iScience, Volume *25*

Supplemental information

Direct reprogramming of adult adipose-derived regenerative cells toward cardiomyocytes using six transcriptional factors

Shingo Narita, Kazumasa Unno, Katsuhiro Kato, Yusuke Okuno, Yoshitaka Sato, Yusuke Tsumura, Yusuke Fujikawa, Yuuki Shimizu, Ryo Hayashida, Kazuhisa Kondo, Rei Shibata, and Toyoaki Murohara

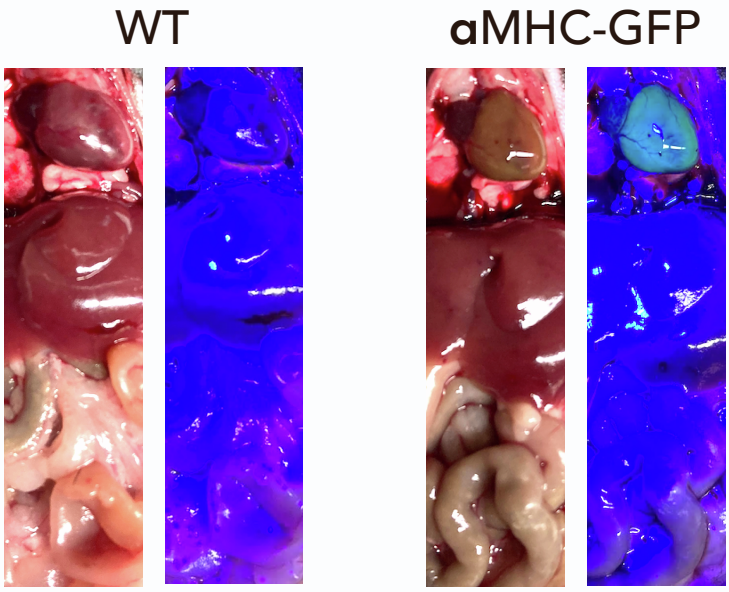

Figure S1 (Related to Figure 1). C57BL/6-Tg (Myh6-EGFP) MG3Tm (αMHC-GFP) and wild-type (WT) heart. Only αMHC-GFP heart organ expressed GFP.

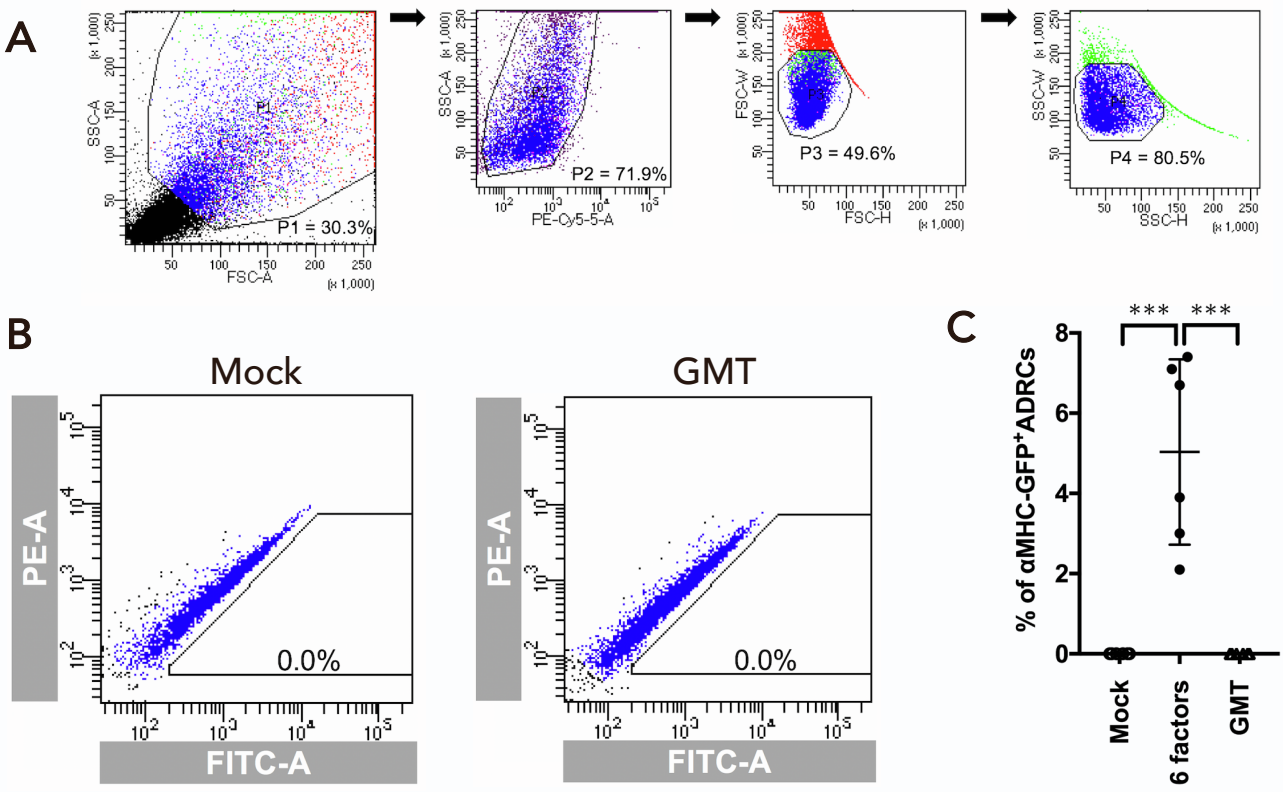

Figure S2 (Related to Figure 2). Flow cytometry analysis of ADRCs three weeks after transfection. (A) Gating strategy for the purification. After initial FSC/SSC gating, live cells were gated as 7-AAD(PE-Cy5-5) negative, and then doublet was excluded. (B) Representative FACS plots of mock induced (left) and GMT (Gata4, Mef2c, Tbx5) induced (right) ADRCs, both of which are incubated three weeks after transduction. The FACS plot of 6 factors ADRCs is shown in Figure 2I. (C) Percentages of αMHC-GFP^{+}ADRCs three weeks after the transfection of each mock control, six factors, and GMT (*$p < 0.1$, **$p < 0.05$, ***$p < 0.01$, n=6).

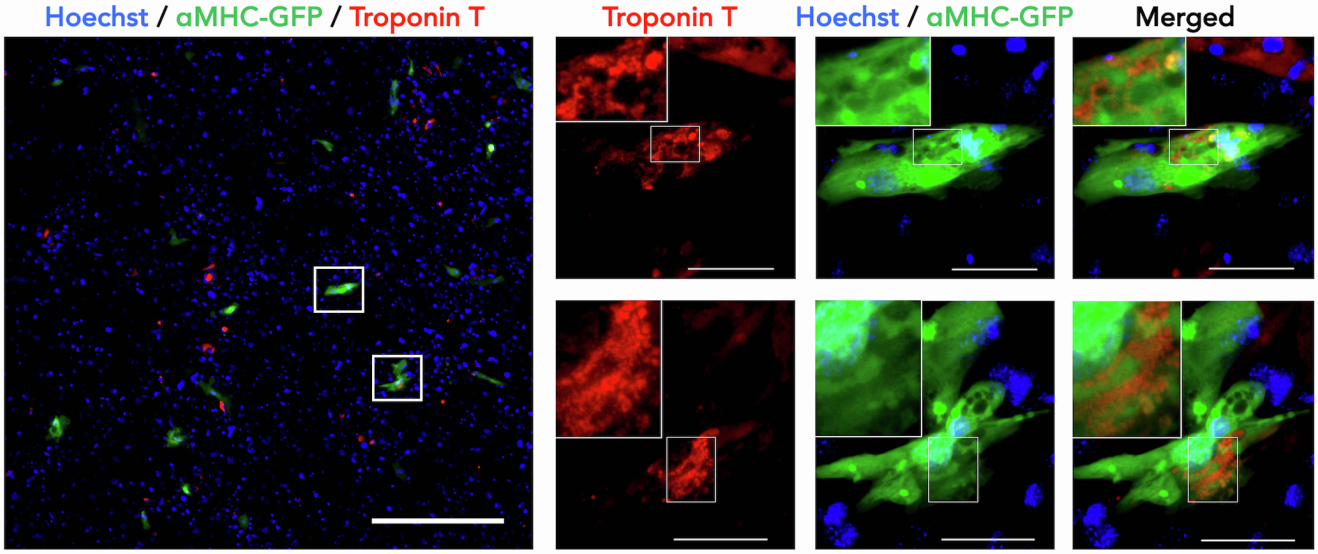

Figure S3 (Related to Figure 3). Representative images of GFP⁺ADRCs immunostaining for cardiac troponin T after induction of six factors.

The secondary antibody was labeled with Alexa 594 (red). Scale Bars = 500 μm (left panel) and 50 μm (right panels)

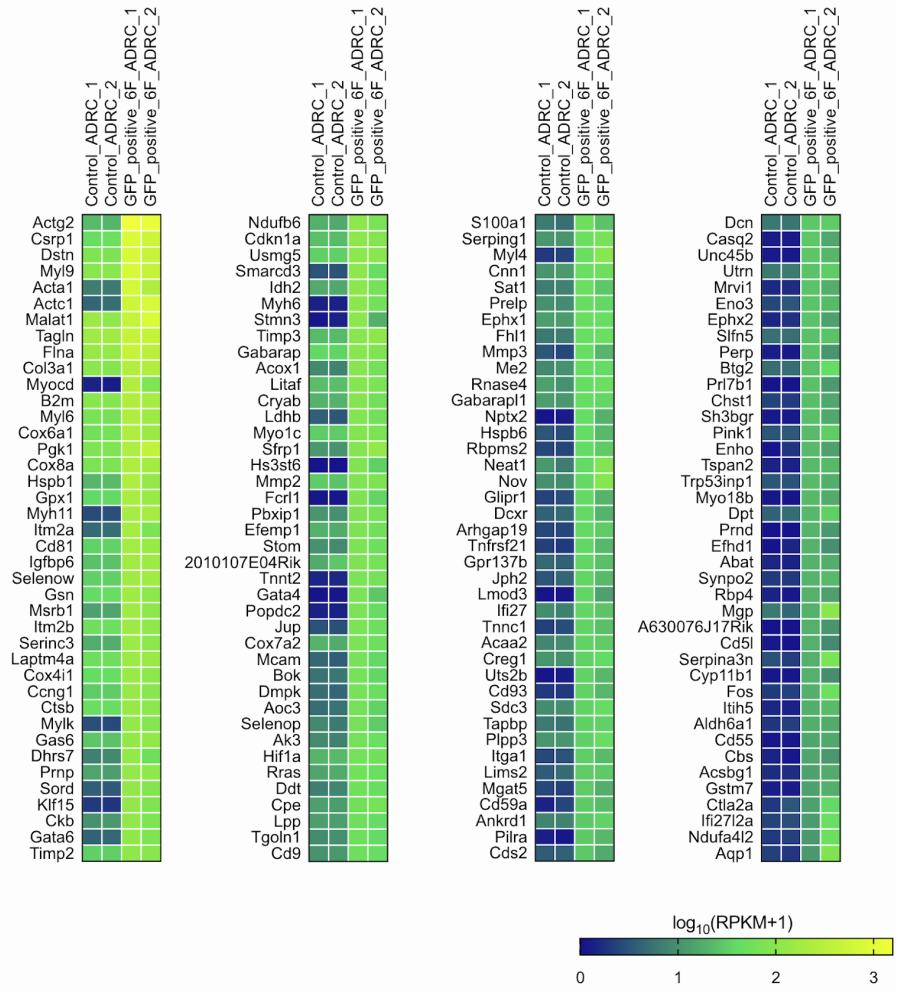

Figure S4 (Related to Figure 3). Heatmap of differentially expressed genes in GFP⁺ADRCs during reprogramming.

Genes upregulated in GFP⁺ADRCs compared with uninduced ADRCs, which are listed in order of decreasing FDR p-value (160 genes).

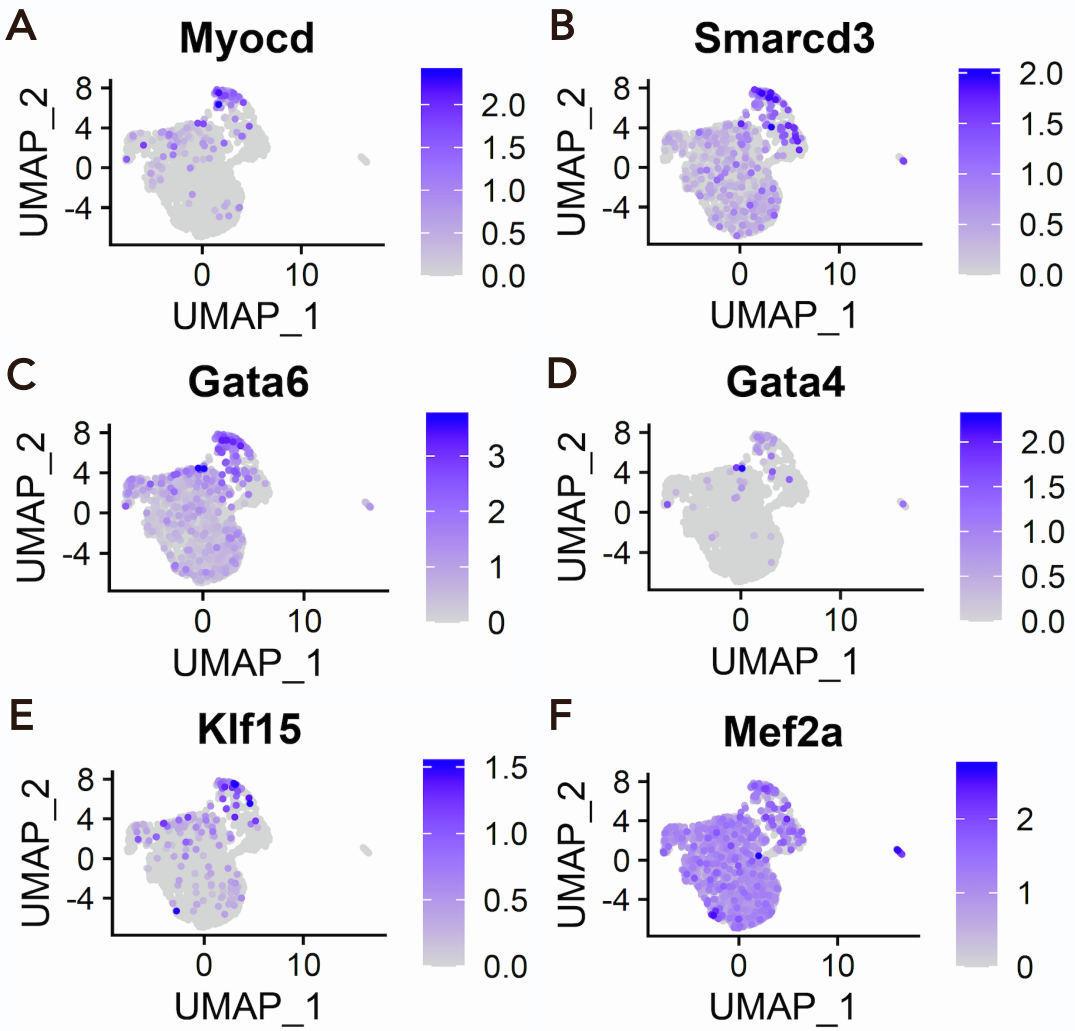

Figure S5 (Related to Figure 3). Single-cell RNA sequence analysis of 6F-ADRCs three weeks after transfection.

FeaturePlots of each exogenous six factor genes expressions showing (A) Myocd, (B) Smarcd3(Baf60c), (C) Gata6, (D) Gata4, (E) Klf15, and (F) Mef2a expression.

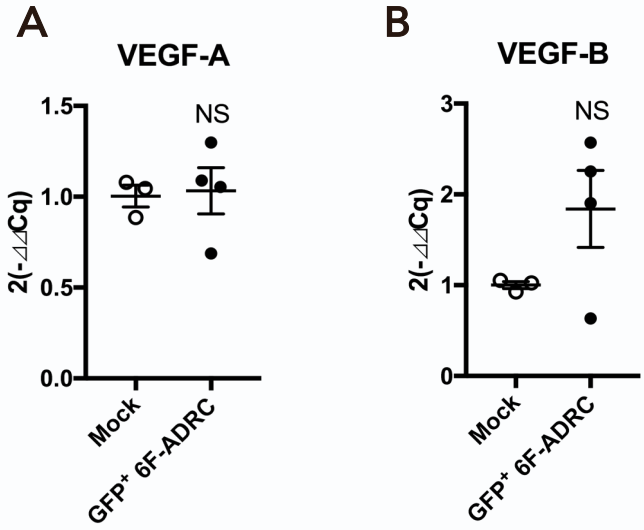

Figure S6 (Related to Figure 4). qPCR analysis of VEGF-A and VEGF-B expression.

(A) VEGF-A and (B) VEGF-B expression levels of GFP$^+$ 6F-ADRCs three weeks after transfection (n=4) relative to mock induced ADRCs (n=3).

Table S1 (Related to STAR Methods). List of primers used for In-fusion cloning.

Target	Primer Sequence
Gata4	5'CGTCAGATCCGCTAGGAGCTTGGGGCGATGTAC3' 5'CTGCAGAATTCTCGATCCAGTGCTCCACCTGGA3'
Mef2c	5'GTCAGATCCGCTAGCAATACATAATTTCAGGGACGA3' 5'GGAGAGGGGCGGATCACTATTAAGTAATAATGTGATCA3'
Tbx5	5'GTCAGATCCGCTAGCAGAATAGAACCTCGCGCG3' 5'GGAGAGGGGCGGATCTGGCACAGGTCAGCCTTT3'
Gata6	5'GTCAGATCCGCTAGCGGACAGTGGATGGCCTTG3' 5'GGAGAGGGGCGGATCGCCTCTTGGTAGCACCAG3'
Mef2a	5'CGCTACCGGTCTCGAGGTGACTGAAAATGGGGC3' 5'GGCCGCTGCAGAATTCCAGGAAGCCTTAGGTCA3'
Baf60c	5'GTCAGATCCGCTAGCTGAGCCCACCCCGATGGC3' 5'GGAGAGGGGCGGATCTGGTCCACCCTGACGCTT3'
Klf15	5'CGTCAGATCCGCTAGCAGCATGGTGGACCACCT3' 5'CTGCAGAATTCTCGATGCGCTCAGTTGATGGCG3'
Myocd	5'CGTCAGATCCGCTAGACTCACCATGACACTCCTG3' 5'CTGCAGAATTCTCGACACTGAGCTCTCGTAGCT3'
Nkx2-5	5'GTCAGATCCGCTAGCGCTCCTGCGTCGCCACCATGT3' 5'GGAGAGGGGCGGATCCAGCTTTCCCATCCCCGGA3'
Hrt2	5'GTCAGATCCGCTAGCTCGTCGCGATGAAGCGCC3' 5'GGAGAGGGGCGGATCACTATTGCAAAGAGATTCAAG3'
Hand2	5'GTCAGATCCGCTAGCCACGGAAGGCACCATGAG3' 5'GGAGAGGGGCGGATCCGGCTCACTGCTCTCCTC3'
Tbx20	5'CGTCAGATCCGCTAGGGAACCATGGAGTTCACG3' 5'CTCGAGACCGGTAGCCCGACTCTCAGTGGAATC3'
Prox1	5'GTCAGATCCGCTAGCTCCAGTGATGCCTGACCA3' 5'GGAGAGGGGCGGATCGAGCGTTGCAATCTCTACT3'
Hey1	5'CGTCAGATCCGCTAGACCATGAAGAGAGCTCACC3' 5'CTGCAGAATTCTCGACAACCGTCCCAACACACC3'
Jarid2	5'CGTCAGATCCGCTAGATCTCAGAATGAGCAAGGAA3' 5'CTCGAGACCGGTAGCATAAGTGACCATGGGTTCG3'

Table S2 (Related to STAR Methods). List of primers used for qPCR.

Target	Primer Sequence
Gapdh	5'AAAGGGTCATCATCTCCGCC3' 5'GCCCTTCCACAATGCCAAAG3'
Myh6 (αMHC)	5'ATCATCCCGGCATGACTGTG3' 5'AGCTCCTCCGTCCTCTGTAT3'
Actc1	5'CCCTGGTATTGCCGATCGTA3' 5'TGGGCCTGCCTCATCATACT3'
Tnnt2	5'TGGTGGAGGAGTACGAGGAG3' 5'ATCCTCAGCCTTCCTCCTGT3'
Ttn	5'CCCTCCATTCAAGGTTGGCT3' 5'GGCCGGCATTGTTAGAAACC3'
Fhod3	5'GCATCAAGAGGGTGGACATT3' 5'TCCTCCTCCCCTAGGTCTGT3'
Actn2	5'ACAATCGACCAACTGCACCT3' 5'GGATCTCCTCGATGCTGTGG3'
VEGF-A	5'AGCACAGCAGATGTGAATGC3' 5'AATGCTTTCTCCGCTCTGAA3'
VEGF-B	5'CCTGGAAGAACACAGCCAAT3' 5'GGAGTGGGATGGATGATGTC3'

Table S3 (Related to STAR Methods). Composition of qPCR reaction mixture.

qPCR Regent	Volume per reaction
TB Green Premix Ex Taq II	12.5μl
Forward Primer (10μM)	1μl
Reverse Primer (10μM)	1μl
Template cDNA (<100ng)	2μl
dH_2O	8.5μl
Total volume	20μl

Table S4 (Related to STAR Methods). qPCR amplification conditions.

Stage	Temperature (℃)	Time
Initial denaturation	95	30 seconds
40 cycles	95	5 seconds
	55	30 seconds
Final extension	72	30 seconds