Supplemental Table S1 Small molecules tested in cardiac reprogramming (Related to Figure1)

Name	Abbreviation	Concentration (μM)	MW	Discription
SB431542	S	2	384.4	ALK4/5/7 inhibitor
CHIR99021	CHIR, C	10	465.3	GSK3 inhibitor
Rolipram		10	275.3	PDE4-inhibitor
5'-Azacytidine		3	244.2	DNA methylation inhibitor
RG108		5	334.3	DNA methyltransferase inhibitor
parnate	P	2.5	364.5	MAO and LSD1 inhibitor
Lithium chloride	LiCL	5k	42.4	GSK3 inhibitor
NaButyrate	NaB	250	110.1	HDAC inhibitor
PS48		5	286.8	PDK1 activator
HX531		1	483.6	RXR antagonist
Carbacyclin		10	350.5	PPARδ agonist
BI-6C9		20	471.6	tBid inhibitor
Purvalanol B		2	432.9	CDK inhibitor
Foskolin	F	10	410.5	adenylate cyclase activator

Supplemental movie S1. GFP+ beating clusters were generated from Isl1-Cre/ROSA^{mTmG} MEFs. (Related to Figure 3)

Beating clusters were generated from Isl-Cre/ROSA^{mTmG} MEFs and video was taken at day 30.

Supplemental movie S2. The same beating cluster was shown in Figure 4A and B. (Related to Figure 4)

Video was taken at day 25 of cardiac reprogramming, just before those cells were fixed for immunochemistry analysis.

Supplemental Experimental Procedures

MEF and TTF preparation

MEFs and TTFs were prepared from C57B6 and OG2 mice (JAX) using established methods (Efe et al., 2011) . Isl1-Cre/ROSAmTmG embryos were obtained by crossing Isl-Cre mice with ROSAmTmG mice (JAX) (Muzumdar et al., 2007; Srinivas et al., 2001). Briefly, MEFs were isolated from E13.5 mouse embryos. The head, internal organs from the abdominal cavity, and heart regions were carefully removed from the embryos, and the tissues left were cut and trypsinized to produce single-cell suspensions. Those cells were expanded in MEF growth medium (MEF-GM) containing DMEM supplemented with 10% FBS, 2 mM Glutamax and 0.1 mM non-essential amino acids (NEAA). To generate TTFs, tail tips from neonatal mice were minced with a sterile razor blade and then evenly put into 10cm culture dish containing 2ml MEF-GM. Next day, additional medium was filled in. Five days later, fibroblasts migrated out of tissue samples were collected and expanded.

Lentivirus production and infection.

Lentivirus encoding Oct4, rtTA and tetO-OKS were prepared in 293T using pSin-EF2-hOCT4 (Addgene #16579), FUW-M2rtTA (Addgene #20342) and pHAGE2-TetO-STEMCCA-redlight (a generous gift from Dr. Gustavo Mostoslavsky) as previously described (Kim et al., 2011; Yu et al., 2007; Zhu et al., 2010). To ensure reproducibility and minimize inter-experimental variation, lentivirus was generated in a large batch and then aliquoted and stored at -80℃. Polybrene (4ug/ml) was added into the medium during virus infection to increase infection efficiency.

Generation of smooth muscle cells and endothelial cells

Smooth muscle cells and endothelial cells were generated following the same procedure as cardiac reprogramming, except after BMP4 treatment, medium was switched into smooth muscle growth medium containing 50% IMDM plus 50% F-12, supplemented with 7ug/ml of insulin, 15ug/ml of transferrin, 450uM of monothioglycerol, 5 mg/ml of bovine serum albumin fraction V and 10ng/ml PDGF-BB(R & D Systems); or endothelial cell growth medium, EGM2 (Lonza)

Immunostaining

Cells were fixed in 4% paraformaldehyde for 15 min and permeabilized in 0.5% Triton X-100 for 15min, then incubated in blocking buffer containing 3% BSA for 1 h at room temperature. Primary antibodies were diluted in blocking buffer and incubated overnight at 4°C. The following antibodies we used: cardiac troponin T (MS-295-P1, Thermo Scientific; 1:500); Gata4 (sc-25310, Santa Cruz Biotechnology; 1:200); MEF2C (#5030s, Cell signaling; 1:200); cardiac myosin heavy chain (ab15, Abcam; 1:400); Nkx2-5 (sc-8697, Santa Cruz Biotechnology; 1:200); Isl1 (DSB; 1:100); myosin light chain 2v (ab15, abcam; 1:300); myosin light chain 2a (# 311011, synaptic systems; 1:100); Connexin-43 (610061, BD Biosciences; 1:100); α-Actinin (A8711, Sigma-Aldrich; 1:200); Ki67 (550609, BD Biosciences; 1:200); SMA (A2547, Sigma-Aldrich; 1:400); Calponin 2(sc-16607, Santa Cruz Biotechnology; 1:100); CD31(550274; BD Biosciences; 1:100); and VE-cadherin (sc-9989; Santa Cruz Biotechnology; 1:100). Following extensive PBS washes (total 1h), cells were incubated with the appropriate Alexa Fluor–conjugated secondary antibodies (Invitrogen) for 1 h at room temperature and

nuclei were stained with DAPI (4,6-diamidino-2-phenylindole; Sigma-Aldrich). Images were acquired by a Zeiss Axioimager Z1 equipped with an Apotome system and processed using Zeiss Axiovision software.

Gene expression analysis by QPCR

Total RNA was extracted from samples at the designated time points using the RNeasy Plus mini kit with QiaShredder (Qiagen). RNA was reverse-transcribed using the iScript cDNA synthesis kit (Bio-Rad). Quantitative PCR was performed with iQSYBR Green Supermix on the 7500 Fast Real-Time PCR System (Applied Biosystems). All qPCR reactions were done in triplicate, and the expression data were normalized to GAPDH levels. Here are the details about the primers we used in this study.

Target	primer	sequences
Gata4	F	CCTGGAAGACACCCCAATCTC
	R	AGGTAGTGTCCCGTCCCATCT
Nkx2.5	F	GGTCTCAATGCCTATGGCTAC
	R	GCCAAAGTTCACGAAGTTGCT
MEF2C	F	AGATACCCACAACACACCACGCGCC
	R	CATTATCCTTCAGAGAGTCGCATGCGCTT
RyR2	F	ACATCATGTTTTACCGCCTGAG
	R	TTTGTGGTTATTGAACTCTGGCT
Myh6	F	GATGCCCAGATGGCTGACTT
	R	GGTCAGCATGGCCATGTCCT
TnnT2	F	GCGGAAGAGTGGGAAGAGACA
	R	CCACAGCTCCTTGGCCTTCT
Nanog	F	TCTTCCTGGTCCCCACAGTTT
	R	GCAAGAATAGTTCTCGGGATGAA
Rex1	F	CCCTCGACAGACTGACCCTAA
	R	TCGGGGCTAATCTCACTTTCAT
GAPDH	F	GTGGCAAAGTGGAGATTGTTG
	R	CTCCTGGAAGATGGTGATGG

Chromatin Immunoprecipitation (ChIP).

Starting MEFs(D0) and induced Cells at day 40 (D40) and minced neonatal heart tissues were used for histone modification analysis using EZ-ChIP Chromatin Immunoprecipitation kit (EMD Millipore). Briefly, histones and DNA were cross-linked with 1% formaldehyde. Chromatin with a DNA fragment length of 200–500 bp was obtained by sonication. Equal amounts of soluble chromatin were incubated with normal rabbit IgG, antitrimethyl-histone H3Lys4 (EMD Millipore), or antitrimethyl-histone H3Lys27 (EMD Millipore). After overnight incubation, protein G beads were added to pull-down interested chromatin. Then DNA fragments were purified and analyzed by qPCR. Primers are the same as previously reported (Efe et al., 2011; Ieda et al., 2010).

Electrophysiology.

Large beating clusters of induced cardiomyocytes were first dispersed by 0.2% collonage II for 25 minutes, trypsinized for 2 minutes, and then replated onto matrigel-coated coverslips in CDM. Two days later, the coverslips were transferred to a superfusion bath (Warner RC-26GLP) on a Nikon TiS inverted microscope equipped with a dual wavelength microfluorometer (IonOptix, Milton, MA). Superfusion solutions were warmed to 30°C using the superfusion system (ValveLink, AutoMate Scientific, Berkeley, CA). Single spontaneously beating cells or small clusters were selected for study, with one cell per cluster being patch-clamped. An Axopatch 200B amplifier (Molecular Devices Inc., Sunnyvale, CA) was coupled via pClamp software (v10) to patch electrodes of 2–5 MegOhms (1B-150F; WPI, Sarasota, FL) filled with intracellular solution containing 120mM KCl, 20mM NaHEPES,10mM

MgATP, 0.1mM K_2EGTA, 2mM $MgCl_2$, set to pH 7.1 with KOH. Myocytes were superfused at constant flow (W2-64, Warner Instruments. Hamden, CT) with modified Tyrode's extracellular solution containing 137mM NaCl, 10mM NaHEPES, 10mM dextrose, 5mM KCl, 2mM $CaCl_2$, 1mM $MgCl_2$, set to pH 7.4 using NaOH. Action potentials were recorded under whole-cell current clamp at zero applied current, soon after rupturing the patched cell membrane.

Intracellular Ca^{2+} measurements.

To record calcium transients, the myocytes were loaded with 5 µM Fluo-4 AM Ca^{2+} indicator plus Powerload™ (Invitrogen Corp), as directed by the manufacturer, for 20 minutes at room temperature followed by 20 minutes in dye-free extracellular solution, to allow for de-esterification of the dye prior to commencing recordings. Spontaneous Ca^{2+} transients were recorded at 30°C via a standard optical filter set (#49011 ET, Chroma Technology Corp., Bellows Falls, VT) and a photomultiplier-based recording system (IonOptix PMT400, Milton, MA). In myocyte clusters, fluorescence was recorded from the entire cluster plus a cell-free border by adjusting a cell-framing adaptor. Between sampling periods, excitation light was blocked by a shutter (CS35; Vincent Associates, Rochester, NY) and background fluorescence was recorded after removing the cell(s) from the field of view at the end. Where appropriate, 1µM isoproterenol, (i.e., (-)-isoproterenol hydrochloride from an aqueous stock produced fresh each day), or 25 µM CCh (carbamylcholine chloride) were applied locally to the cell or cluster of interest using a perfusion pencil (AutoMate Scientific, Berkeley, CA). The agonists were purchased from Sigma Chem. Co. (St Louis, MO, USA).

Electrophysiological recording and analysis.

Action potentials were digitized at 5 kHz and low-pass filtered at 2 kHz. Fluorescence transients were digitized at 1 kHz and low-pass filtered at 0.5 kHz. For analysis, the maximum depolarization rate of the action potential upstroke (V_{max}) was calculated using Clampfit (pClamp 10, Molecular Devices, Sunnydale, CA). Action potential durations, determined between the upstroke (at V_{max}) and 50% or 90% repolarization (APD_{50}/APD_{90}), were determined using in-house analysis routines implemented in Excel 2007 (Microsoft, Redmond, WA). The measured action potential amplitudes include correction for a -5.6 mV liquid junction potential. To confirm the identity of cells and clusters framed by a cell-free border, brightfield images were also recorded. Ca^{2+} transients were calibrated using the pseudo-ratio method (Cheng et al., 1993) assuming an *in situ* dissociation constant of 1.1μM for Fluo-4 (Ljubojević et al., 2011). The resting fluorescence level used in the calculation was determined after the initial arrest of spontaneous beating during treatment with 25 μM CCh. All statistical comparisons were performed using two-tailed, paired or unpaired t-tests. Mean values are presented with standard errors (mean ± SEM).

Supplemental references

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