Supporting Information

Min et al.

Table S1. Description of antibodies used in this study.

Primary antibodies	Manufacturer	Catalog#		Dilution (frozen sections)	Dilution (isolated cells)	Dilution (Flow cytometry)
Twist2	Abcam	Ab66031	Rabbit polyclonal	1:200		1:50
Twist2	Sigma	WH0117581M1	Mouse monoclonal	1:50		
cTnI	Abcam	Ab47003	Rabbit polyclonal	1:100		
cTnT	Invitrogen	MA5-12960	Mouse monoclonal	1:200	1:200	
α-actinin	Abcam	Ab90776	Rabbit polyclonal	1:200		
CD31	BD Biosciences	553370	Mouse monoclonal	1:100	1:200	
vimentin	Sigma	V2258	Mouse monoclonal	1:100		
α-SMA	Sigma	A2547	Mouse monoclonal	1:200		
DDR2	Sigma	SAB1302555	Rabbit polyclonal		1:200	
CD105	BioLegend	120413	CD105-APC Rat IgG2A			1:50
CD73	BD Biosciences	561545	CD73-Alexa Fluor 488 Rat IgG2A			1:50
CD44	BD Biosciences	561862	CD44-APC Rat IgG2B			1:50
c-kit	BD Biosciences	553356	CD117-APC Rat IgG2B			1:50
pdfgra	R&D Systems	FAB1062G	pdgfra-Alexa Fluor 488 Goat IgG			1:50
CD31	BD Biosciences	551262	CD31-APC Rat IgG2A			1:50
CD90.2	BD Biosciences	561974	CD90.2-APC Rat IgG2A			1:50
CD45	R&D Systems	967208	CD45-PerCP Rat IgG2B			1:20
CD29	eBioscience	17-0291-82	CD29-APC Hamster IgG			1:20
Sca-1	BD Bioscience	560654	Ly-6A/E-APC-Cy7 Rat IgG2A			1:20
CD105	R&D Systems	967207	CD105/Endoglin-CFS Rat IgG2A			1:20

Isotype Controls	Manufacturer	Catalog#		Dilution (Flow cytometry)
PerCP Rat IgG2B	R&D Systems	967114	Rat IgG2B-PerCP Isotype Control	1:20
APC Hamster IgG	eBioscience	17-4888	Armenian Hamster IgG-APC Isotype Control	1:20
APC-Cy7 Rat IgG2A	BD Bioscience	552770	Rat IgG2a-APC-Cy7 Isotype Control	1:20
CFS Rat IgG2A	R&D Systems	965715	Rat IgG2A-CFS Isotype Control	1:20
APC Rat IgG2B	BD Biosciences	553991	Rat IgG2b-APC Isotype Control	1:50
APC Rat IgG2A	BD Biosciences	553932	Rat IgG2a-APC Isotype Control	1:50
Alexa Fluor 488 Goat IgG	R&D Systems	IC108G	Goat IgG-Alexa Fluor 488 Isotype Control	1:50
Alexa Fluor 488 Rat IgG2a	BD Biosciences	557676	Rat IgG2a-Alexa Fluor® 488 Isotype Control	1:50

Secondary antibodies	Manufacturer	Catalog#		Dilution (frozen sections)	Dilution (isolated cells)	Dilution (Flow cytometry)
Alexa Fluor 488	Life Technologies	A-11029	Goat anti Mouse IgG (H+L)	1:400	1:400	
Alexa Fluor 488	Life Technologies	A-11008	Goat anti-Rabbit IgG (H+L)	1:400	1:400	
Alexa Fluor 647	Life Technologies	A-21236	Goat anti-Mouse IgG (H+L)			1:200
Alexa Fluor 647	Life Technologies	A-32733	Goat anti-Rabbit IgG (H+L)			1:200

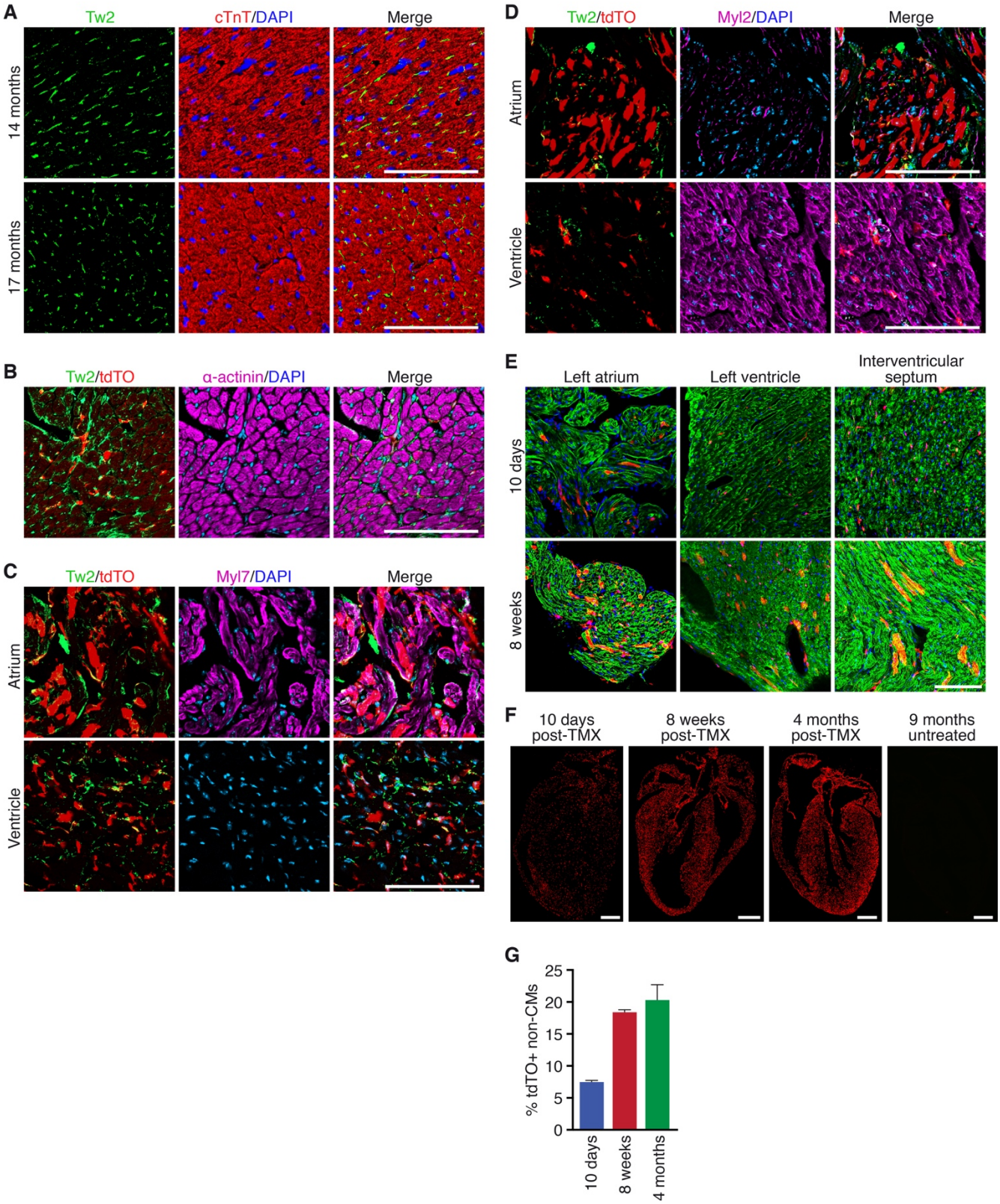
A
Tw2
cTnT/DAPI
Merge
14 months
17 months
B
Tw2/tdTO
α-actinin/DAPI
Merge
C
Tw2/tdTO
Myl7/DAPI
Merge
Atrium
Ventricle
D
Tw2/tdTO
Myl2/DAPI
Merge
Atrium
Ventricle
E
Left atrium
Left ventricle
Interventricular septum
10 days
8 weeks
F
10 days post-TMX
8 weeks post-TMX
4 months post-TMX
9 months untreated
G
% tdTO+ non-CMs
25
20
15
10
5
0
10 days
8 weeks
4 months

Fig. S1. Analysis of Tw2-tdTO+ cells in adult heart.

(A) Immunostaining of Tw2 (green) and cTnT (red) proteins on heart sections of 14- and 17-month old wild-type mice. Scale bar: 100 um.

(B) Tw2 antibody immunostaining (green) and tdTO labeling on heart sections of Tw2-CreERT2; R26-tdTO mice at 10 days post TMX treatment. Scale bar: 100 um.

(C) Tw2 antibody immunostaining (green) and tdTO labeling in atrium and ventricle at 10 days post-TMX treatment. Sections were co-immnuostained with anti-Myl7, an atrial marker (violet). Scale bar: 100 um.

(D) Tw2 antibody immunostaining (green) and tdTO labeling in atrium and ventricle at 10 days post-TMX treatment. Sections were co-immnuostained with anti-Myl2, a ventricular marker (violet). Scale bar: 100 um.

(E) tdTO labeling in left atrium, left ventricle and interventricular septum at 10 days and 8 weeks post-TMX treatment. Sections were immunostained with cTnT. Scale bar: 100 um.

(F) Whole heart imaging at various time points post-TMX treatment. Right panel shows absence of tdTO signal in heart of Tw2-CreERT2; R26-tdTO mice without TMX treatment. Scale bar: 100 um.

(G) Percentage of tdTO+ non-CMs was quantified and averaged at indicated time points. n= 3 mice for each time point. Data are expressed as mean $\pm$ SEM.

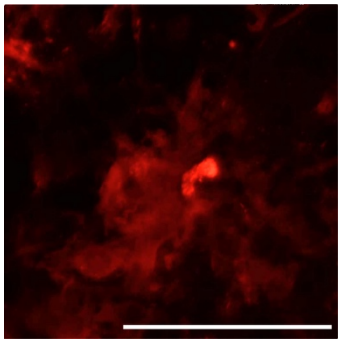

Fig. S2. Image from video (see MovieS1) of Tw2-tdTO+ cells that differentiated into beating CMs in culture. Scale bar: 100 um.

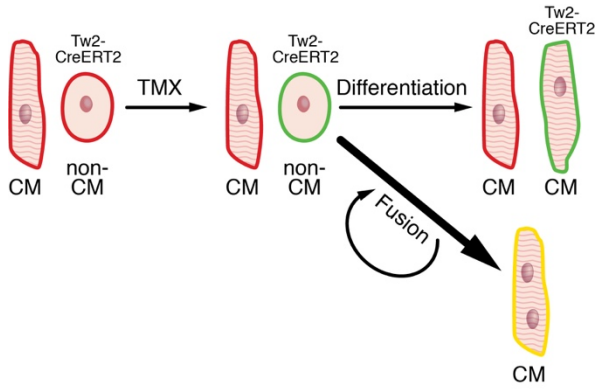

Fig. S3. Schematic of using Tw2-CreERT2; R26-mT/mG/+ mice to distinguish between fusion and differentiation events. CM denotes cardiomyocytes, non-CM represents non-cardiomyocytes. Upon TMX treatment of Tw2-CreERT2; R26-mT/mG/+ mice, a CM with a yellow membrane represents fusion of a non-CM to a CM. On the other hand, a CM with a green membrane represents de novo differentiation of a non-CM to a CM.

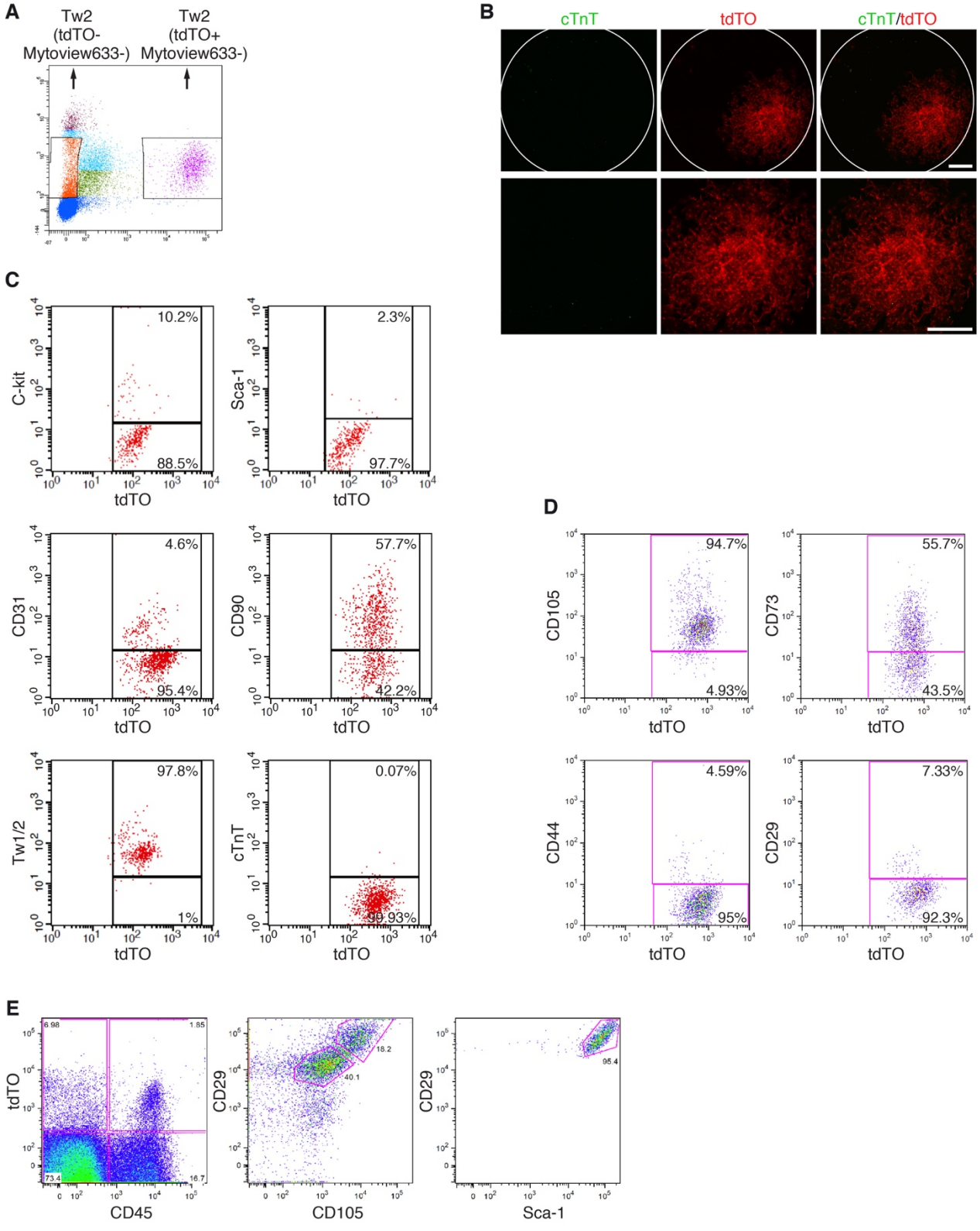
A
Tw2 (tdTO- Mytoview633-)
Tw2 (tdTO+ Mytoview633-)
B
cTnT
tdTO
cTnT/tdTO
C
C-kit
10.2%
88.5%
tdTO
Sca-1
2.3%
97.7%
CD31
4.6%
95.4%
CD90
57.7%
42.2%
Tw1/2
97.8%
1%
cTnT
0.07%
99.93%
D
CD105
94.7%
4.93%
CD73
55.7%
43.5%
CD44
4.59%
95%
CD29
7.33%
92.3%
E
tdTO
CD45
6.98
1.85
73.4
16.7
CD29
CD105
18.2
40.1
CD29
Sca-1
95.4

Fig. S4. FACS analysis of Tw2-tdTO+ cells.

(A) FACS plot showing isolation of Tw2-tdTO+ (tdTO+; Mitoview 633 low) and Tw2-tdTO- (tdTO- Mitoview 633 low) cell populations from ventricles of Tw2-CreERT2; R26-tdTO mice at 10 days post-TMX treatment.

(B) Representative images of a well containing cTnT negative cells from clonal analysis. The images were acquired using an INCell high throughput confocal microscope under 4X lens. Bottom panels show magnified images. Scale bar: 100 um.

(C) FACS analysis for expression of Twist, cTnT, CD31, and CD90 was performed on Tw2-tdTO+ cells isolated from ventricles of Tw2-CreERT2; R26-tdTO mice at 10 days post-TMX treatment.

(D) FACS analysis for expression of CD105, CD73, CD44, and CD29 was performed on Tw2-tdTO+ cells isolated from ventricles of Tw2-CreERT2; R26-tdTO mice at 10 days post-TMX treatment.

(E) FACS plot showing multi-color MSC panel analysis of Tw2-tdTO+ cells isolated from ventricles of Tw2-CreERT2; R26-tdTO mice at 10 days post-TMX treatment. CD45-, CD29+, CD105+, and Sca-1+ mark MSC population.

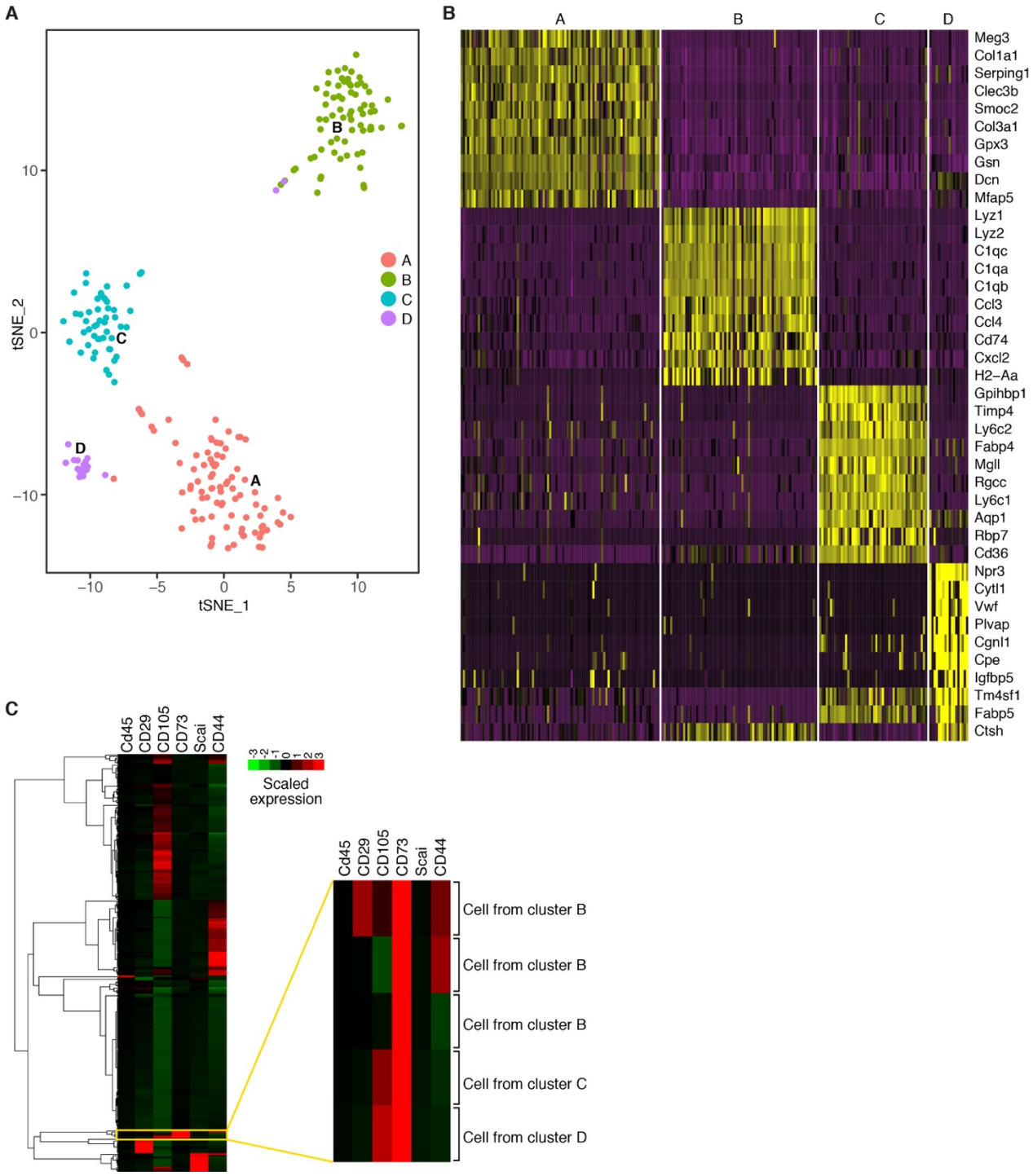

Fig. S5. Single cell RNA-seq of Tw2-tdTO+ cells.

(A) tSNE plot of the Tw2-tdTO+ cells. Each cluster is alphabetically labeled and color coded.

(B) Heat map of the 5 clusters with top 10 enriched marker genes, as identified by single cell RNA-seq.

(C) Heat map showing the expression of 6 MSC marker genes in each of the single Tw2-tdTO+ cells.

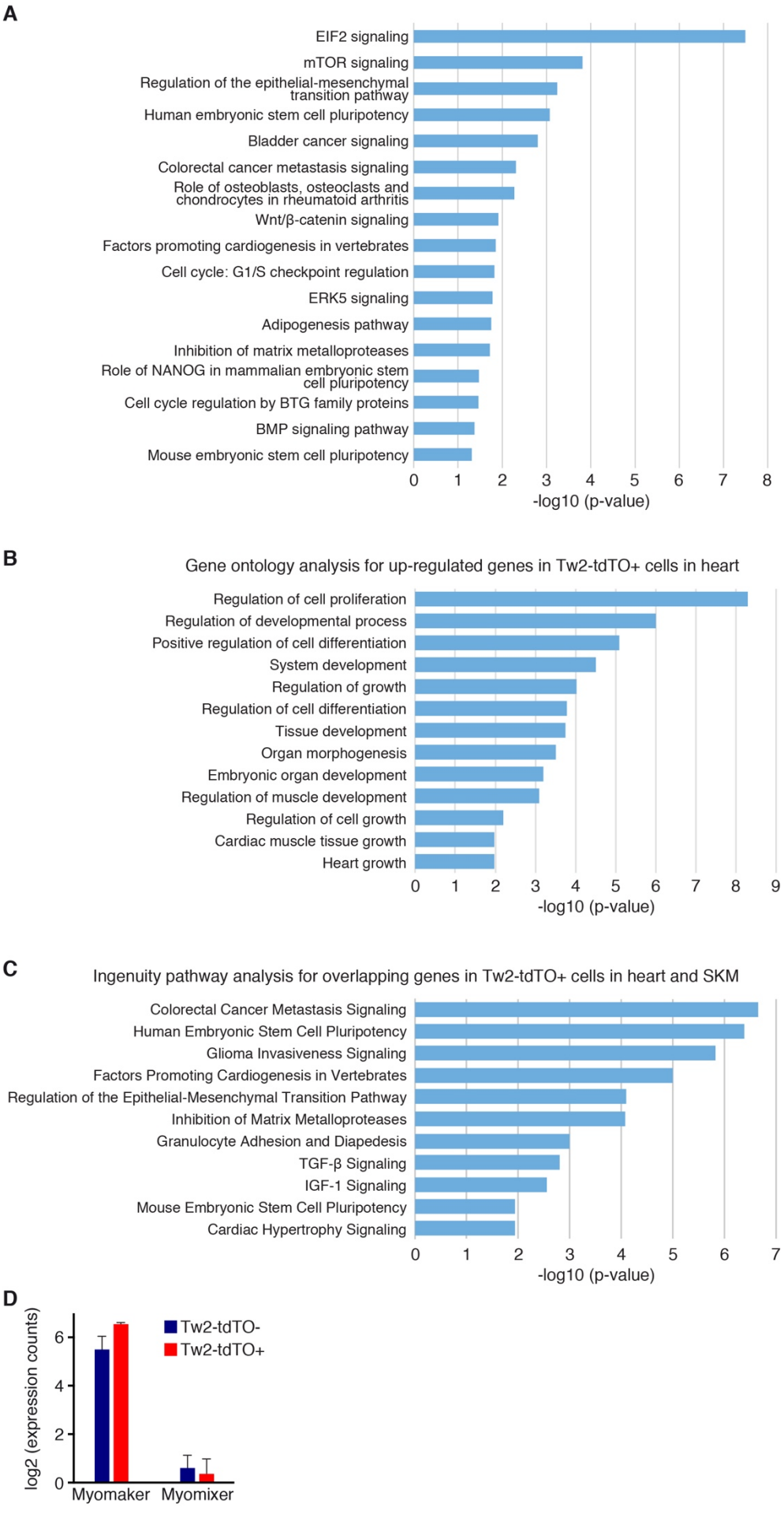
A
EIF2 signaling
mTOR signaling
Regulation of the epithelial-mesenchymal transition pathway
Human embryonic stem cell pluripotency
Bladder cancer signaling
Colorectal cancer metastasis signaling
Role of osteoblasts, osteoclasts and chondrocytes in rheumatoid arthritis
Wnt/β-catenin signaling
Factors promoting cardiogenesis in vertebrates
Cell cycle: G1/S checkpoint regulation
ERK5 signaling
Adipogenesis pathway
Inhibition of matrix metalloproteases
Role of NANOG in mammalian embryonic stem cell pluripotency
Cell cycle regulation by BTG family proteins
BMP signaling pathway
Mouse embryonic stem cell pluripotency
0 1 2 3 4 5 6 7 8
-log10 (p-value)
B
Gene ontology analysis for up-regulated genes in Tw2-tdTO+ cells in heart
Regulation of cell proliferation
Regulation of developmental process
Positive regulation of cell differentiation
System development
Regulation of growth
Regulation of cell differentiation
Tissue development
Organ morphogenesis
Embryonic organ development
Regulation of muscle development
Regulation of cell growth
Cardiac muscle tissue growth
Heart growth
0 1 2 3 4 5 6 7 8 9
-log10 (p-value)
C
Ingenuity pathway analysis for overlapping genes in Tw2-tdTO+ cells in heart and SKM
Colorectal Cancer Metastasis Signaling
Human Embryonic Stem Cell Pluripotency
Glioma Invasiveness Signaling
Factors Promoting Cardiogenesis in Vertebrates
Regulation of the Epithelial-Mesenchymal Transition Pathway
Inhibition of Matrix Metalloproteases
Granulocyte Adhesion and Diapedesis
TGF-β Signaling
IGF-1 Signaling
Mouse Embryonic Stem Cell Pluripotency
Cardiac Hypertrophy Signaling
0 1 2 3 4 5 6 7
-log10 (p-value)
D
log2 (expression counts)
0 2 4 6
Tw2-tdTO-
Tw2-tdTO+
Myomaker
Myomixer

Fig. S6. RNA-seq analysis of Tw2-tdTO+ cells. (A) IPA analysis of genes enriched in Tw2-tdTO+ cells relative to Tw2-tdTO- cells. (B) GO analysis of up-regulated genes in Tw2-tdTO+ cells compared to Tw2-tdTO- interstitial cells. (C) IPA analysis of the 380 overlapping genes in Tw2-tdTO+ cells in heart and skeletal muscle (SKM). (D) Myomaker and myomixer expression profile in Tw2-tdTO+ and Tw2-tdTO- cells.

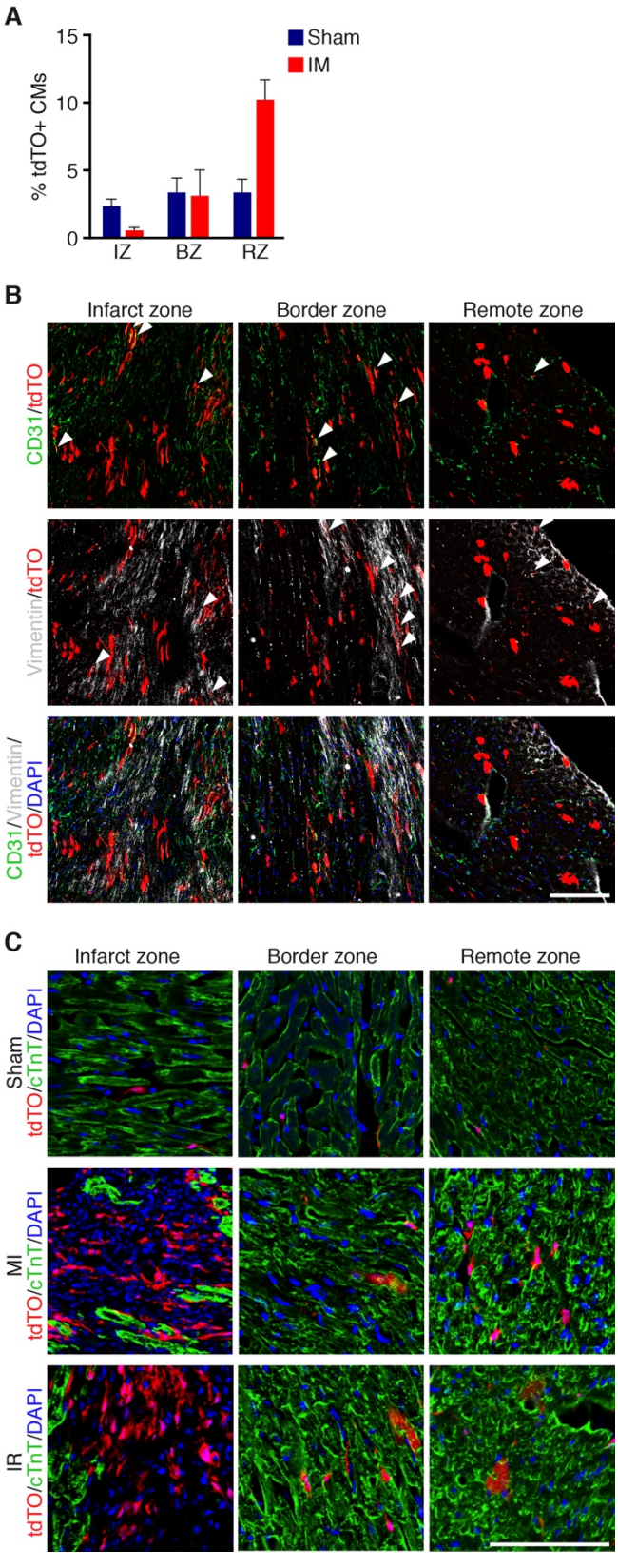
A
15
10
5
0
% tdTO+ CMs
Sham
IM
IZ
BZ
RZ
B
Infarct zone
Border zone
Remote zone
CD31/tdTO
Vimentin/tdTO
CD31/Vimentin/
tdTO/DAPI
C
Infarct zone
Border zone
Remote zone
Sham
tdTO/cTnT/DAPI
MI
tdTO/cTnT/DAPI
IR
tdTO/cTnT/DAPI

Fig. S7. Tw2-tdTO+ cells during myocardial injury after MI and I/R.

(A) Quantification of the Tw2-tdTO+ CMs in infarct zone (IZ), boarder zone (BZ) and remote zone (RZ) after sham and MI injury.

(B) Different areas of the heart after 1 week of IR were co-immnuostained with vimentin (grey) and CD31 (green). Infarct zone in the left panels, border zone in the middle panels, and remote zone in the right panels after 1 week of IR. Scale bar: 100 um.

(C) Immunostaining of different areas of the heart after MI. Infarct zone in the left panels, border zone in the middle panels, and remote zone in the right panels after 2 weeks of MI and I/R. cTnT marks cardiomyocytes in green. Scale bar: 100 um.

Supporting Information – Movie

Movie S1. Tw2-tdTO+ cells differentiated into CMs when maintained in CM induction media, as identified by expression of the CM marker cTnT (red). Movie shows that some of the differentiated CMs began beating after 2 weeks of culture.