SUPPLEMENTAL METHODS

Reprogramming HDFs with 4 individual lentiviruses. Reprogramming of HDFs was carried out on MEF feeder cells. For reprogramming on feeder cells, $8x10^5$ HDFs were seeded in six-well tissue culture dish and maintained with HDF growth medium. On the second day, cells were transduced with individual lentiviruses containing human OCT4, SOX2, KLF4, and c-MYC at a 1:1:1:1 ratio plus 5 μg/ml polybrene (Sigma). The day of this initial transduction was considered as day 0. On day 3, cells were digested off the culture dish with 0.05% trypsin-EDTA (Gibco) and counted with a hemocytometer. Cells (~50,000) were then transferred onto a mouse embryonic fibroblast (MEF) feeder layer in a gelatin-coated 10-cm culture dish and cultured with human embryonic stem cell (ESC) growth medium mTeSR-1. The old medium was aspirated, and the cells were refeeded with new mTeSR-1 medium everyday. Background non-ESC-like colonies usually appeared from days 5–6, while ESC-like colonies with distinct light refractive property appeared as early as on days 12–13. On days 16–20, the living ESC-like colonies were immune-stained with TRA-1–60 mAb (Millipore) and Alexa Fluor 488 secondary antibody (Invitrogen). Positive colonies with ESC-like morphologies were picked out with a glass needle and seeded on Matrigel coated new culture dish. Each single picked colony was then maintained and expanded following routine passaging and culturing protocols and established as one individual HDF derived iPSC line.

Differentiation of pluripotent stem cell-derived cardiomyocytes (CMs). To derive cardiac cells from OKSM-SIRT6-iPSCs, we performed a modified differentiation protocol as previously described (1) In brief, $2x10^6$ undifferentiated ESCs were detached by Accutase (Sigma) and seeded onto Matrigel-coated plates (ES qualified, BD Biosciences, San Diego, CA) using hES mTeSR-1 cell culture medium (StemCell Technologies, Vancouver, Canada) for 1 day. To induce cardiac differentiation, we replaced mTeSR-1 medium with RPMI-B27 medium (Invitrogen) supplemented with the following cytokines: 100 ng/ml human recombinant activin A (R&D Systems) for 24 h, followed by 10 ng/ml human recombinant BMP4 (R&D Systems) for 4 days. The medium was exchanged for RPMI-B27 without supplementary cytokines; cultures were maintained by changing media every 2 days for 13 additional days. Widespread spontaneous beating activity was typically observed by day 14 after addition of activin A.

Differentiation of pluripotent stem cell-derived endothelial cells (ECs). To derive endothelial cells from OKSM-SIRT6-iPSCs, we performed the differentiation protocol as previously described (2). In brief, we cultured undifferentiated ESCs in differentiation medium on ultra-low attachment plates (Corning Incorporated, Corning, NY) for embryoid body formation (EBs). Differentiation medium consisted of Iscove's modified Dulbecco's medium (IMDM) supplemented with 16 BIT (BSA, insulin, transferrin; Stem Cell Technologies), 15% Knockout TM Serum Replacement (KnockoutTM SR) (Invitrogen, Carlsbad, CA), 2 mM L-glutamine, 450 mM monothioglycerol (Sigma, St. Louis, MO), 20 ng/ml bFGF (R&D Systems Inc., Minneapolis), 0.1 mM nonessential amino acids, 50 ng/ml VEGF (R&D Systems Inc.), 50 mg/ml streptomycin, and 50 U/ml penicillin. Twelve days after differentiation, EBs were collected, resuspended in 1.5 mg/ml rat tail collagen type I (Becton Dickinson, San Jose, CA), plated onto six-well plates, and incubated for 30 minutes at 37°C. Upon gel formation, each dish received an addition of EGM-2 medium (Lonza, Basel, Switzerland) with 5% Knockout TM SR, 50 ng/ml VEGF, and 20 ng/ml bFGF, and was then further incubated for 3 days without media change.

Transfection and luciferase assay. HeLa cells were grown in DMEM with high glucose (Gibco Life technologies) and 10% FBS (Gibco Life technologies). These cells were transfected with 100 ng of pMIR-REPORT with or without end-modified microRNA oligonucleotides (Dharmacon, USA) using Lipofectamine 2000 (Invitrogen, USA). Reporter gene assay was performed 24 hours after transfection using Luciferase Assay Kit (Promega, USA). The cells were also co-transfected with 10 ng of firefly construct for normalizing transfection efficiency. The microRNA mimics miR-766 and miR-cel-67 were purchased from Dharmacon.

SUPPLEMENTAL FIGURE LEGENDS

Figure S1: Cloning and expression of SIRT6. **(A)** Vector map showing lentiviral construct encoding SIRT6 generated by cloning its cDNA in pCDH_CMV-MCS-EF1_RFP vector to make pCDH_CMV-SIRT6_EF1-RFP construct. This construct was transfected in HEK cells to validate expression of SIRT6 by **(B)** RT-PCR. **(C)** Schematic representation of HDF reporogramming process.

Figure S2: The iPSC colonies derived from old HDFs using conventional factors OKSM displayed typical morphology when stained for pluripotency factors **(A)** OCT-4, **(B)** Tra-1-60, **(D)** NANOG, **(E)** Tra-1-81, and **(C** & **F)** DAPI for nuclear staining. **(G)** Brdu incorporation assay was performed to confirm any significant difference in proliferation of young and old HDFs. Results are indicated as fold change in absorbance at 450 nm in the young vs old HDFs.

Figure S3: HDFs derived from young subjects were infected with a 4-in-1 OKSM construct together with or without SIRT6 and emerging colonies were stained for Alkaline Phosphatase on day 16. Shown are the representitive results from one of the reprogrammed celllines. In order to quantify our results, we used NIH ImageJ software for automated colony counting.

Figure S4: The iPSC lines derived from older subjects with or without SIRT6 have similar abilities to differentiate into cardiomyocytes, as measured by immunostaining for representative **(A)** α-actinin, **(B)** DAPI, and **(C)** TNNT2. The iPSC lines derived from older subjects with or without SIRT6 have similar abilities to differentiate into endothelial cells as seen by representativie immunostaining for **(D-F)** CD31, DAPI, and CD144. Derived ECs could form **(G)** tubules in cell culture dish and **(H)** uptake LDL. **(I)** Bright field image of SIRT6-ECs.

Figure S5: miR-766 regulates SIRT6 in Hela cells. **(A)** SIRT6 3'UTR/luciferase and miR-766 mimics were co-transfected in HeLa cells and luciferase activity was measured 24 hours after transfection. Specificity of the mimic was confirmed by using miR-cel-67. The results are plotted as relative change in mean luciferase activity from three independent experiments. Results indicate a decrease in luciferase activity when cells are transfected with 50 nM concentration of miR-766 mimic. Increasing the concentration of mimic to 100 nM further reduced luciferase expression. On the other hand, miR-cel-67 had no effect on luciferase expression even at a concentration of 100 nM. **(B)** HeLa cells were transfected with miR-766 and western blot was performed for SIRT-6. normalization was performed with β-actin and specificity was conformed by transfecting with cel-67. miR-766 transfection resulted in reduction of SIRT6 protein expression, whereas miR-cel-67 had no effect on it. **(C)** HeLa cells were co-transfected with SIRT6 3'UTR construct along with miR-766 Zip vector. The results are plotted as relative change in mean luciferase activity from three independent experiments. miR-766 inhibition results in increased luciferase activity in HEK cells.

Figure S6: Schematic representation of TF activation profiling assay. A series of biotin-labeled probes are made based on the consensus sequences of 48 different transcription factor-DNA-binding sites. The probe mix is incubated with nuclear extracts, after which individual probes will find its corresponding TF and form TF/probe complexes, which can be easily separated from free probes through a simple spin column purification. The bound probes are detached from the complex and analyzed through hybridization with a plate; each well is specifically pre-coated with complementary sequences of the probes. The captured DNA probe is further detected with streptavidin-HRP. Luminescence is reported as relative light units (RLUs) on a microplate luminometer.

Figure S7: Transcription factor binding assay at SEPT6/miR-766 promoter. **(A)** TF activation assay was performed according to manufacturer's instructions at the SEPT6 promoter and region R1; higher binding of SP1 and p53 was observed. **(B)** Compilation of results from 2 young and 2 old subjects.

Supplemental Video

Video S1: Video showing cardiomyocytes derived from SIRT6-OKSM-iPSC spontaneously beating observed by day 14 after addition of activin A.

Supplemental References

1. Laflamme MA, Chen KY, Naumova AV, Muskheli V, Fugate JA, Dupras SK, Reinecke H, Xu C, Hassanipour M, Police S, O'Sullivan C, Collins L, Chen Y, Minami E, Gill EA, Ueno S, Yuan C, Gold J, Murry CE. (2007) Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. *Nat Biotechnol*.**25**:1015-1024
2. Li Z, Wilson KD, Smith B, Kraft DL, Jia F, Huang M, Xie X, Robbins RC, Gambhir SS, Weissman IL, Wu JC. (2009) Functional and transcriptional characterization of human embryonic stem cell-derived endothelial cells for treatment of myocardial infarction. *PLoS One*. **4**:e8443