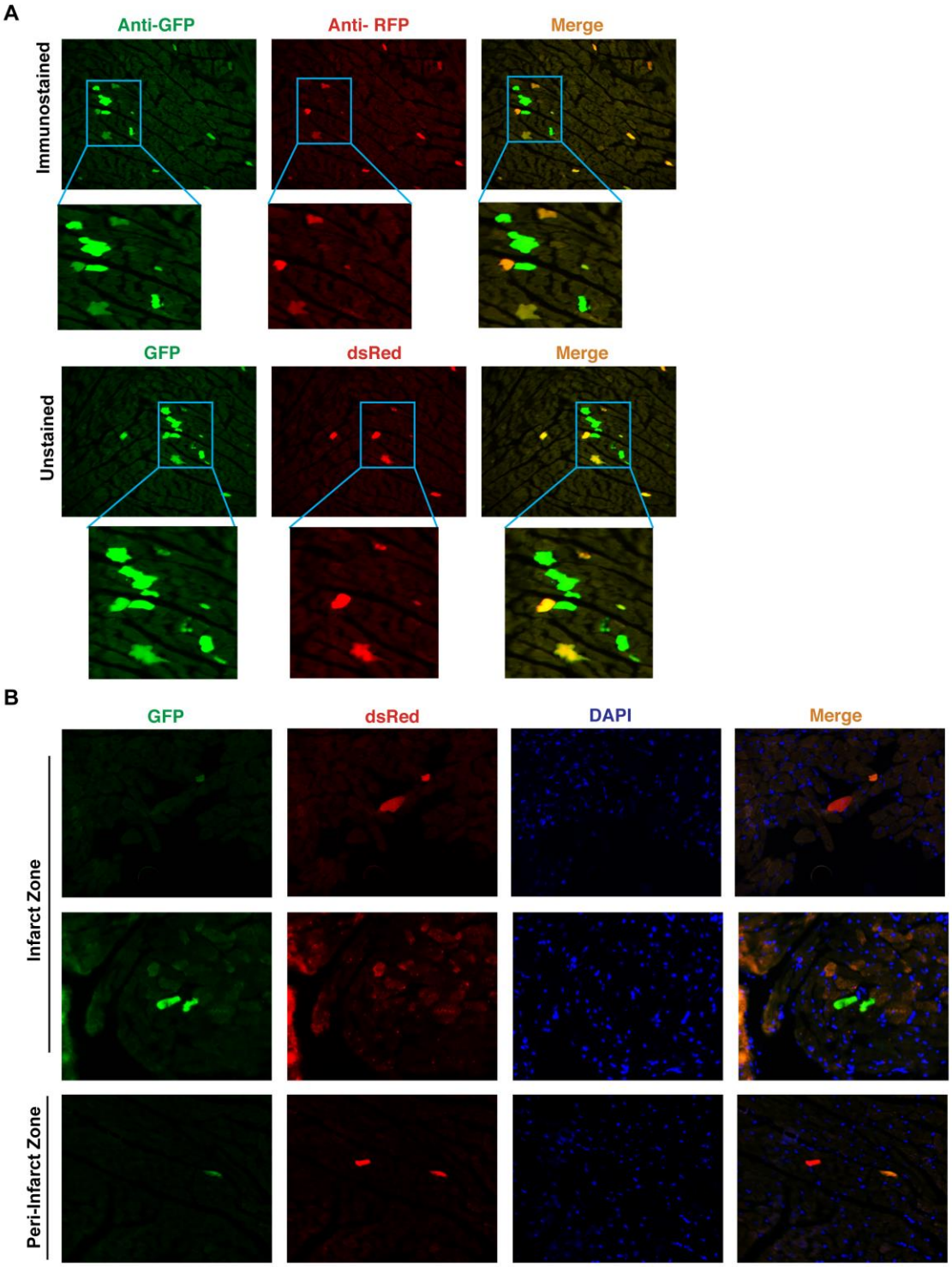

Figure S3. Validation of α-MHC-Cre MADM Fluorescent Reporter and Examples of Single-Colored Cells in Infarct and Peri-Infarct Regions, Related to Figure 3

(A) Representative GFP- or RFP-immunostained and unstained adjacent heart sections from α-MHC-Cre MADM mice showing that the signal intensity was similar in immunostained sections compared to sections visualized by fluorescence, validating use of the fluorescent reporter in this system. Arrows are pointing to two single-colored cells showing similar signal intensities in the two adjacent sections.

(B) Representative images from α-MHC-Cre MADM mouse heart sections treated with 4F showing single-colored cardiomyocytes at the infarct zone (top two panels). Bottom panel shows a representative peri-infarct region without scar where there are many events of recombination including a single-colored cardiomyocyte.

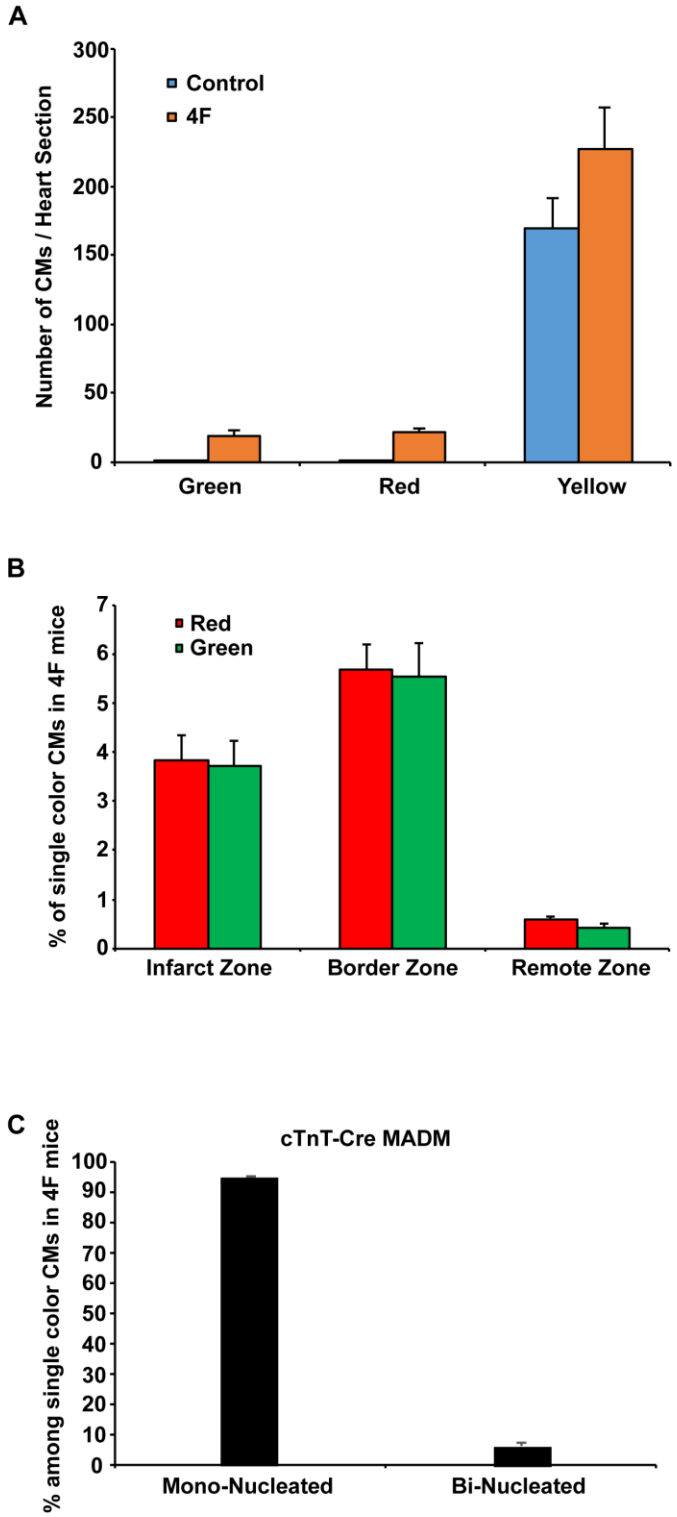

Figure S4. Spatial Location and Nucleation of Divided Cardiomyocytes *In Vivo*, Related to Figure 3

(A) Bar graph shows quantification of the absolute numbers of single-colored and yellow-colored cardiomyocytes in α-MHC-Cre MADM 2 weeks after infarction and injection with either LacZ (Control) or 4F adenoviruses (n=8 animals in each group and 10 sections from each animal).

(B) Bar graph shows quantification of the percent single-colored cells at the infarct, border and remote zones in α-MHC-Cre MADM mice injected with 4F adenoviruses at the time of the infarct and harvested 2 weeks later (n=8000 cells analyzed from 8 animals).

(C) Bar graph shows quantification of single-colored cardiomyocyte nucleation where over 90% of the single-colored cardiomyocytes were mononucleated in cTnT-Cre MADM mice hearts injected with 4F at the time of MI and harvested 2 weeks later (n=2000 cells analyzed from 10 different heart sections from three animals).

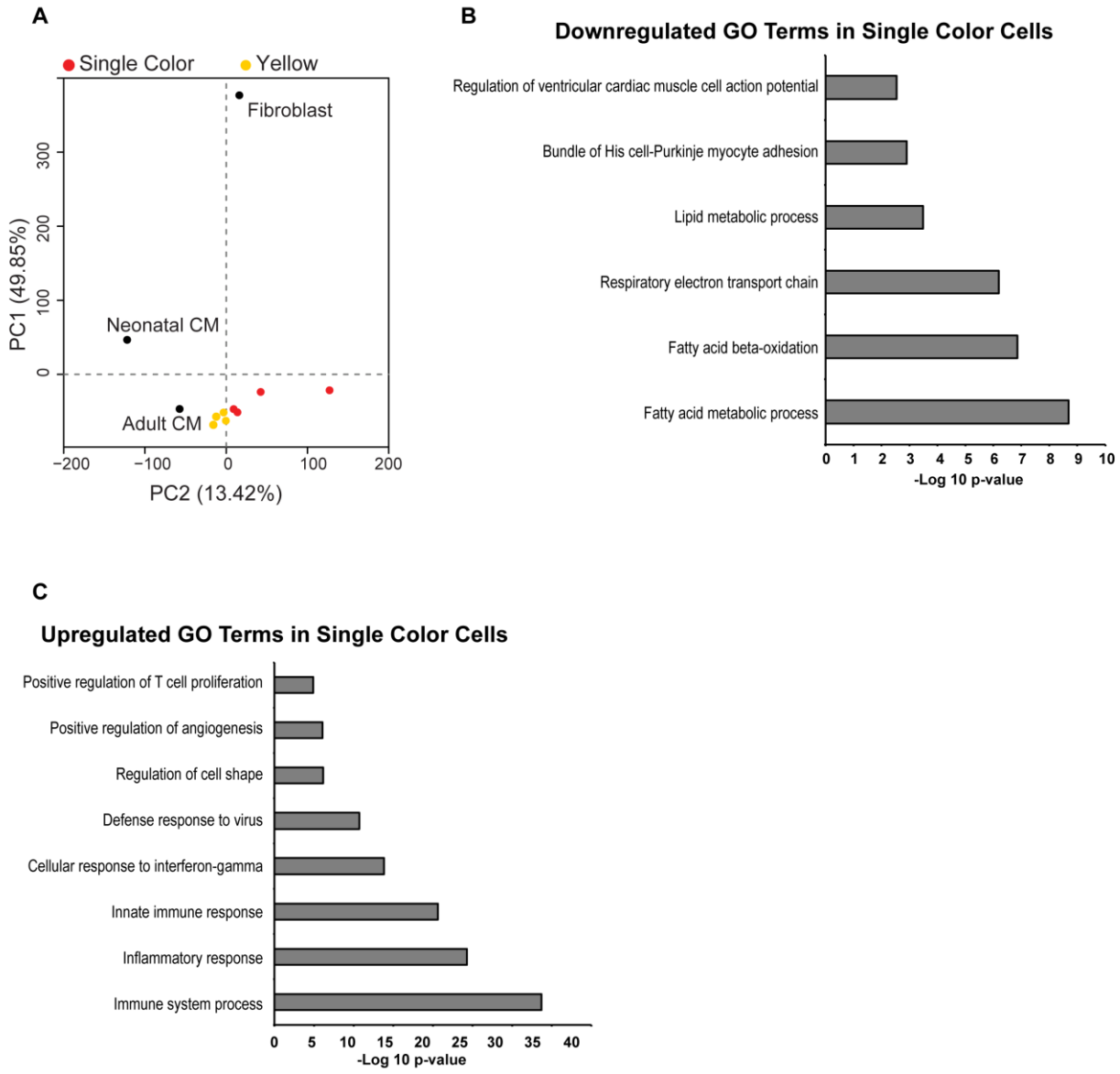

Figure S5. RNA Sequencing of Cardiomyocytes Isolated from MADM Mice after Cytokinesis, Related to Figure 3

(A) Principal component analysis (PCA) of RNA-seq data from isolated single-colored adult cardiomyocytes from MADM mice 2 weeks after injection with the 4F (post-cytokinesis) compared to yellow-colored cardiomyocytes (indeterminate) from the same animals (n=4), and also compared to averages of control adult cardiomyocytes (CM), neonatal CMs and cardiac

fibroblasts (n=3). Adult single-colored cells (red) had greater variability than the yellow cells that were mostly cells that had not undergone cell division.

(B, C) Gene Ontology (GO) term analysis of the genes downregulated (B) and upregulated (C) by more than two-fold with $p<0.05$ significance between the single-colored vs yellow CMs.

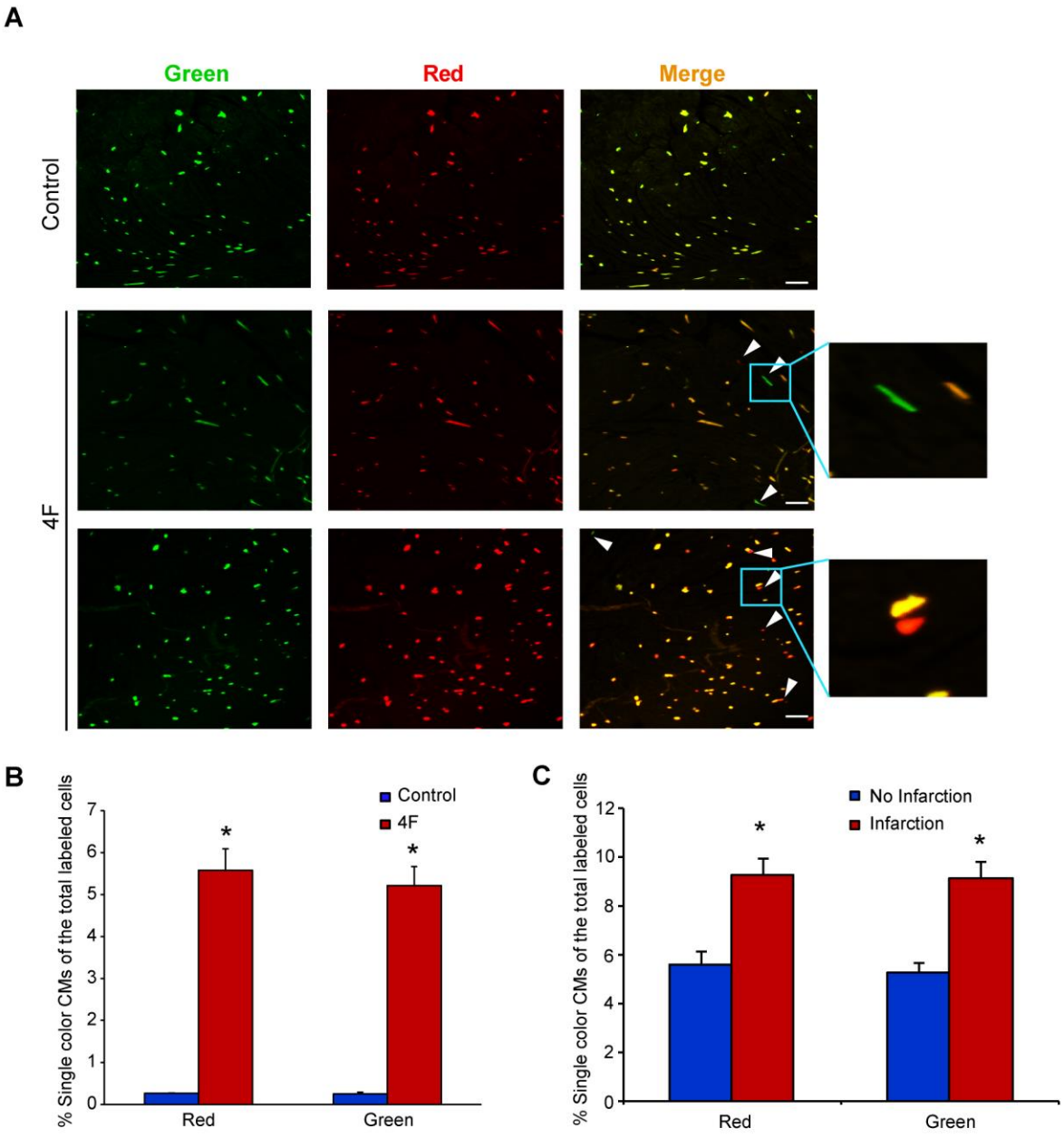

Figure S6. 4F Induces Cardiomyocyte Proliferation in Non-Infarcted α-MHC-Cre MADM Heart, Related to Figure 3

(A, B) Representative immunofluorescence images (A) and quantification of single-colored cells (B), indicating cardiomyocyte cytokinesis, in α-MHC-Cre MADM mice in histological sections without myocardial infarction.

(C) Direct comparison of quantification of the single-colored cells in infarcted and non-infarcted hearts treated with either 4F or control LacZ virus. Low magnification views indicate breadth of recombination in cardiomyocytes, and insets show higher magnification of single- or double-colored cells. Mice were infected intramyocardially with adenoviruses encoding CDK1, CCNB, CDK4, and CCND (4F) or control Lac-Z virus and the hearts were harvested 2 weeks later (n=6 animals in each group, *$p<0.05$). Bars indicate means and SEM.

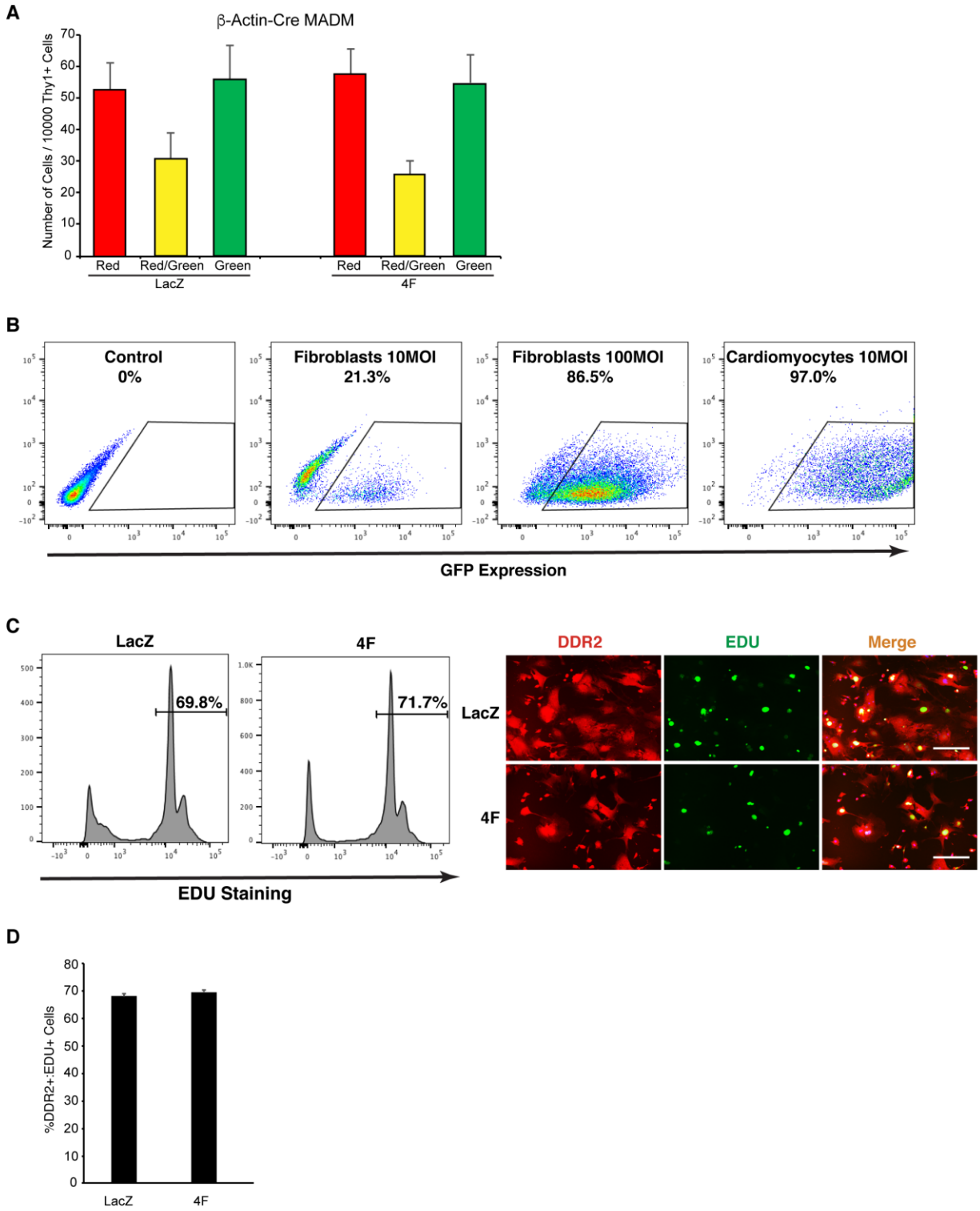

Figure S7. 4F Does Not Affect Fibroblast Proliferation *In Vivo* or *In Vitro* Related to Figure 4

(A) Quantification of isolated $Thy1^+$ cells from ubiquitous β-Actin-Cre-MADM mice by using a Langendorff preparation, digesting the heart, and sorting a cardiac fibroblast-enriched population marked with the APC-conjugated-Thy1 antibody. FACS was used to quantify the number of single-colored fibroblasts and revealed no difference between animals treated with 4F or LacZ control virus (n=4 animals in each group).

(B) Representative FACS plots showing infection efficiency of GFP adenovirus in $Thy1^+$ cardiac fibroblasts infected with 10 or 100 MOI, compared to iPS-CMs infected with 10 MOI of the virus.

(C) Representative FACS plots (left panels) and immunostaining (right panels) of EDU incorporation in $DDR2^+$ cells (pre-sorted for $Thy1^+$) infected with either LacZ control virus, or CDK1-CDK4-CCNB-CCND (4F) for 48 hours (n=3 independent experiments and 3 technical replicates in each).

(D) Quantification of FACS analysis (**C**) from pre-sorted $Thy1^+$cardiac fibroblasts co-stained with DDR2 (fibroblast marker) and EDU and infected with either LacZ control virus, or 4F viruses for 48 hours (n=3 independent experiments with 3 replicates in each). Bars indicate means and SEM.