Stem Cell Reports, Volume *1*

Supplemental Information

Direct Reprogramming of Human Fibroblasts

Toward a Cardiomyocyte-Like State

Ji-Dong Fu, Nicole R. Stone, Lei Liu, C. Ian Spencer, Li Qian, Yohei Hayashi, Paul Delgado-Olguin, Sheng Ding, Benoit G. Bruneau, and Deepak Srivastava

INVENTORY OF SUPPLEMENTAL INFORMATION

Figure S1 (related to Figure 1).

Figure S2 (related to Figure 1).

Figure S3 (related to Figure 2).

Figure S4 (related to Figure 3).

Figure S5 (related to Figure 3).

Figure S6 (related to Figure 4).

Supplemental Movie 1 (related to Figure 5).

Supplemental Movie 2 (related to Figure 5.

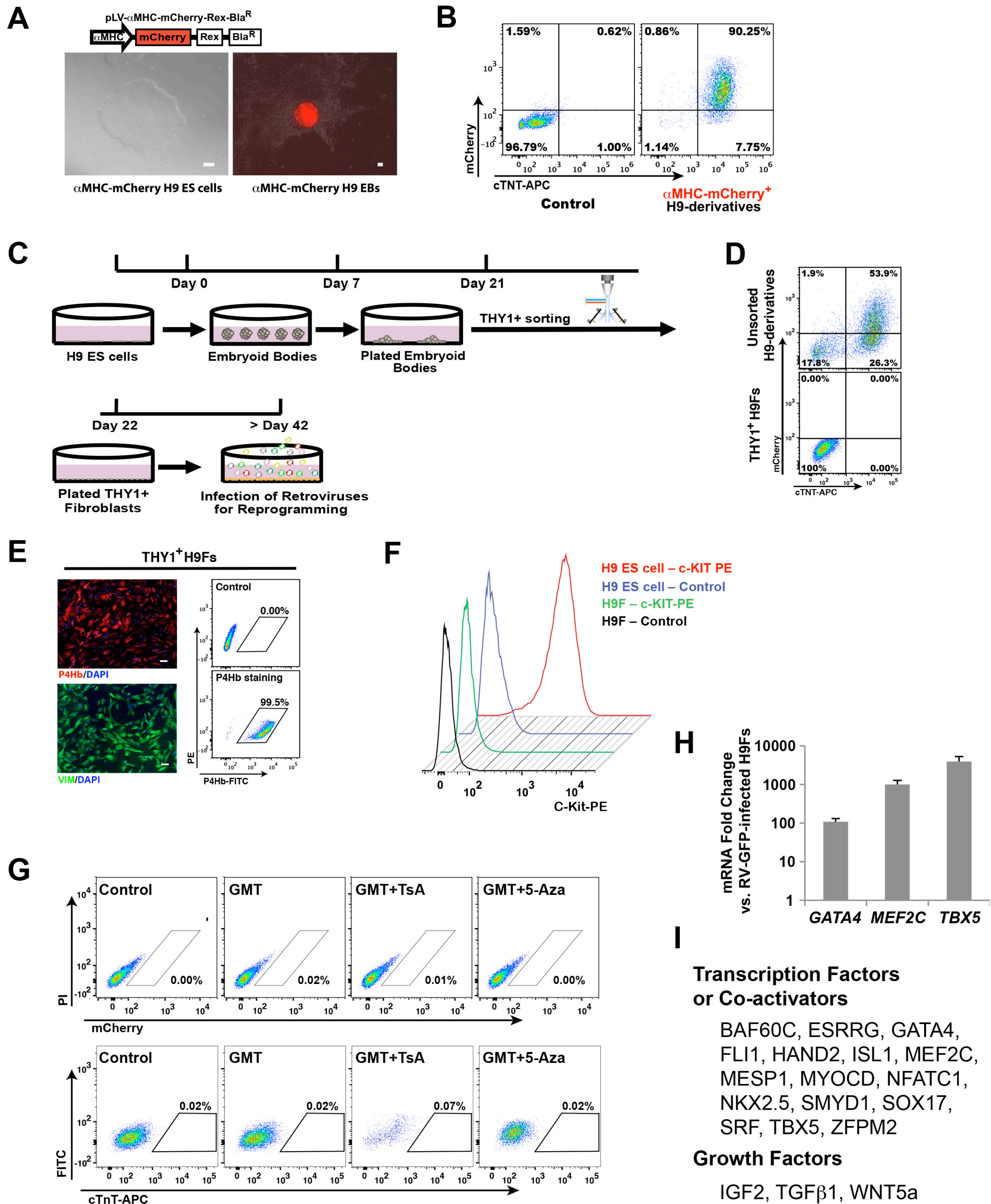

Figure S1. Strategy to identify factors that can reprogram human fibroblasts toward cardiomyocyte (CM)-like cells, related to Figure 1.

(A) Top, schematic of lentiviral cardiac-reporter construct, in which mCherry expression is driven by the mouse αMHC promoter. Bottom, Representative images of undifferentiated (left panel, bright field) and differentiated αMHC-mCherry H9 ES-derived embryoid bodies

(EBs) (right panel) with mCherry (red) being expressed only in beating areas. Scale bars, 50 μm. (B) Histogram of FACS analysis of differentiated human ES cells containing the reporter, showing that nearly all mCherry$^+$ cells, purified by FACS sorter, were also cardiac troponin T (cTNT)$^+$. (C) Scheme of differentiation (EB method) and reprogramming protocol for H9 ES cell–derived fibroblasts (H9Fs). (D) Sorted THY1$^+$ H9Fs had no contamination of mCherry$^+$ and cTNT$^+$ cells. (E) Purified THY1$^+$ H9Fs were positive for fibroblast markers, Prolyl-4-Hydroxylase β (P4Hb) and vimentin (VIM). Scale bars, 20 μm.

(F) THY1$^+$ H9Fs purified by FACS contained no c-KIT$^+$ progenitor/stem cells.

(G) Representative FACS analyses showing that neither mCherry$^+$ nor cTNT$^+$ cells were detected in H9Fs 2 weeks after retroviral infection of GMT, even in the presence of trichostatin A (TsA) or 5-aza-2´-deoxycytidine (5-Aza).

(H) Graph showing mRNA-fold change in expression of human *GATA4*, *MEF2C* and *TBX5* in H9Fs after retroviral infection compared to control retrovirus (n=3).

(I) List of GMT and the other 16 candidate reprogramming factors selected for human cardiac reprogramming.

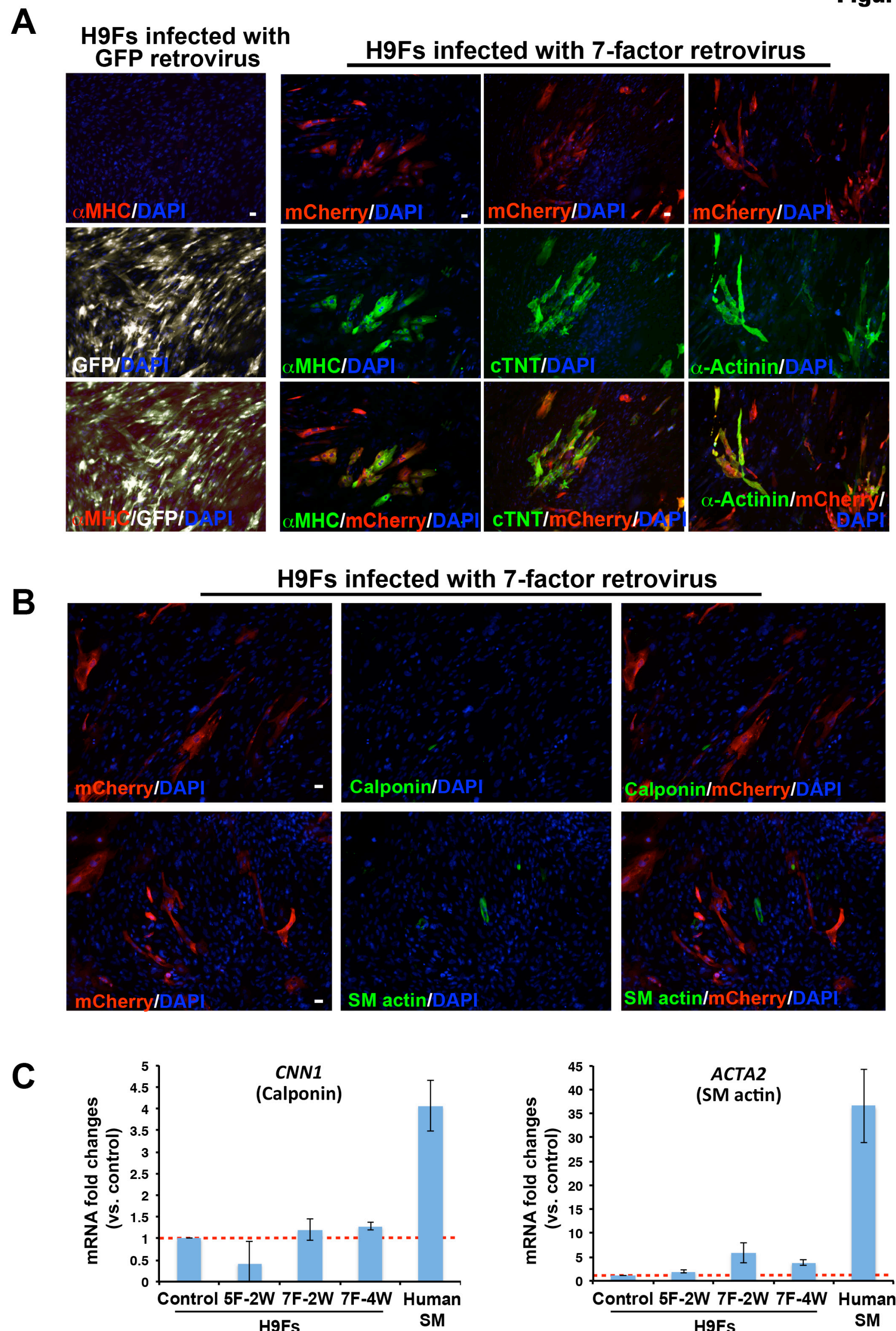

Figure S2. Human fibroblasts reprogrammed by 7 factors express cardiac genes, but not smooth-muscle genes, related to Figure 1.

(A) Immunocytochemistry of H9Fs 2 weeks after retroviral infection with GFP or 7-factors using mCherry, cTNT, or MHC antibodies; merged images in lower panels. (B) Calponin and smooth muscle (SM) actin, markers of smooth-muscle cells assayed by immunocytochemistry, were not present in αMHC-mCherry^{+} cells reprogrammed with 7 factors. Scale bars, 20 µm. (C) The mRNA expression levels of *CNN1* (Calponin) and *ACTA2* (SM actin) had no significant changes in H9Fs (n=4) after 5F- or 7F-retroviral infection. Human smooth muscle (SM) served as the positive control.

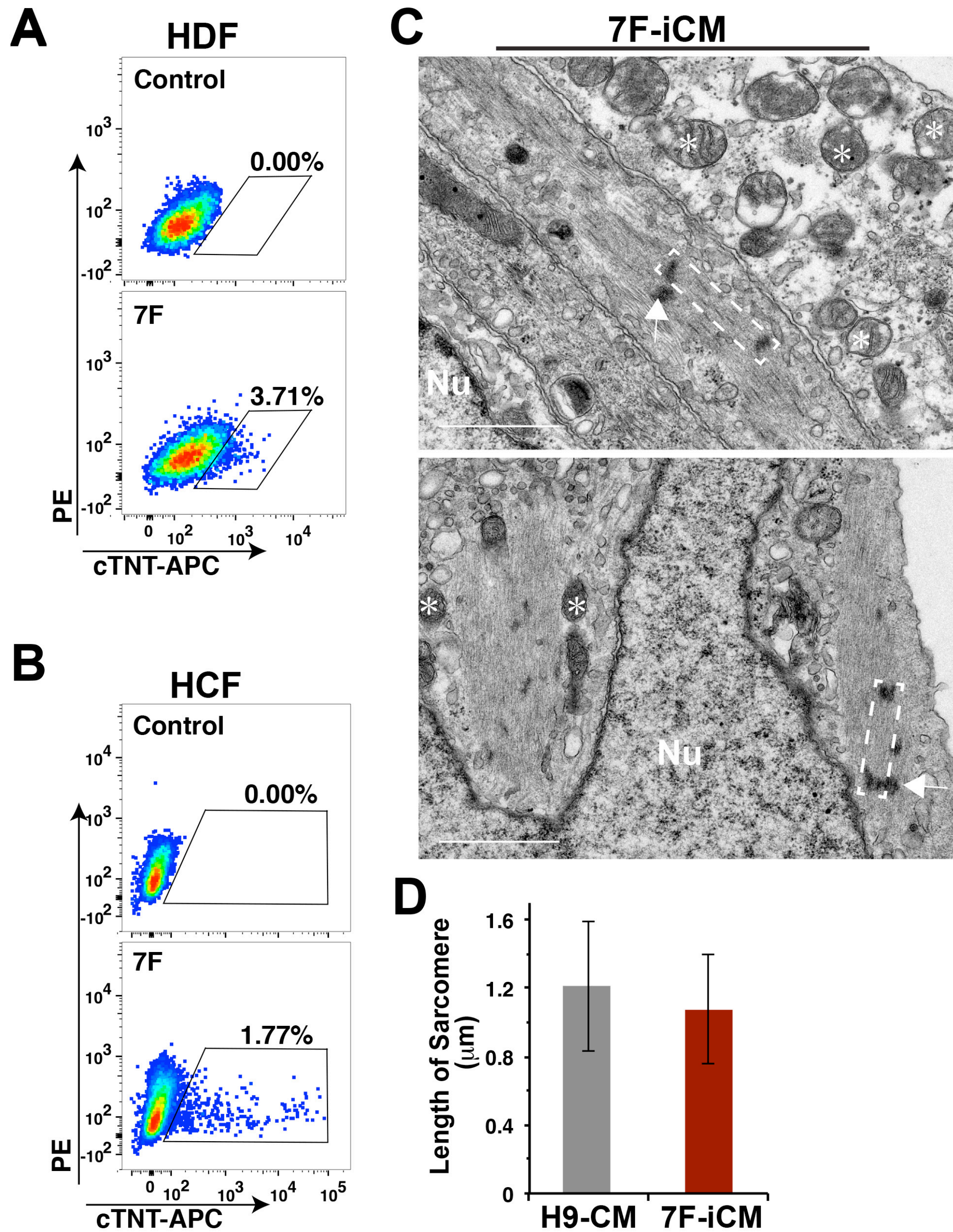

Figure S3. Human fibroblasts reprogrammed by 7 factors express cardiac genes and form sarcomere-like structures, related to Figure 2.

(A–B) Representative FACS plots of cTNT⁺ cells in HDFs and HCFs 2 weeks after infection with 7 factors. (C) Electron microscopic images of αMHC-mCherry⁺ cells revealed enriched mitochondria (star) and sarcomeres (dash area) with Z lines (arrow) in 7-factor reprogrammed iCMs. Scale bars, 1 μm. (D) Summary of the sarcomere length measured from 7-factor-reprogrammed human iCMs (n=81 from 19 cells) and H9-CMs (n=19 from 10 cells).

Figure S4

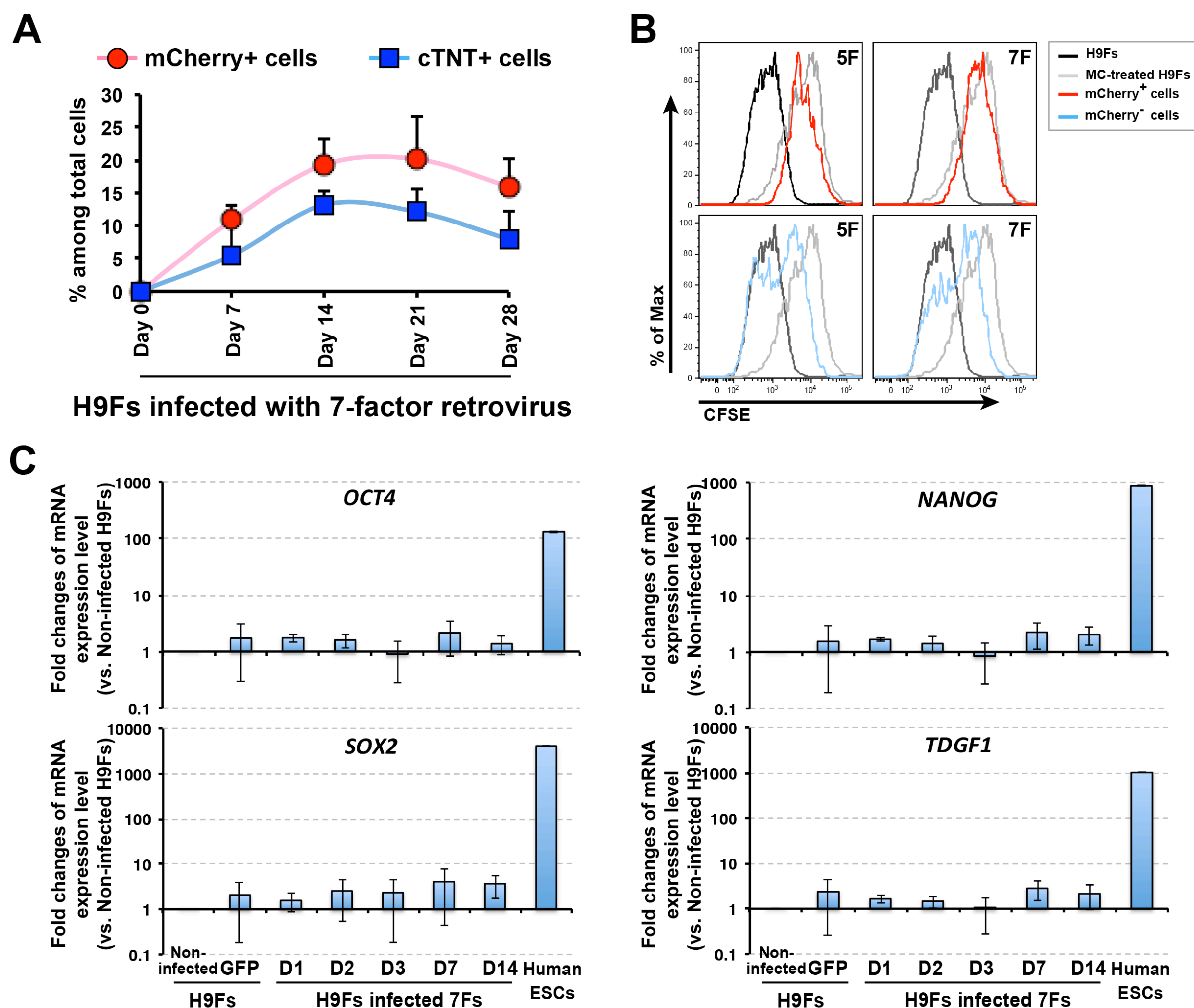

Figure S4. Behavior of human iCMs, related to Figure 3.

(A) The temporal dynamics of reprogramming efficiency of αMHC-mCherry$^+$ (red circle) and cTNT$^+$ (blue square) cells in H9Fs (n=3) in the 4 weeks after 7-factor retroviral infection. (B) Cell proliferation studied by using dilution of carboxyfluorescein diacetate succinimidyl ester (CFSE), showing that cell proliferation was observed in many non-reprogrammed H9Fs, but not in reprogrammed αMHC-mCherry$^+$ cells. Non-infected H9Fs (black line) were used as positive controls for cell proliferation, illustrating cell division by decreased fluorescence intensity. Mitomycin C (MC)-treated H9Fs (gray line) were used as negative controls for proliferation and demonstrated high fluorescence intensity due to lack of dilution. Images are representative of 3 independent experiments. (C) qRT-PCR showing that pluripotent genes were not significantly activated in 2 weeks after 7-factor retroviral infection (n=3). Human ESCs served as the positive control.

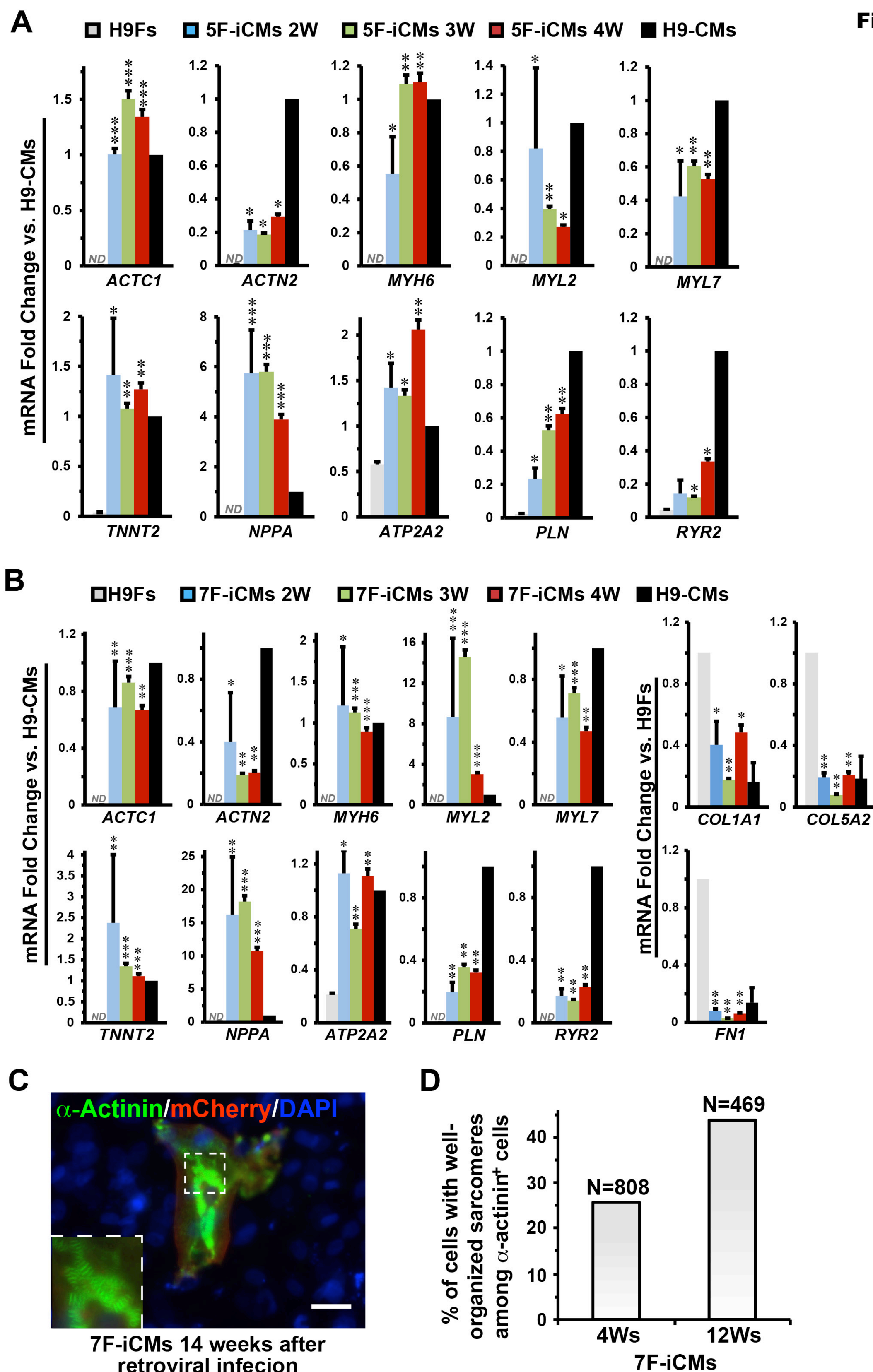

Figure S5. Gene expression of human iCMs, related to Figure 3.

(A) mRNA expression levels of cardiac-specific genes, including *ACTC1*, *ACTN2*, *MYH6*, *MYL2*, *MYL7*, *TNNT2*, *NPPA*, *ATP2A2*, *PLN*, and *RYR2* in H9Fs, 5F-iCMs (2, 3 and 4 weeks

(Ws) after induction), and H9-CMs by qPCR. N=3. (B) mRNA-expression levels of cardiac-specific (*ACTC1*, *ACTN2*, *MYH6*, *MYL2*, *MYL7*, *TNNT2*, *NPPA*, *ATP2A2*, *PLN*, *RYR2)*, and fibroblast-enriched (*COL1A1*, *COL5A2*, *FN1)* genes in H9Fs, 7F-iCMs (at 2, 3, 4 weeks (W) after induction), and H9-CMs. N=3. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$ versus H9Fs. C) Immunocytochemistry of α-actinin revealed sarcomeric organization in 7-factor reprogrammed iCMs over 12 weeks after retroviral infection. Scale bar indicate 20 μm. D) Quantification of cells with well-organized sarcomeric structures among reprogrammed α-actinin^{+} cells 4 weeks (4Ws) or 12 weeks (12Ws) after retroviral infection.

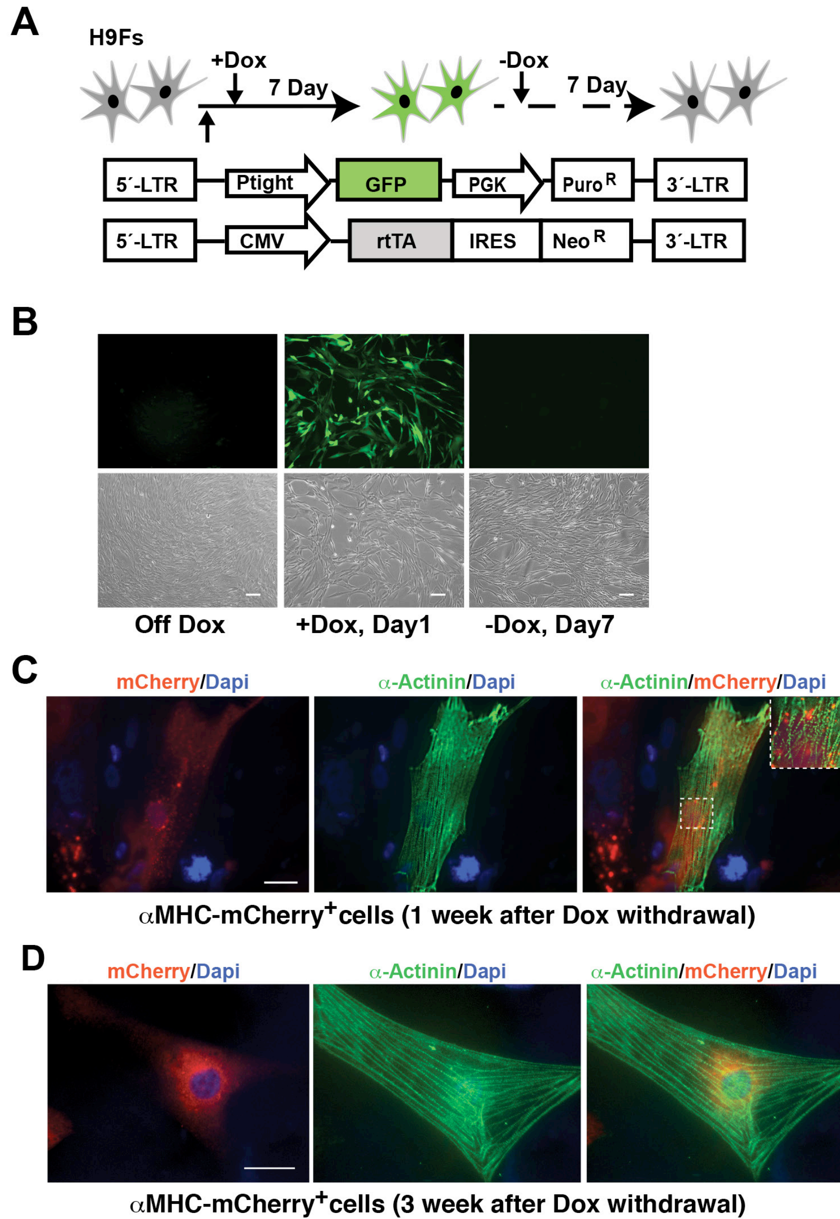

Figure S6. Expression kinetics of doxycycline-inducible gene expression, related to Figure 4.

(A) Scheme of the strategy to test expression kinetics of the doxycycline (Dox)-inducible retroviral system. (B) Images of H9Fs infected with RVX-tetOn-GFP and RVX-rtTA before (off Dox), 1 day after Dox addition (+Dox) and 7 days after Dox withdrawal (–Dox). Upper panel indicates GFP^{+} cells and lower panel is corresponding bright field image. Scale bars, 50 μm. (C-D) iCMs derived from 2-week Dox induction after 7-factor inducible retroviral infection maintained αMHC-mCherry and α-actinin expression 1 week (C) and 3 weeks (D) after Dox withdrawal. Inset represents high magnification of boxed area. Scale bars, 20 μm.