A Novel Transcription Factor Combination for Direct Reprogramming to a Spontaneously Contracting Human Cardiomyocyte-like State

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Short title: A Novel Direct Reprogramming Combination

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HIGHLIGHTS

- Using network-based algorithm Mogrify, acoustic liquid handling, and high-content kinetic imaging cytometry we screened the effect of 4,960 unique transcription factor combinations.

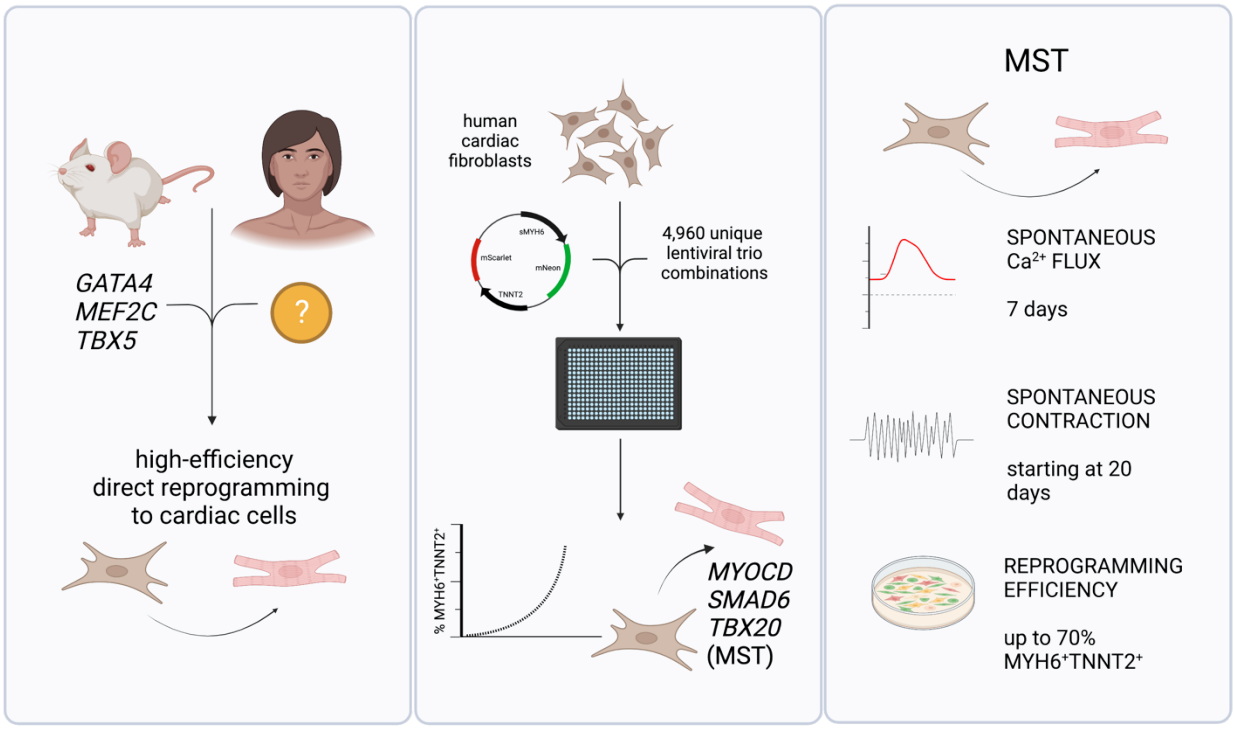
- Using 24 patient-specific human fibroblast samples we identified the combination of *MYOCD*, *SMAD6*, and *TBX20* (MST) as the most successful direct reprogramming combination.

- MST cocktail results in reprogrammed cells with spontaneous contraction, cardiomyocyte-like calcium transients, and expression of cardiomyocyte associated genes.

ABSTRACT

The reprogramming of somatic cells to a spontaneously contracting cardiomyocyte-like state using defined transcription factors has proven successful in mouse fibroblasts. However, this process has been less successful in human cells, thus limiting the potential clinical applicability of this technology in regenerative medicine. We hypothesized that this issue is due to a lack of cross-species concordance between the required transcription factor combinations for mouse and human cells. To address this issue, we identified novel transcription factor candidates to induce cell conversion between human fibroblasts and cardiomyocytes, using the network-based algorithm Mogrify. We developed an automated, high-throughput method for screening transcription factor, small molecule, and growth factor combinations, utilizing acoustic liquid handling and high-content kinetic imaging cytometry. Using this high-throughput platform, we screened the effect of 4,960 unique transcription factor combinations on direct conversion of 24 patient-specific primary human cardiac fibroblast samples to cardiomyocytes. Our screen revealed the combination of *MYOCD*, *SMAD6*, and *TBX20* (MST) as the most successful direct reprogramming combination, which consistently produced up to 40% $TNNT2^{+}$ cells in just 25 days. Addition of FGF2 and XAV939 to the MST cocktail resulted in reprogrammed cells with spontaneous contraction and cardiomyocyte-like calcium transients. Gene expression profiling of the reprogrammed cells also revealed the expression of cardiomyocyte associated genes. Together, these findings indicate that cardiac direct reprogramming in human cells can be achieved at similar levels to those attained in mouse fibroblasts. This progress represents a step forward towards the clinical application of the cardiac direct reprogramming approach.

GRAPHICAL ABSTRACT



KEYWORDS

Direct reprogramming, Fibroblast, Cardiomyocyte, High-throughput, Spontaneous contraction, Calcium cycling

ABBREVIATIONS

MST: *MYOCD*, *SMAD6*, *TBX20*

MI: myocardial infarction

GMT: *GATA4*, *MEF2C*, *TBX5*

GMT-M: *GATA4, MEF2C, TBX5, MYOCD*

iCMs: cardiomyocyte-like cells, induced cardiomyocytes

hiCMs: human cardiomyocyte-like cells, human induced cardiomyocytes

GMTM-H: *GATA4*, *MEF2C*, *TBX5*, *MYOCD*, *HAND2*

PCFs: primary cardiac fibroblasts

hiPSC-CMs: human induced pluripotent stem cell-derived cardiomyocytes

BSA: bovine serum albumin

MOI: multiplicity of infection

MSP: *MYOCD, SMAD6, PBX1*

MSS: *MYOCD, SMAD6, SNAI2*

D-BAITS: DMEM, bovine serum albumin, ascorbic acid, insulin, transferrin, sodium selenite

CTD_{90}: calcium transient duration 90%

INTRODUCTION

The human heart is thought to lose approximately 1 billion cardiomyocytes after a myocardial infarction (MI)[1]. As human adult cardiomyocytes are only minimally proliferative (<1% per year)[2], these lost cardiomyocytes cannot be replaced and instead a fibrotic scar is formed, resulting in reduced cardiac function. The development of approaches to replace the function of these lost cardiomyocytes is therefore key in recovery from MI and heart failure. Existing methods for cardiac regenerative medicine, such as engraftment of 'cardiac progenitor cells,' have largely either proven unsuccessful[3] or, in the case of engraftment of human pluripotent stem cell-derived cardiomyocytes, will require immunosuppression and suppression of arrythmias[4]. Conversely, *in situ* direct reprogramming of cardiac fibroblasts to cardiomyocytes within the heart is potentially an ideal method to replace lost cardiomyocytes in the heart and provide functional recovery from MI or heart failure. However, despite advances in this field over the past decade, the direct reprogramming of human fibroblasts to cardiomyocytes remains inefficient compared to that of mouse.

In mouse cells, the combination of the transcription factors *Gata4*, *Mef2c*, and *Tbx5* (GMT) has been demonstrated by numerous groups to reprogram mouse fibroblasts to cardiomyocyte-like cells (iCMs)[5-10], despite early skepticism[11,12]. The efficiency of this reprogramming has since been further enhanced by supplementing GMT with transcription factors such as *Hand2*[6,7,13,14], *Nkx2-5*[7,13], *Akt1*[14,15], *Znf281*[15], *or Myocd, Srf, Mesp1, and Smarcd3*[10]. Small molecules and microRNAs have likewise been used to augment reprogramming[8,9,13,16] or to induce GMT expression[17] from mouse fibroblasts.

In human cells, several groups have demonstrated that GMT alone is not sufficient for the reprogramming of human fibroblasts to human iCMs (hiCM). Studies show that addition of other

transcription factors such as *MYOCD*, *HAND2*, *MESP1*, *ESSRG*, *ZFPM2*, *ASCL1*, *TBX20*, and/or *NKX2-5* are required. From these transcription factor-based cocktails, the combination of GMT plus *MYOCD* and *HAND2* (GMTM-H) has been demonstrated as the most efficient for reprogramming the human fibroblast to hiCM. GMTM-H generates 7.27-19.6% TNNT2-GFP$^+$ cells after 2 weeks of reprogramming, depending on the fibroblast source[18]. The use of reprogramming enhancers, such as microRNAs *miR-1* or *miR-133*[9,18-22] and small molecules[20] have also been identified to improve the efficiency of human direct reprogramming, based on cardiac promoter activity and gene expression. GMT in the presence of miR-133 can generate 41.8% TNNT2$^+$ cells after 2 weeks of reprogramming with antibiotic selection[22]. Furthermore, optimization of transcription factor stoichiometry using polycistronic vectors with a MEF2C-GATA4-TBX5 splicing order has further improved the reprogramming efficiency 10-fold in mouse cells[23,24]. Nonetheless, despite this progress, generation of hiCMs suffers from lower efficiency than mouse iCMs and the generation of spontaneously contracting cells has not been conclusively proven without co-culture with mouse CMs or hiPSC-CMs (**Table S1**). These difficulties suggest that there may be inter-species differences in the gene regulatory networks that control the fibroblast and/or cardiomyocyte states; therefore, alternative non-GMT-based transcription factor combinations are required for reprogramming.

Previous attempts to find transcription factor combinations to reprogram human fibroblasts to cardiomyocytes have been guided by low-throughput additive approaches using the mouse-discovered GMT as a starting combination and have used limited phenotypic measures of cardiomyocyte identity, such as expression of MYH6 or TNNT2, to measure reprogramming success. However, this 'single gene' methodology potentially may only optimize for transcription factor cocktails that primarily upregulate the expression of MYH6 or TNNT2 themselves rather

than switch on the complete cardiomyocyte transcriptional network or generate hiCMs. Indeed, a combinatorial screen utilizing RT-qPCR to optimize for factors that upregulate simultaneous expression of 5 cardiac genes (*Myh6, Myl2, Actc1, Nkx2.5,* and *Scn5a*) in mouse embryonic fibroblasts revealed that the combination of *Tbx5*, *Mef2c*, and *Myocd* upregulated a broader range of cardiac genes compared to GMT[25]. Understandably, prior studies have selected candidate genes based solely on their high expression in human adult cardiomyocytes and their role during cardiac development. However, existing network-based algorithms that consider the transcription factor network already in place in the starting cell type, such as Mogrify[26], could be used to identify candidate factors for direct reprogramming that specifically restructure the human fibroblast gene regulatory network; thus facilitating more informed transcription factor screens for hiCM generation.

Until now, high-throughput methods have not been applied to screen and identify reprogramming factors in primary human cells. A major limitation for performing high-throughput screens using human fibroblasts is cell availability. Different cell sources have been used in prior studies, including neonatal human foreskin fibroblasts[19], human cardiac fibroblasts[18], dermal fibroblasts[21] or embryonic stem cell-derived fibroblasts[9,20]. However, due to the limited proliferative capacity of primary fibroblasts and the decrease in reprogramming efficiency[20] over multiple passages, only immortalized fibroblasts have been used to perform high-throughput chemical screens for reprogramming enhancers[9] in mouse fibroblasts. Since concordance between immortalized and primary cells has not been well studied, it is unclear whether the optimized condition can be directly translated to primary cells.

Another major barrier for performing high-throughput screening in human cells is the transfection strategy. Retroviruses are used to overexpress factors in most human fibroblast cardiac

direct reprogramming protocols, as early work demonstrated these infect cells at >90% efficiency rather than the <20% achieved with lentiviruses[18]. The amphitropic retrovirus produced by PLAT-A cells[9,19,20] is not able to withstand centrifugation or freeze/thaw cycles without up to a 100-fold reduction in viral titer; therefore, it must be made fresh prior to each experiment. Incorporating viral production into the reprogramming pipeline makes the addition of automation to the transcription factor screening process highly onerous. A major step forward was the use of more stable VSV-G pseudotyped doxycycline-inducible lentiviruses[21] in HEK293T cells, which are compatible with freeze/thaw cycles, express factors at the same level as non-inducible constructs, and have been successfully used to generate cardiac-like cells from human fibroblasts[22].

In this study, we developed a novel high throughput reprogramming strategy using low passage primary fibroblasts derived from 24 pediatric patients undergoing cardiac surgery. Cardiac, rather than dermal, fibroblasts were chosen as a starting cell type due to their central role in decreased heart function following MI, making these cells an attractive target for direct reprogramming *in vivo*. We have combined a lentiviral approach for reproducible gene introduction, a dual TNNT2-mScarlet MYH6-mNeonGreen reporter strategy, automated 384-well virus, small molecule, and growth factor dispensing, and high-content kinetic imaging cytometry to assess for both calcium cycling and cardiac reporter activity. Using this methodology, we found that variations of the traditional GMT-containing transcription factor combinations are not suitable for human direct cardiac reprogramming. Therefore, taking an unbiased approach, we used the Mogrify algorithm to identify new candidate transcription factors. Utilizing our optimized high-throughput screening methodology to assess reprogramming success, we found the combination of *MYOCD*, *SMAD6*, and *TBX20* (MST) as the most efficient combination. In just 6 days, MST produces 33% MYH6$^+$ and 17% TNNT2$^+$ cells on average and can produce upwards to 90%

MYH6$^+$ and 40% TNNT2$^+$ cells. With the addition of a growth factor FGF2 and small molecule XAV939, these cells exhibited cardiomyocyte-like gene and protein expression, spontaneous calcium transients starting at 7 days, and spontaneous contraction at just 25 days. Our efficient reprogramming strategy can open new avenues for therapeutic application of hiCMs.

METHODS

Fibroblast isolation

Deidentified human heart samples were obtained from 24 patients aged 4 days – 21 years undergoing cardiac surgery with informed consent in accordance with Lurie Children's Hospital Institutional Review Board (**Table S2**). Cardiac tissues were collected in RPMI 1640, transferred to a cell culture dish, and minced into small pieces (<1 mm^2) using a sterile scalpel. Tissue pieces were then suspended in IMDM (Hyclone) supplemented with 20% FBS (VWR) and transferred to one well of a 6-well cell culture plate (Greiner) coated with a 1:800 dilution of growth factor-reduced Matrigel (Corning)[27]. All cells were kept at 37 °C and 5% CO_2. Media was changed every 2 days beginning on day 5 and primary cardiac fibroblasts (PCFs) migrated out of the explants within 3-4 weeks. Migrated fibroblasts were passaged once they reached 90% confluence by rinsing with DPBS, then digesting in TrypLE Express (Gibco) for 5 minutes, and suspending with IMDM+20% FBS. Fibroblasts were filtered through a 100 µm cell strainer (Falcon) and transferred to Matrigel-coated 15 cm^2 cell culture plates (Greiner). Cells were grown to 90% confluence and subsequently passaged (1:3 ratio) at least 2 more times before use in direct reprogramming experiments.

Cardiac differentiation from human induced pluripotent stem cell-derived cardiomyocytes

Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) were used as positive controls. Differentiation of human induced pluripotent stem cells into cardiomyocytes was performed according to previously described protocol with slight modifications[28,29]. Briefly, hiPSCs were split at a 1:15 ratio using 0.5 mM EDTA and grown in B8 medium for 4 days reaching ~80% confluence. At the start of differentiation (day 0), B8 medium was changed to R6C, consisting of RPMI 1640 (Corning, 10-040-CM), supplemented with 6 μM of glycogen synthase kinase 3- inhibitor CHIR99021 (LC Labs, C-6556). On day 1, medium was changed to RPMI 1640 basal medium alone, and on day 2 medium was changed to RBA-C59, consisting of RPMI 1640 supplemented with 2 mg/mL fatty acid-free bovine serum albumin (GenDEPOT, A0100), 200 μg/mL L-ascorbic acid 2-phosphate (Wako, 321-44823) and 2 μM Wnt-C59 (Biorbyt, orb181132). Medium was then changed on day 4 and then every other day with RBAI consisting of RPMI 1640 supplemented with 500 μg/mL fatty acid-free bovine serum albumin, 200 μg/mL L-ascorbic acid 2-phosphate, and 1 μg/mL *E. coli*-derived recombinant human insulin (Gibco, A11382IJ). Contracting cells were noted from day 8, differentiated cardiomyocytes were treated with 100 μg/mL of neomycin from day 8 to day 12. On day 16 of differentiation, cardiomyocytes were dissociated using DPBS for 20 min at 37 °C followed by 1:200 Liberase TH (Roche, 5401151001) diluted in DPBS for 20 min at 37 °C, centrifuged at 300 × g for 5 min, and filtered through a 100 μm cell strainer (Falcon). Cells were then plated in RBAI+10% Cosmic Calf Serum (Hyclone) for 2 days on 1:800 Matrigel-coated plates for each assay, media was then switched back to RBAI which was changed every 2-3 days and cells were assayed on day 30.

Flow cytometry

For flow cytometry analysis, fibroblasts were dissociated from cell culture dishes using TrypLE Express (Gibco) for 5 minutes and hiPSC-CMs were dissociated using 1:200 Liberase TH (Roche) in PBS for 20 min. 1×10^6 cells were transferred to round bottom FACS tubes (Falcon) and fixed with 4% PFA in DPBS (100 µL) for 20 min. For analysis of intracellular markers, cells were washed twice using DPBS and permeabilized in 100 µL of DPBS with 0.5% BSA and 0.5% saponin for 15 min. Once fixed and permeabilized, cells were stained for 2 h at room temperature and washed prior to data collection. For analysis of extracellular markers, fixed cells were washed twice and stained for 30 min on ice prior to data collection. Antibodies used are listed in **Table S3.** All data were collected using a CytoFLEX flow cytometer (Beckman Coulter) and analyzed using CytExpert 2.2 software (Beckman Coulter) using isotype controls to set positive gates.

Plasmids

All plasmids used in this study have been deposited in Addgene. To generate doxycycline-inducible lentiviral plasmids for overexpression of candidate factors, ORF cDNA was inserted into the FU-tetO-Gateway (Addgene 43914) backbone using Gateway cloning. First, attB sites were added to factor ORF cDNA via PCR reaction. We amplified the N-terminal region of each gene in our candidate pool by designing forward primers containing the attB1 sequence, Shine-Dalgarno sequence, and Kozak sequence upstream of the transcriptional start site, as well as overlap to the cDNA ORF. We designed reverse primers to amplify the C-terminal of each gene and to include the attB2 site. Primers used for PCR reactions are listed in **Table S4.** PCR reactions were performed by combining 12.5 µL Q5 high-fidelity 2× master mix (NEB), 2.5 µL each of 10 µM forward and reverse primers, and 1 µL of 1 ng/µL of DNA template (**Table S5**). Cycling conditions were as follows: initialization at 98 °C for 30s, followed by 35 cycles of denaturation at 98 °C for

10 seconds, annealing at 60 °C for 10 seconds, and elongation at 72 °C for 1s per KB of template, ending with a final elongation at 72 °C for 5 minutes and a hold at 4 °C. Resulting fragments were purified and recombined with Gateway pDONR221 Vector (Invitrogen) using Gateway BP Clonase II enzyme mix (Invitrogen) to generate entry clones. Purification of attB- containing PCR fragments was performed using PEG 8000/MgCl2, following the Gateway Clonase II enzyme mix protocol. The BP reaction was performed by combining 150 ng of attB-PCR product, 150 ng of pDONR221 vector, and 2 µL of BP Clonase II in TE buffer to a total volume of 8 µL. DNA was allowed to recombine overnight at 25 °C before adding 1 µL of Proteinase K and incubating at 37 °C for 10 min. We then transformed One Shot TOP10 chemically competent *E. coli* cells (Invitrogen) with 1 µL of BP product following the manufacturer's recommended protocol. Transformed bacteria were plated on LB agar plates with Kanamycin (50 µg/mL) and surviving entry clones were selected. We purified plasmid DNA using the ZymoPURE Plasmid Miniprep Kit (Zymo Research) following the manufacturer's protocol. Gateway LR Clonase II Enzyme mix (Invitrogen) was used to catalyze recombination of entry clones with the FU-tetO-Gateway backbone (Addgene plasmid # 43914) using 150 ng of pDONR221-cDNA plasmid, 150 ng of FU-tetO-Gateway plasmid, and 2 µL of LR Clonase II in TE buffer to a total volume of 8 µL. DNA was allowed to recombine, and expression clones were selected using ampicillin (100 µg/mL), following the same protocol described above. Expression plasmids were verified by Sanger sequencing using the T7 forward and GW_R reverse primers (**Table S4**). All expression plasmids were propagated in Stbl2 or NEB Stable bacteria to reduce the occurrence of recombination due to long terminal repeats. Plasmid maps are found in **Figure S1**.

Lentiviral production

To generate lentivirus, Lenti-X 293T packaging cells (Takara, 632180) (passage 2 – 3) were thawed and allowed to recover in DMEM with 10% FBS (Avantor Seradigm, VWR) and 4 mM L-alanyl-L-glutamine (DMEM complete) for 4 days before transection with lentiviral plasmids. After recovery, cells were dissociated using TrypLE Express (Gibco), seeded into uncoated 10 cm^2 cell culture plates at 3.8 million cells per plate in 15 mL of DMEM complete and incubated for 20 h (37 °C, 5% CO_2). The following day, media was replaced with 10 mL of DMEM complete with 25 µM cloroquine diphosphate and incubated for 5 h. To transfect cells, we prepared a mixture of 1.3 pmol psPAX2 (Addgene 12260), 0.72 pmol pMD2.G (Addgene 12259), and 1.64 pmol of transfer plasmid (tet-O-cDNA) in 500 µL of OptiPro SFM (Gibco). Polyethyenimine (PEI) (Polysciences, 23966-1) was used as a transfection reagent. In a separate tube, PEI (1 mg/mL) was further diluted with OptiPro SFM at a DNA to PEI ratio of 1:2 to a final volume of 500 µL. The diluted PEI solution was then added dropwise to the diluted DNA. The mixture was incubated at room temperature for 10 min before being added dropwise to the Lenti-X 293T cells in culture. A media change with 15 mL of DMEM complete was performed the following day. Cells were kept in culture and lentiviral supernatant was collected at 48 h post-transfection and stored at 4 °C until the second collection of supernatant at 72 h post-transfection. The 30 mL of lentiviral supernatant was pooled and concentrated to $1/100^{th}$ of its original volume (300 µL) in DPBS using PEG-iT (System Biosciences), following the manufacturer's protocol. Lentiviral titration was performed by RT-qPCR using the Lenti-X qRT-PCR Titration Kit (Takara) and the concentration of lentivirus used for this paper was found to be above 1×10^7 copies / mL. Concentrated lentivirus was aliquoted and stored at -80 °C until use.

Lentiviral transduction

All PCFs used for direct reprogramming were propagated in culture (37 °C, 5% CO_2) using 15 cm^2 cell culture plates coated in 1:800 growth factor-reduced Matrigel (Corning)[27] and IMDM (Hyclone) supplemented with 20% FBS (VWR). On reprogramming day -1, PCFs were dissociated using TrypLE Express and plated at 600,000 cells per well in Matrigel-coated, non-treated 6-well plates in DMEM+10% FBS (GenDEPOT Opti-Gold) media. To achieve high transduction efficiency, cells were allowed to attach to the plate surface for 2-3 hours and were subsequently transduced using TransDux Max (SBI). For lentiviral transduction, media in each well was aspirated and replaced with 1.5 mL of DMEM+10% FBS media containing TransDux Max (1x), 16 µL of concentrated FUdeltaGW-rtTA lentivirus (7.2×10^7 copies / mL) and 16 µL of concentrated pFU-GW-sMYH6-mScarlet-TNNT2-mNeon (1.84×10^7 copies / mL). Cells were incubated overnight. On reprogramming day 0, cells were dissociated using TrypLE Express, pooled, and replated for reprogramming in 25 µL of DMEM 10% FBS media containing TransDux Max (1x) at 3,000 cells per well in a Matrigel-coated µClear flat bottom black 384-well plates (Greiner) (cell numbers and media volumes were scaled according to surface area for other plate formats). Cells were allowed to attach to the plate surface for 2-3 h before transduction. Plates were briefly centrifuged and 50 nL of each concentrated lentivirus for transcription factor overexpression was dispensed into wells using Echo Acoustic Liquid Handling (LabCyte). Plates were incubated overnight. On reprogramming day 1, a media change was performed using 25 µL of DMEM 10% FBS containing 0.25 µg/mL doxycycline (Sigma-Aldrich). Media was replaced every two days. For reprogramming with small molecules, 5 µM XAV939 (APExBIO) was added to the media starting on reprogramming day 3. In experiments using reprogramming media, DMEM supplemented with 5 mg/mL fatty acid-free bovine serum albumin (BSA), 40 µg/mL

ascorbic acid 2-phosphate, 20 µg/mL insulin, 5 µg/mL transferrin, and 7 µg/L sodium selenite was used starting on reprogramming day 1.

Reporter Fluorescence Quantification

We quantified the fluorescence of cells transduced with pFU-GW-sMYH6-mScarlet-TNNT2-mNeon (Addgene 170712) and ORF cDNA-expressing transcription factors on reprogramming day 6. Media in µClear bottom black 384-well plates (Greiner) was replaced with 25 µL of FluoroBrite DMEM (Thermo Fisher) supplemented with 4 mM L-alanyl-L-glutamine and NucBlue nucleus staining reagent (1 drop per mL) (Invitrogen). Plates were incubated for 15 min (37 °C, 5% CO_2) prior to capturing one image per well using high-content image cytometry (KIC, Vala Sciences). For image analysis, we used an algorithm developed by Vala Sciences to identify nuclear bounds and quantify their FITC and TRITC average pixel intensity (API). We used negative control wells, containing fibroblasts transduced with pFU-GW-sMYH6-mScarlet-TNNT2-mNeon and rTTA alone, to set a negative API threshold. Cells with API above the negative threshold in both the FITC and TRITC channel were counted as positive. Percent positive cells was calculated relative to the total number of nuclei in the well. All experiments were performed with at least three technical replicates and at least three separate patient samples.

Calcium Imaging

We assayed calcium flux in cells on days 7-30 of reprogramming. For calcium imaging, media in flat bottom black 384-well plates (Greiner) was replaced with 25 µL of HBSS (Gibco) supplemented with 0.02 M HEPES (Fisher Scientific), 2.5 mM probenecid (Sigma), 0.04% pluronic F-127 (Sigma), and 10 µM Cal-520 (AAT Bioquest). Following a 1 h incubation (37 °C,

5% CO_2), media was aspirated and replaced with 25 µL of FluoroBrite DMEM (Gibco) supplemented with 4 mM L-alanyl-L-glutamine and NucBlue Live ReadyProbes Reagent (1 drop per mL) (Invitrogen). Plates were incubated for an additional 10 min (37 °C, 5% CO_2) prior to data collection using high-content image cytometry (KIC, Vala Sciences). Images were captured at in the FITC channel for 60 s (50 FPS, 10 ms exposure). We then used an algorithm developed by Vala Sciences to quantify FITC pixel intensity within nuclear and cell borders and to analyze trace parameters.

Stimulation

Reprogrammed cells were differentiated in 384-well plates and stained with Cal-520 and NucBlue Live ReadyProbes Reagent as described above. Following initial data collection, cells were stimulated with 2 µM isoproterenol (Sigma) and incubated for 10 min (37 °C, 5% CO_2) prior to data collection.

Quantitative Real-time PCR

Cell lysates for RT-qPCR were collected in 300 µL TRIzol (Invitrogen) and their total RNA was isolated using Direct-zol RNA Microprep kit (Zymo Research), following the manufacturer's protocol. Reverse transcription was performed from 0.5 µg – 1 µg of RNA using Maxima H Minus master mix (Thermo), following the manufacturer's protocol. cDNA was then diluted 1:10 for 1 µg starting RNA or 1:5 for 0.5 µg starting RNA. RT-qPCR was performed using Taqman gene expression assays (Applied Biosystems) (**Table S6**) and Taqman Fast Advanced Master Mix (Applied Biosystems), following the manufacturer's protocol. All PCR reactions were prepared in triplicate in a 384-well format. Data was collected using the QuantStudio 5 Real-Time

PCR system. Relative quantification of gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method[30], normalized to the reference 18S and fibroblast control sample, as specified in the figure legends.

Immunostaining

Reprogrammed fibroblasts were dissociated at day 30 and plated at 10,000 cells/well of Matrigel-treated Nunc Lab-Tek II 8-chamber slides. After 3 days, cells were fixed with 4% PFA (Electron Microscope Sciences, 15713S) in DPBS for 20 min at RT followed by permeabilization with 0.3% Triton X-100 (Fisher Bioreagents, BP151-100) in DPBS for 10 min at RT. Cells were then blocked with 1 mg/mL BSA in DPBS for 60 min at RT, and stained with rabbit polyclonal IgG TNNT2 (1:200, abcam, ab45932) and mouse monoclonal IgG1 a-Actinin (1:1000, Sigma-Aldrich, A7811) primary antibodies in 1 mg/mL BSA plus 0.1% Tween (Fisher, BP337-100) for 2 h at RT. Cells were then washed for 5 min with DPBS three times, then stained with secondary antibodies Alexa Fluor 488 Goat anti Rabbit IgG (1:500, Invitrogen, A11012) and Alexa Fluor 594 Goat anti Mouse IgG1(1:500, Invitrogen, A21125) in 1 mg/mL BSA plus 0.1% Tween (Fisher, BP337-100) for 2 h at RT. Cells were washed three times for 10 min with 0.1% Tween in DPBS and mounted with ProLong Diamond Antifade Mountant with DAPI (Invitrogen, P36962). Slides were imaged with a Ti-E inverted fluorescence microscope (Nikon Instruments) using NIS-Elements software.

RNA-seq

Three samples of un-transduced cardiac fibroblasts, MST transduced cardiac fibroblasts, and hiPSC-derived cardiomyocytes at d30 were prepared. RNA was extracted using 150 µL/well of TRIzol Reagent (Thermo Fisher, 15596026). RNA was then purified using the Direct-zol RNA

MicroPrep kit (Zymo, R2062) including on-column DNase digestion to remove genomic DNA. Samples were quantified using a Thermo Scientific NanoDrop 8000 and passed QC, and were then shipped in dry ice for library preparation and sequencing

Paired-end (100 bp) RNA-sequencing on hiPSCs was performed using the DNBSEQ sequencing platform at Beijing Genomics Institute. Total RNA was extracted and oligo dT beads were used to enrich mRNA with poly A tail using DNBSEQ Eukaryotic strand-specific mRNA library protocol. mRNA molecules were fragmented into small pieces and the fragmented mRNA was synthesized into first strand cDNA using random primers. The second strand cDNA was synthesized using dUTP instead of dTTP. The synthesized cDNA was subjected to end-repair and 3' adenylated and adaptors were ligated to the ends of these 3' adenylated cDNA fragments. The U-labelled second-strand template was digested with Uracil-DNA-Glycosylase (UDG) and PCR amplification was performed. Following library quality control and circularization, the library was amplified to make DNA nanoball (DNB) and sequenced on DNBSEQ platform.

Using SOAPnuke[31], raw fastq files were processed to trim/remove adaptors, low-quality reads, and N reads. Clean sequencing reads were mapped to the GRCh38 reference genome using STAR[32] and counted using RSEM[33]. Genes with less than 10 counts across all 9 samples were excluded from subsequent analysis. Following variance stabilizing transformation in DESeq2 R package[34], principal component analysis was performed to visualize the clustering of samples. Differential expression analysis was performed using the DESeq2 R package[34] accounting for the matched pair design. Results were shown by a volcano plot generated using the ggplot2 R package[35]. Genes with adjusted *P*-value <0.05 corrected for multiple testing using the Benjamini Hochberg method were considered as differentially expressed. Using the most strongly differentially expressed genes between hiPSC-CM and PCF, and between MST-reprogrammed

hiCM and PCF, hierarchical clustering based on Euclidean distance was performed by the pheatmap R package[36]. Ingenuity Pathway Analysis (IPA) was performed to identify canonical pathways based on the differentially expressed genes (FDR <0.005)[37]. This was used to identify differences in pathways related to biological processes that were relevant between our samples.

To identify relevant gene sets, Gene Set Enrichment Analysis[38] was performed. Outputs from DESeq2 analysis for hiPSC-CM vs PCF and MST-reprogrammed hiCM vs PCF comparisons were used as inputs for GSEA analysis. Human curated gene sets (C2) and cell type signature gene sets (C8) were the target gene sets. Results with FDR < 0.05 were considered as statistically significant.

Statistics

24 primary samples were used to generate data for this study. Specific samples used for each experiment are detailed in **Table S2** and sample size is indicated in each figure legend. All statistical analysis was performed using GraphPad Prism 9 software. Data are shown as mean ± standard deviation. Student's t-test was used for pairwise comparisons in most instances, as indicated in figure legends. However, because gene expression data from RT-qPCR analysis was not normally distributed, a non-parametric Mann-Whitney test was performed. Comparisons among 3 or more groups were performed using One-way ANOVA with subsequent pairwise testing via t-test with Tukey's adjustment for multiple comparisons or Fisher's LSD test.

RESULTS

Platform Optimization for transcription factor screening

We first developed a lentiviral transduction and reporter platform to allow for high-throughput screening of candidate transcription factors for direct reprogramming in primary human cardiac fibroblasts (PCFs) from pediatric patients (**Figure 1A**). These PCFs expressed typical fibroblast markers CD90 and vimentin and did not express endothelial cell marker CD31 by flow cytometry (**Figure S2A**). PCFs expressed fibroblast markers and did not express cardiomyocyte markers by RT-qPCR. hiPSC-derived cardiomyocytes were used as a positive control (**Figure S2B**). To facilitate the screening, PCFs were transduced with a dual-fluorescence reporter (pFU-GW-sMYH6-mScarlet-TNNT2-mNeon), validated using human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) (**Figure S3A**). PCFs were then transduced in multiplex using only 50 nL of concentrated doxycycline-inducible lentivirus (1 x 10^{8-10} copies/mL), delivered in 384-well format via liquid handling, resulting in >90% transduction efficiency (**Figure S3B**). Cell seeding density (3,000 cells per well), MOI (0.17), basal media (DMEM+10% FBS), and doxycycline dose (0.25 μg/mL) were optimized for our system as described in **Figure S3C-S3F**. Reporter activity was measured using kinetic image cytometry on reprogramming day 6, thus facilitating high-throughput screens.

To validate our workflow and the ability of our lentiviral system to induce differentiation towards the cardiac fate in the absence of a transgenic reporter, we transduced PCFs with previously published transcription-factor based cardiac reprogramming cocktails (**Table S1**). As expected, we observed upregulation of endogenous *MYH6* and *TNNT2* transcripts by RT-qPCR within 6 days of reprogramming with each of the tested published factor combinations, (**Figure 1B**). Likewise, the tested combinations induced activation of our dual MYH6/TNNT2 reporter; however, simultaneous cardiac-specific MYH6 and TNNT2 activity was detected in only 2-8% of transduced cells (**Figure 1C-1D). *MYH6* activity was detected in 3-9% of cells and *TNNT2***

activity was detected in 4-12% of cells (Figure S4A-S4C). No reporter activity was observed in control cells (PCFs) transduced with rTTA and reporter alone. Interestingly, overexpression of *MYOCD* alone led to upregulation of endogenous TNNT2 and, to a lesser extent, MYH6, as measured by RT-qPCR as well as activation of our dual fluorescent reporter (**Figure 1B-D**). This finding is consistent with reports of partial activation of the cardiac gene network, including aMHC and cTnT, by overexpression of *MYOCD* alone in human foreskin fibroblasts[39] and in mouse fibroblasts[40].

Nonetheless, despite cardiac promoter activation in cells obtained using our optimized platform, none of the published transcription factor combinations tested was sufficient to generate spontaneously beating cells within a 4-week timeframe.

Identification of novel transcription factor combination *MYOCD*, *SMAD6*, *TBX20* for direct cardiac reprogramming

To increase reprogramming efficiency and generate functional reprogrammed cardiomyocytes, we next used the network-based algorithm Mogrify[26] to identify a novel set of transcription factors whose overexpression is predicted to result in the cardiac reprogramming of PCFs (**Figure S5**). Additionally, transcription factors which were previously reported to reprogram fibroblasts into cardiomyocytes, as well as factors that have been reported to increase cardiac direct reprogramming efficiency were also included in our candidate list (**Figure 1F, Table S5**). We generated doxycycline-inducible lentiviral constructs for overexpression of the 32 candidate factors and used our platform to assay the reprogramming efficiency of all factor trios in PCFs expanded from each of 3 distinct primary samples. Our initial screen of a total of 4,960 distinct combinations identified several factor trios with higher reprogramming efficiency compared to

GMT-containing cocktails or *MYOCD* overexpression alone (**Figure 2A, Table S7**). To ensure reproducibility of the identified combinations, we validated our top 50 hits by transducing PCFs from 13 primary samples in triplicate to identify the top 3 combinations resulting from our screen: (1) *MYOCD*, *SMAD6*, *TBX20* (MST); (2) *MYOCD*, *SMAD6*, *PBX1* (MSP); and (3) *MYOCD*, *SMAD6*, *SNAI2* (MSS) (**Figure 2B**). While reprogramming success among experiments and samples is highly variable, each of these combinations generated $MYH6^{+}TNNT2^{+}$ cells at higher efficiency (up to 70%, 13.3% on average) compared to GMT-based cocktails (up to 20%, 1-5% on average) and to *MYOCD* overexpression alone (up to 21%, 6% on average). (**Figure 2C**) Although MST, MSP, and MSS all produce similar percentages of $TNNT2^{+}$ cells (~20%), MST produces significantly more $MYH6^{+}$ cells (~30%) from which up to 80% are $MYH6^{+}TNNT2^{+}$ cells, as measured by reporter expression (**Figure 2C**). The potential of the MST combination to reprogram PCFs with high efficiency is illustrated in **Fig. 2D**. Likewise, MST-derived cells express higher levels of TNNT2 protein compared to MSP and MSS and *MYOCD* overexpression alone (**Figure S6**) and the combination gives rise to approximately 40% $TNNT2^{+}$ by flow cytometry at reprogramming day 15 (**Figure 2E**).

Optimization of Reprogramming Media and Small Molecule Screen

Culture conditions have been reported to influence reprogramming efficiency and reprogrammed cell function based on cardiac gene expression. We optimized our reprogramming media to provide a more defined serum-free environment in which to assess the effect of different small molecules on cardiac reprogramming. We began by supplementing DMEM with 5 mg/mL bovine serum albumin (BSA), 200 µg/mL ascorbic acid, 10 µg/mL insulin, and 7 µg/L transferrin (D-BAITS). To further optimize this media formulation for direct reprogramming, we performed

a dose optimization for each component. Our findings suggest that using 5 mg/mL BSA, 40 µg/mL ascorbic acid, 20 µg/mL insulin, 5 µg/mL transferrin, and 7 µg/L sodium selenite starting at reprogramming day 0 leads to an increased reprogramming efficiency in MST-transduced cells (**Figure 3A**). However, compared to DMEM+10% FBS, the optimized D-BAITS media decreased MST reprogramming efficiency measured by reporter activity by 15%, resulting in an average of 20.2% ± 5.6% $MYH6^{+}TNNT2^{+}$ reprogrammed cells, compared to 35.8% ± 12.2% in the presence of FBS (**Figure 3B**). To assess the endogenous expression of cardiac genes in D-BAITS media compared to FBS-supplemented media, we performed RT-qPCR at reprogramming day 6 on MST transduced cells with no reporter overexpression. Our data demonstrate that genes related to cardiac structure (*MYH6*, *TNNT2*, *MYL7*) in both conditions were upregulated. However, MST-induced cells derived in D-BAITS media showed significantly higher levels of endogenous sodium and calcium channel-related genes (*SCN5A*, *ATPA2*) compared to those derived cells in DMEM+10% FBS *(P* = 0.0004), suggesting improved cardiac function. Notably, *RYR2,* which encodes the ryanodine receptor essential for cardiomyocyte calcium handling was not upregulated in either condition, suggesting incomplete reprogramming (**Figure 3C**).

We then performed a small molecule screen to identify pathways that can be modulated to further increase the yield of $MYH6^{+}TNNT2^{+}$ cells and improve cardiac function. Small molecules and growth factors were added to cells 3 days after MST transduction, and reporter fluorescence was quantified 6 days after transcription factor induction with doxycycline-containing media. We used PCFs from 4 patient samples to screen 21 modulators of major signaling pathways (Wnt, TGFβ, BMP, and FGF) at a range of concentrations (**Figure S7**). Our findings demonstrated a consistent fold increase in $MYH6^{+}TNNT2^{+}$ cells following Wnt pathway inhibition with XAV939, IWP-L2, or IWP-L6. Whereas no significant change was observed following Wnt activation with

CHIR99021, nor with modulation of BMP or FGF pathways. A slight, but significant increase in $MYH6^{+}TNNT2^{+}$ cells following treatment with the TGFβ inhibitor, SB431542 and JAKi was observed (**Figure 3D**). However, because reprogramming efficiency was most consistently and highly increased by Wnt pathway inhibition, we further characterized cells derived using MST and D-BAITS media supplemented with XAV939 (1 μM). Our data demonstrate that the addition of XAV939 to MST-derived cells significantly increased their expression of endogenous *RYR2* compared to use of MST alone ($P = 0.0004$) (**Figure 3C**). Addition of XAV939 to MST + D-BAITS media also increased expression of all other cardiac genes analyzed, especially that of *ATPA2* and, to a lesser extent, *SCN5A.* Co-expression of endogenous α-Actinin and TNNT2 at reprogramming day 30 was also observed. While levels of protein expression varied in the reprogrammed population, we observed striation patterns in both high- and low- expressing $\alpha\text{-Actinin}^{+}TNNT2^{+}$ cells (**Figure S8A**). MST reprogrammed cells also displayed elevated expression of sarcomere-related genes *MYH6, ACTC1, TNNT2, TPM1, MYBPC3,* and *TTN* compared to PCFs (**Figure S8B**). In addition to upregulation of cardiac genes, we noted downregulation of the fibroblast marker gene *POSTN* in these conditions (**Figure 3C**).

Functional assessment of MST-derived hiCMs

To determine the degree of reprogramming induced by MST and to compare the effect of media and small molecules on the reprogrammed cells' function, we assessed the cells for spontaneous calcium flux. Reprogrammed cells were incubated with Cal-520, a fluorogenic calcium-sensitive dye prior to imaging. Fluorescence was measured for 60 seconds using high-content kinetic imaging at reprogramming day 7, 15, 22, and 30. Although calcium transients were detectable as early as differentiation day 7, they were more pronounced by day 22 (**Video S1**). At

that time point, calcium transients were extremely rare (<0.1%) and slow (~40 s) in cells cultured using DMEM+10% FBS (**Figure 4A-4B**) but were more commonly seen in cells cultured using D-BAITS media, with an average calcium transient duration 90% (CTD_{90}) of 22.4s ± 1.7 s (**Figure 4A-4C**). Although the addition of XAV939 to MST-derived cells in D-BAITS media lowered the total number of cells cycling calcium by 44.7% compared to D-BAITS alone, their flux occurred more rapidly, with a CTD_{90} of 19.4 s ± 2.3 s compared to 22.4 s ± 1.7 s without XAV939 (**Figure 4A ii, 4B-C**). Previous studies using serum-free media and GMT in mouse suggest that FGF signaling improves functionality of directly reprogrammed cells by resulting in a 100-fold increase in beating cells[41]. In our system, addition of FGF2 (100 ng/mL) to D-BAITS resulted in a decrease in CTD_{90} time (14.2 s ± 0.9 s compared to 22.4s ± 1.7s without FGF2) and a 38.3% increase in the total number of cycling cells (**Figure 4B**). Simultaneous treatment with XAV939 and FGF2 did not significantly change the CTD_{90} (12.3 s ± 0.75s) compared to FGF2 alone; however, reproducibility, based on a lower SEM (0.74 s compared to 0.90 s), was increased without a decrease in the total number of cycling cells.

To investigate whether MST-derived cells respond to pharmacological stimulation, we analyzed calcium flux in response to the β-adrenergic agonist isoproterenol (2 μM), as previously described[42]. Treatment with isoproterenol resulted in faster calcium cycling with a CTD_{90} of 2.0 s ± 0.4s compared to 8.9 s ± 0.7s for untreated cells, as would be expected with cardiomyocytes (**Figure 4D-E**). However, peak amplitude as well as maximum upstroke and downstroke velocity were unchanged, suggesting that further optimization of $Ca2^{+}$ signaling machinery is necessary to produce cells that fully emulate cardiac function. (**Figure 4F-I**). We monitored spontaneous cell contraction and observed cell movement as early as day 12 of differentiation, with contraction becoming more evident by day 29 (**Figure 4J, Video S2**).

To determine the extent of reprogramming in MST-derived cells, we performed RNA sequencing and compared the transcriptome of three samples of MST-reprogrammed hiCMs to that of matched cardiac fibroblasts using hiPSC-CMs as a control. PCA of gene expression data showed distinct clustering of individuals into the three sample groups (PCFs, MST-reprogrammed hiCMs, and hiPSC-CMs) based on PC1 (67% of variance) and PC2 (24% of variance) (**Figure 5A**). The top 10 upregulated genes contributing to PC1, based on absolute value of the loadings, are all mitochondrial related (including *MT-CO1*, *MT-RNR2*, *MT-ND4*, etc.). These are followed by cardiac *ACTC1*, *MYL7*, and *MYH6*. For PC2, the top upregulated genes are smooth muscle related *ACTA2*, *MYH11*, *FLNA*, and calcium related *MYLK*. As expected, top downregulated genes for both PC1 and PC2 are fibroblast related (*FN1*, *COL1A1*, and *COL1A2*, and *EEF1A1)* (**File S1**). This independent clustering was further corroborated by hierarchical cluster analysis (**Figure 5B**). Together, these data suggest that MST-reprogrammed hiCMs are in an intermediate state between cardiac fibroblasts and hiPSC-CMs. Nonetheless, gene-set enrichment analysis (GSEA) revealed an upregulation of cardiac gene sets in MST-derived cells compared to PCFs. Enriched gene sets include cardiac conduction, calcium regulation in cardiac cells, and cardiac muscle contraction (**Figure 5C**). Likewise, Ingenuity Pathway Analysis (IPA) identified several significant cardiac-related pathways in MST-derived cells, including cardiac hypertrophy signaling (P = 1.48e-10), dilated cardiomyopathy signaling (P = 1.12e-08), calcium signaling (P = 8.13e-06), cardiac β-adrenergic signaling (P = 4.57e-04), and factors promoting cardiogenesis in vertebrates (P = 0.02) (**File S2**).

Analysis of the 9,293 differentially expressed genes (FDR < 0.05) in MST-derived cells over fibroblasts also suggests a global shift to a cardiac-like state (**Figure 5D, S9A**). As expected, MST-derived hiCMs expressed high levels of *MYOCD*, *SMAD6*, and *TBX20*, even higher than

levels found in hiPSC-CMs (**Figure S9B**). Cardiac fibroblast markers *IL6*, *MMP1* (collagenase 1), *SERPINE1*, *POSTN*, *P4HB*, *COL1A1*, *COL1A2*, and *FN1* were all downregulated after MST reprogramming to levels similar to hiPSC-CMs (**Figure S9C**). Compared to cardiac fibroblasts, MST-derived hiCMs showed significant upregulation of cardiac-specific genes, including *ACTC1, ACTN2, MYH6, MYH7B, MYL2, MYL3, MYL4, MYL7, TNNT2, TNNC1,* and *TTN* (**Figure 5E**). We also noted expression of all cardiac ion channel genes: *KCNQ1, KCNJ2, KCND2, KCNJ4, SCN5A, KCNH2,* and *KCND3* (**Figure 5F**). Upregulated expression of genes encoding cardiac structural proteins (*DES, ACTA1, UNC45B*) and proteins involved in cardiac cell metabolism and signaling (*CKM SMYD1, NPPA*) were also observed in MST-derived hiCMs, (**Figure 5G**). Finally, we noted increased expression of several cardiac calcium-handling related genes (*CASQ2, RYR2, HCN3, HCN4, PLN, CACNA1C, ATP2A2, CAMK2D*) in MST-derived hiCMs relative to PCFs (**Figure 5H**).

While this data demonstrates a transcriptomic shift towards a cardiac state, the overall expression of cardiac-related genes in MST-reprogrammed hiCMs remains lower than that of hiPSC-CMs, suggesting that further optimization, perhaps by inclusion of growth factors, additional reprogramming factors, or small molecules is necessary for complete reprogramming.

DISCUSSION

We have optimized a lentiviral transduction and cardiac reporter platform to efficiently screen combinations of transcription factors for their ability to produce cells with active cardiac MYH6 and TNNT2 promoters and to assay for calcium flux. Using this platform, we identified a novel combination of transcription factors: *MYOCD, SMAD6, TBX20* (MST) that reprograms human PCFs into cardiomyocyte-like cells based on gene expression and sarcomere structure.

MST, in conjunction with FGF2, XAV939, and our dose-optimized media containing BSA, ascorbic acid, insulin, transferrin, and sodium selenite, allows for the derivation of cells with functional cardiac characteristics. We, like others, have found that reprogramming efficiency and calcium flux are enhanced by addition of the Wnt pathway inhibitor, XAV939. In our system, the number of calcium cycling cells is further increased with the addition of FGF2. Together, these conditions result in the reprogramming of human PCFs to cardiac-like cells that are responsive to pharmacological stimulation and spontaneously contract in culture.

We have used MST to successfully reprogram 24 distinct primary samples from patients (aged 0.01 - 21 years) undergoing cardiac biopsy for diverse diagnoses, as well as one healthy tissue (**Figure S2**). To our knowledge, this is the first study to demonstrate direct cardiac reprogramming using cells from numerous patients and diverse cardiac diagnoses. Expectedly, we have found that reprogramming efficiency is variable from patient to patient, with some lines not converting, and have included all such instances in our data. While genetic background may play a role in reprogramming efficiency, we speculate that the inherent diversity in starting fibroblast population and activation state may also contribute to differences in reprogramming outcomes. Further studies will be necessary to determine whether MST-reprogrammed cells retain disease-associated phenotypes and whether reprogramming efficiency differs when using aging tissues. Likewise, we have found that MST-derived cells express both atrial and ventricular-associated genes (**Figure S9D**). Future optimizations focusing on subtype specification may be beneficial for disease modeling. Although hiCM-derived cells express several genes associated with a mature electrophysiological phenotype, including *KCNJ2*, *KCNQ1*, *SCN5A*, *CACNA1C*, *RYR2*, and *ATP2A2*, expression of genes associated with myofibril isoform switching (*MYH7*, *MYL2*, *TNNI3*)

is low relative to fetal-associated *MYH6*, *MYL7*, and *TNNI1 (***Figure S9E***)*; highlighting an area for further improvement of this reprogramming protocol.

MYOCD and T-box factors have been utilized in existing cardiac direct reprogramming cocktails. TBX20 is known to play a role in cardiac development through regulation of GATA4 and NKX2-5 transcription[43]. It is also implicated in maintaining function of the cardiac conduction system[44]. While this manuscript was under review, Tang et. al independently identified TBX20 as a cardiac reprogramming factor[45]. In their study, addition of TBX20 to GMT + miR133 resulted in cells with more fully activated cardiac conduction systems. The reprogrammed cells exhibited increased mitochondrial function and spontaneous calcium flux after 1 month; however, contraction was observed only in co-culture with hiPSC-CMs, limiting the therapeutic applications of the approach.

While overexpression of *MYOCD* alone in PCFs led to activation of our cardiac reporter and to some cardiac gene induction, no spontaneous contraction nor calcium flux was observed in the resulting cells. Partial activation of the cardiac gene network, including aMHC and cTnT, by overexpression of *MYOCD* alone in human foreskin fibroblasts has been previously reported[39]. However, as in our study, the authors conclude that additional factors are necessary to derive cells that are fully committed to the cardiac fate. Similarly, in mouse fibroblasts, addition of *MYOCD* to GMT contributes to improved cardiac gene expression but does not enhance cardiac function[40].

Further studies will be necessary to determine the mechanism of MST-based direct reprogramming; however, we note that the factors *MYOCD* and *SMAD6* were present in each of the top 3 combinations identified in our screen. This may suggest a shared reprogramming mechanism driving activation of our aMHC-TNNT2 transgenic reporter. SMAD6 is known to inhibit TGFβ signaling through inhibition of receptor-associated SMADs (SMAD2/3) and TGFβ

inhibition using SB431542 has been shown to improve the efficiency of GMT-based reprogramming[9]. Therefore, we speculate that SMAD6 allows for direct reprogramming through a similar mechanism. In our system, the addition of TGFβ1 did not affect MST-reprogramming efficiency; however, further TGFβ inhibition using SB431542, A83-01, or ITD-1 did result in a marginal increase in $MYH6^{+}TNNT2^{+}$ cells.

Directly reprogrammed cardiomyocytes have the potential to advance precision medicine by providing an expedient source of patient-matched cells that can be used *in vitro* for drug screening or *in vivo* as therapy for heart failure. This study presents MST as a novel transcription factor combination that is suited for direct reprogramming of human cardiac fibroblasts. Further studies can determine whether efficiency of this system can be improved by using a polycistronic construct and by optimizing the timing of small molecule delivery. Due to the observed functional characteristics of MST-derived cells, future investigational and therapeutic approaches may benefit from the use of this combination.

ACKNOWLEDGEMENTS. The authors would like to thank all patient donors for their contributions to this work. Graphical abstract and Figure 1a were created with BioRender.com.

SOURCES OF FUNDING. This work was supported by NIH grant R01 CA220002, R01CA261898, the Leducq Foundation (P.W.B.), and the Northwestern Molecular and Translational Cardiovascular Research Training Program T32HL134633: SP0040691.

ACCESSION CODES. Gene Expression Omnibus: RNA-seq data have been deposited with accession code GSE218091.

DISCLOSURES. The authors declare no competing interests.

SUPPLEMENTAL MATERIAL

Tables S1-S7

Figures S1-S9

Files S1-S2

Videos S1-S2

References 1-45

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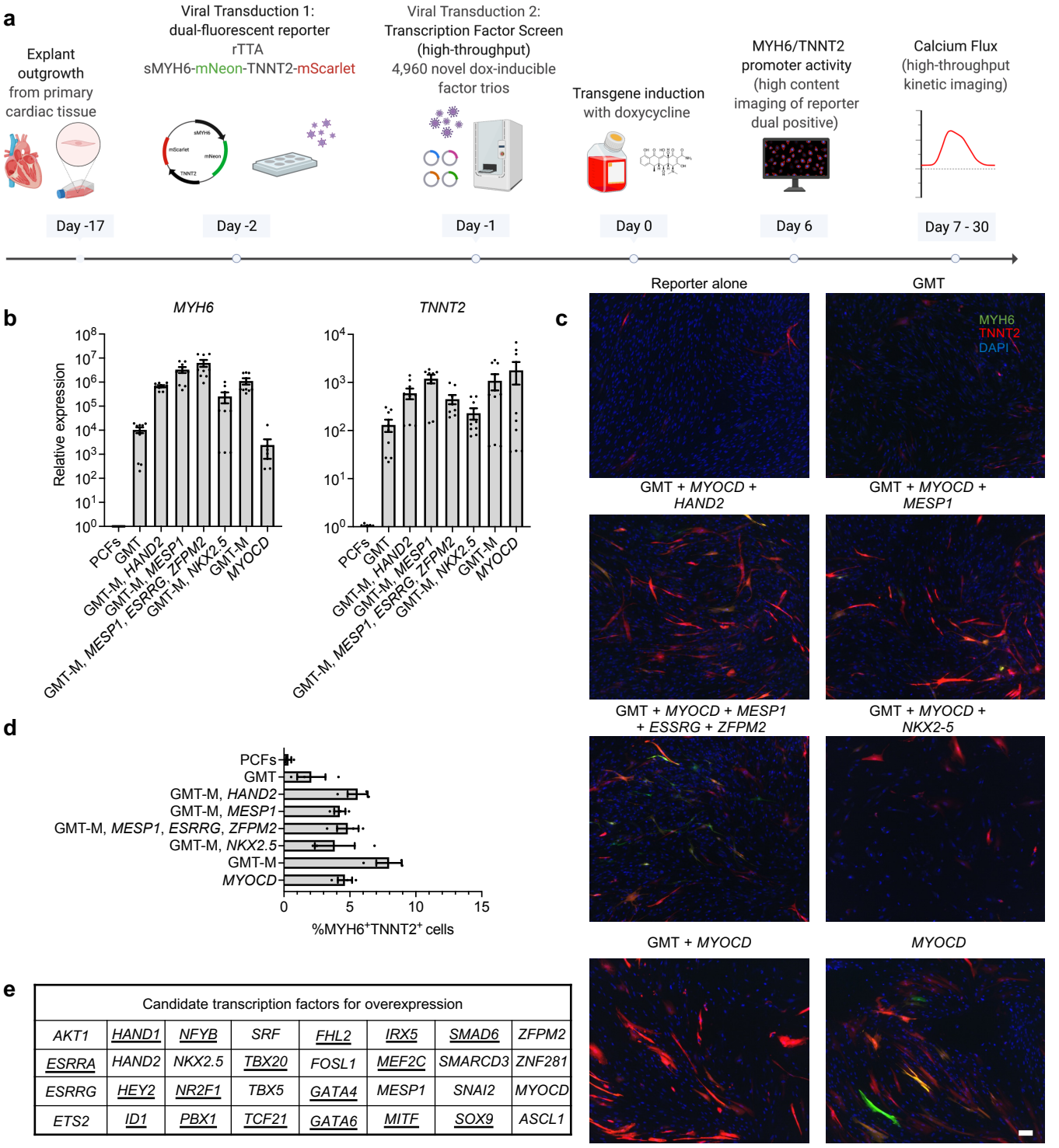

Candidate transcription factors for overexpression							
AKT1	*HAND1*	*NFYB*	*SRF*	*FHL2*	*IRX5*	*SMAD6*	*ZFPM2*
ESRRA	*HAND2*	*NKX2.5*	*TBX20*	*FOSL1*	*MEF2C*	*SMARCD3*	*ZNF281*
ESRRG	*HEY2*	*NR2F1*	*TBX5*	*GATA4*	*MESP1*	*SNAI2*	*MYOCD*
ETS2	*ID1*	*PBX1*	*TCF21*	*GATA6*	*MITF*	*SOX9*	*ASCL1*

Figure 1. Establishment of a high throughput platform to assess the effect of transcription factor combinations on cardiac direct reprogramming. (a) Lentiviral transduction and reporter platform: human cardiac fibroblasts are obtained from discarded primary human cardiac tissue by explant outgrowth. Cells are collected and transduced overnight with rTTA and a MYH6/TNNT2 dual cardiac reporter. The following day, cells are collected and replated. Lentivirus combinations are delivered in multiplex using an acoustic liquid handler. Media containing doxycycline is changed every two days beginning on day two. Fluorescence from cardiac reporters is measured using a high-content kinetic imager 6 days after doxycycline addition. **(b)** Relative expression of *MYH6* and *TNNT2* measured by RT-qPCR in primary fibroblasts transduced only with existing cardiac reprogramming factor cocktails at reprogramming day 6. Expression normalized to PCF control. (*n* = 3 biological replicates, 3 technical replicates each). **(c)** Representative images of primary cardiac fibroblasts transduced with sMYH6-mNeonGreen-TNNT2-mScarlet and reprogrammed using published factor combinations. Scale bar = 100 μM. Images acquired using high content image cytometry at reprogramming day 6 (KIC, VALA Sciences) **(d)** Quantification of MYH6+TNNT2+ cells as measured by pFU-GW-sMYH6-mScarlet-TNNT2-mNeon reporter fluorescence on reprogramming day 6 (*n* = 3 biological replicates). All cells were subject to reporter overexpression **(e)** Candidate transcription factors for overexpression. Factors predicted by Mogrify underlined. Primary cardiac fibroblasts (PCF), *GATA4, MEF2C, TBX5* (GMT), GMT + *MYOCD* (GMT-M). Error bars: mean ± SEM.

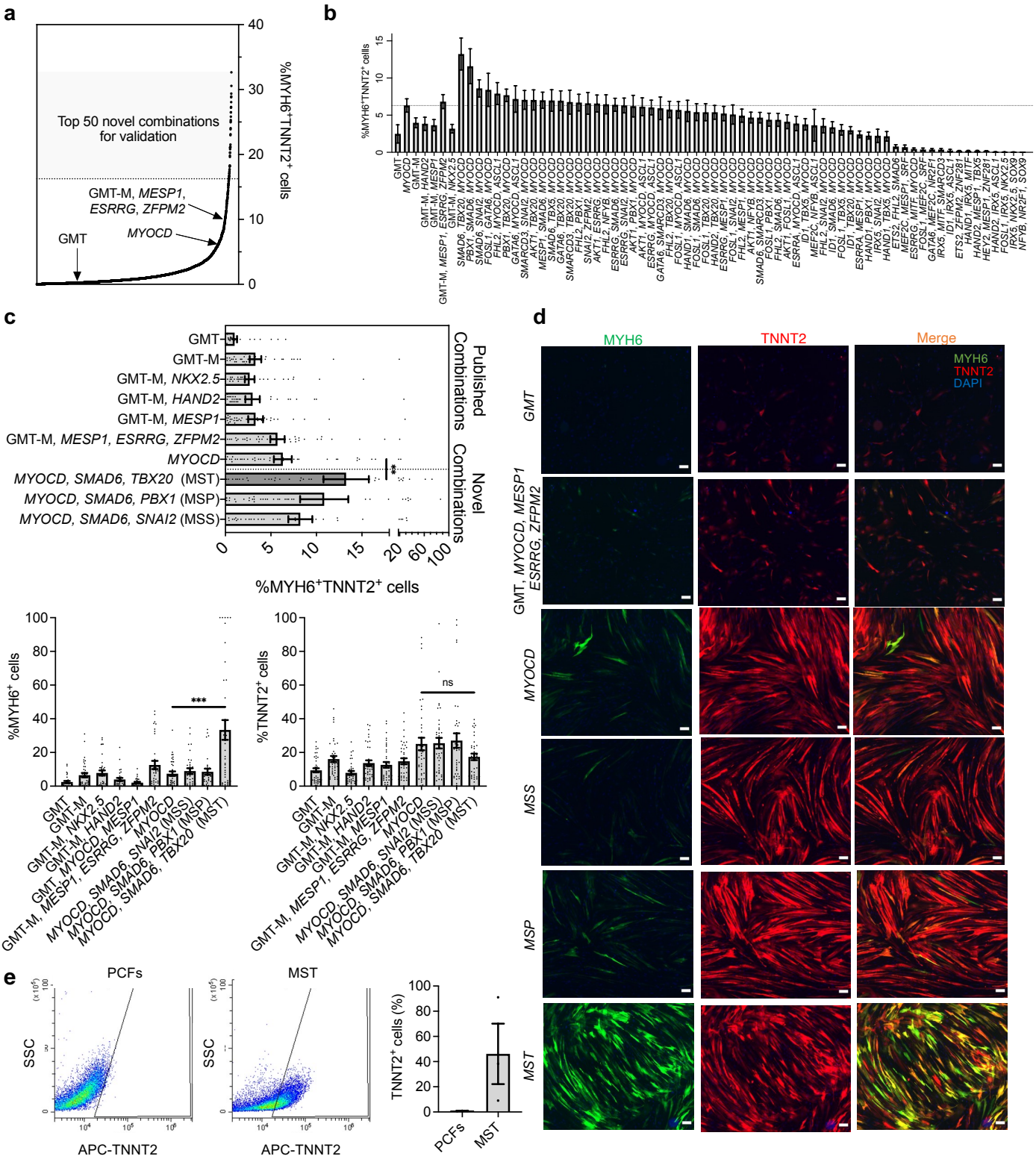

Figure 2. *MYOCD, SMAD6, TBX20* (MST) overexpression generates MYH6⁺TNNT2⁺ cells with higher efficiency compared to existing GMT-based transcription factor combinations. (a) Primary screen of transcription factor trios (*n* = 3 biological replicates). PCFs were transduced with rTTA and sMYH6-mNeon-TNNT2-mScarlet lentivirus and were subsequently transduced with e ach 3-factor combination from our lentiviral library. Cells were cultured in DMEM 10% FBS containing 0.25µg/mL doxycycline and fluorescence was quantified on reprogramming day 6. Distribution of means plotted. Top 50 factors, *MYOCD*, and GMT-M, *MESP1*, *ESRRG*, *ZFPM2* (best-performing published combination) indicated. **(b)** Validation of top 50 factors from lentiviral screen (*n* = 42 wells analyzed from 13 biological replicates) **(c)** Top 3 transcription factor trio combinations (*n* = 38 wells analyzed per condition from 13 biological replicates): %MYH6⁺TNNT2⁺ cells (top) %MYH6⁺ cells (bottom left) and %TNNT2⁺ cells (bottom right), measured by pFU-GW-sMYH6-mNeon-TNNT2-mScarlet fluorescence on reprogramming day 6. *P* values were calculated by paired t-test. **(d)** Images comparing *MYH6* and *TNNT2* reporter expression in novel combinations MST, MST, and MSS with *MYOCD* and published factor cocktails. Scale bar = 100 µM. Images acquired using high content image cytometry at reprogramming day 6 (KIC, Vala Sciences) **(e)** Flow cytometry analysis of TNNT2 expression in MST-transduced cells; reprogramming day 15. Representative data of untransduced and MST-transduced samples (left), quantification (right) (*n* = 3 biological replicates). ns $P > 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$. Error bars: mean ± SEM.

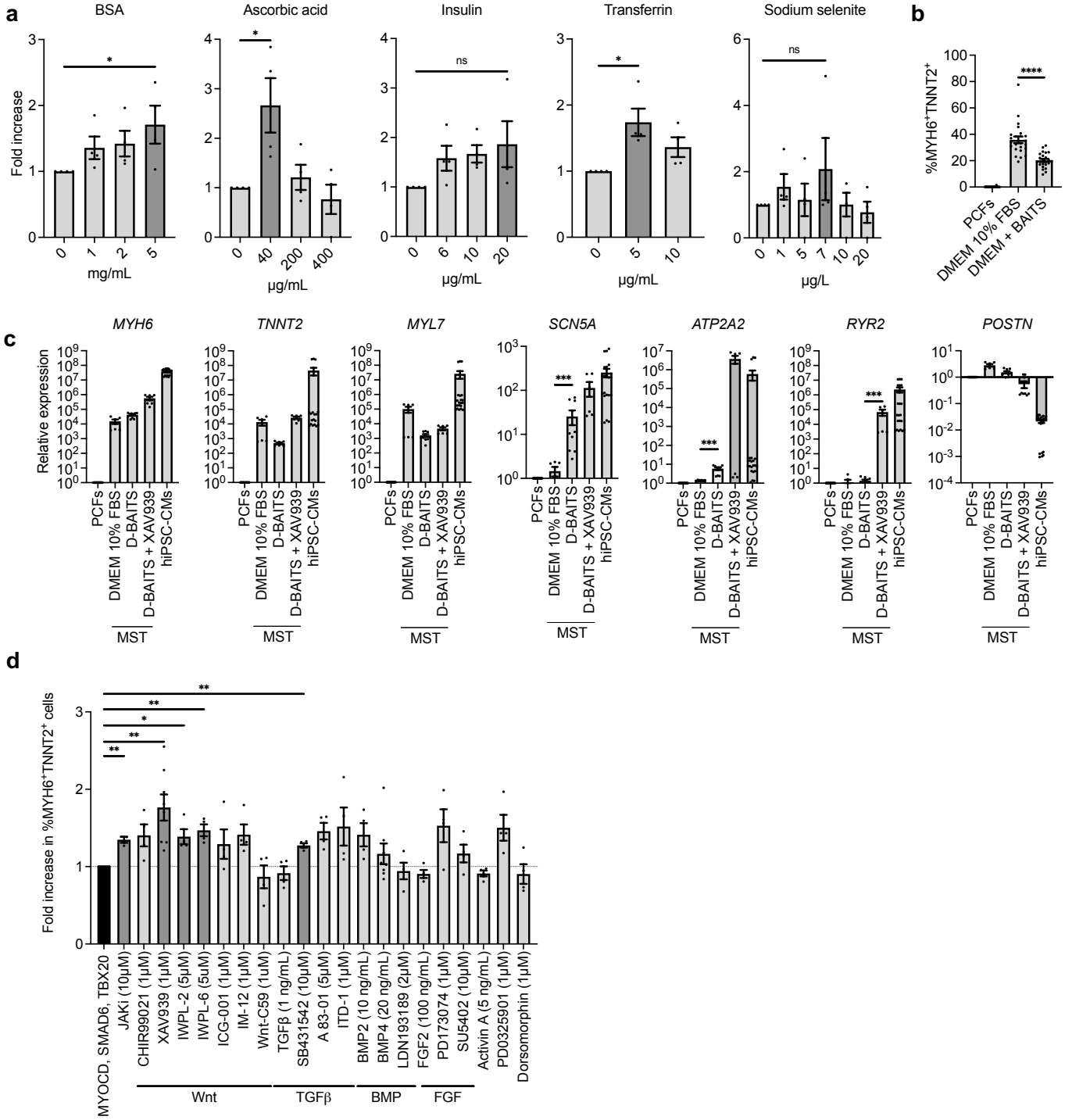

Figure 3. Wnt Inhibition using XAV939 enhances reprogramming efficiency of MST in defined media. (a) Reprogramming media component dose optimization. Fold increase in %MYH6⁺TNNT2⁺ cells, measured by pFU-GW-sMYH6-mNeon-TNNT2-mScarlet fluorescence on reprogramming day 6. Cells were transduced with MST and cultured in DMEM media containing BSA, ascorbic acid, insulin, transferrin, and sodium selenite starting 24h post-transduction. (*n* = 83 wells analyzed for each condition from 3-4 biological replicates; mean of biological replicates plotted). *P* values were calculated by t-test. **(b)** Percent mNeon (MYH6) mScarlet (TNNT2) double positive cells following culture in either DMEM+10% FBS or D-BAITS media following transduction with MST. Flourescence quantified on reprogramming day 6. (*n* = 24 technical replicates). *P* values were calculated by t-test, **(c)** RT-qPCR analysis for cardiac and fibroblast gene expression (*n* = 2-3 biological replicates), reprogramming day 6. *P* values were calculated by Mann-Whitney test. **(d)** Small molecule screen. Fold increase in percent MYH6⁺TNNT2⁺ cells on reprogramming day 6. Cells were transduced with MST and cultured in DMEM media containing 5 mg/mL BSA, 40 µg/mL ascorbic acid, 20 µg/mL insulin, 5 µg/mL transferrin, and 7 µg/L sodium selenite. (*n* = 40 wells analyzed for each condition from 3-4 biological replicates; mean of biological replicates plotted). *P* values were calculated by ANOVA followed by Fisher's LSD test. ns $P > 0.05$, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$. Error bars: mean ± SEM.

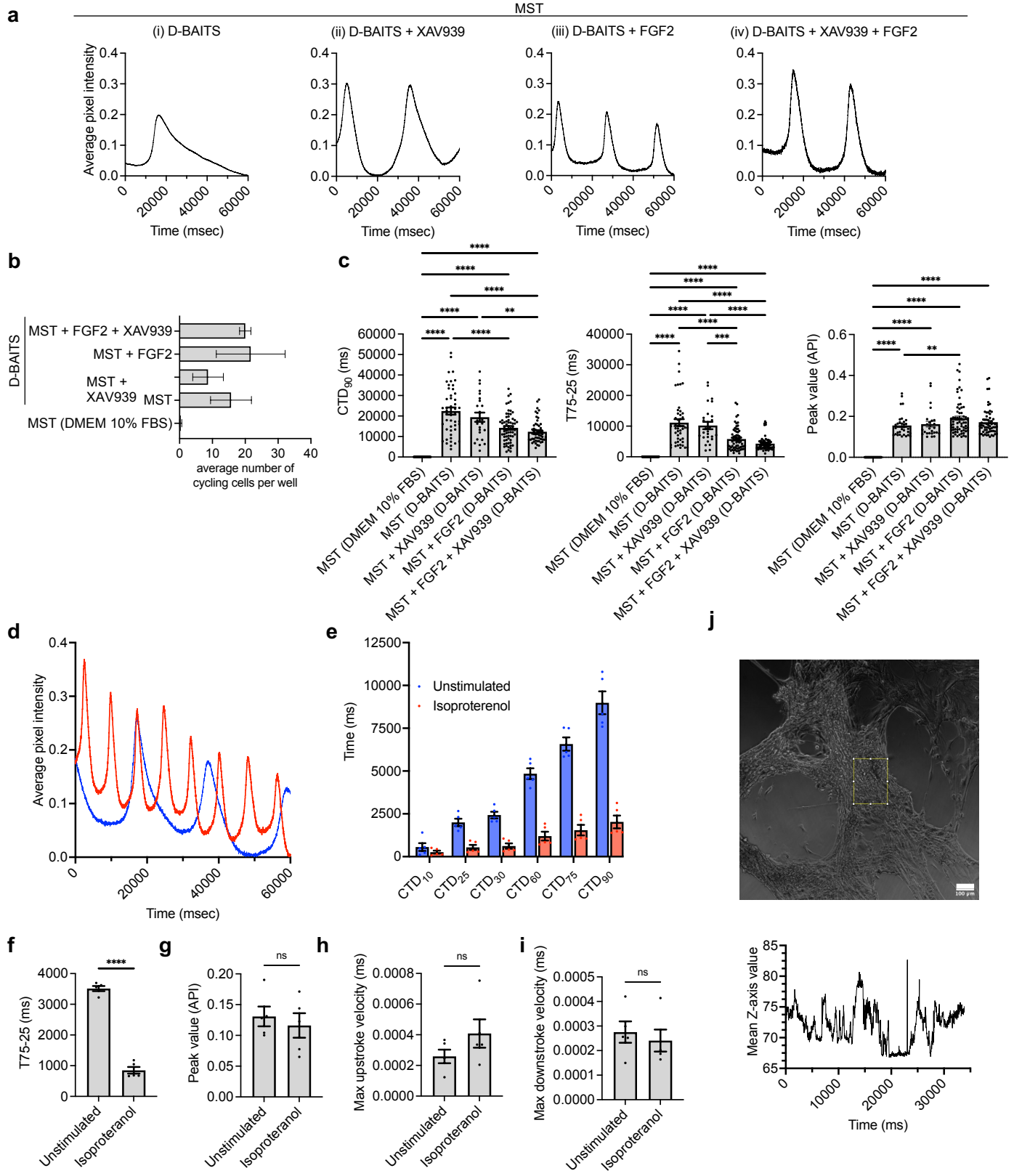

Figure 4. FGF2 enhances calcium cycling in MST-derived cells and produces contractile cells that respond to pharmacological stimulation. **(a)** Spontaneous Ca^{2+} oscillations in reprogrammed cells on reprogramming day 22; representative traces: (i) DMEM + BAITS, (ii) DMEM + BAITS + XAV939, (iii) DMEM + BAITS + FGF2, (iv) DMEM + BAITS + XAV939 + FGF2 **(b)** Aggregate number of cycling cells per well of a 384-well plate detected by analysis software **(c)** CTD_{90} (left), T75-25 (center), peak value (right) per condition (n = 3 wells per condition). P values calculated using one-way ANOVA with subsequent pairwise testing via t-test with Tukey's adjustment for multiple comparisons. **(d)** representative traces of unstimulated (blue) and isoproterenol stimulated (red) cells. Effects of 2 µM isoproterenol stimulation on **(e)** calcium-transient duration of reprogrammed cells **(f)** T75-25 **(g)** peak value **(h)** maximum upstroke velocity **(i)** maximum downstroke velocity (n = 5 cells per condition). P values calculated using t-test. **(j)** Spontaneous contraction in MST-derived cells; reprogramming day 25. Still from video (top), quantification of movement along Z-axis within ROI (yellow) (bottom). ns $P > 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$. Error bars: mean ± SEM.

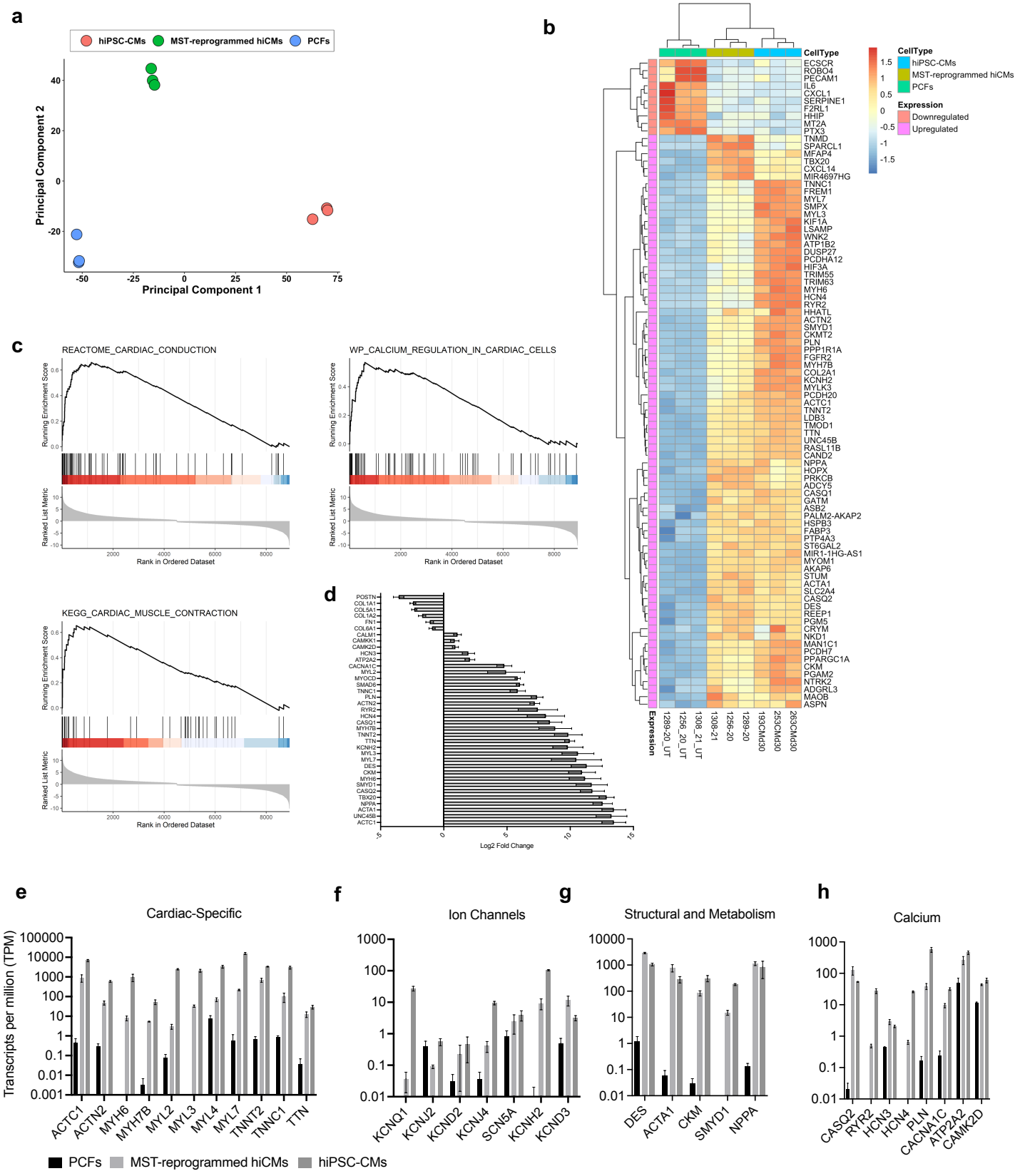

Figure 5. RNA-seq analysis reveals cardiac transcriptome changes in MST-reprogrammed cells. (a) Principal component analysis of MST-reprogrammed hiCMs, PCFs, and hiPSC-CMs (n =3 biological replicates per condition). **(b)** Hierarchical clustering of 86 variable genes significantly upregulated or downregulated in both hiPSC-CMs and MST-derived hiCMs (*n* = 3, P-adjusted value < 0.00001 and abs(log2foldchange) > 5). **(c)** Enrichment plots of the indicated gene sets: cardiac conduction (top left), calcium regulation in cardiac cells (top right), cardiac muscle contraction (bottom). **(d)** Comparative expression of cardiac genes in MST-reprogrammed hiCMs vs. primary cardiac fibroblasts. Transcripts per million for expression of **(e)** cardiac-specific genes, **(f)** ion channels, **(g)** structural and metabolism-related genes **(h)** calcium-related genes in primary cardiac fibroblasts (PCFs) (black), MST-reprogrammed hiCMs (light gray), and hiPSC-CMs (dark gray). Error bars: mean ± SEM.

Publication	Starting human cell type	Virus	Transcription factor cocktail	Additional enhancers	Initial screening reporter/ readout	Cardiac markers	Spontaneous calcium flux	Spontaneous contraction in human cells
Nam/Olson PNAS, 2013	Adult CF Adult DF Neonatal FF	Retrovirus amphotropic fresh	GATA4, TBX5, MYOCD, HAND2 or GATA4, MEF2C, TBX5, MYOCD, HAND2 (in absence of microRNAs)	miR-1, miR-133	TNNT2 expression (FC)	Neonatal FF 2 weeks: TNNT2$^+$ (25.7% with microRNAs, 19.6% transcription factors alone) Adult CF 2 weeks: TNNT2$^+$ (10.4% with microRNAs, 7.27% transcription factors alone) Adult DF 2 weeks: TNNT2$^+$ (4.43% with microRNAs)	8 weeks: 'low percentage'	11 weeks: 'small subset'
Wada/Ieda PNAS, 2013	Adult CF, pediatric CF	Retrovirus VSVG fresh	GATA4, MEF2C, TBX5, MYOCD, MESP1	None	Cardiac gene induction (RT-qPCR)	4 weeks: α-actinin$^+$ (5.5%) 4 weeks: TNNT2$^+$ (5.9%)	4 weeks: ~1%, "low frequency"	Contraction in co-culture with mouse CMs
Fu/Srivastava Stem Cell Rep, 2013	hESC-FB, adult DF, CF	Retrovirus VSVG fresh	GATA4, MEF2C, TBX5, MYOCD, MESP1, ESRRG, ZFPM2	TGFβ1 (20 ng/mL) (in absence of MYOCD and ZFPM2)	MYH6 promoter reporter (FC) TNNT2 expression (FC)	hESC-FB 2 weeks: aMHC-mCherry$^+$ (18.1%), TNNT2$^+$ (10.7%), aMHC-mCherry:cTNT^{++} (13.0%) DF 2 weeks: TNNT2$^+$ (3.71%) CF 2 weeks: TNNT2$^+$ (1.77%)	4 weeks: only with stimulation (20% of mCherry$^+$)	Not described
Christoforou/ Leong Sci Rep, 2017	DF	Lentivirus VSVG fresh	GATA4, MEF2C, TBX5, MYOCD, NKX2-5	miR-1, miR-133a, JAK1i	ACTN2 expression (IF)	2 weeks: TNNT2$^+$ (0.21% transcription factors alone, 3.8% with micro-RNAs, 3.8% with JAK1i)	7 days, but subsided by day 12-14	4 weeks: not detected
Mohamed/ Srivastava Circulation, 2017	Immortalized CF	Retrovirus amphotropic fresh	GATA4, MEF2C, TBX5, MYOCD	SB431542 (2.6 μM) XAV939 (5 μM)	α-MHC-GFP reporter TNT-GCaMP (FC)	3 weeks: TNNT2-GFP$^+$ (13.4% with small molecules)	10 days: spontaneous transients, homogenous transients within 3 weeks	Not described
Zhou/Qian Cell Stem Cell, 2019	Adult CF	Retrovirus VSVG frozen	GATA4, MEF2C, TBX5 (polycistronic)	miR-133	TNNT2 expression (FC)	2 weeks: TNNT2$^+$ (2.83%) (41.8% with antibiotic selection)	20 days: spontaneous transients	Contraction in co-culture with mouse neonatal CMs
Garry/Olson Nat Cell Biol., 2021*	Adult CF	Retrovirus amphotropic fresh	GATA4, MEF2C, TBX5, MYOCD, HAND2, PHF7	None	TNNT2 and α-Actinin expression (FC)	3 weeks: TNNT2$^+$ (~3.5%) α-Actinin$^+$ (~10.0 %) TNNT2+α-Actinin$^+$ (~3.0%)	Not described for human cells	Not described for human cells
Tang/Zhou Circulation, 2022*	hESC-FB, CF, DF	Retrovirus VSVG fresh	GATA4, MEF2C, TBX5, TBX20	miR-133	α-MHC and α-Actinin expression (FC)	2 weeks: α-MHC$^+$ (30.3%) α-Actinin$^+$ (23.8%)	1 month: spontaneous transients in co-culture with hiPSC-CMs	Contraction in co-culture with hiPSC-CMs
Wang/Qian Cell Stem Cell, 2022*	hESC-FB	Lentivirus Retrovirus	MEF2C, ASCL1	None	cTnT and α-Actinin expression (FC)	2 weeks: cTnT$^+$α-Actinin$^+$ (6.53%)	Not described for human cells	Not described for human cells
This work	Pediatric CF	Lentivirus VSVG frozen	MYOCD, SMAD6, TBX20	XAV939 (1 μM) FGF2 (100 ng/ mL)	MYH6/TNNT2 promoter reporter and Ca^{2+} flux (high content kinetic imaging)	6 days: MYH6-mNeon$^+$ (33%) TNNT2-mScarlet$^+$ (17%) MYH6-mNeon-TNNT2-mScarlet^{++} (13%) without antibiotic selection	7 days: spontaneous transients, homogenous transients within 3 weeks	Spontaneous contraction starting at 20 days

Table S1. Published transcription factor combinations for direct differentiation of human fibroblasts into cardiomyocytes. CF: cardiac fibroblast, TTF: tail tip fibroblasts, DF: dermal fibroblasts, FF: foreskin fibroblasts, hESC-FB: human embryonic stem cell-derived fibroblasts, hiPSC-CMs: human iPSC derived cardiomyocytes. FC: flow cytometry, IF: immunofluorescence. * published after completion of our screen.

Sample	Sex	Age at collection (years)	Fundamental Diagnosis	Experiments
1101-19	F	0.01	AVC (AVSD), Complete (CAVSD), Balanced ventricles, Rastelli type C	RT-qPCR (Figure 1B), LV Validation (Figure 2B, 2C)
1184-19	M	7.00	N/A (donor tissue)	LV Validation (Figure 2B, 2C)
1185-19	F	6.36	Total anomalous pulmonary venous connection (TAPVC), Type 4 (mixed)	LV Validation (Figure 2B, 2C)
1213-20	M	2.50	Pulmonary atresia, VSD (Including TOF, PA)	LV Validation (Figure 2B, 2C)
1256-20	F	2.00	N/A (donor tissue)	LV screen (Figure 2A), representative images of direct reprogramming using published combinations (Figure 1C), LV Validation (Figure 2B, 2C)
1269-20	F	12.08	ASD, Secundum	RT-qPCR (Figure 1B, 3C), LV Validation (Figure 2B, 2C), Representative Images of top 3 factor combinations (Figure 2D), media optimization (Figure 3A), Basal media (Figure 3B), RNA-Seq (Figure 5)
1266-20	F	0.40	VSD, Type 2 (Perimembranous) (Paramembranous)	LV screen (Figure 2A)
1274-20	M	0.43	AVC (AVSD), Complete (CAVSD), Balanced ventricles, Rastelli type C	LV Validation (Figure 2B, 2C), RNA-Seq (Figure 5)
1275-20	M	0.28	AVC (AVSD), Complete (CAVSD), Balanced ventricles, Rastelli type A	LV Validation (Figure 2B, 2C)
1281-20	M	14.55	Tetralogy of Fallot, Pulmonary stenosis	LV Validation (Figure 2B, 2C)
1289-20	F	0.34	AVC (AVSD), Complete (CAVSD), Balanced ventricles, Rastelli type A	LV screen (Figure 2A)
1300-21	M	0.84	Pulmonary atresia-IVS	LV Validation (Figure 2B, 2C)
1301-21	M	10.84	Truncus arteriosus, With pulmonary dominance (with obstructed arch) (Van Praagh A4), With interrupted aortic arch	LV validation (Figure 2B, 2C)
1302-21	F	0.52	Tetralogy of Fallot, Pulmonary stenosis	LV validation (Figure 2B, 2C)
1304-21	F	0.74	Single ventricle, Heterotaxy (heterotaxy syndrome) (visceral heterotaxy), DORV + CAVC (CAVSD) + Asplenia (Right isomerism)	LV validation (Figure 2B, 2C)
1308-21	F	0.30	AVC (AVSD), Complete (CAVSD), Balanced ventricles, Rastelli type A	Calcium imaging (Figure 4A-C)
1318-21	M	0.53	Tetralogy of Fallot, Common atrioventricular canal (AVSD)	TNNT2 immunostaining (Figure 2E)
1326-21	M	4.28	Cardiomyopathy, Dilated	Small molecule screen (Figure 3D)
1329-21	M	16.59	DORV, "TGA type" (Subpulmonary VSD), Subpulmonary VSD + No PS (Taussig Bing)	RT-qPCR (Figure 1B, 3C), isoproterenol stimulation (Figure 4B-I)
1336-21	F	0.65	DORV, "TOF type", Subaortic VSD + PS, Nonrestrictive VSD	direct reprogramming using published combinations (Figure 1D), TNNT2 flow cytometry (Figure 2F), RT-qPCR (Figure 3C), small molecule screen (Figure 3D)
1337-21	M	21.27	Hypoplastic left heart syndrome (HLHS), Aortic stenosis + Mitral stenosis	direct reprogramming using published combinations (Figure 1D), TNNT2 flow cytometry (Figure 2F), RT-qPCR (Figure 3C), small molecule screen (Figure 3D), TNNT2 and α-actinin immunostaining (Figure 3E)
1340-21	M	12.31	Hypoplastic left heart syndrome (HLHS), Aortic atresia + Mitral atresia	direct reprogramming using published combinations (Figure 1D), TNNT2 flow cytometry (Figure 2F), RT-qPCR (Figure 3C), small molecule screen (Figure 3D)
1346-21	F	0.40	AVC (AVSD), Intermediate (transitional)	RT-qPCR (Figure 3C), TNNT2 and α-actinin immunostaining (Figure 3E)
1347-21	M	2.54	Hypoplastic left heart syndrome (HLHS), Aortic atresia + Mitral atresia	RT-qPCR (Figure 3C), TNNT2 and α-actinin immunostaining (Figure 3E), cell contraction (Figure 4J)

Table S2. Patient sample information

Marker	Antibody	Source	Application
TNNT2	Alexa Fluor 647 Mouse anti-cardiac troponin T	BD Biosciences #565744	1:33 (FC)
TNNT2	Rabbit anti-cardiac troponin T	Abcam ab45932	1:200 (ICC)
α-actinin	Mouse anti-a-actinin	Sigma A7811	1:500 (ICC)
CD31	Alexa Fluor 647 Mouse anti-Human CD31	BD Pharmingen #558094	1:100 (FC)
CD90	APC Mouse Anti-Human CD90	BD Pharmingen #559869	1:50 (FC)
Vimentin	Mouse anti-vimentin	BD Pharmingen #550513	1:100 (FC)

Table S3. Antibodies for flow cytometry (FC) and immunocytochemistry (ICC).

FACTOR	PCR FORWARD PRIMER SEQUENCE	PCR REVERSE PRIMER SEQUENCE
ASCL1	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACCATG GAAAGCTCTGCCAAGATGGAGAGC	GGGGACCACTTTGTACAAGAAAGCTGGGTTTCAGAACCAGT TGGTGAAGTCGAGAAGC
AKT1	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACCATG AGCGACGTGG	GGGGACCACTTTGTACAAGAAAGCTGGGTTTCAGGCCGTGC CG
ESRRA	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACCATG TCCAGCCAGGTGGTGGGCATTGAGC	GGGGACCACTTTGTACAAGAAAGCTGGGTTTCAGTCCATCA TGGCCTCGAGCA
ESRRG	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACCATG TCAAACAAAGATCGACACA	GGGGACCACTTTGTACAAGAAAGCTGGGTTTTAGCAGACCT TGGCCTCC
ETS2	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACCATG AATGATTTCG	GGGGACCACTTTGTACAAGAAAGCTGGGTTTCAGTCCTCCG TGTCG
FHL2	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACCATG ACTGAGCGCTTTGACTGCCACC	GGGGACCACTTTGTACAAGAAAGCTGGGTTCAGATGTCTTT CCCACAGTCG
FOSL1	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACCATG TTCCGAGACTTCGGGG	GGGGACCACTTTGTACAAGAAAGCTGGGTTTCACAAAGCGA GGAGGGTT
GATA6	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACCATG GCCTTGACTGACGGCGGCTG	GGGGACCACTTTGTACAAGAAAGCTGGGTTTCAGGCCAGG GCCAGGGCGCAC
HAND1	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACCATG AACCTCGTGGGCAGC	GGGGACCACTTTGTACAAGAAAGCTGGGTTTCACTGGTTTA ACTCCAGCG
HAND2	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACCATG AGTCTGGTAGGTGGTTTTCC	GGGGACCACTTTGTACAAGAAAGCTGGGTTTCACTGCTTGA GCTCCAGG
HEY2	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACCATG AAGCGCCCCTGC	GGGGACCACTTTGTACAAGAAAGCTGGGTTTTAAAAAGCTC CAACTTCTGTCC
ID1	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACCATG AAAGTCGCCAGTGGCAGCACC	GGGGACCACTTTGTACAAGAAAGCTGGGTTCAGCGACACAA GATGCGATCGTCC
IRX5	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACCATG TCCTATCCGCAGGGCTA	GGGGACCACTTTGTACAAGAAAGCTGGGTTTTAAATGTCGG ACATACCTTTCTTC
MESP1	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACCATG GCCCAGCCCCTGT	GGGGACCACTTTGTACAAGAAAGCTGGGTTTCACTTGGGCT CCTCAG
MITF	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACCATG CAGTCCGAATCGGG	GGGGACCACTTTGTACAAGAAAGCTGGGTCTAACAAGTGTG CTCCGTCTCTT
MYOCD	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACCATG ACACTCCTGGGGTCTGA	GGGGACCACTTTGTACAAGAAAGCTGGGTTCTACCACTGCT GCAAGTGAAG
NFYB	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACCATG ACAATGGATGGTGACAGT	GGGGACCACTTTGTACAAGAAAGCTGGGTTTCATGAAAACT GAATTTGCTGA
NR2F1	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACCATG GCAATGGTAGTTAGCAGC	GGGGACCACTTTGTACAAGAAAGCTGGGTTCTAGGAGCACT GGATGGACA
PBX1	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACCATG GACGAGCAGCCCAG	GGGGACCACTTTGTACAAGAAAGCTGGGTTTCAGTTGGAGG TATCAGAGTGAA
SMAD6	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACCATG TTCAGGTCCAAACGCTC	GGGGACCACTTTGTACAAGAAAGCTGGGTTCTATCTGGGGT TGTTGAGGAG
SMARCD3	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACCATG ACTCCAGGTCTTCAGCA	GGGGACCACTTTGTACAAGAAAGCTGGGTTCTAGGTGTTGC GCAC
SNAI2	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACCATG CCGCGCTCCTT	GGGGACCACTTTGTACAAGAAAGCTGGGTTTCAGTGTGCTA CACAGCAGCC
SOX9	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACCATG AATCTCCTGGACCCCTT	GGGGACCACTTTGTACAAGAAAGCTGGGTTTCAAGGTCGAG TGAGCTGTG
SRF	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACCATG TTACCGACCCAAGCTGG	GGGGACCACTTTGTACAAGAAAGCTGGGTTTGAAACAGGGA TCTGCACTG
TBX20	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACCATG GAGTTCACGGCGTCCCCC	GGGGACCACTTTGTACAAGAAAGCTGGGTTCATACAAATGG CGTCATCACAGCAGAG
TCF21	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACCATG TCCACCGGCTCCCTCAGC	GGGGACCACTTTGTACAAGAAAGCTGGGTTCAGGACGCGG TGGTTCCACATAAG
ZFPM2	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACCATG TCCCGGCGAAAGCAAAGCAAAC	GGGGACCACTTTGTACAAGAAAGCTGGGTTTCATTTGACAT GTTCTGCTGCATGTGATGAG
ZNF281	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACCATG AAAATCGGCAGTGGGTTCCTGAGTG	GGGGACCACTTTGTACAAGAAAGCTGGGTTTACCTGTAACT CTGGCTGGTGGGTG
T7 (sequencing)	TAATACGACTCACTATAGGG	
GW_R (sequencing)		AATTCCCGCGGGCCCTCTAGATC

Table S4. Primers used for Gateway cloning and Sanger sequencing

Factor	Plasmid Addgene #	Source Sequence	Inclusion Rationale
ASCL1	184382	ORF amplified from tetO-ALN (Addgene Plasmid #43918)	Used in direct reprogramming
AKT1	170682	ORF amplified from pWZL Neo Myr Flag AKT1 (Addgene Plasmid #20422)	Reported to enhance reprogramming of mouse fibroblasts to functional cardiomyocytes (Zhou/Olson 2015)
ESRRA	170683	ORF amplified from MGC Human ESRRA Sequence-Verified cDNA (Dharmacon CloneId:6527166)	Mogrify prediction (fibroblast to adult heart) Mogrify prediction (cardiac fibroblast to adult heart)
ESRRG	170684	ORF amplified from pMX-hESRRG (a gift from Deepak Srivastava)	Used in published cocktail combinations
ETS2	170685	ORF amplified from FLAG-Ets2 (Addgene Plasmid #28128)	Reported role in direct differentiation of human fibroblast to cardiac progenitors (Islas/Schwartz 2012)
FHL2	170686	ORF amplified from pcDNA3.1-C-(k)DYK-FHL2 (Genscript Clone ID #OHu26596)	Mogrify prediction (cardiac fibroblast to adult heart)
FOSL1	170687	ORF amplified from p6599 MSCV-IP N-HAonly FOSL1 (Addgene Plasmid #34897)	Mogrify prediction (whole blood to cardiac myocyte)
GATA4	46030	Plasmid was a gift from John Gearhart (Addgene Plasmid #46030)	Used in published cocktail combinations Mogrify prediction (fibroblast to cardiac myocyte) Mogrify prediction (fibroblast to adult heart)
GATA6	170688	ORF amplified from pEN_TT 3XFLAG-wtGATA6-3XAU1 (Addgene Plasmid #72612)	Mogrify prediction (fibroblast to cardiac myocyte) Mogrify prediction (cardiac fibroblast to cardiac myocyte)
HAND1	170689	ORF amplified from MGC Human HAND1 Sequence-Verified cDNA (Dharmacon CloneId:3162118)	Mogrify prediction (fibroblast to adult heart) Mogrify prediction (cardiac fibroblast to adult heart)
HAND2	170690	ORF amplified from MGC Fully Sequenced Human HAND2 cDNA Dharmacon #40003700	Used in published cocktail combinations
HEY2	170691	ORF amplified from MGC Human HEY2 Sequence-Verified cDNA (Dharmacon CloneId:3945225)	Mogrify prediction (fibroblast to adult heart)
ID1	170692	ORF amplified from pcDNA3 hId1 (Addgene Plasmid #16061)	Mogrify prediction (fibroblast to cardiac myocyte) Mogrify prediction (cardiac fibroblast to cardiac myocyte)
IRX5	170693	ORF amplified from pcDNA3.1-C-(k)DYK-IRX5 (Genscript Clone ID #OHu18967)	Mogrify prediction (fibroblast to adult heart) Mogrify prediction (cardiac fibroblast to adult heart)
MEF2C	46031	Plasmid was a gift from John Gearhart (Addgene Plasmid #46031)	used in published cocktail combinations Mogrify prediction (fibroblast to adult heart) Mogrify prediction (cardiac fibroblast to adult heart)
MESP1	170694	ORF amplified from pSAM2_mCherry_Mesp1 (Addgene #72687)	Used in published cocktail combinations
MITF	170695	ORF amplified from pEGFP-N1-MITF-A (Addgene Plasmid #38132)	Mogrify prediction (cardiac fibroblast to adult heart)
MYOCD	170696	ORF amplified from MGC Human *MYOCD* sequence-verified cDNA (Dharmacon, clone ID 9051897)	Used in published cocktail combinations
NFYB	170697	ORF amplified from MGC Human *NFYB* sequence-verified cDNA (Dharmacon, clone ID 3680937)	Mogrify prediction (fibroblast to cardiac myocyte) Mogrify prediction (cardiac fibroblast to cardiac myocyte)
NKX2-5	46029	Plasmid was a gift from John Gearhart (Addgene Plasmid #46029)	used in published cocktail combinations Mogrify prediction (fibroblast to adult heart) Mogrify prediction (cardiac fibroblast to adult heart)
NR2F1	170698	ORF amplified from MGC Human *NR2F1* sequence-verified cDNA (Dharmacon, clone ID 3912075)	Mogrify prediction (fibroblast to cardiac myocyte) Mogrify prediction (cardiac fibroblast to cardiac myocyte)
PBX1	170699	ORF amplified from PBX1A-pCMV1 (Addgene #21029)	Mogrify prediction (fibroblast to cardiac myocyte) Mogrify prediction (cardiac fibroblast to cardiac myocyte)
SMAD6	170700	ORF amplified from CS2 Smad6 (Addgene #14960)	Mogrify prediction (fibroblast to cardiac myocyte) Mogrify prediction (cardiac fibroblast to cardiac myocyte)
SMARCD3	170701	ORF amplified from pBS-hBAF60c (Addgene #21036)	Reported to enhance reprogramming of mouse fibroblasts to functional cardiomyocytes (Christoforou/Leong 2013)
SNAI2	170702	ORF amplified from MGC Human *SNAI2* Sequence-Verified cDNA (Dharmacon, clone ID 3908245)	Mogrifry prediction (PBMC to adult heart)
SOX9	170703	ORF amplified from MGC Human *SOX9* Sequence-Verified cDNA (Dharmacon, clone ID 6200521)	Mogrify prediction (cardiac fibroblast to cardiac myocyte)
SRF	170704	ORF amplified from pEntr-SRFflbio (Addgene #32971)	Reported to enhance reprogramming of mouse fibroblasts to functional cardiomyocytes (Christoforou/Leong 2013)
TBX20	170705	ORF amplified from pcDNA3.1-C-(k)DYK-TBX20 (Genscript Clone ID #OHu10999)	Mogrify prediction (fibroblast to cardiac myocyte)
TBX5	46032	Plasmid was a gift from John Gearhart (Addgene Plasmid #46032)	used in published cocktail combinations Mogrify prediction (fibroblast to adult heart) Mogrify prediction (cardiac fibroblast to adult heart)
TCF21	170706	ORF amplified from pcDNA3.1-C-(k)DYK-TCF21 (Genscript Clone ID #OHu24607)	Mogrify prediction (cardiac fibroblast to cardiac myocyte)
ZFPM2	170707	ORF amplified from pMX-hZFPM2, a gift from Deepak Srivastava	Used in published cocktail combinations
ZNF281	170708	ORF amplified from MGC Human ZNF281 Sequence-Verified cDNA (Dharmacon CloneId:6527166)	Reported to stimulate cardiac reprogramming of adult mouse fibroblasts (Zhou/Olson 2017)

Table S5. Addgene deposit details of candidate factors for reprogramming

Factor	RT-qPCR Taqman Primer
18S	Hs99999901_s1
MYH6	Hs00411908_m1
TNNT2	Hs00943911_m1
MYL7	Hs01085598_g1
SCN5A	Hs00165693_m1
ATP2A2	Hs00544877_m1
RYR2	Hs00181461_m1
FN1	Hs01549976_m1
POSTN	Hs01566750_m1
COL1A1	Hs00164004_m1
COL1A2	Hs01028956_m1

Table S6. RT-qPCR primers

TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells
ESRRG, MYOCD,ASCL1	32.65679682	*PBX1, SMARCD3,MYOCD*	15.83912715	*HAND1, SMARCD3,MYOCD*	11.11334675
FHL2, NFYB,MYOCD	30.57194663	*SRF, TBX20,MYOCD*	15.58681962	*AKT1, ID1,MYOCD*	10.94331936
FHL2, TBX20,MYOCD	29.37566785	*PBX1, SNAI2,MYOCD*	15.52639092	*ID1, NFYB,MYOCD*	10.89369407
FHL2, MYOCD,ASCL1	28.92895228	*ESRRA, PBX1,MYOCD*	15.47224341	*SMAD6, MYOCD,ASCL1*	10.83207379
HAND1, PBX1,MYOCD	28.8180186	*AKT1, MITF,MYOCD*	15.45882787	*NFYB, TBX5,MYOCD*	10.73413166
AKT1, TBX20,MYOCD	28.06204955	*ID1, SRF,MYOCD*	15.2567319	*HAND2, MEF2C,SRF*	10.6478074
ETS2, FHL2,SMAD6	27.31776352	*MEF2C, MESP1,MYOCD*	15.25149341	*GATA6, SOX9,MYOCD*	10.51163536
HAND1, SMAD6,MYOCD	27.27764741	*IRX5, NKX2.5,SOX9*	15.1935109	*AKT1, HAND1,SNAI2*	10.46901173
GATA6, MYOCD,ASCL1	27.21115227	*NFYB, PBX1,MYOCD*	14.91346352	*IRX5, MITF,TBX5*	10.42460317
PBX1, SMAD6,MYOCD	26.50317144	*AKT1, TBX5,MYOCD*	14.88025514	*HEY2, MESP1,TBX5*	10.41666667
ID1, SMAD6,MYOCD	26.47377256	*GATA4, ZFPM2,MYOCD*	14.53790985	*NKX2.5, PBX1,MYOCD*	10.39558094
SMAD6, SNAI2,MYOCD	25.82412461	*ID1, PBX1,MYOCD*	14.42481071	*FHL2, HAND1,MYOCD*	10.33177612
SMAD6, SMARCD3,MYOCD	25.30253183	*MESP1, PBX1,MYOCD*	14.33059965	*FOSL1, NFYB,SMARCD3*	10.24605601
ESRRA, MESP1,MYOCD	25.1166381	*HEY2, SMAD6,MYOCD*	14.23892657	*HAND2, MITF,SMARCD3*	10.22218932
AKT1, NFYB,MYOCD	24.97332475	*HAND1, ID1,MYOCD*	14.21236621	*MEF2C, NKX2.5,MYOCD*	10.20051432
ESRRG, MITF,MYOCD	24.05143404	*FHL2, ID1,MYOCD*	14.21206084	*HAND2, SMARCD3,MYOCD*	10.17743854
FOSL1, PBX1,MYOCD	23.88103461	*ESRRG, PBX1,MYOCD*	14.14402992	*FHL2, SNAI2,SRF*	10.10264235
ID1, TBX5,MYOCD	23.12159195	*HAND2, MESP1,TBX5*	14.01859306	*NFYB, SMAD6,MYOCD*	9.863475357
HEY2, MESP1,ZNF281	22.96693476	*HAND1, SNAI2,MYOCD*	13.97359418	*GATA4, GATA6,MYOCD*	9.858082418
FHL2, PBX1,MYOCD	21.52331732	*FOSL1, IRX5,NKX2.5*	13.9013418	*HEY2, SNAI2,MYOCD*	9.778496521
AKT1, ESRRG,MYOCD	21.19013247	*FHL2, SMARCD3,MYOCD*	13.88432885	*AKT1, SNAI2,MYOCD*	9.635462574
PBX1, TBX20,MYOCD	21.1443243	*MESP1, TBX5,MYOCD*	13.73938197	*ESRRA, MEF2C,ASCL1*	9.604005954
HAND2, TBX20,MYOCD	21.11641319	*ESRRA, HAND1,MYOCD*	13.48480504	*HEY2, ID1,ASCL1*	9.530855106
FOSL1, TBX20,MYOCD	21.09902528	*GATA6, MEF2C,NR2F1*	13.37235229	*ID1, SMARCD3,MYOCD*	9.527799559
FHL2, MESP1,MYOCD	21.04910878	*ESRRA, MEF2C,MYOCD*	13.33901075	*ZFPM2, MYOCD,ASCL1*	9.496443886
GATA6, TBX20,MYOCD	21.01376197	*ID1, IRX5,ASCL1*	13.19998848	*FHL2, GATA4,SMARCD3*	9.467120181
AKT1, MYOCD,ASCL1	20.81173594	*IRX5, TBX5,MYOCD*	13.14427853	*IRX5, TBX20,MYOCD*	9.421196619
SMAD6, TBX20,MYOCD	20.3114088	*ESRRG, MEF2C,MYOCD*	13.12322027	*GATA6, SRF,ASCL1*	9.345794393
SMARCD3, SNAI2,MYOCD	20.24840164	*MEF2C, NFYB,ASCL1*	13.10487695	*FOSL1, MITF,MYOCD*	9.343104918
ID1, TBX20,MYOCD	19.82178763	*FHL2, SRF,MYOCD*	13.03059869	*FHL2, MEF2C,MYOCD*	9.27685623
SNAI2, ZFPM2,MYOCD	19.62052847	*ID1, MESP1,MYOCD*	12.97336487	*ID1, NKX2.5,MYOCD*	9.239558157
ESRRG, SNAI2,MYOCD	18.31120885	*GATA4, PBX1,MYOCD*	12.92076286	*AKT1, SRF,MYOCD*	9.205790572
MESP1, SMAD6,MYOCD	18.22102504	*HAND1, TBX20,MYOCD*	12.83075616	*IRX5, MITF,MYOCD*	9.188523894
AKT1, FOSL1,MYOCD	18.19186831	*FHL2, TBX5,MYOCD*	12.82927125	*MEF2C, NR2F1,ASCL1*	9.065968391
ESRRG, SMAD6,MYOCD	18.15999791	*NFYB, TBX20,MYOCD*	12.80793353	*ESRRA, SOX9,MYOCD*	9.060869072
HAND1, IRX5,MITF	18.15401163	*MESP1, TBX20,MYOCD*	12.69417406	*IRX5, MITF,SMAD6*	8.9376176
SMAD6, TBX5,MYOCD	17.73227255	*GATA4, TBX5,MYOCD*	12.47080817	*ID1, MEF2C,MYOCD*	8.762344636
FHL2, SMAD6,MYOCD	17.64523538	*MEF2C, MESP1,SRF*	12.44871387	*HAND1, IRX5,MYOCD*	8.757724446
GATA6, SMARCD3,MYOCD	17.40340657	*ETS2, ZFPM2,ZNF281*	12.44763615	*MESP1, SMARCD3,MYOCD*	8.698411718
IRX5, MITF,SMARCD3	17.33207103	*PBX1, TBX5,MYOCD*	12.36177611	*MEF2C, NFYB,MYOCD*	8.632488141
AKT1, PBX1,MYOCD	17.13085278	*HEY2, SMARCD3,MYOCD*	12.18676311	*MESP1, SNAI2,MYOCD*	8.62092715
FOSL1, SNAI2,MYOCD	17.07521691	*HAND2, IRX5,ASCL1*	12.09338577	*ESRRG, NFYB,MYOCD*	8.518932147
FOSL1, MYOCD,ASCL1	17.05385177	*HEY2, TBX20,MYOCD*	11.92818062	*IRX5, PBX1,ASCL1*	8.509907672
ESRRG, MESP1,MYOCD	16.75698356	*FOSL1, MEF2C,SRF*	11.90905572	*IRX5, MESP1,MYOCD*	8.463941261
FOSL1, SMAD6,MYOCD	16.69322925	*IRX5, NR2F1,SNAI2*	11.89669168	*IRX5, SMARCD3,MYOCD*	8.378979819
FOSL1, GATA6,MYOCD	16.6388835	*ESRRG, SMARCD3,MYOCD*	11.68034929	*ESRRA, NR2F1,TBX5*	8.373478373
NFYB, NR2F1,SOX9	16.63638405	*IRX5, SMAD6,MYOCD*	11.33049645	*AKT1, FHL2,MYOCD*	8.353924711
SMARCD3, TBX20,MYOCD	16.5160179	*HEY2, PBX1,MYOCD*	11.32823291	*SMARCD3, TBX5,MYOCD*	8.33442337
IRX5, SNAI2,MYOCD	16.35825073	*NFYB, SNAI2,MYOCD*	11.31750803	*GATA4, PBX1,SRF*	8.312735926
ESRRA, MYOCD,ASCL1	16.2563617	*HAND2, MEF2C,SMAD6*	11.29568106	*FHL2, GATA6,MYOCD*	8.281129384
HAND1, TBX5,MYOCD	16.18496688	*HAND2, TBX5,MYOCD*	11.22600633	*MITF, SMARCD3,TBX5*	8.26873385
FOSL1, TBX5,MYOCD	16.03670269	*SNAI2, TBX5,MYOCD*	11.2206549	*HAND1, ID1,SMAD6*	8.220968406
FHL2, SNAI2,MYOCD	16.00392343	*SNAI2, TBX20,MYOCD*	11.16936178	*FHL2, SMAD6,SRF*	8.192463399
ESRRG, TBX20,MYOCD	15.89660316	*GATA4, SRF,MYOCD*	11.1142854	*IRX5, MEF2C,MYOCD*	8.155298655

Table S7. List of all 4,960 combinations tested and their respective reprogramming efficiency (MYH6^{+}TNNT2^{+})

TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells
GATA4, HAND1,MEF2C	8.145014023	*SOX9, TBX5,MYOCD*	6.843188972	*FOSL1, MEF2C,TBX20*	5.95699812
FHL2, HEY2,MYOCD	8.096382803	*MEF2C, MESP1,SMARCD3*	6.84073716	*TBX20, MYOCD,ASCL1*	5.954215276
NFYB, ZNF281,MYOCD	8.088313871	*ESRRA, IRX5,MYOCD*	6.830601497	*FOSL1, SRF,MYOCD*	5.941328251
HAND2, MEF2C,NFYB	8.085393719	*GATA4, MESP1,SNAI2*	6.799553797	*GATA4, SMARCD3,MYOCD*	5.925870585
GATA4, GATA6,SMAD6	8.048720542	*FHL2, NFYB,SRF*	6.788911475	*TBX20, TBX5,MYOCD*	5.88905844
GATA4, MYOCD,ASCL1	8.047981737	*AKT1, SMAD6,MYOCD*	6.78564355	*HAND1, NFYB,MYOCD*	5.882814935
GATA4, HAND1,SRF	8.047115976	*HAND2, ID1,MYOCD*	6.751936667	*ESRRG, FOSL1,NR2F1*	5.8729483
ESRRG, FOSL1,NKX2.5	8.045825523	*FOSL1, IRX5,SRF*	6.737372903	*GATA4, NKX2.5,SRF*	5.857105966
NR2F1, SRF,ASCL1	8.019525802	*ESRRA, NFYB,MYOCD*	6.734274438	*GATA6, MEF2C,SMARCD3*	5.844492295
GATA6, PBX1,MYOCD	7.989543894	*FHL2, NKX2.5,SMAD6*	6.731246895	*HEY2, SNAI2,TCF21*	5.82010582
FOSL1, HAND2,MYOCD	7.954934021	*ESRRA, MITF,MYOCD*	6.717409549	*ESRRA, HAND1,MESP1*	5.817324828
IRX5, SRF,MYOCD	7.953006114	*GATA6, NKX2.5,SNAI2*	6.711528488	*FOSL1, SMAD6,SRF*	5.758179151
ESRRG, TBX5,MYOCD	7.936815496	*AKT1, SMARCD3,MYOCD*	6.710582481	*FOSL1, HEY2,SMARCD3*	5.756358768
FHL2, NKX2.5,TCF21	7.890499195	*HAND1, IRX5,SMAD6*	6.69123926	*IRX5, NFYB,MYOCD*	5.742964089
ID1, SNAI2,MYOCD	7.870472071	*NKX2.5, TBX20,MYOCD*	6.687601681	*GATA4, SRF,TBX5*	5.741690528
HAND1, NKX2.5,SMARCD3	7.85849708	*GATA6, SNAI2,MYOCD*	6.64218126	*AKT1, GATA6,PBX1*	5.737112135
NFYB, SMARCD3,MYOCD	7.78540136	*GATA6, SRF,MYOCD*	6.636835901	*GATA4, HEY2,MYOCD*	5.735626197
FHL2, MEF2C,SMAD6	7.768346028	*FOSL1, NFYB,MYOCD*	6.619657898	*GATA6, ID1,MYOCD*	5.71332414
GATA4, HAND2,SRF	7.767379797	*HAND1, NFYB,SMAD6*	6.549903847	*GATA4, ID1,SMAD6*	5.685666259
HAND2, IRX5,MYOCD	7.763016371	*ESRRG, ETS2,NKX2.5*	6.528032134	*ESRRG, GATA6,HAND1*	5.682315507
FHL2, HAND2,MYOCD	7.735033677	*PBX1, SRF,MYOCD*	6.512775903	*ETS2, IRX5,NR2F1*	5.663850985
ID1, MITF,SRF	7.723577236	*MEF2C, MESP1,ASCL1*	6.484396141	*IRX5, SNAI2,SRF*	5.661881528
GATA4, NFYB,MYOCD	7.679146559	*AKT1, MESP1,MYOCD*	6.46313198	*ESRRG, FHL2,TBX20*	5.638043723
AKT1, GATA4,SMARCD3	7.656145174	*GATA4, GATA6,ASCL1*	6.458797327	*HAND1, ID1,NKX2.5*	5.633347969
HAND1, IRX5,SNAI2	7.649681529	*GATA4, HEY2,SRF*	6.435915548	*HAND1, NFYB,NKX2.5*	5.627183439
HEY2, TBX5,MYOCD	7.629035455	*GATA6, SMARCD3,SRF*	6.422939068	*MITF, SMAD6,MYOCD*	5.610967648
FHL2, GATA6,MEF2C	7.62610268	*GATA4, SMAD6,TBX5*	6.414124909	*GATA4, HAND1,TBX5*	5.604644397
ETS2, MESP1,TBX5	7.623643066	*GATA4, MEF2C,NKX2.5*	6.362563365	*GATA4, SNAI2,MYOCD*	5.58801827
FHL2, MITF,MYOCD	7.509577362	*MEF2C, PBX1,ASCL1*	6.338004009	*GATA6, TBX5,MYOCD*	5.586912789
HEY2, NFYB,MYOCD	7.44967198	*GATA4, NFYB,NR2F1*	6.336645873	*FOSL1, SMAD6,TCF21*	5.577172503
MEF2C, NFYB,SMAD6	7.414806521	*HAND1, NFYB,SMARCD3*	6.336233962	*MEF2C, MESP1,NFYB*	5.556892007
GATA4, SMAD6,MYOCD	7.40361198	*ESRRG, HAND1,SMARCD3*	6.322769789	*SMAD6, TBX5,ASCL1*	5.555555556
GATA6, HAND2,ZFPM2	7.369606249	*HAND1, MEF2C,MYOCD*	6.283091496	*HAND1, SOX9,SRF*	5.554507338
ESRRG, SMAD6,ZNF281	7.333333333	*ESRRG, ETS2,FHL2*	6.280281447	*HAND1, HAND2,MYOCD*	5.549465025
SNAI2, SRF,MYOCD	7.304544483	*GATA4, GATA6,MEF2C*	6.251382641	*FHL2, IRX5,MYOCD*	5.543633493
HEY2, MITF,SRF	7.287705957	*HAND1, SMAD6,ASCL1*	6.241293254	*GATA4, MESP1,TBX5*	5.527606721
HAND1, HAND2,MEF2C	7.22765022	*TBX20, ZNF281,MYOCD*	6.225840271	*AKT1, GATA6,MYOCD*	5.508072175
PBX1, SMARCD3,SRF	7.221351542	*ESRRG, MEF2C,MESP1*	6.179971678	*MEF2C, MESP1,PBX1*	5.499573453
ESRRG, FOSL1,MYOCD	7.185609267	*MESP1, SRF,MYOCD*	6.172215696	*ESRRG, HEY2,MYOCD*	5.482848683
MESP1, NFYB,SOX9	7.125205042	*AKT1, ESRRG,SRF*	6.156114465	*ETS2, GATA6,MYOCD*	5.46478611
MITF, MYOCD,ASCL1	7.089808551	*GATA4, MEF2C,SRF*	6.134655552	*HAND1, MEF2C,TBX20*	5.454545455
FHL2, MEF2C,SRF	7.059260278	*MEF2C, MITF,NKX2.5*	6.129764375	*GATA6, MEF2C,PBX1*	5.43071161
ESRRA, FHL2,MESP1	7.052252092	*MEF2C, SMARCD3,SRF*	6.125515387	*MEF2C, NKX2.5,TCF21*	5.338541667
ESRRG, HAND2,MYOCD	7.049427504	*GATA4, MESP1,SRF*	6.087671053	*MEF2C, TBX5,MYOCD*	5.32880728
AKT1, FOSL1,SRF	7.047392094	*GATA4, MEF2C,MESP1*	6.08680056	*MESP1, SOX9,ASCL1*	5.311653117
ESRRG, IRX5,SMARCD3	7.008290769	*ESRRA, ESRRG,ID1*	6.076388889	*FOSL1, ID1,SRF*	5.300584809
HAND2, SOX9,ASCL1	7.001103306	*MEF2C, TBX20,TBX5*	6.070624686	*ESRRG, ID1,MYOCD*	5.300209003
ID1, MITF,TBX5	6.986899563	*GATA4, SMAD6,SRF*	6.038475125	*GATA4, ID1,SMARCD3*	5.282007789
FHL2, FOSL1,MYOCD	6.967461532	*ESRRG, FHL2,MYOCD*	6.008964694	*ETS2, IRX5,ZNF281*	5.277279676
MEF2C, NFYB,SMARCD3	6.938169257	*FHL2, GATA4,MYOCD*	6.007626386	*GATA4, HEY2,MEF2C*	5.27231908
HAND2, MEF2C,MYOCD	6.932874636	*GATA4, GATA6,TBX5*	6.002550035	*ESRRA, SMAD6,MYOCD*	5.268170531
HEY2, IRX5,MYOCD	6.895638675	*HEY2, SOX9,MYOCD*	5.98756411	*MESP1, TBX5,TCF21*	5.236486486
GATA6, SOX9,TBX5	6.882673291	*NKX2.5, SMAD6,MYOCD*	5.987554747	*GATA4, TBX20,MYOCD*	5.236167023
ESRRA, ETS2,ID1	6.870229008	*GATA6, HAND2,NR2F1*	5.964912281	*FHL2, MESP1,SMAD6*	5.235228102

Table S7. List of all 4,960 combinations tested and their respective reprogramming efficiency (MYH6$^+$TNNT2$^+$)

TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells
AKT1, ESRRA,NR2F1	5.21188194	AKT1, GATA4,MYOCD	4.607616017	HEY2, MESP1,SRF	4.140990148
GATA4, HAND2,SMAD6	5.204167863	GATA6, MEF2C,TBX20	4.604331457	MITF, ZFPM2,MYOCD	4.132408819
FHL2, PBX1,SMAD6	5.192370682	FOSL1, SMAD6,TBX20	4.600900189	GATA6, MEF2C,NFYB	4.125410846
ESRRG, SRF,MYOCD	5.183829374	GATA6, SMAD6,SMARCD3	4.560442873	PBX1, MYOCD,ASCL1	4.111420557
ESRRA, ID1,MYOCD	5.178902627	FHL2, MESP1,SNAI2	4.54865797	TBX5, ZNF281,MYOCD	4.089376536
MEF2C, NR2F1,TBX20	5.172002251	ETS2, TBX5,ZFPM2	4.545454545	ESRRG, GATA4,MYOCD	4.080912939
ESRRA, HEY2,MEF2C	5.162945242	AKT1, ESRRA,MYOCD	4.54108891	HAND1, MEF2C,SMARCD3	4.054272094
ESRRG, GATA6,MYOCD	5.157291265	HAND1, IRX5,SMARCD3	4.539554423	SMARCD3, SRF,MYOCD	4.041922284
FHL2, NKX2.5,SRF	5.155275693	ID1, IRX5,MYOCD	4.537193774	NFYB, SMAD6,TCF21	4.035874439
ETS2, NKX2.5,PBX1	5.144032922	AKT1, PBX1,SMAD6	4.520547945	MEF2C, SMAD6,MYOCD	4.028334075
NKX2.5, SMARCD3,MYOCD	5.136128192	GATA4, MEF2C,SMAD6	4.518523458	ETS2, FHL2,ID1	4.022928147
MEF2C, MESP1,TBX20	5.108225108	NR2F1, SRF,TBX20	4.506882395	FOSL1, MEF2C,TBX5	4.011396011
IRX5, MEF2C,TBX20	5.08928782	ETS2, FHL2,MEF2C	4.494954761	FOSL1, HEY2,SOX9	4
IRX5, SNAI2,ZFPM2	5.069708492	MEF2C, NR2F1,SMARCD3	4.464825357	AKT1, MEF2C,MESP1	3.991028432
MEF2C, SOX9,TBX5	5.063841064	MEF2C, ZFPM2,MYOCD	4.461538462	GATA4, SNAI2,TBX20	3.977857692
MEF2C, MESP1,SOX9	5.063637815	MEF2C, SMAD6,SMARCD3	4.460523989	FOSL1, PBX1,TBX5	3.977668192
ETS2, NR2F1,SOX9	5.062118744	GATA4, SMAD6,SMARCD3	4.447493005	ESRRA, MESP1,SRF	3.958089078
HAND1, IRX5,SOX9	5.054464565	ETS2, IRX5,ASCL1	4.433309185	ESRRA, GATA4,SRF	3.957087682
FHL2, MESP1,NFYB	5.050860023	NFYB, SRF,ASCL1	4.393356334	FOSL1, ID1,MYOCD	3.951965229
AKT1, FHL2,GATA6	5.026461402	HAND2, ZFPM2,MYOCD	4.39305338	GATA6, NFYB,MYOCD	3.942564027
HAND2, ID1,SRF	5.024347036	MESP1, NR2F1,PBX1	4.38247012	HAND1, MYOCD,ASCL1	3.939010878
HAND1, HEY2,MEF2C	5.015349551	FHL2, MEF2C,SNAI2	4.381534645	GATA6, NR2F1,PBX1	3.931704261
HAND2, HEY2,ASCL1	5.010224949	MEF2C, MESP1,SMAD6	4.375298977	ESRRG, ZFPM2,MYOCD	3.930987857
MESP1, NFYB,MYOCD	4.973523027	SMAD6, TBX5,TCF21	4.373177843	GATA6, SMAD6,TBX5	3.930806036
GATA6, TBX20,TBX5	4.954229952	HAND2, MITF,SOX9	4.369538077	HAND1, MEF2C,NFYB	3.93062878
NKX2.5, SRF,MYOCD	4.925406955	HAND2, NR2F1,ZFPM2	4.366347178	HAND1, MESP1,ZFPM2	3.923853924
HAND1, MITF,TBX20	4.892473118	GATA4, NR2F1,TBX5	4.366002215	AKT1, GATA6,HAND1	3.911977687
FOSL1, SMARCD3,MYOCD	4.873889918	IRX5, MITF,PBX1	4.355177239	ESRRA, ETS2,IRX5	3.904067892
AKT1, MEF2C,SMARCD3	4.873840743	GATA4, TBX20,TBX5	4.338070391	FHL2, GATA6,SRF	3.902553272
GATA6, MEF2C,SMAD6	4.864810552	HAND1, NKX2.5,TBX5	4.325133224	GATA4, MEF2C,ASCL1	3.902141892
NFYB, MYOCD,ASCL1	4.864003921	GATA4, MEF2C,NFYB	4.31915341	FOSL1, MESP1,MITF	3.900730122
FHL2, MESP1,SRF	4.861976948	MESP1, NKX2.5,MYOCD	4.317334326	ESRRA, NR2F1,SMAD6	3.899484503
SMAD6, SRF,TBX20	4.821597736	MEF2C, MITF,SRF	4.301075269	ESRRA, SRF,TBX5	3.899285413
ID1, SOX9,MYOCD	4.814897691	GATA6, ZFPM2,MYOCD	4.298152255	GATA4, MEF2C,SMARCD3	3.898710031
NKX2.5, NR2F1,MYOCD	4.81092437	ESRRA, NKX2.5,MYOCD	4.283454657	HAND1, ID1,SRF	3.897090486
TBX5, MYOCD,ASCL1	4.809203143	PBX1, SNAI2,SRF	4.269266358	MEF2C, NKX2.5,TBX5	3.89524411
NKX2.5, SNAI2,MYOCD	4.782779998	GATA6, HAND1,MEF2C	4.264566225	FHL2, MESP1,PBX1	3.893153195
ESRRA, MEF2C,MESP1	4.769355164	PBX1, SMAD6,SRF	4.260346657	HAND2, SNAI2,MYOCD	3.883346075
IRX5, NR2F1,MYOCD	4.764038232	SNAI2, MYOCD,ASCL1	4.250323106	ESRRG, ETS2,MEF2C	3.879075291
SMAD6, ZFPM2,MYOCD	4.728766362	FOSL1, GATA6,ZFPM2	4.247226423	HAND1, SOX9,ZNF281	3.873239437
HAND1, SMAD6,TBX20	4.725223813	SMAD6, SRF,MYOCD	4.245015599	SRF, TBX5,MYOCD	3.873023672
AKT1, GATA4,SMAD6	4.708348024	MITF, SRF,TBX20	4.241406292	MEF2C, MITF,MYOCD	3.861442362
MEF2C, SMARCD3,MYOCD	4.70192665	HAND1, ID1,NFYB	4.239117129	GATA4, MEF2C,PBX1	3.84084621
MEF2C, SMARCD3,TBX20	4.699005213	ESRRA, SMARCD3,MYOCD	4.229669885	MEF2C, NR2F1,TBX5	3.832384689
FHL2, SMARCD3,SRF	4.682574075	HAND1, MEF2C,SOX9	4.229302832	AKT1, MEF2C,SMAD6	3.832167425
FOSL1, MEF2C,MYOCD	4.674418447	ESRRA, GATA6,IRX5	4.225142153	MESP1, SMAD6,SRF	3.827838828
FHL2, NFYB,SMAD6	4.66775009	NFYB, NR2F1,ASCL1	4.211277659	HAND2, SRF,MYOCD	3.813772323
ESRRA, MESP1,TBX20	4.653851385	HAND1, HEY2,NR2F1	4.210324523	FHL2, FOSL1,SRF	3.804378823
GATA6, SRF,TBX20	4.641830184	MEF2C, NKX2.5,SMARCD3	4.203748981	FOSL1, SNAI2,SOX9	3.797814743
FHL2, SNAI2,ZNF281	4.636768768	HAND1, IRX5,NFYB	4.182403346	GATA6, PBX1,SMAD6	3.791943036
GATA4, MEF2C,TBX20	4.635449867	MEF2C, SMARCD3,SOX9	4.175948021	FHL2, ZFPM2,ZNF281	3.787878788
ESRRG, IRX5,TCF21	4.634581105	FOSL1, SNAI2,SRF	4.163238077	MESP1, MITF,NKX2.5	3.776352506
FHL2, MESP1,SMARCD3	4.627426946	FOSL1, SMAD6,TBX5	4.156085394	GATA4, MITF,MYOCD	3.754404988
GATA4, IRX5,MYOCD	4.622476422	ESRRA, TBX20,MYOCD	4.150667377	SOX9, TBX5,ASCL1	3.738294888

Table S7. List of all 4,960 combinations tested and their respective reprogramming efficiency (MYH6^{+}TNNT2^{+})

TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells
FOSL1, MESP1,PBX1	3.733833959	*ESRRA, FHL2,SMARCD3*	3.446883804	*AKT1, NKX2.5,PBX1*	3.204563981
GATA6, SRF,ZFPM2	3.728070175	*FHL2, MESP1,TBX20*	3.442740602	*AKT1, ETS2,GATA6*	3.191214202
FHL2, PBX1,ZNF281	3.724928367	*FHL2, MEF2C,TBX20*	3.439261081	*AKT1, ETS2,HEY2*	3.185874535
GATA4, HAND1,PBX1	3.717053645	*ESRRA, FHL2,GATA4*	3.438006924	*PBX1, TBX20,TBX5*	3.184264589
AKT1, ETS2,PBX1	3.712127014	*FHL2, SRF,TBX5*	3.426173477	*GATA4, MESP1,NFYB*	3.183623806
FHL2, NKX2.5,MYOCD	3.71017871	*SMAD6, SMARCD3,SRF*	3.423357173	*MEF2C, SMAD6,SRF*	3.174782709
GATA4, MESP1,MYOCD	3.706701277	*AKT1, SMAD6,SRF*	3.411527575	*GATA6, ID1,MEF2C*	3.174603175
ESRRG, ETS2,HEY2	3.703942117	*ETS2, SNAI2,SOX9*	3.397745572	*AKT1, SMARCD3,SRF*	3.171312306
AKT1, HAND1,NFYB	3.70251695	*HEY2, SNAI2,SRF*	3.390957702	*ESRRA, MEF2C,SOX9*	3.165182987
GATA4, MEF2C,MYOCD	3.700589902	*AKT1, GATA4,SRF*	3.389830508	*IRX5, TBX5,ZNF281*	3.164556962
GATA6, ID1,SRF	3.698065747	*HAND1, IRX5,TCF21*	3.378378378	*ESRRA, HAND2,MYOCD*	3.16370179
IRX5, SOX9,MYOCD	3.69672844	*FHL2, IRX5,MEF2C*	3.370163649	*AKT1, MEF2C,MYOCD*	3.163211058
HAND2, IRX5,TBX5	3.696369597	*HEY2, ID1,NKX2.5*	3.363143631	*GATA6, MESP1,MYOCD*	3.157337186
MEF2C, MESP1,NKX2.5	3.693674828	*SMAD6, SRF,ASCL1*	3.36017094	*IRX5, MEF2C,PBX1*	3.152278495
HAND1, TBX20,ASCL1	3.691171499	*MESP1, SNAI2,SOX9*	3.35919278	*GATA4, HEY2,NKX2.5*	3.139971054
NFYB, NR2F1,TBX5	3.687315634	*HAND2, IRX5,NKX2.5*	3.359173127	*IRX5, SMARCD3,ZFPM2*	3.129313278
HEY2, NFYB,ZFPM2	3.685397523	*ESRRA, GATA4,NKX2.5*	3.358630251	*ESRRA, IRX5,SMAD6*	3.117535421
ESRRG, NFYB,ZFPM2	3.68161421	*FOSL1, GATA6,PBX1*	3.352677397	*HAND2, MITF,NR2F1*	3.112639849
SMAD6, SNAI2,SRF	3.681581096	*FHL2, PBX1,SRF*	3.344302574	*GATA4, HAND2,TBX20*	3.107047064
HAND2, NFYB,ZNF281	3.673469388	*FHL2, ZFPM2,MYOCD*	3.342468463	*GATA6, HAND1,MYOCD*	3.105735134
GATA4, SMAD6,SNAI2	3.661971582	*ETS2, HAND2,MESP1*	3.336353431	*ETS2, MESP1,SOX9*	3.104335474
HAND2, MITF,PBX1	3.657978967	*ESRRA, ESRRG,MYOCD*	3.336019071	*AKT1, IRX5,MEF2C*	3.095725828
AKT1, NFYB,NKX2.5	3.656882797	*GATA6, SNAI2,TBX5*	3.33563377	*TBX20, TCF21,ZFPM2*	3.094462541
HAND2, MESP1,MYOCD	3.655986253	*GATA6, HEY2,TBX5*	3.328035234	*GATA6, IRX5,SMARCD3*	3.091397849
GATA6, NR2F1,SMAD6	3.655844753	*GATA4, PBX1,SOX9*	3.323281946	*FOSL1, GATA4,SMAD6*	3.090470648
HAND1, IRX5,NKX2.5	3.650912728	*GATA4, HAND2,NKX2.5*	3.318912048	*ETS2, HAND2,NFYB*	3.090286884
FHL2, MEF2C,NKX2.5	3.645279653	*HAND1, NFYB,SNAI2*	3.316433595	*ESRRA, HEY2,NKX2.5*	3.089429811
HAND1, SRF,ASCL1	3.637741569	*GATA4, HAND1,SMAD6*	3.305899585	*FOSL1, GATA4,IRX5*	3.087084645
FHL2, SMAD6,SNAI2	3.63435465	*SMAD6, SOX9,SRF*	3.305808415	*MESP1, TCF21,MYOCD*	3.080725831
MEF2C, NKX2.5,TBX20	3.633665958	*AKT1, NFYB,SMAD6*	3.299422499	*GATA4, SMARCD3,SNAI2*	3.076538979
HAND2, PBX1,SRF	3.628117914	*HAND2, NR2F1,TBX5*	3.297291864	*ETS2, MEF2C,SRF*	3.075122366
ESRRA, MEF2C,SRF	3.627231722	*GATA4, HAND1,SNAI2*	3.293776472	*MEF2C, MESP1,SNAI2*	3.074164393
GATA4, MEF2C,ZFPM2	3.624425547	*GATA6, NFYB,TBX5*	3.286149562	*GATA4, GATA6,PBX1*	3.061695197
FHL2, MEF2C,PBX1	3.621810016	*AKT1, FHL2,MESP1*	3.281893004	*IRX5, MEF2C,TBX5*	3.060569492
FOSL1, MESP1,MYOCD	3.619760209	*HAND1, SNAI2,TBX5*	3.276823959	*GATA4, ID1,SRF*	3.059833378
FHL2, SMAD6,SMARCD3	3.619216935	*IRX5, NKX2.5,ASCL1*	3.275529865	*GATA4, SOX9,SRF*	3.044291056
GATA4, IRX5,SNAI2	3.617079408	*AKT1, HAND2,MYOCD*	3.27216335	*GATA4, SNAI2,SRF*	3.036542834
FHL2, HEY2,TCF21	3.616636528	*GATA4, HAND1,SMARCD3*	3.265850582	*HAND2, MYOCD,ASCL1*	3.036445329
HAND2, HEY2,SOX9	3.612479475	*GATA4, IRX5,SRF*	3.252075121	*FOSL1, NFYB,PBX1*	3.026586457
HAND2, HEY2,SNAI2	3.576537911	*HAND2, SMAD6,MYOCD*	3.250792012	*ESRRG, HAND1,MYOCD*	3.012978786
IRX5, NKX2.5,MYOCD	3.570563187	*HAND1, MESP1,MYOCD*	3.250246867	*FOSL1, NFYB,SRF*	3.007742086
HAND2, ZNF281,MYOCD	3.557682084	*ESRRA, SMAD6,SRF*	3.243040681	*AKT1, PBX1,SMARCD3*	3.004882109
ESRRA, TBX5,MYOCD	3.55431318	*ETS2, TBX20,MYOCD*	3.237071246	*IRX5, MEF2C,MESP1*	3.001112195
GATA6, HAND2,NKX2.5	3.53539224	*FHL2, GATA6,SMAD6*	3.231412899	*GATA6, PBX1,SRF*	2.998130342
AKT1, NKX2.5,SRF	3.532172784	*ESRRA, GATA6,MEF2C*	3.230148048	*ETS2, MEF2C,MYOCD*	2.993495566
HAND1, ID1,IRX5	3.527478932	*AKT1, FOSL1,SMAD6*	3.224455611	*NKX2.5, SRF,TBX20*	2.993159501
ESRRG, HAND1,NFYB	3.517630017	*MITF, PBX1,ZFPM2*	3.224155578	*GATA4, IRX5,MESP1*	2.98732302
GATA4, GATA6,SRF	3.511296158	*GATA4, SNAI2,TBX5*	3.218317855	*GATA4, HAND1,MYOCD*	2.98476366
FHL2, MEF2C,MESP1	3.487291604	*MESP1, NR2F1,ASCL1*	3.215434084	*SMAD6, TCF21,ASCL1*	2.983293556
GATA6, MITF,MYOCD	3.477302815	*GATA4, NR2F1,PBX1*	3.210717482	*HAND1, NKX2.5,MYOCD*	2.980774421
MESP1, MYOCD,ASCL1	3.469221095	*IRX5, MITF,NR2F1*	3.210089779	*HAND1, NKX2.5,PBX1*	2.978033721
HAND1, MEF2C,SRF	3.463456444	*ESRRA, IRX5,SRF*	3.209840339	*FOSL1, SRF,TBX5*	2.97339698
GATA4, IRX5,MEF2C	3.461106351	*IRX5, MITF,SRF*	3.209109731	*FHL2, SMAD6,TBX5*	2.972657592
HAND2, IRX5,NR2F1	3.454757993	*ESRRA, TBX5,TCF21*	3.20855615	*GATA4, ID1,MYOCD*	2.969644603

Table S7. List of all 4,960 combinations tested and their respective reprogramming efficiency (MYH6^{+}TNNT2^{+})

TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells
MEF2C, SNAI2,MYOCD	2.965026795	*SMARCD3, MYOCD,ASCL1*	2.825790664	*GATA4, GATA6,HEY2*	2.622898544
ESRRG, MITF,TCF21	2.962962963	*MITF, TBX5,MYOCD*	2.825353575	*GATA6, SMAD6,MYOCD*	2.621379612
HAND2, NKX2.5,SRF	2.961548256	*FHL2, SRF,TBX20*	2.819396051	*ESRRG, SOX9,ZNF281*	2.617857451
ETS2, HEY2,TCF21	2.959821197	*FHL2, GATA4,ASCL1*	2.817816899	*ETS2, FHL2,TBX20*	2.609890896
IRX5, MEF2C,NR2F1	2.957779174	*GATA4, SOX9,MYOCD*	2.812246379	*GATA4, NKX2.5,SMARCD3*	2.608653159
MEF2C, PBX1,SMARCD3	2.956167176	*MITF, SNAI2,MYOCD*	2.810723153	*MEF2C, NFYB,SRF*	2.605019242
GATA6, HAND2,TBX5	2.955748413	*FOSL1, PBX1,SNAI2*	2.80500963	*AKT1, MEF2C,ASCL1*	2.601809955
FHL2, SMARCD3,SNAI2	2.954194133	*SOX9, TBX20,MYOCD*	2.804691085	*AKT1, FHL2,IRX5*	2.597465887
FOSL1, MEF2C,PBX1	2.952487733	*IRX5, MESP1,SNAI2*	2.799465571	*HEY2, ZNF281,MYOCD*	2.597402597
GATA4, MESP1,TBX20	2.951003149	*FOSL1, SMAD6,SNAI2*	2.796847684	*ESRRA, SMAD6,TBX5*	2.592859998
MITF, SMARCD3,SRF	2.949608781	*HEY2, MEF2C,SNAI2*	2.791216276	*GATA6, MEF2C,SNAI2*	2.591760422
HAND1, MEF2C,PBX1	2.93969993	*FHL2, GATA6,HEY2*	2.787108589	*ESRRA, FOSL1,SMARCD3*	2.590839447
HAND1, MITF,SMAD6	2.939620319	*ESRRA, GATA4,SNAI2*	2.785831682	*ESRRG, FOSL1,MESP1*	2.589576001
FHL2, SMARCD3,TBX5	2.937191483	*GATA4, NFYB,TBX5*	2.779251295	*FHL2, ID1,SRF*	2.587429689
AKT1, IRX5,SNAI2	2.936597349	*AKT1, NKX2.5,MYOCD*	2.77611302	*NKX2.5, TBX5,ASCL1*	2.582159624
MESP1, TBX5,ASCL1	2.933832709	*HEY2, TCF21,MYOCD*	2.772092321	*MESP1, SOX9,MYOCD*	2.58077401
ETS2, MEF2C,SMAD6	2.932405518	*GATA4, HAND2,HEY2*	2.765763575	*GATA6, MITF,NKX2.5*	2.580645161
AKT1, SMARCD3,SNAI2	2.931737634	*ETS2, MESP1,SMARCD3*	2.765339368	*HAND1, ID1,MESP1*	2.579789219
HAND2, HEY2,SMARCD3	2.927927928	*NR2F1, TBX20,ASCL1*	2.762762763	*HAND2, MESP1,ASCL1*	2.579329419
NKX2.5, SMARCD3,SRF	2.91975719	*ESRRG, GATA6,NKX2.5*	2.761211323	*AKT1, ESRRA,HAND1*	2.578531886
FHL2, HAND1,TBX5	2.918569308	*SMARCD3, SRF,TBX20*	2.759800806	*SMARCD3, SRF,TCF21*	2.564102564
MEF2C, MYOCD,ASCL1	2.917974825	*MITF, TBX20,MYOCD*	2.757264089	*AKT1, IRX5,MYOCD*	2.562957178
IRX5, PBX1,MYOCD	2.917910769	*MITF, NFYB,SOX9*	2.75456515	*GATA6, IRX5,MESP1*	2.557892961
ETS2, PBX1,SMARCD3	2.915716375	*FHL2, ID1,TBX5*	2.754228464	*SRF, ZFPM2,ZNF281*	2.553978463
FHL2, NFYB,SMARCD3	2.912562508	*IRX5, SMAD6,SRF*	2.73634118	*ESRRA, IRX5,ZNF281*	2.553763441
AKT1, MEF2C,SRF	2.910937135	*FHL2, GATA6,TBX5*	2.720368165	*GATA4, NFYB,SMARCD3*	2.551522018
FHL2, FOSL1,SNAI2	2.910931984	*HAND2, SMAD6,TBX20*	2.717752266	*ESRRA, ESRRG,SMARCD3*	2.550724638
SMARCD3, TBX20,ZFPM2	2.910713431	*HAND2, MESP1,NKX2.5*	2.715918464	*HAND1, SOX9,ZFPM2*	2.547018639
NKX2.5, SNAI2,SRF	2.910160064	*GATA6, HAND2,MEF2C*	2.705615712	*ESRRG, GATA4,TBX5*	2.545003104
ETS2, FHL2,HAND1	2.905597292	*GATA4, HEY2,PBX1*	2.704025997	*MEF2C, NKX2.5,SRF*	2.534467494
MESP1, SMAD6,TBX20	2.905469118	*AKT1, ID1,NKX2.5*	2.702702703	*MESP1, NFYB,ZFPM2*	2.513464991
ETS2, TBX5,MYOCD	2.903441752	*GATA4, HAND2,MYOCD*	2.702354179	*GATA4, HAND2,SOX9*	2.513413179
ESRRA, SNAI2,SRF	2.901801609	*ETS2, IRX5,TBX20*	2.692852596	*HAND2, MITF,SMAD6*	2.512077295
AKT1, NFYB,PBX1	2.894980677	*AKT1, FHL2,GATA4*	2.689920393	*MITF, NKX2.5,PBX1*	2.511728551
HEY2, NKX2.5,MYOCD	2.894120362	*PBX1, SMAD6,SMARCD3*	2.689445599	*GATA6, ID1,SOX9*	2.507396821
ESRRG, IRX5,MYOCD	2.894080063	*AKT1, MESP1,NFYB*	2.689123031	*GATA4, HEY2,SMAD6*	2.488680924
MEF2C, NR2F1,TCF21	2.888814496	*AKT1, GATA4,TBX5*	2.686050466	*ETS2, NR2F1,TBX20*	2.48447205
ESRRA, NR2F1,ZNF281	2.888086643	*NFYB, NKX2.5,MYOCD*	2.682926829	*ETS2, SNAI2,MYOCD*	2.48227647
PBX1, SOX9,SRF	2.880589216	*ID1, MITF,SMARCD3*	2.682226338	*ESRRA, FOSL1,MYOCD*	2.480369678
GATA6, MEF2C,SRF	2.880572881	*MITF, TBX20,ZNF281*	2.680893985	*HAND2, SMARCD3,TBX5*	2.477596992
AKT1, HEY2,MEF2C	2.876962211	*SMARCD3, SRF,TBX5*	2.680876002	*NFYB, SMAD6,SRF*	2.472747389
ESRRG, FHL2,MEF2C	2.876431736	*MEF2C, MESP1,ZNF281*	2.67531169	*MESP1, MITF,MYOCD*	2.467398906
ESRRG, ETS2,IRX5	2.864601945	*GATA6, MESP1,PBX1*	2.673395647	*AKT1, ETS2,TCF21*	2.4600246
AKT1, SMAD6,ASCL1	2.861714034	*AKT1, ETS2,MESP1*	2.668808884	*ETS2, GATA4,SNAI2*	2.455431992
ETS2, FOSL1,TBX5	2.859576864	*ETS2, FHL2,IRX5*	2.667451362	*ETS2, FHL2,SNAI2*	2.454774667
AKT1, MESP1,SNAI2	2.849066617	*FHL2, NFYB,PBX1*	2.667428972	*AKT1, MESP1,PBX1*	2.452088806
FHL2, GATA4,SMAD6	2.84866862	*ESRRA, SNAI2,MYOCD*	2.667304445	*HAND1, PBX1,TBX20*	2.449277376
AKT1, HAND1,MYOCD	2.847663251	*MESP1, NKX2.5,NR2F1*	2.666666667	*FHL2, GATA6,PBX1*	2.448739719
MEF2C, SNAI2,SOX9	2.842313499	*GATA4, MESP1,NKX2.5*	2.653753027	*FHL2, GATA6,NR2F1*	2.444319284
FOSL1, GATA4,SNAI2	2.839521814	*GATA4, PBX1,SMAD6*	2.648026316	*GATA4, IRX5,PBX1*	2.443280977
NKX2.5, NR2F1,SMAD6	2.838127813	*GATA4, HAND2,SNAI2*	2.639234398	*ESRRA, FHL2,HAND1*	2.44027461
MEF2C, SMARCD3,SNAI2	2.837912207	*MEF2C, SMAD6,ZNF281*	2.632583257	*ESRRG, ETS2,MYOCD*	2.431119642
ETS2, SMAD6,MYOCD	2.830956353	*GATA4, SRF,TBX20*	2.629606729	*ETS2, PBX1,SMAD6*	2.429937878
HAND2, NFYB,MYOCD	2.830764167	*GATA4, NR2F1,TBX20*	2.626570196	*PBX1, SOX9,MYOCD*	2.429806119

Table S7. List of all 4,960 combinations tested and their respective reprogramming efficiency (MYH6^{+}TNNT2^{+})

TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells
GATA4, SMARCD3,TBX20	2.423130852	*FHL2, MITF,SOX9*	2.272727273	*FOSL1, SOX9,SRF*	2.159788293
FHL2, GATA4,SNAI2	2.420427947	*GATA6, MESP1,SRF*	2.265372168	*IRX5, MESP1,TBX5*	2.158565559
IRX5, NFYB,TBX5	2.418725948	*GATA4, MESP1,SOX9*	2.264141653	*ID1, MEF2C,PBX1*	2.157476555
HEY2, MESP1,MYOCD	2.41698866	*ID1, MITF,NFYB*	2.26022417	*FHL2, FOSL1,GATA6*	2.156831043
ID1, SMAD6,SRF	2.414346454	*IRX5, ZNF281,ASCL1*	2.259036145	*MITF, NKX2.5,MYOCD*	2.154391991
GATA4, HAND1,TBX20	2.406808877	*HAND1, MESP1,TBX5*	2.258281309	*NFYB, SMAD6,SNAI2*	2.15153052
FOSL1, NR2F1,ZNF281	2.405498282	*AKT1, ETS2,ID1*	2.257403056	*GATA6, ID1,ASCL1*	2.14814081
ETS2, SRF,TBX5	2.401070199	*ESRRA, GATA6,SOX9*	2.25047409	*IRX5, MITF,TBX20*	2.139406487
ID1, SRF,TBX20	2.388139777	*GATA4, NKX2.5,SMAD6*	2.249711306	*ETS2, NFYB,SMAD6*	2.138149014
AKT1, GATA6,TBX5	2.3872903	*HAND1, HEY2,MYOCD*	2.246758078	*GATA4, ID1,PBX1*	2.135733925
GATA4, IRX5,TBX5	2.386503343	*ID1, MEF2C,SMAD6*	2.243113696	*AKT1, FOSL1,NFYB*	2.130190021
AKT1, ID1,SRF	2.382996718	*HAND2, MESP1,NFYB*	2.237047792	*HEY2, MESP1,SMAD6*	2.127836121
NR2F1, TBX20,MYOCD	2.381048044	*ETS2, HEY2,ID1*	2.236099282	*GATA4, NFYB,SRF*	2.125435373
FOSL1, IRX5,ZNF281	2.380952381	*ESRRG, GATA6,HEY2*	2.233676976	*AKT1, GATA6,HEY2*	2.122681914
HAND1, NKX2.5,SMAD6	2.378510379	*NFYB, NKX2.5,SNAI2*	2.232664577	*ETS2, MESP1,SRF*	2.120427397
MEF2C, SMAD6,TBX20	2.376863647	*HAND2, MESP1,ZFPM2*	2.232439651	*PBX1, SMARCD3,TBX5*	2.116346176
MEF2C, NFYB,PBX1	2.376316909	*MESP1, ZFPM2,MYOCD*	2.221931256	*ESRRA, ESRRG,MESP1*	2.115755979
FHL2, SOX9,SRF	2.375737041	*AKT1, HEY2,TBX5*	2.219050785	*FHL2, MEF2C,TCF21*	2.113746959
FHL2, GATA6,SMARCD3	2.374366957	*NKX2.5, SMAD6,SRF*	2.217288039	*ESRRA, HAND2,MEF2C*	2.108979705
AKT1, HAND2,TBX20	2.372598162	*AKT1, SMAD6,SNAI2*	2.209994519	*GATA4, NR2F1,MYOCD*	2.107823604
GATA4, SMARCD3,TBX5	2.371645221	*FHL2, SMARCD3,TBX20*	2.207052347	*AKT1, SNAI2,SRF*	2.10290564
MEF2C, PBX1,SOX9	2.369289068	*GATA4, MESP1,SMARCD3*	2.207024196	*GATA6, NFYB,SNAI2*	2.102536078
GATA4, MEF2C,SNAI2	2.362761077	*AKT1, ETS2,NKX2.5*	2.207005397	*MITF, NFYB,MYOCD*	2.101142371
ESRRG, NKX2.5,MYOCD	2.362510175	*GATA6, MESP1,TBX5*	2.204617032	*GATA4, HAND2,TBX5*	2.09916322
ESRRA, GATA6,MYOCD	2.354502595	*HAND1, SRF,TBX5*	2.203442042	*NFYB, ZFPM2,MYOCD*	2.098367879
GATA4, MESP1,PBX1	2.353689567	*AKT1, NR2F1,ZNF281*	2.201257862	*ESRRG, ETS2,SMARCD3*	2.0940772
IRX5, PBX1,SMAD6	2.352893196	*ETS2, IRX5,SMAD6*	2.198691339	*MEF2C, SOX9,SRF*	2.09122947
FOSL1, MEF2C,NKX2.5	2.352614038	*ESRRA, TBX20,TCF21*	2.197802198	*FOSL1, NKX2.5,MYOCD*	2.090400427
FOSL1, GATA6,SRF	2.352150538	*MESP1, PBX1,SMARCD3*	2.191464821	*HAND2, PBX1,MYOCD*	2.086593636
ESRRA, MEF2C,NR2F1	2.352034205	*ESRRA, ETS2,SRF*	2.189794222	*HAND1, MESP1,SRF*	2.086318516
ESRRA, ZNF281,ASCL1	2.35042735	*FHL2, SNAI2,TBX5*	2.188353957	*ESRRG, ETS2,HAND2*	2.082410061
HAND1, HEY2,TCF21	2.345058626	*FHL2, IRX5,SRF*	2.186767949	*IRX5, MEF2C,SMARCD3*	2.081661808
HAND2, MITF,NFYB	2.341137124	*HEY2, SMARCD3,TBX20*	2.184843146	*IRX5, MESP1,SMARCD3*	2.079880872
HAND1, MEF2C,TBX5	2.333122846	*SNAI2, SRF,TBX5*	2.184087724	*ESRRA, MEF2C,SNAI2*	2.079147411
FOSL1, HAND2,TCF21	2.33029382	*HAND1, PBX1,SMARCD3*	2.183697678	*MEF2C, SRF,MYOCD*	2.074288862
GATA6, HAND1,HEY2	2.329458905	*FHL2, GATA4,NR2F1*	2.182952183	*ESRRG, FOSL1,SRF*	2.073849128
FHL2, HEY2,ASCL1	2.325950215	*AKT1, GATA4,MESP1*	2.180248773	*SMARCD3, SNAI2,TBX5*	2.069293026
ESRRG, PBX1,SOX9	2.325581395	*GATA4, PBX1,TBX5*	2.180152792	*FOSL1, GATA4,MYOCD*	2.068440972
FHL2, MESP1,ZNF281	2.322097378	*GATA4, MEF2C,NR2F1*	2.178986755	*GATA6, SNAI2,ASCL1*	2.062826655
HAND1, NR2F1,SMARCD3	2.32172471	*MEF2C, SNAI2,TBX20*	2.178576863	*HAND1, IRX5,MEF2C*	2.061087061
ESRRA, IRX5,NKX2.5	2.31884058	*GATA4, HEY2,TBX5*	2.176838022	*HAND1, ID1,MEF2C*	2.058673783
HAND2, MESP1,SMARCD3	2.317115646	*IRX5, MEF2C,SRF*	2.175795827	*GATA4, GATA6,SOX9*	2.056785155
NFYB, SRF,MYOCD	2.312066498	*ETS2, FOSL1,IRX5*	2.175727701	*ESRRA, ID1,MESP1*	2.056204017
ETS2, MEF2C,SOX9	2.311546765	*HAND1, NKX2.5,SOX9*	2.173101022	*MEF2C, NKX2.5,SMAD6*	2.049475014
ETS2, IRX5,MYOCD	2.307150191	*FOSL1, GATA4,HAND2*	2.171977469	*AKT1, FHL2,FOSL1*	2.048508901
PBX1, ZNF281,ASCL1	2.306102455	*HAND1, SRF,TBX20*	2.17054655	*HAND1, SMARCD3,ASCL1*	2.046475464
ETS2, TCF21,ASCL1	2.304964539	*PBX1, SRF,TBX20*	2.169495646	*HAND1, HAND2,SNAI2*	2.046186274
GATA4, HEY2,NR2F1	2.30241344	*IRX5, MEF2C,NFYB*	2.168587183	*FOSL1, HAND2,ZFPM2*	2.044444444
HAND2, MEF2C,ZFPM2	2.298850575	*NR2F1, TCF21,ASCL1*	2.168367347	*HAND1, HAND2,NFYB*	2.042567982
FOSL1, HAND1,TCF21	2.287581699	*FOSL1, PBX1,SRF*	2.16802168	*TCF21, ZNF281,MYOCD*	2.040816327
AKT1, ID1,MESP1	2.281302195	*GATA6, SRF,TBX5*	2.167241473	*NKX2.5, SMAD6,ASCL1*	2.04052913
ESRRG, SMARCD3,SRF	2.279643097	*MEF2C, SNAI2,TBX5*	2.166897188	*HAND1, SMARCD3,SRF*	2.038568157
GATA6, HEY2,SMAD6	2.275523332	*GATA6, NR2F1,TBX20*	2.16210903	*ID1, MEF2C,MESP1*	2.037650974
MEF2C, TBX5,ZFPM2	2.275098056	*HAND2, IRX5,SNAI2*	2.160493827	*ETS2, FOSL1,PBX1*	2.037310916

Table S7. List of all 4,960 combinations tested and their respective reprogramming efficiency (MYH6^{+}TNNT2^{+})

TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells
ETS2, ID1,NFYB	2.036953904	*ESRRA, HAND2,PBX1*	1.928374656	*IRX5, NFYB,NKX2.5*	1.802554375
MESP1, SMAD6,ZFPM2	2.036890877	*ETS2, GATA4,SMAD6*	1.928057659	*SOX9, SRF,MYOCD*	1.801338567
FOSL1, SMAD6,SMARCD3	2.035516582	*HAND2, MEF2C,SMARCD3*	1.925925926	*ESRRA, MESP1,SMAD6*	1.796920098
HAND1, NR2F1,ASCL1	2.034509	*NFYB, NKX2.5,PBX1*	1.92531847	*ESRRA, HEY2,MYOCD*	1.79457987
FOSL1, SRF,TBX20	2.033502297	*ESRRA, SMARCD3,SOX9*	1.917566131	*AKT1, HEY2,MYOCD*	1.794219976
MESP1, PBX1,SRF	2.032997107	*FOSL1, GATA6,SNAI2*	1.914708442	*GATA6, NKX2.5,MYOCD*	1.793536254
FHL2, GATA6,NFYB	2.028176169	*ETS2, SMARCD3,MYOCD*	1.914267016	*FOSL1, ID1,MESP1*	1.790528233
AKT1, MEF2C,TBX5	2.02764223	*ETS2, MEF2C,ZFPM2*	1.911240687	*ESRRA, NFYB,NKX2.5*	1.79005647
ESRRG, ETS2,ID1	2.025440347	*IRX5, NFYB,SMARCD3*	1.908185924	*NKX2.5, PBX1,SRF*	1.788664952
IRX5, SRF,TBX5	2.023193496	*HEY2, MESP1,SNAI2*	1.906707324	*GATA6, NFYB,PBX1*	1.787046856
IRX5, SOX9,SRF	2.021809614	*ETS2, IRX5,NKX2.5*	1.906009367	*FOSL1, TBX5,ZNF281*	1.782945736
MEF2C, SRF,TBX20	2.02020202	*GATA4, MESP1,SMAD6*	1.902907753	*AKT1, ESRRG,HAND2*	1.781696643
HAND1, HEY2,SNAI2	2.01988251	*ETS2, SMAD6,ZFPM2*	1.900567687	*ID1, SNAI2,SRF*	1.780689991
ETS2, IRX5,NFYB	2.019080287	*HAND1, MESP1,NFYB*	1.898845164	*AKT1, GATA4,HAND1*	1.780669355
AKT1, ESRRG,SOX9	2.01754386	*NFYB, TCF21,ASCL1*	1.898734177	*HAND2, IRX5,SRF*	1.779245849
ESRRG, ETS2,SNAI2	2.00930942	*GATA6, NR2F1,MYOCD*	1.89701897	*AKT1, ESRRA,MEF2C*	1.77897346
ETS2, MESP1,PBX1	2.004865035	*MEF2C, NFYB,SNAI2*	1.896654719	*FHL2, SOX9,ZNF281*	1.777059774
MESP1, SNAI2,SRF	2.003881737	*NR2F1, SMAD6,MYOCD*	1.895322249	*ESRRG, HAND1,ID1*	1.775956284
ESRRA, ID1,TBX20	1.992450932	*FOSL1, GATA4,TBX5*	1.891352748	*NKX2.5, SRF,TBX5*	1.774555183
MESP1, PBX1,ZNF281	1.989106414	*ID1, MEF2C,SRF*	1.882115897	*HAND1, MESP1,SMARCD3*	1.772810886
ESRRG, MESP1,SMARCD3	1.988756471	*FHL2, MEF2C,NFYB*	1.882036939	*HAND2, NFYB,SOX9*	1.771324686
NR2F1, TBX5,MYOCD	1.98716148	*ESRRA, FOSL1,SRF*	1.877625724	*HAND2, ID1,TBX5*	1.769914835
GATA4, HAND1,IRX5	1.986632438	*FOSL1, MEF2C,NFYB*	1.876153562	*HAND2, SMARCD3,TBX20*	1.769689678
FOSL1, NR2F1,SOX9	1.986195521	*ETS2, HAND2,PBX1*	1.875782149	*GATA4, NKX2.5,NR2F1*	1.768270521
ETS2, IRX5,MEF2C	1.983906271	*AKT1, MESP1,TBX20*	1.874140203	*PBX1, SRF,TBX5*	1.766016133
GATA6, IRX5,TBX5	1.983117941	*HAND1, TBX5,TCF21*	1.874094129	*FOSL1, NR2F1,TBX5*	1.764014423
FOSL1, SMARCD3,SRF	1.980406338	*FHL2, NFYB,NKX2.5*	1.873439726	*AKT1, ETS2,HAND1*	1.762820513
HAND1, SMARCD3,ZFPM2	1.979430091	*HEY2, NR2F1,PBX1*	1.866975183	*AKT1, GATA4,SNAI2*	1.759838513
ETS2, MESP1,TBX20	1.979420114	*ETS2, FOSL1,TCF21*	1.866151866	*FHL2, NKX2.5,NR2F1*	1.758742825
FHL2, FOSL1,PBX1	1.979107836	*MESP1, NR2F1,SOX9*	1.862617072	*HEY2, SMARCD3,SNAI2*	1.755804654
HAND2, ID1,MESP1	1.978402335	*AKT1, PBX1,SRF*	1.861519127	*FHL2, ID1,MESP1*	1.753805877
GATA4, NFYB,SNAI2	1.976911977	*FHL2, NFYB,SNAI2*	1.853802933	*ETS2, MEF2C,SNAI2*	1.7530943
HAND1, SRF,MYOCD	1.976545368	*MESP1, SMARCD3,TBX20*	1.851145533	*PBX1, SMAD6,SNAI2*	1.752254491
ETS2, HAND1,TBX5	1.97596047	*GATA4, HEY2,NFYB*	1.851003274	*NFYB, PBX1,SNAI2*	1.749803567
GATA4, NFYB,TBX20	1.975811985	*FHL2, HAND2,MEF2C*	1.85092848	*ETS2, NFYB,SOX9*	1.747815231
ESRRA, MESP1,SMARCD3	1.974602622	*GATA6, HAND2,SRF*	1.847869375	*FOSL1, GATA4,HAND1*	1.747782825
ETS2, FHL2,TBX5	1.974177703	*FOSL1, SMARCD3,SNAI2*	1.835183518	*FHL2, MESP1,MITF*	1.747058824
FOSL1, GATA4,NKX2.5	1.973735484	*MESP1, MITF,ASCL1*	1.834862385	*FHL2, NR2F1,TBX5*	1.745200698
GATA6, SOX9,ASCL1	1.972157773	*SMAD6, SOX9,MYOCD*	1.834399659	*GATA4, ZFPM2,ZNF281*	1.742160279
ESRRG, HAND1,SMAD6	1.964446629	*SMAD6, SMARCD3,SNAI2*	1.831167017	*ESRRA, MEF2C,NFYB*	1.741240581
ETS2, MEF2C,TBX5	1.962851017	*HAND2, IRX5,TCF21*	1.830808081	*ESRRG, MESP1,NKX2.5*	1.737516869
AKT1, GATA6,HAND2	1.962748389	*FHL2, HAND2,ASCL1*	1.83027307	*HAND2, IRX5,MITF*	1.736111111
ESRRA, HAND1,SRF	1.960386589	*AKT1, ID1,TBX20*	1.82575345	*HAND1, IRX5,PBX1*	1.730981201
ETS2, IRX5,SNAI2	1.950730851	*MEF2C, PBX1,MYOCD*	1.821887936	*HAND1, MITF,SMARCD3*	1.73062453
ESRRA, ZFPM2,MYOCD	1.949583705	*AKT1, NKX2.5,SOX9*	1.821117244	*PBX1, ZFPM2,MYOCD*	1.725639446
FHL2, MEF2C,TBX5	1.946636902	*ETS2, GATA6,SMAD6*	1.817188984	*HAND2, SMARCD3,TCF21*	1.720430108
HAND1, MITF,SOX9	1.945003353	*GATA4, HAND2,NR2F1*	1.817099849	*ESRRA, GATA4,HEY2*	1.719460737
SNAI2, SRF,TBX20	1.936072253	*GATA4, NKX2.5,TBX5*	1.814612917	*HAND2, MESP1,TBX20*	1.719376998
ETS2, ID1,IRX5	1.934794146	*HEY2, MEF2C,NFYB*	1.810699588	*AKT1, MEF2C,TBX20*	1.717034594
AKT1, SRF,TBX20	1.934503087	*GATA4, SMARCD3,SRF*	1.809208685	*ESRRG, ETS2,TBX5*	1.714122605
SMARCD3, SNAI2,SRF	1.933083363	*FOSL1, GATA4,ASCL1*	1.808892952	*AKT1, MESP1,SMARCD3*	1.711829203
MEF2C, SMAD6,TBX5	1.931457431	*HEY2, SMAD6,ASCL1*	1.805894805	*ETS2, FOSL1,TBX20*	1.711386058
GATA6, TBX20,ZNF281	1.931150294	*ID1, SNAI2,SOX9*	1.805880113	*AKT1, FHL2,SMARCD3*	1.7100582
ESRRA, HEY2,NFYB	1.930365325	*FOSL1, NKX2.5,SMARCD3*	1.805642199	*IRX5, SOX9,TCF21*	1.703940362

Table S7. List of all 4,960 combinations tested and their respective reprogramming efficiency (MYH6^{+}TNNT2^{+})

TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells
FOSL1, GATA4,TCF21	1.702513928	*AKT1, GATA6,NKX2.5*	1.611940625	*AKT1, FHL2,SRF*	1.507383966
AKT1, FOSL1,TBX20	1.702006689	*ID1, MESP1,SRF*	1.611393599	*AKT1, NFYB,SRF*	1.507269224
AKT1, GATA6,SMARCD3	1.701647195	*GATA6, NFYB,SRF*	1.610804731	*ETS2, FOSL1,SNAI2*	1.503267929
ESRRA, NR2F1,SRF	1.699936368	*ESRRG, MEF2C,ZNF281*	1.610305958	*ESRRA, MEF2C,SMAD6*	1.503094607
FHL2, NFYB,TBX20	1.699134499	*FOSL1, HEY2,ASCL1*	1.603907893	*FOSL1, HEY2,SRF*	1.502720543
FOSL1, PBX1,SMAD6	1.697480685	*ESRRA, FHL2,HEY2*	1.600338191	*FHL2, NR2F1,MYOCD*	1.502613834
ID1, MEF2C,NKX2.5	1.696796601	*HAND1, HEY2,PBX1*	1.598793363	*FHL2, ID1,NKX2.5*	1.502591288
HEY2, SRF,ASCL1	1.695906433	*IRX5, MEF2C,SNAI2*	1.5977367	*ID1, MITF,PBX1*	1.501501502
MEF2C, NR2F1,SNAI2	1.695775489	*HAND2, MITF,MYOCD*	1.597444089	*HAND2, IRX5,MEF2C*	1.500276114
ETS2, FHL2,MESP1	1.695525173	*HAND1, TBX20,TBX5*	1.597125097	*ESRRA, HAND2,SRF*	1.49961846
HEY2, TBX20,TCF21	1.694290976	*HAND1, MEF2C,NR2F1*	1.595752971	*AKT1, FOSL1,NKX2.5*	1.499518701
HEY2, SRF,TBX5	1.693717608	*FOSL1, PBX1,SMARCD3*	1.594929039	*ESRRG, GATA6,NR2F1*	1.498519047
HAND2, HEY2,TCF21	1.691162457	*AKT1, ESRRA,GATA6*	1.58722716	*HAND2, NKX2.5,ASCL1*	1.495162709
HEY2, MEF2C,SRF	1.690894111	*NFYB, SRF,TBX20*	1.583661746	*FOSL1, GATA4,SRF*	1.493942552
ESRRA, NKX2.5,NR2F1	1.690767248	*AKT1, MEF2C,PBX1*	1.583609904	*GATA6, NR2F1,TBX5*	1.493834893
NFYB, TBX20,TBX5	1.684000803	*TBX20, ZFPM2,MYOCD*	1.581902181	*IRX5, PBX1,TBX5*	1.491411257
ESRRA, MITF,NR2F1	1.682267649	*ETS2, GATA4,SRF*	1.581445929	*FOSL1, NFYB,SMAD6*	1.490244915
MEF2C, NFYB,SOX9	1.678803843	*ETS2, GATA6,TBX5*	1.580163632	*FHL2, GATA4,GATA6*	1.489516216
HAND1, ZFPM2,MYOCD	1.677432457	*GATA6, HEY2,SRF*	1.579514619	*FOSL1, GATA6,TBX20*	1.48914932
ESRRG, SMAD6,SRF	1.676204033	*PBX1, TBX20,ASCL1*	1.577669903	*FHL2, HAND1,SOX9*	1.488782689
ESRRA, FHL2,MYOCD	1.674130347	*FHL2, FOSL1,MESP1*	1.575794626	*HAND2, NFYB,TBX5*	1.488095238
ETS2, FHL2,MYOCD	1.665929501	*HEY2, MEF2C,MESP1*	1.572264778	*SMAD6, SOX9,TBX5*	1.485332522
HEY2, MESP1,NR2F1	1.664774877	*ESRRA, SRF,MYOCD*	1.567272892	*MESP1, SMARCD3,TBX5*	1.484042231
ESRRA, HEY2,PBX1	1.664397917	*GATA4, NR2F1,SMARCD3*	1.566413029	*FOSL1, GATA4,MESP1*	1.483897763
ESRRA, MESP1,NFYB	1.659259971	*ESRRA, GATA4,MYOCD*	1.564993451	*FHL2, FOSL1,SMAD6*	1.48306469
GATA6, SOX9,TBX20	1.65298418	*GATA4, HAND1,NR2F1*	1.562382094	*ESRRG, HAND1,SOX9*	1.482445312
ESRRG, MEF2C,MITF	1.65059889	*GATA6, HAND1,NKX2.5*	1.560808036	*GATA4, NR2F1,SMAD6*	1.481828556
FHL2, HAND1,PBX1	1.650432194	*GATA6, HAND1,TBX20*	1.559070128	*GATA4, NR2F1,SNAI2*	1.480372895
ETS2, FHL2,SOX9	1.650358962	*FHL2, MESP1,TBX5*	1.559050964	*NKX2.5, MYOCD,ASCL1*	1.476880876
ETS2, IRX5,MESP1	1.650288954	*NKX2.5, TBX5,MYOCD*	1.559013135	*ESRRG, ETS2,SMAD6*	1.472354669
GATA4, SOX9,TBX5	1.650165017	*AKT1, GATA6,MESP1*	1.557909605	*MEF2C, NKX2.5,SNAI2*	1.471961944
IRX5, MEF2C,NKX2.5	1.648438288	*AKT1, HAND1,MESP1*	1.556302192	*HAND1, MEF2C,SNAI2*	1.470908695
FOSL1, HAND2,SRF	1.647821136	*ETS2, ID1,PBX1*	1.55262266	*GATA6, HEY2,ZNF281*	1.470694301
NFYB, SMARCD3,TBX5	1.643982195	*GATA4, HAND2,PBX1*	1.549875349	*ESRRA, PBX1,SMARCD3*	1.470394652
GATA4, NR2F1,SRF	1.642702169	*GATA4, IRX5,NKX2.5*	1.547733575	*ESRRA, HEY2,SRF*	1.469738553
IRX5, NFYB,SRF	1.642600359	*ETS2, HAND1,SNAI2*	1.547468662	*GATA6, HAND2,SNAI2*	1.46968487
ESRRG, GATA6,MEF2C	1.642025743	*ETS2, HAND2,MEF2C*	1.547325103	*ESRRA, HEY2,SOX9*	1.46422837
AKT1, ID1,ZNF281	1.641414141	*IRX5, TBX20,TCF21*	1.547116737	*ESRRA, SRF,TBX20*	1.463488469
AKT1, GATA4,TCF21	1.640566741	*IRX5, MESP1,TBX20*	1.546401402	*AKT1, FOSL1,MESP1*	1.46051161
GATA6, ID1,TBX5	1.63945328	*NFYB, SMAD6,TBX5*	1.546278431	*HAND1, IRX5,TBX5*	1.458344672
ETS2, GATA4,MEF2C	1.639437236	*MEF2C, ZFPM2,ASCL1*	1.545253863	*ETS2, HAND2,SMAD6*	1.458228371
GATA6, IRX5,SMAD6	1.639344262	*MEF2C, NFYB,TBX5*	1.539163015	*MEF2C, PBX1,SMAD6*	1.4571949
ETS2, FHL2,HAND2	1.638464442	*HAND1, SOX9,MYOCD*	1.538356996	*HEY2, ID1,MYOCD*	1.455675423
HAND1, NR2F1,SRF	1.629613378	*ID1, NFYB,SRF*	1.538233582	*HAND1, PBX1,SNAI2*	1.455515104
FHL2, SMARCD3,ASCL1	1.624986792	*GATA4, PBX1,SMARCD3*	1.532788818	*ESRRG, GATA6,TBX20*	1.454138702
SMAD6, SOX9,ZFPM2	1.622718053	*FHL2, MESP1,SOX9*	1.529715762	*AKT1, NFYB,NR2F1*	1.450383917
FHL2, GATA6,SNAI2	1.621404917	*HAND1, PBX1,SRF*	1.524420649	*ETS2, PBX1,MYOCD*	1.449502878
GATA6, SOX9,SRF	1.619339182	*ESRRA, NFYB,TBX20*	1.522254385	*SMAD6, SRF,TBX5*	1.448602476
GATA4, HAND1,MESP1	1.618191089	*AKT1, GATA6,NR2F1*	1.520858301	*ID1, PBX1,SRF*	1.446229084
ESRRA, FHL2,NR2F1	1.616963925	*GATA4, GATA6,HAND2*	1.515483958	*FHL2, TBX5,ZNF281*	1.445681291
HAND2, NKX2.5,MYOCD	1.616902392	*HAND2, ID1,MEF2C*	1.514124294	*ESRRG, MEF2C,NKX2.5*	1.44444034
MEF2C, MESP1,NR2F1	1.616576724	*IRX5, SMAD6,SMARCD3*	1.513687601	*MEF2C, PBX1,SNAI2*	1.444205881
AKT1, GATA4,HAND2	1.615508885	*ETS2, HEY2,NFYB*	1.511842783	*ETS2, MEF2C,MESP1*	1.443929259
HAND1, SMAD6,TBX5	1.612769044	*FHL2, GATA6,MESP1*	1.511328705	*HAND2, IRX5,NFYB*	1.443326271
ESRRG, MITF,ZNF281	1.612241114	*HEY2, SMAD6,SRF*	1.509484748	*ETS2, NFYB,SNAI2*	1.443256903

Table S7. List of all 4,960 combinations tested and their respective reprogramming efficiency (MYH6^{+}TNNT2^{+})

TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells
AKT1, SMAD6,SMARCD3	1.442963132	*IRX5, SMARCD3,SRF*	1.372450511	*HAND2, IRX5,SMARCD3*	1.294875278
ETS2, FOSL1,NFYB	1.441534199	*SRF, MYOCD,ASCL1*	1.368925394	*AKT1, FHL2,MEF2C*	1.294758247
NR2F1, SNAI2,ASCL1	1.439263097	*FOSL1, GATA4,MEF2C*	1.367211976	*SMAD6, SRF,TCF21*	1.294498382
IRX5, NKX2.5,SRF	1.437913343	*ETS2, MEF2C,NFYB*	1.365510053	*FHL2, TCF21,ASCL1*	1.293133741
MEF2C, NKX2.5,PBX1	1.437064814	*ETS2, ID1,MESP1*	1.36408069	*ESRRG, SNAI2,SRF*	1.291989664
IRX5, NR2F1,ZNF281	1.436580238	*NR2F1, PBX1,SRF*	1.362495757	*NFYB, SMAD6,TBX20*	1.291440099
ID1, IRX5,NFYB	1.434113242	*AKT1, FHL2,PBX1*	1.361033948	*ETS2, HAND2,SMARCD3*	1.290088171
HAND2, NFYB,NKX2.5	1.433857539	*GATA6, SMAD6,ASCL1*	1.358695652	*FHL2, NFYB,TBX5*	1.289299059
FOSL1, MESP1,TBX5	1.433566434	*GATA4, GATA6,SMARCD3*	1.357669731	*FOSL1, GATA4,NR2F1*	1.2856291
FHL2, SMAD6,TBX20	1.432260537	*GATA6, HEY2,MEF2C*	1.354637469	*ETS2, SNAI2,TBX20*	1.282870246
HAND1, SMARCD3,SNAI2	1.430514622	*GATA6, HEY2,SMARCD3*	1.354087297	*HAND1, NR2F1,PBX1*	1.280934552
GATA4, NFYB,PBX1	1.42621532	*ETS2, MYOCD,ASCL1*	1.352177736	*ESRRG, FOSL1,GATA6*	1.280846075
GATA4, IRX5,SMAD6	1.424959096	*TBX5, ZFPM2,MYOCD*	1.352067869	*ESRRA, PBX1,SMAD6*	1.278918491
HAND1, HAND2,TBX5	1.423268659	*MITF, ZNF281,ASCL1*	1.351351351	*HAND1, MEF2C,NKX2.5*	1.277790254
ETS2, HEY2,SRF	1.421688382	*MEF2C, SNAI2,ASCL1*	1.348017977	*MEF2C, NR2F1,MYOCD*	1.274816763
GATA4, NFYB,NKX2.5	1.420879747	*NR2F1, PBX1,MYOCD*	1.34576661	*AKT1, PBX1,SOX9*	1.272654048
ESRRA, NKX2.5,SRF	1.419258899	*ESRRA, GATA4,NFYB*	1.344656759	*MESP1, PBX1,SNAI2*	1.272077399
FHL2, FOSL1,GATA4	1.418439716	*NFYB, PBX1,SOX9*	1.344357243	*MESP1, NFYB,NKX2.5*	1.26762445
GATA4, NKX2.5,TBX20	1.418356639	*GATA4, HAND1,ID1*	1.340629275	*ID1, MESP1,SMAD6*	1.266953905
GATA4, HAND2,ID1	1.414580026	*HEY2, ID1,SOX9*	1.339067608	*GATA6, NKX2.5,SOX9*	1.266779364
ETS2, ID1,SNAI2	1.410209248	*ESRRA, NKX2.5,SMAD6*	1.338077311	*GATA6, IRX5,NFYB*	1.265822785
ESRRG, IRX5,SMAD6	1.409023935	*ID1, SMARCD3,SNAI2*	1.332753263	*ETS2, FHL2,NFYB*	1.264755754
GATA6, SMAD6,SOX9	1.408874035	*HAND2, ID1,SNAI2*	1.33015397	*AKT1, FOSL1,GATA6*	1.261488556
FHL2, ID1,NFYB	1.407004066	*MITF, PBX1,MYOCD*	1.327573757	*GATA6, SMAD6,SRF*	1.260713397
HAND2, HEY2,MEF2C	1.406219053	*ID1, NFYB,SNAI2*	1.327509707	*HAND2, MEF2C,PBX1*	1.259554233
ETS2, HAND2,NR2F1	1.405439802	*AKT1, FHL2,ZFPM2*	1.326854149	*NR2F1, SRF,TBX5*	1.254762843
ID1, SMARCD3,SRF	1.405136461	*HAND1, MITF,MYOCD*	1.326370786	*FHL2, HEY2,SMAD6*	1.253508087
HAND2, MEF2C,MESP1	1.404406539	*FHL2, PBX1,SNAI2*	1.325347516	*ETS2, FHL2,PBX1*	1.252871145
ETS2, NFYB,MYOCD	1.404261642	*GATA4, ID1,SNAI2*	1.323225565	*GATA6, HAND2,MYOCD*	1.252847012
ESRRA, TBX5,ZFPM2	1.403508772	*HAND2, HEY2,SRF*	1.323084808	*FOSL1, SNAI2,TBX20*	1.252723312
ESRRA, MESP1,TBX5	1.403442174	*ID1, SMARCD3,TBX20*	1.322673837	*ESRRG, NKX2.5,NR2F1*	1.252236136
ESRRG, ETS2,PBX1	1.402297601	*FHL2, GATA4,HAND2*	1.321585903	*ETS2, FOSL1,MESP1*	1.25216731
ETS2, FHL2,FOSL1	1.400059916	*HAND1, ID1,PBX1*	1.320980184	*FHL2, MEF2C,SOX9*	1.250707414
ESRRG, IRX5,ZNF281	1.397205589	*ETS2, FOSL1,SRF*	1.318627266	*NFYB, PBX1,SRF*	1.250695576
HAND2, ID1,SMARCD3	1.397030342	*ESRRG, ETS2,HAND1*	1.316718704	*ID1, MESP1,TBX20*	1.250078453
IRX5, NR2F1,SRF	1.396478446	*HEY2, SRF,TBX20*	1.31629402	*ID1, MEF2C,SOX9*	1.247370516
ETS2, FOSL1,SMAD6	1.393611414	*HAND1, SMAD6,SNAI2*	1.31594993	*NFYB, TCF21,ZNF281*	1.245989182
ETS2, MITF,SRF	1.39289122	*ETS2, MEF2C,PBX1*	1.31378564	*MITF, PBX1,TBX5*	1.244474342
PBX1, SNAI2,TBX5	1.392295843	*HAND1, HEY2,MITF*	1.31147541	*HAND2, SRF,ZFPM2*	1.243169036
FHL2, IRX5,SMAD6	1.391860641	*ID1, MESP1,TBX5*	1.311035426	*GATA6, NKX2.5,NR2F1*	1.24180023
ETS2, ID1,MEF2C	1.391074586	*ETS2, MEF2C,TBX20*	1.310541311	*HAND1, ID1,SNAI2*	1.234567901
GATA4, SNAI2,ZFPM2	1.388888889	*ESRRG, ETS2,FOSL1*	1.30985833	*GATA6, HAND1,NFYB*	1.232504496
GATA6, HAND1,HAND2	1.386483498	*ETS2, FHL2,SRF*	1.308440464	*AKT1, GATA6,ID1*	1.226757738
ESRRA, PBX1,ZNF281	1.383399209	*ID1, NR2F1,MYOCD*	1.307565975	*NR2F1, SMARCD3,MYOCD*	1.226541935
AKT1, ESRRG,HAND1	1.382018643	*FOSL1, SMAD6,SOX9*	1.307341152	*HAND1, ID1,NR2F1*	1.221982842
ID1, MEF2C,SMARCD3	1.381300564	*FHL2, PBX1,SMARCD3*	1.305626184	*ESRRG, ETS2,SRF*	1.221307113
FHL2, ID1,ASCL1	1.381121054	*ETS2, NR2F1,SRF*	1.304595912	*ESRRG, NKX2.5,ASCL1*	1.219015542
AKT1, GATA4,MEF2C	1.379780122	*IRX5, MESP1,SMAD6*	1.301569036	*NKX2.5, NR2F1,SRF*	1.21720402
FOSL1, IRX5,TBX20	1.379310345	*HAND2, NR2F1,SMARCD3*	1.301345405	*ESRRA, IRX5,TBX5*	1.217135503
IRX5, SMAD6,SNAI2	1.37557703	*HEY2, PBX1,SRF*	1.300971956	*ETS2, HAND1,IRX5*	1.216964162
ETS2, HAND1,NFYB	1.37532603	*ETS2, NFYB,SRF*	1.300644863	*AKT1, GATA4,SOX9*	1.214812762
AKT1, NFYB,ASCL1	1.37524558	*NFYB, SMARCD3,SRF*	1.299236248	*ESRRA, HAND2,IRX5*	1.21378317
IRX5, PBX1,TBX20	1.374935243	*NR2F1, SRF,TCF21*	1.298701299	*AKT1, PBX1,TBX5*	1.212316968
ETS2, GATA4,GATA6	1.373352833	*MESP1, NFYB,SMARCD3*	1.297805182	*GATA4, HEY2,MESP1*	1.210919614
MEF2C, NR2F1,SMAD6	1.372699387	*GATA6, HEY2,ID1*	1.297359291	*ESRRG, GATA4,SMARCD3*	1.208981002

Table S7. List of all 4,960 combinations tested and their respective reprogramming efficiency (MYH6^{+}TNNT2^{+})

TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells
FHL2, IRX5,PBX1	1.208977816	*HAND2, NKX2.5,PBX1*	1.15942029	*MEF2C, NR2F1,SOX9*	1.100110011
ETS2, GATA6,SRF	1.207452738	*GATA6, NKX2.5,SMARCD3*	1.153146217	*ESRRA, HAND1,HEY2*	1.098901099
ESRRA, SMAD6,TBX20	1.206791456	*ETS2, TBX20,TBX5*	1.152534539	*IRX5, NKX2.5,ZFPM2*	1.098039216
ESRRG, FHL2,NKX2.5	1.205003051	*ESRRA, MEF2C,NKX2.5*	1.151046107	*ESRRG, FHL2,IRX5*	1.097219103
ESRRA, NKX2.5,TBX5	1.203506638	*GATA6, MEF2C,TCF21*	1.149425287	*ESRRA, MEF2C,ZNF281*	1.096956829
GATA4, ID1,NFYB	1.203455152	*ETS2, FOSL1,ZNF281*	1.149425287	*GATA6, IRX5,NR2F1*	1.094520671
AKT1, HAND2,MEF2C	1.203421218	*SOX9, SRF,TBX20*	1.149133561	*AKT1, ESRRA,MESP1*	1.09375
HAND2, PBX1,SMAD6	1.202749141	*FOSL1, GATA6,NFYB*	1.14772591	*GATA6, HAND2,NFYB*	1.092926581
ESRRG, NFYB,TBX5	1.201329187	*ID1, TBX20,TBX5*	1.145361593	*HAND1, MEF2C,ZFPM2*	1.089638376
FHL2, HAND2,SMARCD3	1.199732022	*ETS2, SMAD6,SOX9*	1.144865841	*AKT1, IRX5,SRF*	1.089509186
ETS2, FHL2,HEY2	1.199582596	*FOSL1, NKX2.5,SNAI2*	1.144323901	*GATA6, HAND2,IRX5*	1.08868993
ETS2, HEY2,MESP1	1.195814649	*ID1, MEF2C,NR2F1*	1.143859649	*GATA6, HAND1,TBX5*	1.088263588
NR2F1, SMARCD3,TBX5	1.195569406	*ESRRG, FHL2,FOSL1*	1.143814573	*ESRRA, HEY2,MESP1*	1.085683696
ESRRA, NR2F1,SNAI2	1.195219124	*ESRRG, SOX9,MYOCD*	1.143728555	*FOSL1, IRX5,SNAI2*	1.08401084
HAND1, MEF2C,MESP1	1.194553418	*ESRRA, NR2F1,MYOCD*	1.143464379	*ID1, SRF,TBX5*	1.079376424
ID1, MYOCD,ASCL1	1.193113916	*HAND1, SNAI2,SRF*	1.143415872	*ETS2, MITF,MYOCD*	1.079038715
ETS2, HAND1,MEF2C	1.193011193	*ESRRG, MITF,NKX2.5*	1.142359747	*GATA4, HAND2,MITF*	1.075268817
FHL2, GATA6,TBX20	1.19120394	*SMAD6, SNAI2,SOX9*	1.141587484	*ETS2, HAND1,SOX9*	1.075268817
HAND1, HEY2,SRF	1.191001062	*NR2F1, PBX1,SNAI2*	1.141537161	*ESRRG, FHL2,SRF*	1.0742254
HEY2, ID1,SRF	1.190610399	*ID1, NKX2.5,SRF*	1.139217225	*FHL2, GATA4,MEF2C*	1.073097913
ID1, SMAD6,TBX5	1.188975629	*GATA4, MEF2C,TCF21*	1.137014439	*ETS2, GATA4,TBX5*	1.072725471
ETS2, ID1,SMAD6	1.188441793	*HAND1, NFYB,NR2F1*	1.135859795	*ETS2, PBX1,TBX5*	1.069876169
ESRRG, FHL2,TBX5	1.188368991	*GATA4, PBX1,TBX20*	1.134390427	*MEF2C, PBX1,TBX5*	1.069556835
AKT1, ESRRA,TCF21	1.18314396	*AKT1, FHL2,SNAI2*	1.130682821	*HAND1, ID1,SOX9*	1.069114301
ETS2, PBX1,SOX9	1.18286035	*AKT1, IRX5,MITF*	1.127811871	*FOSL1, MESP1,SNAI2*	1.068521624
IRX5, SMARCD3,TBX5	1.181096286	*HAND2, MEF2C,TBX20*	1.126981141	*SMAD6, SNAI2,TBX20*	1.065516386
ETS2, MITF,PBX1	1.180257511	*GATA6, HAND1,PBX1*	1.125948864	*ID1, SOX9,ASCL1*	1.064712097
ESRRA, PBX1,SRF	1.177851903	*NFYB, PBX1,SMAD6*	1.125735872	*SNAI2, SOX9,SRF*	1.062834439
MITF, TBX5,ASCL1	1.175656985	*ESRRG, HAND1,MEF2C*	1.123553565	*ESRRA, MESP1,SNAI2*	1.060453477
NR2F1, TBX20,TBX5	1.175340887	*ID1, MITF,MYOCD*	1.122961833	*MESP1, MITF,TBX20*	1.059747243
GATA4, GATA6,IRX5	1.175330183	*GATA4, IRX5,SOX9*	1.120624672	*FOSL1, NR2F1,SRF*	1.056676273
ETS2, NKX2.5,TBX5	1.174731701	*ETS2, ID1,TBX20*	1.11977381	*NFYB, SNAI2,SRF*	1.05622656
NKX2.5, NR2F1,SMARCD3	1.174261819	*ESRRG, FHL2,SMARCD3*	1.118568233	*AKT1, FOSL1,SMARCD3*	1.054436875
MEF2C, NKX2.5,SOX9	1.174247421	*FHL2, ID1,ZNF281*	1.118387306	*FOSL1, NFYB,NR2F1*	1.054018445
HAND1, MESP1,SMAD6	1.172885496	*FOSL1, MEF2C,NR2F1*	1.117318436	*ESRRA, ID1,NR2F1*	1.048951049
FHL2, PBX1,SOX9	1.172788237	*AKT1, MESP1,SMAD6*	1.115541327	*GATA4, GATA6,ID1*	1.044796084
HAND2, SRF,TBX20	1.172169336	*ESRRG, FHL2,NFYB*	1.113801453	*GATA6, HAND2,SMAD6*	1.044543176
ESRRA, HAND2,TCF21	1.171458999	*NR2F1, SNAI2,SRF*	1.113483146	*GATA6, HAND1,ZNF281*	1.044408775
ESRRA, ESRRG,NKX2.5	1.17048253	*ESRRA, ETS2,NFYB*	1.113107344	*ETS2, GATA4,HEY2*	1.040973451
GATA4, HAND1,NKX2.5	1.169849563	*NFYB, SMARCD3,ASCL1*	1.112537441	*ESRRA, MESP1,ZFPM2*	1.03950104
FHL2, HEY2,MESP1	1.169556842	*ESRRA, ESRRG,GATA4*	1.112484549	*HAND1, HEY2,SOX9*	1.039401264
AKT1, ID1,NFYB	1.168431881	*ETS2, IRX5,PBX1*	1.110946282	*ESRRA, HEY2,SMARCD3*	1.038961039
HEY2, ZFPM2,MYOCD	1.168014376	*ETS2, HAND2,TBX20*	1.109744168	*MITF, SRF,TCF21*	1.038631819
MEF2C, NFYB,ZFPM2	1.167914028	*ESRRA, NKX2.5,SNAI2*	1.109175742	*ESRRG, MEF2C,TBX20*	1.03735381
FOSL1, ZFPM2,MYOCD	1.16692609	*ETS2, ID1,SMARCD3*	1.107270865	*ID1, MEF2C,TBX20*	1.035598706
GATA4, SMAD6,TBX20	1.166633328	*FOSL1, HEY2,MEF2C*	1.106257199	*ETS2, HEY2,NKX2.5*	1.034503559
ESRRG, FHL2,HAND2	1.166505151	*FOSL1, GATA6,TBX5*	1.106189081	*FHL2, NKX2.5,SOX9*	1.033069944
FOSL1, HAND1,IRX5	1.165501166	*AKT1, MESP1,TBX5*	1.105407938	*ETS2, MESP1,SNAI2*	1.03241223
ESRRA, SMAD6,SOX9	1.165432435	*ETS2, HEY2,SOX9*	1.105080093	*IRX5, PBX1,SRF*	1.031568546
FHL2, MITF,SMARCD3	1.165092218	*GATA6, HEY2,MESP1*	1.104862838	*GATA6, MEF2C,MESP1*	1.030993611
HAND1, IRX5,NR2F1	1.163101604	*IRX5, NR2F1,SMAD6*	1.104349786	*ETS2, SRF,TBX20*	1.028351045
PBX1, SOX9,ZFPM2	1.162790698	*ETS2, FHL2,GATA4*	1.103974204	*FOSL1, HAND2,TBX20*	1.027777778
MEF2C, SMAD6,SOX9	1.162224546	*HEY2, MESP1,NKX2.5*	1.102308652	*IRX5, MYOCD,ASCL1*	1.024035861
HAND2, MEF2C,TBX5	1.161952025	*MITF, SMAD6,TCF21*	1.101928375	*ETS2, NFYB,TBX5*	1.0232573
AKT1, NFYB,ZNF281	1.160337553	*PBX1, SNAI2,TCF21*	1.100236184	*ESRRG, MITF,SNAI2*	1.021162451

Table S7. List of all 4,960 combinations tested and their respective reprogramming efficiency (MYH6^{+}TNNT2^{+})

TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells
AKT1, ETS2,TBX5	1.020292664	*NR2F1, SOX9,ZFPM2*	0.977096446	*MITF, SMARCD3,MYOCD*	0.936817809
ETS2, IRX5,SMARCD3	1.01987531	*NFYB, SMAD6,SMARCD3*	0.97608378	*HAND2, MESP1,NR2F1*	0.936371401
ID1, SOX9,SRF	1.01980877	*ETS2, PBX1,SRF*	0.975261985	*ETS2, NFYB,SMARCD3*	0.935546906
GATA4, ID1,MESP1	1.019150115	*ESRRA, IRX5,NFYB*	0.974703262	*AKT1, ESRRG,NFYB*	0.934259312
HEY2, NFYB,SNAI2	1.019014288	*AKT1, HEY2,MESP1*	0.973553633	*AKT1, FOSL1,IRX5*	0.934003227
ETS2, HAND2,ID1	1.018591462	*AKT1, MEF2C,SOX9*	0.973234795	*ETS2, FOSL1,ID1*	0.933604724
MESP1, NFYB,SRF	1.018329939	*ESRRG, MESP1,PBX1*	0.972664618	*ESRRG, PBX1,ZFPM2*	0.931677019
NR2F1, SOX9,ASCL1	1.018220793	*ETS2, HAND1,PBX1*	0.967121324	*HEY2, IRX5,SMAD6*	0.931588903
GATA4, HAND1,HAND2	1.016791407	*NR2F1, PBX1,TBX5*	0.964180909	*ETS2, NKX2.5,ZNF281*	0.930064274
AKT1, SNAI2,SOX9	1.016260163	*MESP1, NKX2.5,SRF*	0.962059621	*ESRRA, GATA4,MESP1*	0.929279422
GATA4, SRF,ZFPM2	1.015228426	*ETS2, IRX5,MITF*	0.961957311	*HAND1, NFYB,TBX5*	0.928862081
GATA4, IRX5,TBX20	1.014492754	*MESP1, NR2F1,SMAD6*	0.9611891	*ETS2, SNAI2,SRF*	0.928400928
ETS2, FHL2,SMARCD3	1.01448632	*HEY2, MESP1,SMARCD3*	0.960384154	*HEY2, NR2F1,MYOCD*	0.925890305
ESRRA, GATA4,TCF21	1.012225982	*ESRRA, GATA4,IRX5*	0.960260672	*ESRRA, IRX5,PBX1*	0.925066669
FHL2, IRX5,NKX2.5	1.012041008	*ETS2, GATA4,HAND1*	0.959989334	*GATA4, GATA6,MESP1*	0.925056264
ESRRA, SMAD6,ZNF281	1.011561321	*ID1, IRX5,MEF2C*	0.959594404	*MEF2C, SMAD6,SNAI2*	0.923200968
ESRRA, NKX2.5,PBX1	1.01010101	*ESRRG, FOSL1,GATA4*	0.958702065	*FHL2, GATA6,MITF*	0.923076923
PBX1, SMAD6,TBX5	1.009721066	*ESRRG, IRX5,NFYB*	0.958393985	*MEF2C, TBX20,ZNF281*	0.922330097
MESP1, SNAI2,TBX5	1.008702532	*ETS2, NFYB,TBX20*	0.957340387	*ESRRG, GATA4,IRX5*	0.922197208
IRX5, SMARCD3,SOX9	1.008507469	*ETS2, SMARCD3,TBX5*	0.956914953	*AKT1, HAND1,SRF*	0.921273032
ESRRA, HAND2,ID1	1.006416935	*ESRRA, SOX9,SRF*	0.956618231	*FHL2, MITF,NKX2.5*	0.920810313
HAND2, MEF2C,SOX9	1.006207236	*AKT1, NFYB,TCF21*	0.954198473	*GATA4, NFYB,ASCL1*	0.920810313
GATA6, MEF2C,SOX9	1.005747126	*ETS2, HEY2,IRX5*	0.953985062	*AKT1, FHL2,NFYB*	0.920663624
FOSL1, HAND1,ZFPM2	1.005025126	*ETS2, GATA6,HAND1*	0.952998975	*ETS2, HEY2,MEF2C*	0.920428845
GATA6, MEF2C,TBX5	1.002506266	*AKT1, GATA6,SNAI2*	0.95292894	*HAND1, ID1,TBX20*	0.920038754
FHL2, MITF,SMAD6	1.001540832	*HAND1, MITF,NFYB*	0.952380952	*ESRRA, MITF,NFYB*	0.918836141
AKT1, GATA6,ZNF281	1	*GATA4, ID1,TBX20*	0.952380952	*NFYB, SRF,TBX5*	0.917946004
ETS2, HAND1,ID1	0.999583222	*GATA6, IRX5,MYOCD*	0.952380952	*ESRRG, ETS2,NFYB*	0.91716672
ID1, MEF2C,TBX5	0.999246465	*AKT1, GATA6,SRF*	0.952277813	*ESRRA, MEF2C,SMARCD3*	0.916075367
ESRRA, ESRRG,MEF2C	0.996594006	*ESRRA, FHL2,SOX9*	0.951649173	*FOSL1, HAND2,MEF2C*	0.910625122
ETS2, FOSL1,MYOCD	0.996473607	*ID1, NFYB,PBX1*	0.950531711	*GATA4, IRX5,SMARCD3*	0.910268552
ESRRG, ETS2,NR2F1	0.995024876	*ESRRA, SOX9,TBX5*	0.950472228	*IRX5, NFYB,PBX1*	0.910184318
HEY2, MITF,MYOCD	0.994865212	*ESRRA, GATA4,ZFPM2*	0.950212462	*AKT1, GATA6,SOX9*	0.910184153
HEY2, IRX5,MESP1	0.994831766	*FHL2, TBX20,ASCL1*	0.949432696	*FOSL1, SNAI2,TBX5*	0.910029329
NR2F1, SMAD6,SMARCD3	0.993334967	*ETS2, SOX9,TBX20*	0.948216682	*ETS2, FOSL1,MEF2C*	0.909257868
GATA6, SNAI2,SRF	0.993258053	*FOSL1, MESP1,SMARCD3*	0.947645155	*ESRRA, SNAI2,TBX5*	0.908598483
ESRRG, ETS2,MESP1	0.99286033	*GATA4, HAND2,IRX5*	0.945970288	*TBX20, ZFPM2,ASCL1*	0.907441016
MESP1, PBX1,SMAD6	0.991730611	*ETS2, NKX2.5,SMARCD3*	0.944792132	*ESRRA, GATA4,MEF2C*	0.906655244
ESRRA, FOSL1,NR2F1	0.991681454	*FHL2, IRX5,TBX20*	0.944097718	*ESRRA, HAND2,TBX5*	0.906028652
ESRRA, HAND1,NR2F1	0.990951225	*AKT1, MESP1,NKX2.5*	0.944055944	*HAND1, NFYB,TBX20*	0.905764537
ESRRG, GATA6,SMAD6	0.99009901	*AKT1, FOSL1,ZNF281*	0.943891352	*ETS2, SMAD6,SRF*	0.904521355
ESRRA, FOSL1,GATA4	0.98934551	*FOSL1, HAND1,NKX2.5*	0.943639821	*AKT1, GATA6,TBX20*	0.903540422
IRX5, SOX9,ZNF281	0.988467875	*AKT1, FHL2,MITF*	0.943396226	*AKT1, FOSL1,GATA4*	0.902060941
FOSL1, MESP1,SMAD6	0.988012296	*ETS2, SMAD6,TBX20*	0.943327493	*ETS2, HEY2,MYOCD*	0.901552017
GATA6, HEY2,SNAI2	0.987654321	*FHL2, NR2F1,SOX9*	0.943273484	*ESRRG, NFYB,SRF*	0.901356837
HEY2, SRF,MYOCD	0.986970131	*ESRRG, ETS2,ZFPM2*	0.94236677	*FHL2, PBX1,TBX5*	0.898197802
FOSL1, NFYB,ASCL1	0.985545335	*GATA6, ID1,SNAI2*	0.941777044	*ETS2, HAND1,SRF*	0.897833022
GATA6, HAND2,HEY2	0.983661377	*HAND2, NKX2.5,SMAD6*	0.940292025	*AKT1, ESRRA,FHL2*	0.897663609
ETS2, SRF,MYOCD	0.982867179	*FOSL1, NKX2.5,TBX5*	0.940204435	*ETS2, ID1,TBX5*	0.89540658
ESRRA, IRX5,SNAI2	0.982682114	*HAND1, TBX5,ASCL1*	0.940144441	*FOSL1, GATA4,PBX1*	0.892376066
ETS2, IRX5,SRF	0.98256282	*ETS2, GATA6,TBX20*	0.93983448	*FHL2, FOSL1,NKX2.5*	0.892268109
ETS2, MESP1,SMAD6	0.981200958	*AKT1, SNAI2,TBX20*	0.939542484	*AKT1, ESRRG,SMAD6*	0.892153703
HAND1, HAND2,MESP1	0.980339251	*GATA6, HEY2,IRX5*	0.939288127	*GATA6, NKX2.5,SRF*	0.891036715
GATA4, NR2F1,SOX9	0.979694362	*FHL2, GATA4,TBX20*	0.937950938	*AKT1, FOSL1,SNAI2*	0.888356371
ETS2, FOSL1,SMARCD3	0.978514324	*ETS2, SMAD6,TBX5*	0.937785351	*HEY2, IRX5,ZFPM2*	0.887925842

Table S7. List of all 4,960 combinations tested and their respective reprogramming efficiency (MYH6^{+}TNNT2^{+})

TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells
HEY2, MEF2C,TBX5	0.887902331	*HEY2, MEF2C,MITF*	0.841750842	*ESRRG, HAND2,MEF2C*	0.810296796
FHL2, TCF21,MYOCD	0.886524823	*HAND1, HAND2,NR2F1*	0.841750842	*ESRRA, ETS2,TBX5*	0.810185185
ESRRA, HAND1,ID1	0.88593577	*IRX5, SMAD6,TBX5*	0.841085765	*NKX2.5, ZNF281,ASCL1*	0.809061489
ESRRG, GATA4,HAND2	0.885416667	*FHL2, ID1,SMARCD3*	0.840422931	*ETS2, GATA4,NR2F1*	0.80686312
MEF2C, MITF,TCF21	0.885121913	*ETS2, GATA4,PBX1*	0.839677152	*FHL2, SMARCD3,SOX9*	0.806721762
GATA4, HEY2,SOX9	0.884915277	*SMAD6, SMARCD3,TBX5*	0.839623885	*AKT1, NKX2.5,SMAD6*	0.8050293
HEY2, SNAI2,TBX20	0.883971145	*IRX5, SNAI2,TBX5*	0.839333586	*HAND2, TCF21,MYOCD*	0.804513133
AKT1, ETS2,NFYB	0.881976584	*GATA4, SMAD6,ZNF281*	0.839117933	*GATA6, NFYB,TBX20*	0.803916325
MESP1, SRF,ZFPM2	0.881410256	*GATA4, GATA6,NKX2.5*	0.837678667	*ETS2, FOSL1,HAND1*	0.803743163
GATA4, IRX5,ASCL1	0.879765396	*IRX5, ZFPM2,MYOCD*	0.837116327	*AKT1, SOX9,MYOCD*	0.802948867
FOSL1, ID1,IRX5	0.878776054	*NFYB, PBX1,ZNF281*	0.836120401	*NR2F1, SMAD6,TBX20*	0.802682778
FHL2, HEY2,SNAI2	0.874820881	*FOSL1, GATA6,HEY2*	0.835378074	*NFYB, PBX1,SMARCD3*	0.801991296
FOSL1, MESP1,TBX20	0.874769797	*NKX2.5, SMARCD3,SNAI2*	0.834824091	*AKT1, SMAD6,TBX20*	0.80034265
GATA4, ID1,MEF2C	0.87465346	*HAND2, TBX20,ZFPM2*	0.833333333	*GATA6, NFYB,NR2F1*	0.8
GATA6, NR2F1,SNAI2	0.873995557	*AKT1, TCF21,ASCL1*	0.831443689	*ESRRG, FOSL1,TBX20*	0.8
ETS2, HEY2,PBX1	0.872545184	*FHL2, IRX5,SMARCD3*	0.831329165	*ETS2, MEF2C,SMARCD3*	0.799485957
ESRRA, SMARCD3,TBX5	0.872239993	*FOSL1, GATA4,NFYB*	0.831022538	*NKX2.5, SOX9,SRF*	0.798383703
GATA4, HAND2,MESP1	0.871574441	*FHL2, GATA6,HAND2*	0.830913503	*ESRRA, MEF2C,TBX5*	0.797948243
ESRRG, MITF,TBX20	0.871305225	*FHL2, ID1,PBX1*	0.830639252	*GATA4, NFYB,SOX9*	0.796359499
IRX5, MESP1,NFYB	0.870247454	*NFYB, SOX9,MYOCD*	0.829776355	*ESRRG, FOSL1,ID1*	0.795366795
GATA4, NKX2.5,MYOCD	0.869486261	*ESRRG, GATA4,GATA6*	0.829473704	*GATA6, HEY2,MYOCD*	0.795012953
HAND2, NFYB,SRF	0.868621064	*PBX1, SMAD6,TBX20*	0.829352757	*NKX2.5, NR2F1,SOX9*	0.793650794
ID1, NFYB,SMARCD3	0.86835512	*AKT1, ESRRA,FOSL1*	0.82883231	*ETS2, MEF2C,NR2F1*	0.793650794
FHL2, TBX20,TBX5	0.865712585	*ESRRA, ESRRG,HAND2*	0.828499666	*MEF2C, PBX1,SRF*	0.793282254
MEF2C, SMARCD3,TBX5	0.865181552	*ESRRG, SRF,TBX5*	0.827521683	*HAND2, SMARCD3,SOX9*	0.791334398
PBX1, SOX9,TBX5	0.862522254	*ETS2, HAND1,MESP1*	0.82732664	*ETS2, MITF,NR2F1*	0.790513834
ETS2, SMARCD3,TBX20	0.860215054	*NR2F1, SRF,MYOCD*	0.826923077	*AKT1, ID1,SMAD6*	0.789562382
GATA4, SMARCD3,ZNF281	0.858704137	*GATA4, MITF,TBX5*	0.826446281	*ETS2, HAND1,HEY2*	0.78927074
AKT1, GATA4,PBX1	0.858012912	*GATA4, HAND1,SOX9*	0.826446281	*AKT1, ID1,SNAI2*	0.789204571
FOSL1, SMARCD3,TBX5	0.857414191	*ETS2, GATA6,SOX9*	0.825912696	*FOSL1, GATA4,SMARCD3*	0.789013274
HAND2, ID1,NKX2.5	0.857363426	*AKT1, HAND1,TBX20*	0.82426059	*AKT1, PBX1,TBX20*	0.788131664
HAND2, HEY2,TBX5	0.857361447	*PBX1, TBX5,TCF21*	0.824175824	*SMARCD3, TBX20,TBX5*	0.787943342
FHL2, NFYB,ASCL1	0.857360679	*ETS2, MITF,NFYB*	0.822894197	*MEF2C, NR2F1,PBX1*	0.787739648
FHL2, GATA6,ASCL1	0.855063939	*GATA4, HEY2,TBX20*	0.822831443	*FOSL1, NR2F1,ZFPM2*	0.784593438
PBX1, SMAD6,SOX9	0.854700855	*FHL2, ID1,SMAD6*	0.822265311	*SMARCD3, ZFPM2,MYOCD*	0.784106969
GATA4, NFYB,SMAD6	0.853837959	*HAND2, NR2F1,SNAI2*	0.821018062	*ESRRG, ETS2,TBX20*	0.783807063
ETS2, HAND2,HEY2	0.852500689	*ESRRA, TBX20,TBX5*	0.820820561	*AKT1, ESRRG,TBX5*	0.78369906
HAND1, NKX2.5,SRF	0.852272727	*GATA6, HAND1,IRX5*	0.818471894	*ID1, PBX1,TBX5*	0.78320715
AKT1, ETS2,FHL2	0.851055309	*ESRRA, NKX2.5,SOX9*	0.81842487	*ETS2, SMAD6,SNAI2*	0.782933877
TBX5, TCF21,MYOCD	0.851017715	*ETS2, FHL2,GATA6*	0.817472023	*ESRRA, NFYB,NR2F1*	0.782608696
IRX5, NFYB,TBX20	0.850969913	*HAND2, SNAI2,ASCL1*	0.817279626	*HEY2, NFYB,TBX5*	0.781798283
HEY2, MEF2C,TCF21	0.850734725	*HAND2, MESP1,MITF*	0.817214235	*MESP1, PBX1,TCF21*	0.78115456
ESRRA, IRX5,SOX9	0.850106809	*AKT1, IRX5,SMAD6*	0.817044469	*HAND2, MESP1,SNAI2*	0.780662645
HAND2, NR2F1,TBX20	0.849317848	*ID1, IRX5,TBX5*	0.816448123	*HEY2, NKX2.5,SNAI2*	0.780394137
ID1, SMAD6,ZFPM2	0.84777326	*FHL2, MESP1,NKX2.5*	0.816244702	*GATA6, NR2F1,SOX9*	0.780059607
ETS2, HAND1,MYOCD	0.847516258	*ESRRG, NR2F1,SRF*	0.815620366	*AKT1, FOSL1,PBX1*	0.777579844
SMARCD3, SOX9,TBX20	0.846856837	*ESRRG, ETS2,MITF*	0.815512465	*ETS2, NR2F1,SMAD6*	0.776870748
ETS2, GATA4,HAND2	0.846701699	*HAND2, SNAI2,TBX20*	0.814998359	*HAND2, ID1,IRX5*	0.774197116
IRX5, NR2F1,SOX9	0.846023689	*GATA4, SMARCD3,SOX9*	0.81300813	*MEF2C, SRF,TBX5*	0.773152184
AKT1, ZFPM2,MYOCD	0.845577761	*HEY2, PBX1,TBX5*	0.812982176	*HAND1, NFYB,PBX1*	0.771955244
AKT1, HAND2,SMARCD3	0.845009957	*FOSL1, HEY2,SMAD6*	0.812457753	*ETS2, GATA6,HEY2*	0.769869005
ETS2, HAND1,NKX2.5	0.844475721	*FHL2, GATA6,IRX5*	0.811767121	*MITF, NR2F1,ZNF281*	0.769230769
ESRRA, MEF2C,PBX1	0.842728322	*IRX5, MESP1,SRF*	0.811423014	*GATA4, HAND2,NFYB*	0.768882858
NR2F1, ZFPM2,ZNF281	0.842696629	*HAND2, ID1,NFYB*	0.810445552	*AKT1, ETS2,SMAD6*	0.768548101

Table S7. List of all 4,960 combinations tested and their respective reprogramming efficiency (MYH6^{+}TNNT2^{+})

TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells
FHL2, HAND2,SRF	0.76762398	*FOSL1, SOX9,ZFPM2*	0.73117939	*ESRRA, PBX1,TBX20*	0.697098976
FOSL1, ID1,ZFPM2	0.76645587	*NR2F1, SNAI2,MYOCD*	0.731176515	*HAND1, MESP1,TBX20*	0.695577544
HAND1, HEY2,MESP1	0.765098069	*ESRRA, NFYB,SNAI2*	0.729993541	*FOSL1, GATA6,NKX2.5*	0.694444444
FHL2, HEY2,TBX20	0.764069828	*HAND2, NFYB,SMARCD3*	0.729527581	*FHL2, ID1,MEF2C*	0.693676817
HAND2, NR2F1,SRF	0.763643059	*ETS2, NFYB,ZFPM2*	0.729525136	*ESRRA, MESP1,NKX2.5*	0.693540584
GATA4, NKX2.5,SOX9	0.762490226	*NFYB, SMAD6,SOX9*	0.728403811	*NFYB, TBX5,TCF21*	0.692840647
ETS2, FOSL1,NKX2.5	0.761481345	*ETS2, GATA4,NFYB*	0.728362221	*HAND2, MITF,SNAI2*	0.692383778
MESP1, SMARCD3,SOX9	0.761460761	*FOSL1, NFYB,TBX5*	0.72753901	*FOSL1, HAND2,NFYB*	0.691913779
AKT1, HEY2,NR2F1	0.76142132	*MESP1, SMARCD3,SNAI2*	0.727313819	*FOSL1, MESP1,SOX9*	0.69034033
GATA4, NKX2.5,ZNF281	0.760456274	*ETS2, MITF,TBX20*	0.72655218	*ESRRA, HAND1,MEF2C*	0.689506572
ESRRA, HAND2,ZFPM2	0.759734093	*ESRRG, MITF,ASCL1*	0.725639946	*ESRRA, SMARCD3,SRF*	0.688561886
ETS2, HAND1,SMAD6	0.758410478	*IRX5, SNAI2,TBX20*	0.725502472	*HAND2, NR2F1,MYOCD*	0.688419231
MEF2C, NKX2.5,NR2F1	0.757745389	*HAND2, NKX2.5,TBX5*	0.725257845	*HAND2, MEF2C,NR2F1*	0.687089443
HAND2, ID1,SOX9	0.757575758	*ETS2, ID1,SRF*	0.724641312	*SMAD6, TBX20,TBX5*	0.686929971
HAND1, HAND2,PBX1	0.757575758	*SRF, TBX20,TBX5*	0.723633436	*ESRRG, SMAD6,TBX5*	0.686868687
IRX5, NR2F1,PBX1	0.757520031	*AKT1, MESP1,SRF*	0.7227267	*GATA6, SMAD6,SNAI2*	0.686844538
ETS2, ID1,MYOCD	0.755492603	*ESRRA, MESP1,NR2F1*	0.722168188	*ESRRA, NKX2.5,TBX20*	0.685652609
IRX5, PBX1,SMARCD3	0.755128721	*GATA6, ZNF281,MYOCD*	0.722127463	*ETS2, HAND2,SOX9*	0.685540335
ID1, SMARCD3,SOX9	0.754771129	*IRX5, SMARCD3,SNAI2*	0.721727371	*FHL2, NR2F1,SNAI2*	0.685420956
PBX1, SMARCD3,ASCL1	0.75436115	*GATA6, MESP1,SMARCD3*	0.721299819	*ETS2, GATA4,MESP1*	0.684974832
GATA4, ID1,SOX9	0.752854766	*ESRRA, GATA4,HAND2*	0.721277692	*NKX2.5, ZFPM2,MYOCD*	0.68414143
ESRRG, ZNF281,MYOCD	0.752445448	*ETS2, ID1,NKX2.5*	0.720512491	*ESRRG, SRF,TBX20*	0.683341178
ESRRG, GATA6,IRX5	0.751802867	*SNAI2, SOX9,TBX20*	0.719420402	*FOSL1, SMARCD3,TBX20*	0.683071813
FOSL1, HEY2,ID1	0.751091034	*ETS2, HAND2,TBX5*	0.718518907	*ESRRG, HAND1,SRF*	0.681924988
ETS2, HEY2,NR2F1	0.75070028	*MESP1, SRF,TBX5*	0.715568867	*ETS2, HAND2,SNAI2*	0.68158701
ETS2, FHL2,ASCL1	0.75	*FHL2, HAND1,MEF2C*	0.715279971	*AKT1, SRF,TBX5*	0.681061014
ETS2, MITF,ASCL1	0.74875208	*MESP1, SMAD6,SNAI2*	0.714884972	*IRX5, NFYB,SNAI2*	0.680310508
ETS2, GATA6,SNAI2	0.748378555	*ETS2, ID1,NR2F1*	0.714399904	*GATA6, ID1,IRX5*	0.678057575
IRX5, SNAI2,SOX9	0.747188623	*HAND1, PBX1,SOX9*	0.714371114	*ETS2, NKX2.5,MYOCD*	0.677631754
SMAD6, SMARCD3,TBX20	0.746744381	*HEY2, SMAD6,SMARCD3*	0.713632959	*ESRRA, ETS2,MESP1*	0.677475301
ESRRG, NFYB,NR2F1	0.745303475	*FHL2, SRF,ZNF281*	0.713012478	*ESRRG, SMAD6,TBX20*	0.676977344
NKX2.5, NR2F1,PBX1	0.744366384	*NKX2.5, SMAD6,TBX20*	0.713012478	*GATA4, GATA6,TBX20*	0.676797718
ESRRA, FHL2,MEF2C	0.744047619	*HAND1, MESP1,NR2F1*	0.712250712	*HAND2, SMAD6,SMARCD3*	0.676328502
GATA6, NFYB,SMAD6	0.743464052	*HEY2, MEF2C,SMAD6*	0.71212648	*FHL2, NKX2.5,PBX1*	0.67612591
MEF2C, NR2F1,SRF	0.743023374	*ESRRG, NKX2.5,PBX1*	0.71123257	*FHL2, ID1,SNAI2*	0.675802543
FOSL1, SNAI2,ZFPM2	0.742300359	*GATA4, GATA6,SNAI2*	0.710606039	*FHL2, HEY2,SOX9*	0.675318554
ETS2, HAND1,NR2F1	0.740747118	*SMAD6, SNAI2,TCF21*	0.709219858	*ESRRA, ETS2,SOX9*	0.673605479
FHL2, MEF2C,SMARCD3	0.740605109	*AKT1, IRX5,SMARCD3*	0.708045238	*FHL2, FOSL1,HAND2*	0.673513255
ESRRG, FOSL1,ZNF281	0.740380631	*FHL2, NKX2.5,ASCL1*	0.706713781	*AKT1, HAND2,SMAD6*	0.67257393
HAND1, HEY2,SMAD6	0.740302433	*ETS2, HAND2,MYOCD*	0.70666517	*ETS2, GATA6,SMARCD3*	0.672188168
AKT1, HAND2,MESP1	0.740278466	*ETS2, GATA6,MEF2C*	0.705853718	*ETS2, HAND1,TCF21*	0.672167339
AKT1, ETS2,MYOCD	0.738611519	*ETS2, HAND2,NKX2.5*	0.70575056	*FHL2, PBX1,TBX20*	0.672145702
GATA4, SOX9,ZFPM2	0.737612613	*FHL2, GATA4,HAND1*	0.705518642	*NFYB, NR2F1,ZNF281*	0.672043011
GATA4, SMAD6,SOX9	0.737100737	*GATA6, MESP1,NR2F1*	0.705467372	*IRX5, MESP1,NKX2.5*	0.671243515
MEF2C, MESP1,ZFPM2	0.73664825	*ESRRA, FHL2,TBX20*	0.704721635	*ETS2, MEF2C,NKX2.5*	0.67114094
ESRRA, GATA6,PBX1	0.73664825	*ETS2, HEY2,SMAD6*	0.704288989	*ESRRA, MESP1,SOX9*	0.670039781
FHL2, NKX2.5,TBX5	0.736585366	*AKT1, IRX5,NFYB*	0.704194127	*SNAI2, TBX20,ZNF281*	0.668896321
ESRRA, HEY2,TBX5	0.736076257	*AKT1, FHL2,ID1*	0.703044137	*SRF, TBX5,ZFPM2*	0.668449198
AKT1, HEY2,ID1	0.735787311	*FHL2, GATA4,SRF*	0.702576112	*HAND1, IRX5,MESP1*	0.667859273
AKT1, SNAI2,TBX5	0.733466934	*ESRRA, HAND2,ZNF281*	0.701754386	*GATA6, PBX1,TBX5*	0.666512077
NR2F1, SNAI2,SOX9	0.732600733	*IRX5, SOX9,TBX20*	0.700280112	*GATA6, IRX5,MEF2C*	0.665396129
NR2F1, PBX1,TBX20	0.731955781	*MITF, PBX1,SNAI2*	0.700015731	*HEY2, ID1,SMAD6*	0.664384977
AKT1, HAND2,TBX5	0.731595793	*FOSL1, GATA6,SMARCD3*	0.699992082	*AKT1, NFYB,SOX9*	0.663805487
ESRRG, HAND1,TBX5	0.731257763	*FOSL1, GATA6,HAND1*	0.699112979	*GATA4, TBX20,ZFPM2*	0.663716814

Table S7. List of all 4,960 combinations tested and their respective reprogramming efficiency (MYH6^{+}TNNT2^{+})

TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells
FHL2, HAND2,SMAD6	0.662883461	*ETS2, SOX9,TBX5*	0.632562102	*NFYB, PBX1,ASCL1*	0.602409639
ID1, MESP1,PBX1	0.662432768	*GATA4, GATA6,NFYB*	0.632511069	*FHL2, IRX5,NFYB*	0.602409639
GATA6, TBX20,ASCL1	0.662251656	*FHL2, HAND2,TBX5*	0.63213798	*GATA6, ID1,NKX2.5*	0.602079912
IRX5, MITF,SNAI2	0.661853189	*FHL2, FOSL1,ID1*	0.631617927	*NKX2.5, SNAI2,TBX20*	0.601242149
ETS2, GATA6,HAND2	0.661480309	*GATA4, MESP1,ZFPM2*	0.630417652	*MEF2C, TCF21,MYOCD*	0.600985065
FHL2, MITF,ASCL1	0.660603339	*IRX5, SMAD6,ASCL1*	0.630228667	*HAND2, MEF2C,ASCL1*	0.598802395
MESP1, SOX9,TBX20	0.660561661	*AKT1, SRF,TCF21*	0.629115504	*ESRRA, GATA4,SOX9*	0.598531535
AKT1, HAND1,NKX2.5	0.660066007	*FHL2, PBX1,TCF21*	0.628930818	*HEY2, IRX5,MEF2C*	0.598020794
ESRRG, SMARCD3,SNAI2	0.659454464	*IRX5, NFYB,NR2F1*	0.627540024	*NKX2.5, SRF,ZFPM2*	0.597609562
ESRRA, ESRRG,ETS2	0.658761528	*SMARCD3, SNAI2,TBX20*	0.627159538	*ESRRG, ETS2,GATA4*	0.5968566
ESRRA, HAND2,NKX2.5	0.658761528	*FOSL1, HEY2,PBX1*	0.624355005	*FOSL1, SOX9,TBX5*	0.596114189
ETS2, NR2F1,TBX5	0.658761528	*HAND1, MEF2C,SMAD6*	0.623781676	*PBX1, SMARCD3,TBX20*	0.595940811
ESRRG, HAND2,ID1	0.658343724	*HEY2, MITF,TBX5*	0.623699351	*ETS2, SMARCD3,SRF*	0.595565382
HAND1, HAND2,SOX9	0.657894737	*FHL2, SRF,ASCL1*	0.623441397	*NFYB, SNAI2,TBX5*	0.593828254
IRX5, SMAD6,TBX20	0.656331408	*HAND2, SOX9,SRF*	0.622471211	*HEY2, SMARCD3,SRF*	0.593605316
ETS2, SRF,ZFPM2	0.656019186	*AKT1, GATA6,IRX5*	0.620905237	*HEY2, MEF2C,TBX20*	0.593151498
ETS2, HEY2,ZFPM2	0.655347036	*HEY2, IRX5,TCF21*	0.620732464	*ID1, SNAI2,TBX5*	0.592780972
IRX5, NFYB,SMAD6	0.65509564	*AKT1, MESP1,SOX9*	0.620567376	*ID1, NFYB,SMAD6*	0.592032334
ESRRG, GATA6,HAND2	0.6544399	*ESRRG, NR2F1,SOX9*	0.620277285	*ESRRG, PBX1,TBX20*	0.591868349
HAND1, SMAD6,SRF	0.654234858	*HAND1, MESP1,SNAI2*	0.619195046	*ESRRA, HAND2,SMAD6*	0.59127864
HAND1, IRX5,SRF	0.654131485	*ESRRG, FOSL1,TCF21*	0.61842919	*AKT1, PBX1,ZNF281*	0.590551181
ESRRA, ESRRG,TBX20	0.653704216	*SNAI2, SRF,ZFPM2*	0.618376928	*GATA4, ID1,NKX2.5*	0.59054614
HAND2, PBX1,TBX5	0.653594771	*ESRRA, SMARCD3,SNAI2*	0.61819335	*MESP1, MITF,TBX5*	0.589390963
ETS2, ID1,SOX9	0.652304705	*AKT1, NKX2.5,SNAI2*	0.617634626	*AKT1, IRX5,MESP1*	0.588235294
SOX9, SRF,TBX5	0.650598179	*FOSL1, NR2F1,MYOCD*	0.617575428	*ETS2, HAND1,SMARCD3*	0.58819453
GATA4, HAND2,MEF2C	0.650019776	*HAND1, SMARCD3,TBX5*	0.617365091	*ESRRG, MESP1,TBX20*	0.587597592
GATA6, HAND1,NR2F1	0.649350649	*HEY2, SMARCD3,SOX9*	0.617283951	*MEF2C, SMAD6,TCF21*	0.587371512
SNAI2, SOX9,MYOCD	0.648995444	*ESRRG, HAND2,SMARCD3*	0.617021277	*AKT1, GATA6,MEF2C*	0.586550383
GATA4, ID1,TBX5	0.646745628	*ETS2, GATA4,MYOCD*	0.615815892	*MITF, NKX2.5,TBX20*	0.584795322
ETS2, FOSL1,GATA4	0.646260063	*FOSL1, GATA6,ID1*	0.614622873	*GATA6, SMARCD3,SNAI2*	0.584783507
NFYB, NKX2.5,SRF	0.645693413	*GATA6, HAND1,SMARCD3*	0.613949766	*MESP1, NFYB,PBX1*	0.583800626
ID1, MESP1,SOX9	0.644972181	*FHL2, NKX2.5,TBX20*	0.613496933	*ESRRA, FHL2,IRX5*	0.583687172
SMARCD3, SOX9,MYOCD	0.644560637	*AKT1, ID1,ZFPM2*	0.61302682	*NR2F1, SMAD6,TCF21*	0.583090379
GATA6, HAND1,MITF	0.644539848	*FHL2, GATA4,NFYB*	0.612608184	*FHL2, HAND2,NFYB*	0.582765846
FHL2, GATA4,MESP1	0.643932264	*HAND1, PBX1,SMAD6*	0.612468607	*HAND1, HAND2,TBX20*	0.582322955
GATA4, MESP1,NR2F1	0.642978856	*GATA4, HEY2,SNAI2*	0.612423447	*ESRRA, GATA4,ZNF281*	0.58224163
HAND1, SNAI2,ASCL1	0.642562503	*HAND1, NKX2.5,SNAI2*	0.611519802	*ETS2, GATA6,NR2F1*	0.582094274
ETS2, NKX2.5,NR2F1	0.642049825	*FHL2, SMAD6,ASCL1*	0.611440088	*HAND2, NR2F1,PBX1*	0.581740616
AKT1, HAND1,SOX9	0.640632264	*ESRRG, SOX9,SRF*	0.611066263	*HAND2, MESP1,SRF*	0.581166273
ID1, SMARCD3,TBX5	0.640433828	*FOSL1, SNAI2,TCF21*	0.610997963	*FOSL1, MESP1,SRF*	0.581050716
SNAI2, SOX9,TBX5	0.638071167	*HAND1, IRX5,TBX20*	0.610640634	*GATA6, ID1,MESP1*	0.581030721
HAND1, MITF,TCF21	0.637958533	*FOSL1, HAND1,NR2F1*	0.609756098	*ESRRG, FOSL1,MEF2C*	0.579527999
ESRRA, ETS2,FOSL1	0.63785129	*ETS2, ID1,MITF*	0.609756098	*ETS2, NKX2.5,TBX20*	0.579312865
HAND2, HEY2,MESP1	0.637067476	*ESRRG, FHL2,HEY2*	0.609184403	*GATA6, MEF2C,ASCL1*	0.579150579
ESRRG, FHL2,SMAD6	0.63657113	*NR2F1, PBX1,SOX9*	0.608567489	*FHL2, GATA6,NKX2.5*	0.578034682
ESRRA, ETS2,SMAD6	0.636425955	*AKT1, FOSL1,HAND1*	0.608371517	*FHL2, HAND2,NR2F1*	0.577655523
ESRRG, HAND1,IRX5	0.636349339	*AKT1, NR2F1,MYOCD*	0.608284637	*HEY2, IRX5,SRF*	0.577517925
HAND2, MEF2C,SNAI2	0.63559322	*HEY2, TCF21,ZNF281*	0.607552343	*AKT1, PBX1,TCF21*	0.576701269
FOSL1, TCF21,MYOCD	0.635208711	*HEY2, MYOCD,ASCL1*	0.60754507	*FOSL1, MESP1,ZFPM2*	0.576701269
MESP1, SMAD6,SMARCD3	0.635045363	*NKX2.5, NR2F1,SNAI2*	0.606514511	*ESRRA, HEY2,SMAD6*	0.57621665
FHL2, HAND1,TCF21	0.634920635	*NKX2.5, SMAD6,SOX9*	0.606240934	*GATA6, HAND1,SOX9*	0.575818116
ETS2, PBX1,SNAI2	0.634661661	*FHL2, HAND2,ID1*	0.606060606	*SMAD6, SMARCD3,SOX9*	0.575659426
FHL2, FOSL1,SOX9	0.634163312	*GATA4, PBX1,SNAI2*	0.604125083	*FHL2, FOSL1,NFYB*	0.575177443
FOSL1, NKX2.5,SRF	0.632711602	*PBX1, SOX9,TBX20*	0.603626628	*HAND1, IRX5,ZFPM2*	0.574712644

Table S7. List of all 4,960 combinations tested and their respective reprogramming efficiency (MYH6^{+}TNNT2^{+})

TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells
AKT1, SMARCD3,TBX5	0.57397806	*AKT1, TBX20,TBX5*	0.544303797	*HAND1, NR2F1,MYOCD*	0.515773179
FOSL1, PBX1,SOX9	0.573487728	*FHL2, HEY2,SRF*	0.54391508	*SOX9, TBX20,TBX5*	0.514800515
ESRRA, ETS2,NKX2.5	0.572246066	*AKT1, FOSL1,ID1*	0.543620774	*HEY2, NFYB,SRF*	0.514800515
ID1, MEF2C,SNAI2	0.57185958	*GATA6, HAND2,SMARCD3*	0.543334916	*HEY2, MEF2C,NKX2.5*	0.51342812
FOSL1, NKX2.5,NR2F1	0.571428571	*HEY2, SMAD6,SOX9*	0.542783405	*ID1, NR2F1,SNAI2*	0.513264649
ESRRA, ID1,NFYB	0.570934028	*HAND1, SMAD6,TCF21*	0.54200542	*HAND1, TCF21,MYOCD*	0.513017752
FHL2, HAND1,NKX2.5	0.56980057	*ESRRA, ETS2,FHL2*	0.541913792	*FHL2, NR2F1,PBX1*	0.512820513
FOSL1, NR2F1,TBX20	0.568444359	*ETS2, FOSL1,MITF*	0.541790126	*MITF, NKX2.5,ZFPM2*	0.512477718
ESRRA, MESP1,ASCL1	0.568181818	*GATA6, HEY2,NR2F1*	0.541407219	*FHL2, MEF2C,MITF*	0.511945392
AKT1, IRX5,NR2F1	0.568181818	*AKT1, ESRRG,SMARCD3*	0.540775051	*GATA6, IRX5,TCF21*	0.511544562
ID1, MESP1,NFYB	0.566382461	*HEY2, MEF2C,SOX9*	0.540540541	*MEF2C, PBX1,TBX20*	0.510912772
GATA6, NR2F1,SRF	0.566325061	*ETS2, FOSL1,SOX9*	0.539260038	*HAND2, MEF2C,ZNF281*	0.509349859
MEF2C, TBX20,MYOCD	0.566179768	*AKT1, ESRRG,NKX2.5*	0.538899688	*FOSL1, MEF2C,MITF*	0.508269463
MESP1, SMAD6,TBX5	0.565887667	*HAND2, ID1,PBX1*	0.537946865	*MEF2C, NFYB,NKX2.5*	0.508002479
ESRRA, SMAD6,SMARCD3	0.565755636	*HAND1, ID1,TCF21*	0.537634409	*ESRRG, FHL2,PBX1*	0.507942041
ESRRA, ETS2,HEY2	0.562601951	*MEF2C, SNAI2,ZNF281*	0.536480687	*FOSL1, NR2F1,SNAI2*	0.507614213
HAND2, TBX20,TBX5	0.562587904	*ESRRA, ETS2,HAND2*	0.534624054	*FHL2, SOX9,MYOCD*	0.50731419
FHL2, GATA6,ID1	0.562368923	*FOSL1, ID1,NR2F1*	0.534402138	*FHL2, GATA4,SOX9*	0.506756757
AKT1, FHL2,HAND1	0.561963311	*ESRRG, NFYB,TCF21*	0.534402138	*FOSL1, GATA6,ASCL1*	0.506329114
HAND2, MESP1,PBX1	0.561748178	*MITF, TCF21,MYOCD*	0.533348457	*FOSL1, HAND1,MESP1*	0.506206442
ETS2, IRX5,TBX5	0.560826481	*MESP1, SRF,TBX20*	0.533251309	*AKT1, NFYB,SNAI2*	0.505747126
MEF2C, SRF,ZNF281	0.559701493	*FHL2, FOSL1,TBX20*	0.532407554	*MITF, SOX9,ZFPM2*	0.504574267
FOSL1, SNAI2,ASCL1	0.559701493	*AKT1, ID1,SMARCD3*	0.532270134	*ESRRG, GATA4,MEF2C*	0.504540308
ETS2, HAND2,IRX5	0.558771558	*MEF2C, SRF,TCF21*	0.530683204	*IRX5, NKX2.5,PBX1*	0.504504505
IRX5, NKX2.5,SMAD6	0.558589179	*ESRRG, NKX2.5,SRF*	0.530322404	*AKT1, NFYB,TBX20*	0.503784888
ESRRG, FOSL1,SMARCD3	0.558477232	*ID1, SOX9,TBX20*	0.529102001	*HEY2, SMAD6,TBX20*	0.503542348
PBX1, SNAI2,SOX9	0.558329246	*FHL2, HEY2,NKX2.5*	0.529100529	*NR2F1, SNAI2,TBX20*	0.502507932
ETS2, FOSL1,GATA6	0.558019289	*ETS2, NR2F1,PBX1*	0.529100529	*AKT1, ESRRG,MITF*	0.502232143
NKX2.5, SOX9,MYOCD	0.558000978	*SMAD6, SNAI2,TBX5*	0.528704808	*HAND1, PBX1,ASCL1*	0.501792115
ID1, SOX9,TCF21	0.555555556	*FHL2, MESP1,NR2F1*	0.527795204	*HAND2, HEY2,PBX1*	0.501253133
ETS2, GATA4,IRX5	0.554624569	*AKT1, ETS2,TBX20*	0.527577938	*AKT1, GATA4,NR2F1*	0.501253133
HEY2, NR2F1,TCF21	0.55392636	*ESRRA, GATA6,HAND2*	0.527413961	*IRX5, MESP1,NR2F1*	0.501111182
HAND2, SMAD6,TBX5	0.553556567	*PBX1, SMARCD3,ZFPM2*	0.526824772	*FHL2, GATA6,SOX9*	0.500752934
FOSL1, SOX9,MYOCD	0.553516068	*GATA6, SNAI2,SOX9*	0.526590772	*AKT1, FOSL1,ASCL1*	0.500575503
GATA4, IRX5,NFYB	0.55342699	*ESRRA, ESRRG,HAND1*	0.524109015	*AKT1, SMAD6,ZFPM2*	0.500500501
MEF2C, NFYB,TBX20	0.553244867	*FOSL1, ID1,SOX9*	0.523864781	*SRF, TBX20,ZNF281*	0.500417014
FHL2, NFYB,NR2F1	0.553204546	*FHL2, GATA6,ZNF281*	0.523166048	*AKT1, ESRRA,SMARCD3*	0.499545867
MITF, ZFPM2,ZNF281	0.551470588	*ESRRA, ID1,NKX2.5*	0.522875817	*GATA6, MITF,SNAI2*	0.499001996
MESP1, PBX1,TBX20	0.551077393	*ETS2, SRF,TCF21*	0.522088353	*HAND1, HEY2,NFYB*	0.498755013
NKX2.5, SMARCD3,TBX20	0.55068393	*NFYB, SMARCD3,TBX20*	0.521672307	*HAND2, ID1,SMAD6*	0.498667856
NR2F1, SMARCD3,SRF	0.550293854	*GATA6, IRX5,PBX1*	0.520935004	*HAND1, MEF2C,ASCL1*	0.49833887
AKT1, HEY2,SRF	0.550151717	*AKT1, ETS2,MEF2C*	0.520509194	*MEF2C, SOX9,MYOCD*	0.497139146
IRX5, PBX1,SNAI2	0.549940005	*FOSL1, HEY2,IRX5*	0.519833674	*ID1, MESP1,SMARCD3*	0.49712996
FHL2, NR2F1,ASCL1	0.54976329	*NKX2.5, ZFPM2,ASCL1*	0.519480519	*ESRRG, ID1,NKX2.5*	0.496861076
ESRRA, SRF,ZFPM2	0.549688488	*ESRRG, MESP1,SRF*	0.519296899	*SMAD6, TCF21,MYOCD*	0.496639891
HAND2, IRX5,MESP1	0.549644931	*IRX5, MEF2C,ZNF281*	0.519256211	*HEY2, SNAI2,TBX5*	0.49634105
HAND2, SMAD6,SRF	0.548998401	*NR2F1, SMARCD3,SNAI2*	0.518402275	*HAND2, HEY2,SMAD6*	0.495662949
ESRRA, ESRRG,SRF	0.548803626	*AKT1, ESRRA,SMAD6*	0.516795866	*FHL2, HEY2,NFYB*	0.495458409
MESP1, NKX2.5,ZNF281	0.548694492	*ESRRG, TBX5,ASCL1*	0.516795866	*ESRRA, ETS2,MEF2C*	0.495282123
FOSL1, HAND1,MYOCD	0.548486073	*NKX2.5, SMAD6,SMARCD3*	0.516795866	*ESRRG, FHL2,SOX9*	0.495049505
HAND1, HAND2,SMAD6	0.547939858	*ETS2, HAND2,SRF*	0.516387976	*GATA4, MEF2C,SOX9*	0.494960354
NKX2.5, SMAD6,SNAI2	0.546448087	*ETS2, GATA6,MESP1*	0.516231326	*ID1, ZFPM2,MYOCD*	0.494606733
HAND1, SOX9,TBX5	0.546030184	*GATA6, SMARCD3,SOX9*	0.516224189	*ID1, SMAD6,TBX20*	0.494490003
AKT1, FOSL1,ZFPM2	0.544781929	*ESRRA, HAND1,NKX2.5*	0.515995872	*ESRRA, ETS2,SNAI2*	0.494321292

Table S7. List of all 4,960 combinations tested and their respective reprogramming efficiency (MYH6^{+}TNNT2^{+})

TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells
FHL2, SOX9,TBX20	0.493096647	*ETS2, NFYB,PBX1*	0.468386408	*ETS2, HAND1,HAND2*	0.442445126
ESRRA, ESRRG,FHL2	0.492984452	*FHL2, MEF2C,NR2F1*	0.468340749	*ESRRG, SMAD6,SMARCD3*	0.442234556
AKT1, SMAD6,TBX5	0.492610837	*FOSL1, MESP1,NR2F1*	0.468021118	*ETS2, NFYB,NKX2.5*	0.441830756
AKT1, ESRRG,FOSL1	0.490890119	*AKT1, ESRRG,ID1*	0.46728972	*ESRRA, NFYB,ASCL1*	0.441102389
FOSL1, HAND2,PBX1	0.490196078	*PBX1, TBX20,ZNF281*	0.46728972	*FHL2, FOSL1,MEF2C*	0.440528634
SOX9, MYOCD,ASCL1	0.489795474	*AKT1, ETS2,FOSL1*	0.467016929	*AKT1, NR2F1,TCF21*	0.440044004
ESRRA, SMARCD3,TBX20	0.489123165	*ESRRA, HAND2,SMARCD3*	0.466977668	*NFYB, NR2F1,SNAI2*	0.440044004
FOSL1, NR2F1,SMAD6	0.48909549	*NFYB, SNAI2,SOX9*	0.466565523	*FHL2, HAND2,MITF*	0.439963773
HAND1, HEY2,IRX5	0.488400488	*HEY2, SOX9,SRF*	0.465889664	*HAND1, MESP1,SOX9*	0.438925903
HEY2, MESP1,PBX1	0.488400488	*FHL2, HAND1,IRX5*	0.46530182	*ID1, NKX2.5,TBX5*	0.438180427
SMARCD3, SOX9,SRF	0.487804878	*NR2F1, SMARCD3,TBX20*	0.464807437	*FHL2, NR2F1,SMAD6*	0.437842551
HAND2, SRF,ZNF281	0.487012987	*AKT1, ESRRG,ETS2*	0.462896109	*AKT1, NFYB,TBX5*	0.437779324
AKT1, HAND1,HAND2	0.485639533	*ETS2, MESP1,NKX2.5*	0.462780826	*AKT1, NR2F1,TBX5*	0.43765375
AKT1, IRX5,SOX9	0.485267421	*HAND2, SRF,TBX5*	0.46271542	*AKT1, FOSL1,TCF21*	0.436723602
HEY2, SNAI2,SOX9	0.485001241	*HAND2, ID1,ZNF281*	0.461680517	*ESRRA, GATA4,TBX20*	0.435695304
FHL2, MITF,ZFPM2	0.484670742	*GATA6, PBX1,SNAI2*	0.461205775	*HAND1, HAND2,SRF*	0.435341193
ESRRG, MEF2C,TBX5	0.48462125	*FHL2, IRX5,TBX5*	0.460932432	*AKT1, HAND1,ZFPM2*	0.434310532
ID1, IRX5,SRF	0.484224421	*FHL2, HAND2,TBX20*	0.460522603	*ESRRG, HAND1,HAND2*	0.433301383
GATA6, HEY2,TBX20	0.483864735	*HAND2, SOX9,TCF21*	0.460430713	*NFYB, NKX2.5,SMAD6*	0.432505883
ID1, SMAD6,SNAI2	0.483091787	*NFYB, NR2F1,PBX1*	0.460405157	*FHL2, MESP1,ASCL1*	0.432054452
HAND2, SRF,ASCL1	0.483091787	*GATA4, TCF21,ASCL1*	0.459770115	*HAND2, HEY2,TBX20*	0.4311509
ESRRA, HAND1,HAND2	0.482523516	*HAND1, SMARCD3,TBX20*	0.459317585	*ESRRA, FHL2,ASCL1*	0.429526018
GATA4, HEY2,ID1	0.48161708	*FHL2, ID1,TBX20*	0.458809589	*FHL2, HAND1,SRF*	0.429395166
IRX5, MEF2C,ZFPM2	0.481596147	*HAND1, SOX9,ASCL1*	0.458590169	*AKT1, GATA4,HEY2*	0.42878901
ESRRG, IRX5,PBX1	0.481441414	*ETS2, FHL2,NR2F1*	0.457939981	*HEY2, SNAI2,ZNF281*	0.428449015
IRX5, SMARCD3,TCF21	0.480294551	*IRX5, TCF21,MYOCD*	0.457533846	*ESRRG, NKX2.5,SNAI2*	0.426742532
SMAD6, ZNF281,MYOCD	0.479742723	*ESRRA, GATA6,HEY2*	0.457124842	*GATA4, ID1,NR2F1*	0.425250175
NFYB, PBX1,TBX20	0.479479281	*FHL2, NFYB,TCF21*	0.456621005	*GATA6, SMARCD3,TBX5*	0.424083383
ESRRA, PBX1,SOX9	0.479467501	*GATA6, HAND1,SMAD6*	0.456621005	*ESRRG, GATA4,ID1*	0.423728814
MITF, SMARCD3,SNAI2	0.479217309	*ESRRA, FOSL1,ID1*	0.456621005	*AKT1, HEY2,SNAI2*	0.423728814
NR2F1, SMAD6,ASCL1	0.478524743	*FOSL1, MEF2C,SMAD6*	0.456104476	*AKT1, ETS2,MITF*	0.423728814
GATA4, SNAI2,SOX9	0.477897252	*ETS2, NR2F1,SNAI2*	0.45540797	*AKT1, GATA6,SMAD6*	0.423280423
AKT1, HEY2,ZNF281	0.477466701	*GATA6, MESP1,TCF21*	0.455373406	*GATA4, IRX5,NR2F1*	0.422687992
ESRRA, HAND1,TBX20	0.476303626	*AKT1, FHL2,TBX20*	0.452178111	*IRX5, NKX2.5,SNAI2*	0.422463381
FHL2, NKX2.5,SNAI2	0.476190476	*AKT1, MEF2C,NR2F1*	0.451511007	*ESRRG, NR2F1,MYOCD*	0.421940928
ESRRG, MESP1,TBX5	0.476190476	*FHL2, HAND1,MESP1*	0.44982699	*AKT1, HAND1,TCF21*	0.421940928
ETS2, GATA4,NKX2.5	0.476190476	*GATA6, HEY2,PBX1*	0.449577745	*MESP1, NR2F1,SRF*	0.421940928
NKX2.5, PBX1,SMARCD3	0.475009857	*ETS2, MESP1,MYOCD*	0.449504845	*FHL2, HEY2,MEF2C*	0.421940928
AKT1, MEF2C,NKX2.5	0.474683544	*GATA6, NKX2.5,TBX20*	0.449383079	*ESRRG, TCF21,MYOCD*	0.4218507
FHL2, MEF2C,ZFPM2	0.474495848	*HAND1, ID1,TBX5*	0.448868492	*FOSL1, GATA6,SOX9*	0.421052632
ETS2, GATA6,PBX1	0.473197335	*ID1, SMAD6,SMARCD3*	0.447793303	*ETS2, TBX20,ASCL1*	0.421052632
ID1, NFYB,NKX2.5	0.472813239	*ESRRG, GATA4,NKX2.5*	0.447776418	*FOSL1, GATA4,ID1*	0.420121719
AKT1, ESRRA,TBX20	0.472519688	*ETS2, SOX9,SRF*	0.447449557	*HEY2, ID1,PBX1*	0.419947507
HAND2, ID1,TBX20	0.471561981	*ESRRG, NKX2.5,SMAD6*	0.447196422	*GATA6, ID1,TCF21*	0.418410042
GATA6, MESP1,SOX9	0.471320095	*HAND2, HEY2,ID1*	0.446648021	*AKT1, ID1,TBX5*	0.418303431
FOSL1, MITF,ASCL1	0.470957614	*FOSL1, GATA4,HEY2*	0.446591353	*HAND2, HEY2,MYOCD*	0.418300654
HEY2, IRX5,SNAI2	0.470922334	*FHL2, GATA4,PBX1*	0.445996276	*AKT1, ETS2,HAND2*	0.41825309
HAND2, NKX2.5,ZNF281	0.470588235	*HAND2, SNAI2,SOX9*	0.44525891	*FHL2, MEF2C,ASCL1*	0.418111899
AKT1, ESRRG,SNAI2	0.470431908	*ESRRG, FHL2,ID1*	0.44523758	*AKT1, FOSL1,TBX5*	0.418001526
ETS2, FHL2,MITF	0.469964515	*ESRRG, ID1,ASCL1*	0.444830034	*ETS2, HEY2,TBX20*	0.417800091
AKT1, ESRRG,PBX1	0.469777202	*HEY2, NR2F1,SOX9*	0.444425016	*PBX1, SNAI2,TBX20*	0.417020666
ETS2, GATA6,MITF	0.469483568	*ETS2, NKX2.5,SNAI2*	0.443201823	*HAND2, NFYB,SMAD6*	0.416995277
AKT1, HEY2,NKX2.5	0.469441258	*HAND1, MITF,NR2F1*	0.442967885	*MESP1, NKX2.5,TBX20*	0.416666667
NFYB, SMARCD3,SOX9	0.4684952	*FOSL1, MITF,ZFPM2*	0.442477876		

Table S7. List of all 4,960 combinations tested and their respective reprogramming efficiency (MYH6^{+}TNNT2^{+})

TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells
AKT1, MEF2C,MITF	0.416400596	*ETS2, SRF,ASCL1*	0.396825397	*ESRRG, IRX5,MITF*	0.368550369
ESRRG, IRX5,MESP1	0.416367265	*ESRRG, MESP1,SMAD6*	0.395941446	*MESP1, NFYB,TBX5*	0.368273625
ESRRG, ID1,MESP1	0.416224224	*SMAD6, ZNF281,ASCL1*	0.395916141	*FHL2, HAND2,SOX9*	0.368258652
ESRRA, NFYB,SRF	0.415895307	*ID1, NKX2.5,SOX9*	0.395256917	*MEF2C, ZFPM2,ZNF281*	0.368033649
HEY2, SOX9,TBX5	0.414737004	*HAND1, SNAI2,ZNF281*	0.394944708	*HAND1, HAND2,HEY2*	0.367937724
ETS2, GATA6,ASCL1	0.414593698	*ID1, SMAD6,SOX9*	0.394322644	*AKT1, MITF,TCF21*	0.367917586
HAND1, HEY2,NKX2.5	0.414499411	*FHL2, IRX5,SOX9*	0.394085234	*GATA4, GATA6,NR2F1*	0.367640215
IRX5, SRF,TBX20	0.413666793	*ESRRA, ZFPM2,ASCL1*	0.393479483	*NKX2.5, SMAD6,ZFPM2*	0.36687631
FOSL1, IRX5,NR2F1	0.413153171	*IRX5, NKX2.5,NR2F1*	0.393123792	*ESRRG, IRX5,MEF2C*	0.366177498
FOSL1, ZFPM2,ZNF281	0.411522634	*ID1, SMAD6,TCF21*	0.392490907	*GATA4, HAND1,ASCL1*	0.364737839
ID1, NKX2.5,SMAD6	0.411387309	*AKT1, ESRRA,SNAI2*	0.392274972	*ESRRG, PBX1,TBX5*	0.364621828
FHL2, HAND2,SNAI2	0.409884233	*MESP1, PBX1,TBX5*	0.391968868	*ESRRG, GATA4,SOX9*	0.364298725
FHL2, FOSL1,HEY2	0.409276944	*ESRRA, HAND1,SNAI2*	0.391154184	*ESRRA, NFYB,SMARCD3*	0.364236093
ID1, PBX1,SMARCD3	0.409210136	*ESRRA, NFYB,TBX5*	0.390673165	*FHL2, SNAI2,TBX20*	0.363043658
ESRRA, TBX20,ZNF281	0.408163265	*HEY2, NFYB,NR2F1*	0.389984645	*AKT1, IRX5,NKX2.5*	0.362608978
ESRRA, IRX5,SMARCD3	0.407925408	*ESRRA, ESRRG,MITF*	0.38961039	*HEY2, SMAD6,TBX5*	0.362578721
ID1, IRX5,SNAI2	0.407921945	*SRF, ZFPM2,MYOCD*	0.38961039	*MEF2C, NFYB,NR2F1*	0.362498822
ESRRA, ESRRG,SNAI2	0.407322692	*ETS2, PBX1,TBX20*	0.389105058	*ESRRG, SMAD6,SOX9*	0.361917851
FHL2, HAND1,HEY2	0.407128067	*AKT1, ESRRA,SRF*	0.388241819	*AKT1, HAND1,TBX5*	0.361662041
ESRRG, GATA6,ASCL1	0.406504065	*FOSL1, ID1,MEF2C*	0.388161087	*HAND1, SNAI2,SOX9*	0.361590038
ESRRG, SRF,ASCL1	0.406504065	*ESRRA, GATA4,SMARCD3*	0.387960062	*ESRRG, MEF2C,SNAI2*	0.361263758
FOSL1, GATA6,HAND2	0.406504065	*ESRRA, ESRRG,NFYB*	0.387596899	*ESRRG, HAND1,TBX20*	0.360800378
HAND2, NR2F1,SOX9	0.406504065	*NFYB, PBX1,TBX5*	0.386159618	*SMAD6, TBX20,ASCL1*	0.359590079
FOSL1, SMARCD3,ZFPM2	0.406504065	*ETS2, GATA6,NKX2.5*	0.384981763	*ESRRG, NFYB,SMAD6*	0.358585623
ESRRG, GATA6,SOX9	0.406504065	*ETS2, NR2F1,SMARCD3*	0.384615385	*MESP1, NFYB,NR2F1*	0.358422939
NKX2.5, PBX1,TBX5	0.40626211	*ESRRG, NFYB,NKX2.5*	0.383877159	*ID1, NFYB,TBX5*	0.356728292
AKT1, GATA4,TBX20	0.405882865	*FHL2, HAND2,IRX5*	0.383385678	*FOSL1, MESP1,TCF21*	0.356506239
MESP1, NKX2.5,PBX1	0.405813206	*HAND1, MITF,TBX5*	0.382165605	*HAND1, NFYB,SRF*	0.356506239
ESRRG, MEF2C,SRF	0.40578023	*AKT1, IRX5,PBX1*	0.381770091	*FHL2, HEY2,PBX1*	0.356421234
GATA6, IRX5,NKX2.5	0.405690427	*GATA6, SNAI2,TBX20*	0.381370044	*ETS2, GATA4,TCF21*	0.356325427
IRX5, MESP1,PBX1	0.405530568	*SMARCD3, SNAI2,SOX9*	0.38091612	*FOSL1, GATA6,MEF2C*	0.355797009
NFYB, SMARCD3,ZFPM2	0.405340963	*FHL2, HAND2,HEY2*	0.38082254	*GATA6, PBX1,TBX20*	0.355555556
FHL2, IRX5,SNAI2	0.404891463	*HAND2, PBX1,SOX9*	0.380677993	*FOSL1, HAND2,NR2F1*	0.355500063
ESRRG, HEY2,IRX5	0.404804691	*ESRRG, HAND1,MITF*	0.380228137	*FOSL1, NFYB,NKX2.5*	0.35502974
FHL2, HAND1,SMARCD3	0.40371875	*ESRRG, HAND2,SMAD6*	0.378877685	*ESRRA, NR2F1,TBX20*	0.354143182
ESRRG, GATA4,NR2F1	0.403694266	*AKT1, ESRRA,HEY2*	0.377417587	*ESRRG, MESP1,ZNF281*	0.35335689
IRX5, SMARCD3,TBX20	0.403414679	*ESRRG, SMARCD3,TBX20*	0.376849971	*NFYB, SNAI2,ZNF281*	0.35335689
ESRRA, ESRRG,IRX5	0.403244631	*ESRRA, HAND1,IRX5*	0.376239647	*FOSL1, NFYB,TBX20*	0.353317221
ESRRG, ETS2,GATA6	0.402823141	*ID1, NKX2.5,TBX20*	0.375659958	*ESRRG, HEY2,TBX5*	0.352945094
ID1, NKX2.5,PBX1	0.401967678	*GATA4, NKX2.5,SNAI2*	0.37552784	*TCF21, ZFPM2,MYOCD*	0.352941176
MEF2C, TCF21,ASCL1	0.401606426	*SNAI2, TBX20,TBX5*	0.37541501	*FOSL1, PBX1,ASCL1*	0.352733686
ESRRG, FHL2,GATA4	0.401606426	*HAND2, NKX2.5,SMARCD3*	0.374816593	*FHL2, NR2F1,SMARCD3*	0.352633478
HEY2, PBX1,TBX20	0.401314372	*AKT1, ESRRA,PBX1*	0.374531835	*HEY2, MESP1,NFYB*	0.351624689
HEY2, NKX2.5,SRF	0.401131436	*MESP1, NR2F1,MYOCD*	0.374430845	*ESRRA, FHL2,NFYB*	0.351149119
ID1, NFYB,SOX9	0.400801603	*ESRRG, HEY2,SOX9*	0.373095815	*FOSL1, IRX5,MYOCD*	0.350877193
ID1, SRF,ASCL1	0.400641026	*AKT1, HEY2,PBX1*	0.372530972	*GATA6, HEY2,NFYB*	0.349134499
HEY2, PBX1,SMARCD3	0.400184603	*ETS2, MITF,NKX2.5*	0.372439479	*FOSL1, SOX9,TCF21*	0.34904014
FHL2, FOSL1,TBX5	0.4	*FHL2, SOX9,TBX5*	0.370624008	*AKT1, HEY2,IRX5*	0.3480514
GATA6, HAND1,MESP1	0.399201597	*FHL2, HEY2,SMARCD3*	0.370457532	*ESRRG, SOX9,ASCL1*	0.346620451
NFYB, PBX1,TCF21	0.398722758	*NFYB, NKX2.5,TBX5*	0.37037037	*HAND2, NFYB,PBX1*	0.345132932
ID1, NKX2.5,NR2F1	0.398428732	*HAND2, MEF2C,NKX2.5*	0.37037037	*ESRRG, GATA4,HAND1*	0.344902368
FOSL1, SMARCD3,ASCL1	0.398373371	*ESRRG, NKX2.5,SMARCD3*	0.370343056	*ESRRA, IRX5,NR2F1*	0.344860166
MESP1, NKX2.5,SMARCD3	0.39750142	*MITF, SMAD6,SNAI2*	0.369344414	*SNAI2, SOX9,TCF21*	0.344827586
GATA4, HAND2,ZNF281	0.397192262	*HAND2, MITF,TBX20*	0.369300096	*HAND2, ID1,TCF21*	0.34466235

Table S7. List of all 4,960 combinations tested and their respective reprogramming efficiency (MYH6^{+}TNNT2^{+})

TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells
AKT1, SOX9,ZFPM2	0.344638141	*NFYB, NR2F1,SMAD6*	0.324981379	*ETS2, FOSL1,HAND2*	0.306845756
GATA6, HAND2,MESP1	0.343304435	*ETS2, SMARCD3,SNAI2*	0.324361629	*AKT1, HAND2,NFYB*	0.306438405
GATA6, SMARCD3,ZFPM2	0.343267557	*GATA6, NKX2.5,TCF21*	0.324348017	*IRX5, MEF2C,ASCL1*	0.305810398
NR2F1, SOX9,TCF21	0.343249428	*GATA6, SMAD6,TCF21*	0.324141018	*ESRRG, HAND1,NR2F1*	0.305810398
NR2F1, SMARCD3,SOX9	0.342338252	*GATA4, MITF,SMARCD3*	0.323275862	*HEY2, TBX5,ZNF281*	0.305810398
NR2F1, PBX1,SMAD6	0.341880342	*ESRRG, ID1,TBX20*	0.322759735	*ESRRA, ESRRG,HEY2*	0.305143854
FHL2, NKX2.5,ZFPM2	0.341880342	*ESRRA, ID1,SRF*	0.322729368	*FOSL1, MEF2C,ZFPM2*	0.304692261
AKT1, HEY2,SMARCD3	0.341756665	*ID1, MESP1,SNAI2*	0.322723065	*HAND2, TCF21,ZFPM2*	0.304429847
ESRRG, FHL2,ASCL1	0.341685649	*HAND1, HEY2,TBX20*	0.322415439	*AKT1, HAND2,TCF21*	0.304414003
ETS2, GATA4,MITF	0.341685649	*ESRRA, GATA6,MESP1*	0.322004708	*AKT1, NFYB,SMARCD3*	0.304295282
ESRRA, IRX5,TBX20	0.341630063	*ESRRG, GATA6,MITF*	0.321543408	*HEY2, PBX1,SNAI2*	0.304097666
MESP1, NR2F1,ZFPM2	0.341530055	*ID1, MEF2C,NFYB*	0.320820535	*AKT1, HEY2,TBX20*	0.303883283
HEY2, SMAD6,SNAI2	0.34111497	*ESRRA, GATA4,NR2F1*	0.320088548	*NFYB, SMARCD3,SNAI2*	0.303508009
ESRRG, FHL2,SNAI2	0.340597677	*PBX1, ZNF281,MYOCD*	0.319364544	*ESRRG, ETS2,SOX9*	0.303424361
ESRRA, HEY2,TBX20	0.340550283	*FOSL1, NFYB,SOX9*	0.319197446	*GATA4, HAND1,NFYB*	0.303145219
PBX1, SMARCD3,SOX9	0.340136054	*ESRRA, NR2F1,SOX9*	0.318979266	*FOSL1, IRX5,SMARCD3*	0.303078726
ESRRA, PBX1,ASCL1	0.340096132	*NFYB, SOX9,TCF21*	0.318471338	*ESRRA, GATA6,TBX5*	0.303030303
ESRRA, HEY2,ID1	0.33970908	*ESRRG, IRX5,TBX20*	0.318350809	*NR2F1, SMAD6,SRF*	0.302357152
GATA6, HAND1,SNAI2	0.339563587	*FHL2, HAND2,NKX2.5*	0.317460317	*ESRRA, MESP1,TCF21*	0.302114804
FOSL1, HAND1,TBX20	0.337642901	*ID1, MITF,TBX20*	0.317460317	*HAND2, MITF,TCF21*	0.302114804
AKT1, HAND1,MEF2C	0.337552743	*SRF, TBX20,TCF21*	0.316797712	*ESRRG, HEY2,SRF*	0.301659125
MEF2C, SNAI2,SRF	0.336700337	*AKT1, HAND2,HEY2*	0.315955766	*ID1, SMARCD3,ZFPM2*	0.301659125
ESRRA, FOSL1,SMAD6	0.335768685	*FOSL1, ID1,NFYB*	0.315873016	*AKT1, MESP1,NR2F1*	0.300808709
ID1, PBX1,TBX20	0.335187787	*IRX5, TBX5,TCF21*	0.315457413	*FHL2, HAND1,ASCL1*	0.300601202
HAND2, MESP1,SOX9	0.335008375	*HAND2, NFYB,ASCL1*	0.315208826	*FHL2, HEY2,ID1*	0.300576498
IRX5, NR2F1,SMARCD3	0.334966057	*ESRRA, MITF,TBX5*	0.31512605	*ID1, MITF,TCF21*	0.300497647
MEF2C, NFYB,TCF21	0.334900231	*GATA6, HAND2,PBX1*	0.314841384	*AKT1, MESP1,ASCL1*	0.300009639
GATA6, PBX1,SMARCD3	0.334554682	*MITF, SRF,MYOCD*	0.314729054	*AKT1, MITF,NKX2.5*	0.299401198
FOSL1, SMARCD3,TCF21	0.334448161	*IRX5, MITF,TCF21*	0.314465409	*ESRRA, IRX5,MITF*	0.299401198
NFYB, SOX9,SRF	0.333354283	*NKX2.5, SMARCD3,SOX9*	0.314232791	*GATA4, MITF,NR2F1*	0.298210736
ETS2, IRX5,ZFPM2	0.333333333	*ESRRA, FOSL1,NFYB*	0.313824181	*ESRRA, MEF2C,TBX20*	0.297642618
NR2F1, SMAD6,SOX9	0.332813819	*ESRRA, PBX1,TBX5*	0.313620072	*MEF2C, SOX9,TBX20*	0.297619048
AKT1, MEF2C,SNAI2	0.332502078	*AKT1, ETS2,SOX9*	0.313601063	*ETS2, FOSL1,NR2F1*	0.297619048
GATA6, NKX2.5,SMAD6	0.332225914	*IRX5, SMAD6,TCF21*	0.313479624	*NKX2.5, PBX1,SNAI2*	0.297526848
HEY2, MEF2C,PBX1	0.332225914	*ETS2, IRX5,SOX9*	0.312989045	*ETS2, FOSL1,HEY2*	0.297505566
HEY2, MESP1,SOX9	0.332016283	*ETS2, SMAD6,SMARCD3*	0.312931204	*NFYB, SOX9,ASCL1*	0.296625881
ESRRG, FHL2,ZFPM2	0.332005312	*AKT1, ESRRA,TBX5*	0.312779267	*ESRRA, HAND2,HEY2*	0.2962209
ESRRG, HAND2,MESP1	0.331271018	*ESRRA, ESRRG,GATA6*	0.3125	*HEY2, NFYB,SOX9*	0.296183313
ESRRG, MITF,SOX9	0.331125828	*ESRRA, SOX9,TBX20*	0.31225605	*GATA6, MESP1,ASCL1*	0.295857988
ESRRG, MITF,TBX5	0.331125828	*ETS2, GATA4,SMARCD3*	0.311640775	*PBX1, SMARCD3,SNAI2*	0.29523991
FHL2, IRX5,MITF	0.330396476	*ESRRA, FHL2,SRF*	0.311546841	*GATA6, SMARCD3,TBX20*	0.295053366
AKT1, MITF,ASCL1	0.330250991	*HAND1, NR2F1,ZFPM2*	0.31152648	*IRX5, TBX5,ZFPM2*	0.294985251
ESRRG, MEF2C,SMARCD3	0.330120995	*NKX2.5, TBX20,TCF21*	0.310077519	*ESRRG, GATA4,MESP1*	0.294985251
FHL2, FOSL1,TCF21	0.330033003	*NFYB, NKX2.5,ASCL1*	0.310077519	*SNAI2, TCF21,MYOCD*	0.294860994
ID1, NR2F1,SMARCD3	0.329777442	*FOSL1, GATA4,SOX9*	0.30989142	*FOSL1, GATA6,MESP1*	0.294117647
AKT1, FHL2,SMAD6	0.329566855	*GATA4, TBX5,ASCL1*	0.309178836	*FOSL1, HAND2,IRX5*	0.29358607
FHL2, GATA4,HEY2	0.329034967	*AKT1, ESRRA,ASCL1*	0.309119011	*ESRRA, ID1,PBX1*	0.293480615
ESRRA, NKX2.5,ASCL1	0.328947368	*HEY2, MEF2C,MYOCD*	0.309050773	*FHL2, GATA4,TBX5*	0.29337232
ESRRA, ETS2,TBX20	0.327674855	*HAND2, MITF,ASCL1*	0.308641975	*ESRRA, GATA6,TCF21*	0.293255132
HAND1, ID1,SMARCD3	0.327330388	*AKT1, SNAI2,TCF21*	0.308641975	*ESRRA, FOSL1,SNAI2*	0.292991838
GATA4, IRX5,ZFPM2	0.326797386	*GATA4, HAND1,HEY2*	0.307933212	*ESRRG, NR2F1,TBX20*	0.292158249
MESP1, NKX2.5,SNAI2	0.326797386	*ESRRA, NFYB,PBX1*	0.307482135	*FHL2, NKX2.5,SMARCD3*	0.292087542
ESRRG, PBX1,SMAD6	0.325732899	*NKX2.5, SMARCD3,TCF21*	0.307469057	*AKT1, ESRRG,MEF2C*	0.291851309
ESRRA, ETS2,GATA6	0.325333027	*GATA6, HAND2,TBX20*	0.307467171	*ETS2, GATA4,ASCL1*	0.29179578

Table S7. List of all 4,960 combinations tested and their respective reprogramming efficiency (MYH6^{+}TNNT2^{+})

TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells
ID1, PBX1,SMAD6	0.291414581	*IRX5, PBX1,SOX9*	0.276243094	*AKT1, TCF21,MYOCD*	0.259401018
GATA4, HEY2,ASCL1	0.291262136	*ETS2, SMARCD3,SOX9*	0.276176054	*PBX1, SOX9,ASCL1*	0.259067358
AKT1, ZFPM2,ZNF281	0.291120815	*FHL2, SMAD6,SOX9*	0.276166196	*AKT1, FOSL1,HEY2*	0.258768013
ESRRA, SMAD6,SNAI2	0.290136203	*ETS2, SNAI2,TCF21*	0.275482094	*AKT1, TCF21,ZNF281*	0.258397933
NFYB, NKX2.5,SMARCD3	0.290120244	*ESRRG, HEY2,NKX2.5*	0.275482094	*IRX5, NR2F1,ASCL1*	0.258397933
ETS2, TCF21,ZNF281	0.289859929	*FOSL1, NFYB,SNAI2*	0.275471115	*GATA6, SMARCD3,TCF21*	0.258262266
ESRRA, SMARCD3,ZFPM2	0.289855072	*FOSL1, SMAD6,ASCL1*	0.275229358	*NFYB, NKX2.5,TBX20*	0.256793559
MESP1, TBX20,TBX5	0.289855072	*HEY2, ID1,MEF2C*	0.274348422	*ETS2, TBX20,ZNF281*	0.256410256
FHL2, TBX5,ASCL1	0.28957529	*ESRRG, MITF,SRF*	0.274348422	*AKT1, NR2F1,SMARCD3*	0.256081946
HAND2, SMARCD3,SRF	0.289256198	*FHL2, HAND1,HAND2*	0.274348422	*AKT1, ESRRA,ESRRG*	0.255767791
ESRRA, HAND1,PBX1	0.288653223	*SNAI2, ZFPM2,ASCL1*	0.274348422	*ETS2, MITF,ZFPM2*	0.255427842
HEY2, MITF,ASCL1	0.288600289	*FHL2, MITF,SNAI2*	0.274223035	*HEY2, IRX5,NKX2.5*	0.255355852
SOX9, ZNF281,MYOCD	0.287749174	*ESRRA, HAND2,NFYB*	0.272676633	*SMAD6, TBX5,ZFPM2*	0.255102041
ESRRG, MEF2C,PBX1	0.287545186	*GATA6, SOX9,ZNF281*	0.272108844	*HEY2, IRX5,SMARCD3*	0.254920665
AKT1, NKX2.5,NR2F1	0.287521639	*ESRRG, NR2F1,SMAD6*	0.2720942	*NR2F1, SNAI2,TBX5*	0.254862703
AKT1, MESP1,ZNF281	0.287356322	*FHL2, TBX5,TCF21*	0.272039072	*AKT1, FOSL1,NR2F1*	0.254722594
GATA6, ID1,NR2F1	0.287356322	*ESRRG, HAND2,TBX20*	0.272014065	*GATA6, MESP1,SMAD6*	0.254452926
FHL2, GATA4,MITF	0.287356322	*HAND2, TCF21,ZNF281*	0.27173913	*AKT1, GATA4,GATA6*	0.254065041
HAND1, ZNF281,ASCL1	0.287356322	*GATA6, MITF,SRF*	0.27173913	*HEY2, NFYB,NKX2.5*	0.253921663
ESRRA, GATA4,ASCL1	0.286944046	*FHL2, HEY2,IRX5*	0.271444083	*MEF2C, MITF,ASCL1*	0.253485425
AKT1, HEY2,SMAD6	0.286650287	*HEY2, SMARCD3,TBX5*	0.271402921	*NKX2.5, TBX5,TCF21*	0.253164557
SMARCD3, SOX9,TCF21	0.286532951	*GATA6, NFYB,SOX9*	0.27100271	*FHL2, SMARCD3,ZNF281*	0.253164557
ETS2, HAND2,MITF	0.286532951	*FOSL1, HAND1,MITF*	0.27100271	*ESRRG, FHL2,MESP1*	0.253077643
ESRRG, MEF2C,SOX9	0.285854719	*GATA6, ID1,NFYB*	0.269909957	*ID1, NR2F1,TBX20*	0.25273781
HAND1, TBX20,ZNF281	0.284900285	*ETS2, HEY2,TBX5*	0.269500634	*GATA6, SMAD6,ZFPM2*	0.252525253
ETS2, SMARCD3,ASCL1	0.284875241	*FOSL1, MESP1,NFYB*	0.268393433	*AKT1, PBX1,SNAI2*	0.251305656
ESRRG, GATA4,SNAI2	0.284814815	*GATA6, MESP1,TBX20*	0.268096515	*FHL2, HEY2,TBX5*	0.25086789
HEY2, IRX5,PBX1	0.284324729	*FHL2, PBX1,ASCL1*	0.267887041	*ESRRG, GATA6,ID1*	0.250626566
HEY2, MESP1,TBX20	0.28399006	*AKT1, ETS2,SNAI2*	0.267522739	*FOSL1, NKX2.5,TCF21*	0.250626566
AKT1, HAND2,SNAI2	0.28293035	*ESRRA, MITF,TCF21*	0.266666667	*FHL2, HAND1,NR2F1*	0.248756219
ID1, PBX1,SOX9	0.282576837	*FHL2, MESP1,TCF21*	0.266666667	*IRX5, SOX9,TBX5*	0.248550321
GATA4, TCF21,ZNF281	0.282485876	*ESRRA, GATA4,HAND1*	0.264933297	*FOSL1, HEY2,MITF*	0.247524752
HAND1, SRF,ZNF281	0.282485876	*GATA6, PBX1,ZNF281*	0.264685792	*HAND2, SNAI2,SRF*	0.247371676
MESP1, NR2F1,TBX20	0.282485876	*ESRRG, MESP1,NFYB*	0.264550265	*FHL2, HAND1,ID1*	0.24703255
HAND2, HEY2,IRX5	0.282485876	*HAND2, PBX1,TBX20*	0.264513825	*NR2F1, PBX1,ZNF281*	0.24691358
ESRRG, FHL2,HAND1	0.282446312	*ESRRA, ESRRG,ZFPM2*	0.264026403	*GATA4, HEY2,IRX5*	0.246891296
GATA4, MEF2C,TBX5	0.281930954	*NKX2.5, SOX9,ZFPM2*	0.263620387	*PBX1, TCF21,MYOCD*	0.246761601
FOSL1, GATA6,SMAD6	0.281709813	*FHL2, SNAI2,ASCL1*	0.263230721	*GATA6, TCF21,MYOCD*	0.246710526
ETS2, FOSL1,ZFPM2	0.281689004	*FOSL1, GATA6,IRX5*	0.262467192	*HAND2, TBX5,ASCL1*	0.246457178
GATA6, NR2F1,SMARCD3	0.281547451	*ESRRA, SNAI2,TBX20*	0.262322056	*AKT1, HAND2,SRF*	0.246345366
ESRRG, NKX2.5,SOX9	0.281504867	*NKX2.5, PBX1,TBX20*	0.262094631	*ESRRA, ID1,MEF2C*	0.246305419
GATA6, HAND1,ASCL1	0.28139891	*GATA6, HAND2,ID1*	0.262040163	*GATA4, HEY2,MITF*	0.246305419
MITF, NR2F1,TBX20	0.281258702	*ESRRG, HEY2,ASCL1*	0.261780105	*FHL2, SRF,ZFPM2*	0.245821042
ETS2, FHL2,NKX2.5	0.281056773	*FOSL1, ID1,ASCL1*	0.261780105	*ETS2, GATA6,IRX5*	0.245098039
ESRRA, ID1,TCF21	0.280898876	*NFYB, SOX9,TBX20*	0.261763981	*HAND1, MESP1,PBX1*	0.245098039
FHL2, HAND1,TBX20	0.280112045	*IRX5, TBX20,TBX5*	0.261600225	*FOSL1, PBX1,TBX20*	0.245084528
MITF, NR2F1,SMARCD3	0.279993108	*FOSL1, MEF2C,ZNF281*	0.261437908	*FHL2, HAND1,SNAI2*	0.244817213
FOSL1, ID1,ZNF281	0.279601548	*HAND1, SNAI2,TBX20*	0.26141316	*ESRRA, FHL2,NKX2.5*	0.243753809
NFYB, NR2F1,SMARCD3	0.279329609	*AKT1, HAND2,ID1*	0.260379273	*ESRRA, ZNF281,MYOCD*	0.243542196
FOSL1, HAND2,TBX5	0.278940028	*HEY2, ID1,IRX5*	0.260067502	*ID1, TBX20,ASCL1*	0.243309002
MEF2C, SMAD6,ZFPM2	0.278092439	*ETS2, MESP1,NFYB*	0.259999438	*GATA6, ID1,SMAD6*	0.243309002
SMAD6, SRF,ZFPM2	0.277686941	*FOSL1, GATA4,TBX20*	0.259774541	*GATA4, SMAD6,ASCL1*	0.242718447
ESRRG, FOSL1,SNAI2	0.277024868	*HAND1, MESP1,ZNF281*	0.25974026	*ID1, IRX5,ZNF281*	0.241746327
HEY2, TBX20,TBX5	0.276634138	*ESRRA, SRF,ZNF281*	0.25974026	*AKT1, TCF21,ZFPM2*	0.241545894

Table S7. List of all 4,960 combinations tested and their respective reprogramming efficiency (MYH6^{+}TNNT2^{+})

TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells
ESRRA, FOSL1,HEY2	0.241189436	*FOSL1, HEY2,SNAI2*	0.22675737	*ESRRG, NR2F1,ZNF281*	0.213675214
FOSL1, HAND2,SMARCD3	0.240963855	*FHL2, GATA4,NKX2.5*	0.22675737	*GATA4, ZNF281,MYOCD*	0.213675214
ESRRG, GATA6,MESP1	0.240673887	*HAND2, HEY2,NFYB*	0.226637325	*FOSL1, NR2F1,PBX1*	0.213477461
FOSL1, MEF2C,SNAI2	0.24054763	*ESRRG, GATA6,NFYB*	0.226393933	*ESRRA, IRX5,ASCL1*	0.21312873
HAND1, HEY2,ID1	0.239808153	*SOX9, TCF21,ASCL1*	0.226244344	*GATA6, MEF2C,MYOCD*	0.212992545
ESRRA, NR2F1,ZFPM2	0.239808153	*AKT1, ESRRG,MESP1*	0.226144324	*FOSL1, MITF,NKX2.5*	0.212765957
FOSL1, SRF,ZNF281	0.239808153	*ESRRA, SNAI2,TCF21*	0.225988701	*ID1, IRX5,SMARCD3*	0.212618073
AKT1, FHL2,TBX5	0.239808153	*NR2F1, SOX9,SRF*	0.225881138	*IRX5, SRF,ZNF281*	0.212284623
GATA6, HAND2,ASCL1	0.239241255	*AKT1, ESRRG,TBX20*	0.22515641	*SMARCD3, SOX9,TBX5*	0.211640212
NKX2.5, NR2F1,TBX5	0.238234317	*ESRRG, HAND2,HEY2*	0.224845419	*HEY2, NKX2.5,TBX20*	0.211640212
ESRRG, HAND2,NR2F1	0.238095238	*ETS2, GATA4,ID1*	0.224435957	*ESRRG, FOSL1,IRX5*	0.211640212
HAND2, TBX5,TCF21	0.237987007	*ESRRG, ETS2,ASCL1*	0.224270492	*FOSL1, TBX20,TBX5*	0.211532851
FOSL1, HAND1,SRF	0.237958872	*FHL2, ID1,NR2F1*	0.223713647	*ESRRA, IRX5,ZFPM2*	0.210970464
HAND1, HAND2,SMARCD3	0.23795669	*HAND2, IRX5,SOX9*	0.223713647	*ESRRG, NKX2.5,TBX5*	0.210970464
ESRRA, HAND1,SMAD6	0.237762238	*ESRRG, NFYB,TBX20*	0.223713647	*HAND2, TBX20,TCF21*	0.210970464
HAND2, MESP1,SMAD6	0.237733221	*ESRRA, HEY2,IRX5*	0.223071119	*FHL2, SOX9,ASCL1*	0.210970464
MEF2C, TBX20,ASCL1	0.237529691	*ESRRG, HAND2,ASCL1*	0.222717149	*GATA4, PBX1,ASCL1*	0.210970464
ID1, PBX1,SNAI2	0.236554038	*MITF, PBX1,SMAD6*	0.222398379	*AKT1, GATA4,NFYB*	0.210703751
HAND1, HEY2,TBX5	0.236406619	*NKX2.5, ZFPM2,ZNF281*	0.222222222	*SRF, TCF21,MYOCD*	0.210402692
MESP1, SOX9,SRF	0.236406619	*AKT1, ETS2,SMARCD3*	0.221483942	*FHL2, SMARCD3,ZFPM2*	0.210304942
FOSL1, GATA4,GATA6	0.236252939	*MITF, SMARCD3,SOX9*	0.221270704	*ESRRA, HAND2,MESP1*	0.209994552
ETS2, HEY2,SNAI2	0.23557126	*ID1, PBX1,ASCL1*	0.221034182	*FHL2, FOSL1,NR2F1*	0.209810875
FHL2, MEF2C,ZNF281	0.23557126	*FHL2, SNAI2,SOX9*	0.220691333	*FOSL1, ID1,PBX1*	0.209757349
ESRRG, HEY2,PBX1	0.235458522	*HEY2, ID1,SNAI2*	0.220353752	*ESRRG, NKX2.5,TBX20*	0.209643606
AKT1, HEY2,SOX9	0.235404896	*ESRRG, PBX1,SNAI2*	0.220091982	*ETS2, NKX2.5,SOX9*	0.208333333
MEF2C, MITF,ZNF281	0.234756545	*ESRRA, MESP1,PBX1*	0.21999686	*ID1, NR2F1,SOX9*	0.20804049
ESRRG, NR2F1,PBX1	0.234711309	*AKT1, HEY2,ZFPM2*	0.219659528	*ESRRG, HAND2,ZFPM2*	0.20768432
ESRRG, HAND2,NKX2.5	0.233918129	*ESRRG, GATA6,SRF*	0.21955256	*HEY2, SNAI2,ZFPM2*	0.207223209
FHL2, GATA6,HAND1	0.233918129	*MESP1, NKX2.5,TBX5*	0.219298246	*FHL2, ID1,SOX9*	0.206897879
IRX5, NKX2.5,TBX20	0.233678055	*HAND2, MITF,ZNF281*	0.219298246	*MEF2C, NKX2.5,ASCL1*	0.206851972
ETS2, TBX20,ZFPM2	0.233624326	*ESRRG, FHL2,ZNF281*	0.219298246	*GATA6, MEF2C,ZNF281*	0.20683052
IRX5, NKX2.5,TBX5	0.233100233	*ESRRG, SOX9,TBX20*	0.219298246	*ETS2, SNAI2,ASCL1*	0.206043956
HAND1, MITF,SNAI2	0.232693426	*PBX1, ZFPM2,ASCL1*	0.218579235	*AKT1, GATA4,ASCL1*	0.205761317
ESRRG, SMAD6,ZFPM2	0.23255814	*GATA6, MESP1,NKX2.5*	0.217885496	*GATA6, ID1,SMARCD3*	0.205444273
ESRRA, IRX5,TCF21	0.232288037	*ESRRG, HEY2,TBX20*	0.217813399	*GATA6, NFYB,SMARCD3*	0.204328325
FOSL1, PBX1,ZNF281	0.232268721	*ESRRA, GATA6,SRF*	0.217095531	*HAND1, NR2F1,SMAD6*	0.203665988
GATA4, NR2F1,ASCL1	0.232198142	*ESRRA, FHL2,ZNF281*	0.216919047	*ID1, MITF,NKX2.5*	0.203252033
GATA4, MITF,TBX20	0.231493674	*AKT1, HEY2,NFYB*	0.216731686	*ESRRG, SMARCD3,ZFPM2*	0.202020202
AKT1, ETS2,IRX5	0.231481481	*NKX2.5, SNAI2,TBX5*	0.216718495	*HAND1, NKX2.5,ZNF281*	0.202020202
MITF, SMAD6,SRF	0.231435941	*NKX2.5, SMARCD3,ASCL1*	0.216450216	*ESRRA, GATA6,ID1*	0.202020202
AKT1, HAND1,HEY2	0.230880404	*ETS2, HAND1,TBX20*	0.216099406	*IRX5, MEF2C,SOX9*	0.201952319
GATA6, SNAI2,TCF21	0.23071244	*FOSL1, HAND1,SMARCD3*	0.215812331	*NFYB, SNAI2,TBX20*	0.201737025
AKT1, IRX5,TBX5	0.230680507	*NFYB, SNAI2,ASCL1*	0.215653008	*ESRRA, PBX1,ZFPM2*	0.201409869
ESRRA, FHL2,FOSL1	0.23045517	*ID1, IRX5,PBX1*	0.215610177	*HAND1, SMAD6,SMARCD3*	0.201104282
FHL2, FOSL1,SMARCD3	0.230429381	*ID1, IRX5,NKX2.5*	0.215257043	*AKT1, FHL2,HEY2*	0.200998472
ESRRA, GATA6,ZFPM2	0.229885057	*GATA4, NR2F1,ZFPM2*	0.215053763	*ETS2, SNAI2,TBX5*	0.200803213
SMARCD3, TCF21,ASCL1	0.229885057	*ESRRA, NKX2.5,SMARCD3*	0.215053763	*ID1, MEF2C,ASCL1*	0.200803213
FOSL1, SRF,ASCL1	0.229357798	*NFYB, TBX20,ASCL1*	0.214788598	*GATA6, MITF,TBX20*	0.200803213
ESRRA, ETS2,TCF21	0.229095074	*AKT1, ETS2,GATA4*	0.21475078	*TBX5, ZFPM2,ASCL1*	0.200267023
GATA6, TCF21,ZFPM2	0.228832952	*ESRRA, GATA4,TBX5*	0.214703425	*ETS2, GATA4,TBX20*	0.199913029
GATA6, IRX5,TBX20	0.227531286	*NFYB, NR2F1,TCF21*	0.214592275	*HAND2, SMAD6,ASCL1*	0.199625337
HEY2, NR2F1,TBX20	0.227272727	*GATA4, MITF,SRF*	0.214592275	*NR2F1, SOX9,TBX5*	0.199600798
NKX2.5, PBX1,SMAD6	0.226948457	*ESRRG, FOSL1,HAND1*	0.214362272	*ETS2, NKX2.5,SRF*	0.199600798
ESRRG, GATA4,ZFPM2	0.22675737	*ESRRG, GATA4,ASCL1*	0.214132762	*FOSL1, TBX20,TCF21*	0.199600798

Table S7. List of all 4,960 combinations tested and their respective reprogramming efficiency (MYH6^{+}TNNT2^{+})

TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells
AKT1, PBX1,ASCL1	0.199600798	*ESRRG, NFYB,PBX1*	0.189363417	*MITF, NFYB,ZNF281*	0.174216028
NKX2.5, SRF,TCF21	0.199203187	*AKT1, GATA6,NFYB*	0.189281691	*ID1, TCF21,MYOCD*	0.173815667
ESRRG, GATA6,PBX1	0.1991946	*IRX5, NKX2.5,ZNF281*	0.189107413	*ETS2, ID1,TCF21*	0.173611111
ESRRA, NR2F1,PBX1	0.199135159	*ETS2, MITF,SMARCD3*	0.189035917	*FHL2, HAND2,PBX1*	0.173160173
HEY2, SRF,TCF21	0.199004975	*HEY2, TBX20,ASCL1*	0.188925998	*FOSL1, HAND2,HEY2*	0.173160173
ESRRA, MITF,SNAI2	0.198807157	*MITF, PBX1,SMARCD3*	0.188454842	*ID1, SMARCD3,ZNF281*	0.172855007
GATA4, NR2F1,ZNF281	0.198807157	*ESRRA, IRX5,MESP1*	0.188323917	*NFYB, NKX2.5,SOX9*	0.172803209
ESRRG, ID1,MEF2C	0.198412698	*FHL2, SMAD6,ZNF281*	0.188323917	*HAND2, NFYB,NR2F1*	0.172711572
IRX5, TCF21,ZNF281	0.198412698	*SRF, TBX5,ASCL1*	0.188323917	*MITF, SMAD6,SMARCD3*	0.172037102
ESRRG, HAND1,NKX2.5	0.198412698	*AKT1, ESRRA,IRX5*	0.187265918	*HEY2, NFYB,TBX20*	0.171431086
ESRRG, MEF2C,ZFPM2	0.198019802	*HAND2, SRF,TCF21*	0.187098503	*ESRRG, PBX1,TCF21*	0.171379606
MESP1, NR2F1,TBX5	0.197238659	*SRF, ZNF281,ASCL1*	0.186858974	*HEY2, NFYB,PBX1*	0.171115674
ESRRG, FOSL1,ZFPM2	0.197086368	*AKT1, NKX2.5,TBX5*	0.186741363	*MESP1, NKX2.5,ZFPM2*	0.170648464
GATA6, SRF,ZNF281	0.196078431	*HAND2, SMARCD3,ASCL1*	0.186259829	*ESRRG, GATA4,SMAD6*	0.170648464
ETS2, MESP1,NR2F1	0.19590443	*ID1, TBX5,ZFPM2*	0.186219739	*ESRRA, FOSL1,TCF21*	0.170502984
ETS2, NKX2.5,SMAD6	0.195888364	*HEY2, TBX5,ZFPM2*	0.185701021	*AKT1, FHL2,SOX9*	0.170271489
HEY2, ZFPM2,ZNF281	0.195694716	*HEY2, PBX1,SOX9*	0.185185185	*ESRRG, SOX9,ZFPM2*	0.170068027
AKT1, FHL2,TCF21	0.194931774	*FOSL1, ID1,SMARCD3*	0.185185185	*HAND2, NR2F1,ASCL1*	0.169779287
NKX2.5, TBX20,TBX5	0.194931774	*FHL2, ID1,IRX5*	0.185096833	*AKT1, GATA4,ID1*	0.169419737
ESRRG, SMARCD3,TCF21	0.194931774	*ESRRG, GATA4,MITF*	0.184501845	*ESRRG, HEY2,ID1*	0.169413946
FOSL1, HEY2,NR2F1	0.194931774	*HEY2, NFYB,ASCL1*	0.184162063	*ESRRG, SNAI2,TBX5*	0.169334463
AKT1, ID1,MEF2C	0.194790319	*ESRRA, SNAI2,ZNF281*	0.184162063	*ID1, MEF2C,ZNF281*	0.169229956
ESRRG, MITF,SMARCD3	0.194615082	*GATA4, HEY2,SMARCD3*	0.184122508	*ESRRA, NR2F1,SMARCD3*	0.169204738
HAND2, ZFPM2,ASCL1	0.194174757	*SMAD6, SOX9,TBX20*	0.183908046	*ESRRG, MITF,ZFPM2*	0.168918919
MESP1, NR2F1,SMARCD3	0.19379845	*ID1, MESP1,NR2F1*	0.183828178	*ESRRA, ETS2,NR2F1*	0.168712986
ESRRG, GATA4,SRF	0.19379845	*HEY2, NKX2.5,NR2F1*	0.183823529	*GATA6, NKX2.5,ZFPM2*	0.168496927
FHL2, IRX5,NR2F1	0.19379845	*FOSL1, HAND1,ASCL1*	0.183823529	*ETS2, HEY2,SMARCD3*	0.168350168
ESRRA, SNAI2,ZFPM2	0.193564506	*MEF2C, PBX1,ZFPM2*	0.18367937	*ESRRA, ETS2,PBX1*	0.168350168
GATA4, HAND2,SMARCD3	0.193348801	*FHL2, IRX5,TCF21*	0.183150183	*FOSL1, SMARCD3,ZNF281*	0.168350168
FOSL1, TBX20,ASCL1	0.193050193	*ESRRG, IRX5,ASCL1*	0.183150183	*IRX5, NKX2.5,SMARCD3*	0.168009857
HAND1, MEF2C,TCF21	0.192772551	*AKT1, SNAI2,ASCL1*	0.182481752	*ESRRA, NR2F1,TCF21*	0.166666667
AKT1, ESRRA,NFYB	0.192678227	*AKT1, ESRRA,ETS2*	0.181488203	*NKX2.5, SMAD6,TBX5*	0.166666667
FHL2, NR2F1,ZNF281	0.192678227	*AKT1, ESRRG,GATA6*	0.181377516	*ID1, TCF21,ASCL1*	0.165283355
FOSL1, MEF2C,TCF21	0.192678227	*FOSL1, HAND1,HEY2*	0.18115942	*ETS2, SNAI2,ZFPM2*	0.165016502
IRX5, SOX9,ASCL1	0.192167577	*GATA6, NKX2.5,TBX5*	0.18018018	*GATA6, MITF,NR2F1*	0.165016502
AKT1, ETS2,SRF	0.19197995	*IRX5, MITF,NKX2.5*	0.18018018	*MESP1, ZFPM2,ZNF281*	0.164473684
AKT1, HAND1,SMAD6	0.191732558	*ESRRG, SMARCD3,TBX5*	0.179887134	*NR2F1, SNAI2,ZNF281*	0.164405453
FOSL1, GATA6,NR2F1	0.191570881	*FHL2, GATA4,TCF21*	0.179476825	*ESRRG, HEY2,SMAD6*	0.164203612
AKT1, SRF,ZFPM2	0.191570881	*ETS2, MEF2C,MITF*	0.178571429	*FHL2, HAND2,ZNF281*	0.164203612
FOSL1, MEF2C,SOX9	0.191570881	*ID1, NFYB,ASCL1*	0.178253119	*AKT1, HAND1,ID1*	0.163934426
HAND1, TBX5,ZNF281	0.191570881	*GATA6, NKX2.5,ZNF281*	0.178253119	*GATA6, TBX20,TCF21*	0.163398693
ESRRA, ETS2,MITF	0.190884187	*NKX2.5, SMARCD3,TBX5*	0.177304965	*HAND2, SNAI2,ZNF281*	0.162601626
ID1, IRX5,SMAD6	0.190849094	*GATA4, MITF,SNAI2*	0.177304965	*AKT1, HAND1,ZNF281*	0.162601626
FOSL1, MEF2C,SMARCD3	0.190723488	*ESRRA, MITF,ASCL1*	0.17699115	*AKT1, SMARCD3,SOX9*	0.16200891
ESRRA, HAND2,NR2F1	0.190672495	*GATA4, PBX1,ZNF281*	0.176678445	*MESP1, TCF21,ASCL1*	0.161812298
ID1, NFYB,TBX20	0.190564261	*AKT1, NR2F1,SNAI2*	0.176316739	*GATA6, HAND1,SRF*	0.161812298
MITF, NFYB,NKX2.5	0.19047619	*ESRRG, GATA4,NFYB*	0.176108695	*SMAD6, SNAI2,ASCL1*	0.161812298
HEY2, ID1,SMARCD3	0.190467671	*ESRRA, FOSL1,MESP1*	0.176049796	*MITF, SMARCD3,TBX20*	0.161706814
GATA4, MITF,NFYB	0.190328569	*ID1, SNAI2,ASCL1*	0.175901495	*GATA4, MESP1,ASCL1*	0.161550889
HEY2, NFYB,SMARCD3	0.190168382	*ESRRA, ID1,SMAD6*	0.175746924	*FOSL1, ID1,TBX20*	0.161290323
FOSL1, HAND1,NFYB	0.19015594	*HAND1, SRF,TCF21*	0.175438596	*GATA6, TBX20,ZFPM2*	0.16064257
ETS2, SMAD6,ASCL1	0.190107288	*ETS2, NKX2.5,ASCL1*	0.174825175	*ESRRG, ETS2,TCF21*	0.160097407
ESRRG, NKX2.5,ZNF281	0.189393939	*ESRRA, ESRRG,ASCL1*	0.17452007	*HAND1, NR2F1,TBX20*	0.159872102
NKX2.5, SNAI2,ASCL1	0.189393939	*AKT1, ESRRG,HEY2*	0.174259058	*GATA6, ID1,PBX1*	0.159872102

Table S7. List of all 4,960 combinations tested and their respective reprogramming efficiency (MYH6^{+}TNNT2^{+})

TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells
ID1, SMARCD3,ASCL1	0.159744409	*IRX5, TBX5,ASCL1*	0.148148148	*AKT1, ID1,SOX9*	0.139470014
MEF2C, PBX1,TCF21	0.159103105	*HAND1, SMAD6,ZFPM2*	0.148148148	*GATA4, MITF,SMAD6*	0.139275766
HEY2, IRX5,TBX5	0.159020155	*GATA4, NKX2.5,ASCL1*	0.147928994	*IRX5, NR2F1,TBX20*	0.139178845
IRX5, PBX1,ZFPM2	0.158982512	*FHL2, NR2F1,SRF*	0.14781966	*MESP1, NFYB,SNAI2*	0.139082058
NR2F1, SMAD6,TBX5	0.158730159	*ESRRG, NKX2.5,ZFPM2*	0.147623265	*HEY2, NR2F1,TBX5*	0.139033716
ESRRG, FHL2,NR2F1	0.158730159	*FOSL1, HAND1,TBX5*	0.147492625	*AKT1, IRX5,ASCL1*	0.138888889
ID1, NR2F1,ZNF281	0.158730159	*HAND2, SMAD6,SOX9*	0.147356264	*ESRRG, MITF,PBX1*	0.138888889
ESRRA, MITF,SMARCD3	0.158730159	*ESRRA, IRX5,MEF2C*	0.147275405	*ESRRA, ID1,SOX9*	0.138888889
AKT1, ETS2,NR2F1	0.158478605	*ESRRA, GATA6,ASCL1*	0.147058824	*SOX9, SRF,ASCL1*	0.138600139
HAND1, HEY2,SMARCD3	0.157977883	*PBX1, SNAI2,ZNF281*	0.147058824	*NFYB, NR2F1,MYOCD*	0.138312586
HEY2, SOX9,TBX20	0.15778779	*ETS2, TBX5,ZNF281*	0.146842878	*ESRRG, HAND2,NFYB*	0.138312586
MITF, SOX9,MYOCD	0.157604413	*GATA6, MITF,TCF21*	0.146842878	*FOSL1, NR2F1,SMARCD3*	0.138277973
FHL2, GATA4,ID1	0.157232704	*IRX5, NR2F1,TBX5*	0.146713615	*IRX5, NFYB,TCF21*	0.138217001
FOSL1, ZNF281,ASCL1	0.157164118	*SNAI2, TBX20,TCF21*	0.146627566	*PBX1, SRF,ASCL1*	0.138026225
ESRRG, MEF2C,SMAD6	0.156996446	*FOSL1, MITF,PBX1*	0.146627566	*MESP1, MITF,ZFPM2*	0.137598899
AKT1, MITF,PBX1	0.156985871	*ESRRA, HEY2,SNAI2*	0.146359312	*FHL2, HEY2,ZNF281*	0.137174211
GATA6, NFYB,TCF21	0.156494523	*ID1, IRX5,TBX20*	0.146287328	*ESRRA, HAND1,ASCL1*	0.136986301
SMAD6, SNAI2,ZFPM2	0.15625	*AKT1, FHL2,HAND2*	0.146116181	*FOSL1, IRX5,SOX9*	0.136612022
ESRRG, GATA6,TCF21	0.15576324	*IRX5, SMAD6,ZFPM2*	0.145979152	*GATA6, TBX5,ZFPM2*	0.136612022
HEY2, NR2F1,SMAD6	0.155607975	*ESRRA, NFYB,ZNF281*	0.145560408	*FOSL1, IRX5,MESP1*	0.136612022
GATA4, IRX5,TCF21	0.155400155	*FOSL1, SRF,ZFPM2*	0.145560408	*HAND2, IRX5,SMAD6*	0.136612022
ESRRA, FHL2,SMAD6	0.15503876	*HEY2, NKX2.5,SMAD6*	0.145401672	*HAND2, IRX5,ZNF281*	0.136279792
ETS2, GATA6,ID1	0.154978081	*ESRRG, HEY2,MITF*	0.144927536	*AKT1, HAND2,ZFPM2*	0.136054422
ESRRG, FOSL1,ASCL1	0.154798762	*HAND2, IRX5,PBX1*	0.14461316	*HAND1, NR2F1,ZNF281*	0.136054422
ESRRG, FOSL1,TBX5	0.154080736	*TCF21, MYOCD,ASCL1*	0.144369586	*IRX5, MESP1,ZFPM2*	0.135501355
HAND1, NFYB,SOX9	0.154011154	*IRX5, NR2F1,ZFPM2*	0.144300144	*GATA6, MEF2C,MITF*	0.134770889
NKX2.5, TBX5,ZNF281	0.153846154	*HAND1, PBX1,ZNF281*	0.144300144	*HAND1, NR2F1,TBX5*	0.133868809
ESRRG, ID1,ZNF281	0.153778054	*GATA6, HAND2,TCF21*	0.144300144	*FOSL1, MITF,TCF21*	0.13368984
FOSL1, HAND1,MEF2C	0.153609831	*ETS2, SOX9,ZNF281*	0.144126862	*HEY2, TCF21,ASCL1*	0.133333333
MESP1, SNAI2,TBX20	0.153566769	*ESRRG, SNAI2,TCF21*	0.143988481	*HAND1, SMARCD3,SOX9*	0.133333333
ESRRG, NR2F1,TBX5	0.152905199	*FOSL1, ID1,SMAD6*	0.143833154	*HAND2, NR2F1,SMAD6*	0.133333333
FOSL1, IRX5,ASCL1	0.152905199	*AKT1, SMARCD3,ASCL1*	0.143678161	*GATA4, MITF,NKX2.5*	0.133333333
GATA4, IRX5,ZNF281	0.152459694	*ETS2, GATA6,NFYB*	0.143342803	*MEF2C, SOX9,ASCL1*	0.133100532
TBX20, ZFPM2,ZNF281	0.152439024	*AKT1, SRF,ASCL1*	0.143266476	*ESRRA, SNAI2,SOX9*	0.132978723
ESRRA, ESRRG,TCF21	0.152207002	*SNAI2, SRF,ZNF281*	0.143266476	*MEF2C, SNAI2,ZFPM2*	0.132802125
ESRRA, ETS2,HAND1	0.151751387	*NR2F1, SOX9,ZNF281*	0.142993327	*MESP1, NFYB,TBX20*	0.132291667
AKT1, HAND1,SMARCD3	0.151542735	*ESRRG, NFYB,SNAI2*	0.142653352	*ETS2, SOX9,MYOCD*	0.131947177
TBX20, TCF21,ZNF281	0.151515152	*ID1, NFYB,ZFPM2*	0.142450142	*HEY2, MITF,TBX20*	0.131752306
GATA6, HAND2,SOX9	0.151515152	*ETS2, HAND1,ASCL1*	0.142450142	*ESRRG, HAND2,SNAI2*	0.131752306
FHL2, FOSL1,HAND1	0.151515152	*GATA4, HAND2,TCF21*	0.142450142	*SNAI2, TCF21,ZNF281*	0.131578947
AKT1, SNAI2,ZNF281	0.15117158	*FHL2, TBX20,TCF21*	0.142450142	*NKX2.5, SOX9,TBX20*	0.131476928
ETS2, GATA4,SOX9	0.150674716	*HAND2, TBX20,ASCL1*	0.142247511	*GATA4, TCF21,ZFPM2*	0.131233596
ZNF281, MYOCD,ASCL1	0.15060241	*HAND2, IRX5,ZFPM2*	0.141843972	*SMARCD3, SOX9,ASCL1*	0.131233596
NKX2.5, SOX9,ZNF281	0.15060241	*FHL2, TBX20,ZFPM2*	0.141543455	*ETS2, HEY2,ASCL1*	0.130890052
IRX5, MESP1,ASCL1	0.15037594	*NFYB, SMARCD3,ZNF281*	0.14137606	*AKT1, NKX2.5,SMARCD3*	0.130718954
MESP1, TCF21,ZNF281	0.15037594	*ESRRG, GATA6,TBX5*	0.141242938	*ESRRG, HEY2,NFYB*	0.130718954
ESRRA, ESRRG,PBX1	0.150296448	*AKT1, SOX9,SRF*	0.141208267	*GATA4, TBX5,ZNF281*	0.130718954
AKT1, MITF,NR2F1	0.149700599	*AKT1, PBX1,ZFPM2*	0.141147106	*MESP1, TBX20,ASCL1*	0.130318512
HAND1, NFYB,ZFPM2	0.149476831	*ID1, MESP1,MITF*	0.140646976	*ESRRG, GATA6,SMARCD3*	0.130311807
ESRRG, MESP1,NR2F1	0.149476831	*HAND2, NR2F1,ZNF281*	0.140056022	*ID1, NR2F1,TBX5*	0.130309993
FHL2, NFYB,ZNF281	0.148809524	*FHL2, HAND1,SMAD6*	0.140056022	*HEY2, SMAD6,ZFPM2*	0.130215178
ESRRG, HAND1,SNAI2	0.148809524	*MEF2C, ZNF281,MYOCD*	0.139794967	*FOSL1, PBX1,ZFPM2*	0.129954516
AKT1, ID1,PBX1	0.14858841	*ESRRA, ID1,SNAI2*	0.139762404	*ESRRA, SOX9,ZFPM2*	0.129701686
ESRRA, NFYB,SMAD6	0.148367953	*ESRRA, GATA6,SMAD6*	0.139599874	*ESRRG, TBX5,TCF21*	0.129701686

Table S7. List of all 4,960 combinations tested and their respective reprogramming efficiency (MYH6^{+}TNNT2^{+})

TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells
ESRRG, ID1,SRF	0.129701686	*MEF2C, TBX20,TCF21*	0.119474313	*ETS2, HAND2,ASCL1*	0.109170306
ETS2, ZNF281,MYOCD	0.129701686	*AKT1, GATA6,ASCL1*	0.118906064	*FOSL1, MITF,SMARCD3*	0.109170306
GATA6, NFYB,ZNF281	0.129539295	*HEY2, SMARCD3,ZNF281*	0.118483412	*ESRRG, MEF2C,NFYB*	0.108932462
ESRRA, NKX2.5,TCF21	0.129198966	*HAND2, SOX9,MYOCD*	0.118483412	*ESRRA, GATA6,MITF*	0.108695652
GATA6, MESP1,ZNF281	0.129198966	*GATA4, SMARCD3,ASCL1*	0.118483412	*AKT1, TBX20,ZFPM2*	0.108577633
ETS2, NR2F1,ZFPM2	0.128700129	*ESRRG, FOSL1,NFYB*	0.11820331	*GATA6, MESP1,MITF*	0.10845987
NKX2.5, SRF,ASCL1	0.128700129	*HAND2, SMAD6,ZNF281*	0.117647059	*MEF2C, MESP1,TBX5*	0.108401084
FOSL1, HAND2,SNAI2	0.128162025	*ID1, MITF,ZFPM2*	0.117577895	*TCF21, ZNF281,ASCL1*	0.108342362
NR2F1, PBX1,ZFPM2	0.12784728	*HAND2, HEY2,NKX2.5*	0.117370892	*GATA4, ID1,TCF21*	0.108225108
ESRRA, GATA6,NKX2.5	0.127713921	*PBX1, SMAD6,TCF21*	0.117370892	*ESRRA, FHL2,SNAI2*	0.108225108
FOSL1, ZNF281,MYOCD	0.127713921	*MITF, PBX1,ASCL1*	0.117315669	*AKT1, ESRRA,ZFPM2*	0.107874865
ESRRA, ETS2,GATA4	0.12756585	*FOSL1, NKX2.5,TBX20*	0.116959064	*ESRRA, FHL2,MITF*	0.107526882
ESRRG, IRX5,NKX2.5	0.126984127	*MITF, TCF21,ZFPM2*	0.116414435	*ID1, NR2F1,SMAD6*	0.107181136
FOSL1, GATA4,MITF	0.126903553	*ETS2, PBX1,TCF21*	0.116144019	*ESRRG, HAND1,PBX1*	0.107181136
ESRRG, ID1,SMAD6	0.126742712	*HAND1, MEF2C,MITF*	0.11554015	*ETS2, MESP1,ASCL1*	0.106837607
ESRRG, GATA6,SNAI2	0.126742712	*AKT1, ESRRA,HAND2*	0.11554015	*GATA4, TBX20,ASCL1*	0.106609808
FOSL1, HEY2,TBX20	0.126262626	*MITF, SMAD6,ZFPM2*	0.115503204	*IRX5, MESP1,TCF21*	0.106157113
MITF, SNAI2,TBX20	0.126023945	*ESRRA, MITF,ZNF281*	0.114942529	*MEF2C, SOX9,ZNF281*	0.105820106
ESRRG, SMAD6,SNAI2	0.125446936	*ESRRA, FHL2,GATA6*	0.114942529	*ESRRA, SMARCD3,ZNF281*	0.105820106
NR2F1, SMARCD3,ASCL1	0.125313283	*ESRRA, TCF21,MYOCD*	0.114942529	*MEF2C, MITF,SNAI2*	0.105820106
ESRRA, HAND2,SNAI2	0.125262889	*FHL2, IRX5,ZNF281*	0.114942529	*AKT1, GATA4,IRX5*	0.105485232
HEY2, PBX1,ZNF281	0.124843945	*AKT1, HAND1,PBX1*	0.114744693	*AKT1, ID1,ASCL1*	0.105042017
AKT1, HAND2,PBX1	0.124610592	*IRX5, MITF,SOX9*	0.114744693	*FOSL1, IRX5,NFYB*	0.105042017
FOSL1, HEY2,NFYB	0.124378109	*FHL2, MITF,PBX1*	0.114678899	*IRX5, MEF2C,SMAD6*	0.104986877
AKT1, ID1,IRX5	0.124378109	*FOSL1, HEY2,MESP1*	0.114547537	*ESRRG, FOSL1,HAND2*	0.104821803
MEF2C, NKX2.5,ZNF281	0.123915737	*ID1, ZNF281,MYOCD*	0.114416476	*ID1, SNAI2,TBX20*	0.104633573
AKT1, FHL2,ZNF281	0.123915737	*FHL2, ID1,MITF*	0.114416476	*MESP1, SMAD6,TCF21*	0.10460251
AKT1, MESP1,TCF21	0.123915737	*AKT1, HEY2,MITF*	0.114155251	*MITF, NFYB,TBX20*	0.104493208
AKT1, FOSL1,MEF2C	0.123680583	*NKX2.5, PBX1,TCF21*	0.114025086	*GATA6, MITF,SMARCD3*	0.104384134
HEY2, MITF,NFYB	0.12345679	*SRF, TBX5,TCF21*	0.113765643	*AKT1, MITF,SMAD6*	0.104166667
ESRRA, MITF,ZFPM2	0.12345679	*ESRRG, FHL2,GATA6*	0.113571834	*MITF, NKX2.5,SNAI2*	0.104166667
FOSL1, HAND2,ZNF281	0.12345679	*ESRRA, ID1,TBX5*	0.113378685	*NFYB, NR2F1,SRF*	0.104166667
GATA6, ZFPM2,ASCL1	0.12300123	*ESRRA, FOSL1,ASCL1*	0.112871993	*GATA4, HEY2,ZFPM2*	0.10384216
SMARCD3, TBX5,ZNF281	0.122699387	*MESP1, PBX1,SOX9*	0.112803158	*GATA4, MITF,ZNF281*	0.10384216
GATA4, ID1,IRX5	0.122599142	*HEY2, NR2F1,SNAI2*	0.112739572	*GATA6, IRX5,ASCL1*	0.103519669
ESRRA, HAND2,ASCL1	0.12254902	*HEY2, TBX5,ASCL1*	0.112612613	*GATA6, SMARCD3,ZNF281*	0.103405679
HAND2, SMAD6,TCF21	0.122508053	*PBX1, TBX20,ZFPM2*	0.112517581	*ESRRG, MITF,NFYB*	0.103305785
AKT1, SMAD6,SOX9	0.12240818	*ESRRG, ID1,SNAI2*	0.112233446	*HEY2, SMARCD3,ZFPM2*	0.103287761
AKT1, ESRRG,ASCL1	0.12195122	*FOSL1, GATA6,MITF*	0.112107623	*ESRRA, ESRRG,SMAD6*	0.103199174
HAND2, ID1,NR2F1	0.121654501	*AKT1, ID1,NR2F1*	0.111755856	*ESRRG, TBX20,ASCL1*	0.103092784
HEY2, ID1,TBX20	0.121583554	*MEF2C, SMAD6,ASCL1*	0.11148272	*SNAI2, ZFPM2,ZNF281*	0.102986612
HEY2, NFYB,SMAD6	0.121236281	*AKT1, TBX20,ASCL1*	0.111111111	*FHL2, MITF,TBX5*	0.102880658
ESRRG, NKX2.5,TCF21	0.121212121	*AKT1, HAND2,MITF*	0.111111111	*ETS2, TBX20,TCF21*	0.102880658
MESP1, MITF,SMARCD3	0.121212121	*SMARCD3, ZNF281,MYOCD*	0.110864745	*HAND1, MITF,PBX1*	0.102564103
HEY2, MEF2C,ZNF281	0.121065375	*HAND1, NFYB,ASCL1*	0.11055832	*ESRRA, HEY2,NR2F1*	0.102564103
HAND1, SMARCD3,TCF21	0.121065375	*ETS2, NR2F1,ZNF281*	0.110375276	*ETS2, TCF21,MYOCD*	0.102564103
ESRRG, FOSL1,SMAD6	0.120798886	*ESRRA, GATA4,MITF*	0.110132159	*AKT1, MITF,TBX20*	0.102459016
FHL2, SOX9,TCF21	0.120772947	*MESP1, NKX2.5,SOX9*	0.110011001	*MEF2C, SMARCD3,TCF21*	0.102459016
HEY2, ZNF281,ASCL1	0.120481928	*ETS2, ZFPM2,ASCL1*	0.110011001	*FHL2, FOSL1,ASCL1*	0.102459016
FOSL1, SOX9,ASCL1	0.120481928	*FOSL1, HEY2,TBX5*	0.109649123	*SOX9, TBX20,ASCL1*	0.102396967
IRX5, NFYB,ZFPM2	0.120418473	*MITF, ZNF281,MYOCD*	0.10940919	*MITF, NFYB,SMAD6*	0.102249489
FOSL1, GATA6,ZNF281	0.120192308	*HAND1, MITF,ASCL1*	0.10940919	*IRX5, SMAD6,SOX9*	0.102249489
ESRRG, MESP1,TCF21	0.119904077	*ESRRG, FOSL1,HEY2*	0.109305249	*IRX5, PBX1,TCF21*	0.101936799
ESRRA, FOSL1,NKX2.5	0.119904077	*GATA6, HAND1,ID1*	0.109289617	*AKT1, HAND1,NR2F1*	0.101626016

Table S7. List of all 4,960 combinations tested and their respective reprogramming efficiency (MYH6^{+}TNNT2^{+})

TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells
FOSL1, HAND2,SMAD6	0.101317123	*MITF, SNAI2,ZNF281*	0.093632959	*MEF2C, SMARCD3,ASCL1*	0.083963056
HAND1, ZNF281,MYOCD	0.101317123	*IRX5, NKX2.5,TCF21*	0.093457944	*MESP1, MITF,SMAD6*	0.083963056
MITF, NFYB,SNAI2	0.10129168	*FOSL1, GATA4,ZFPM2*	0.093370682	*MITF, PBX1,SOX9*	0.083963056
ESRRA, SOX9,TCF21	0.101010101	*FOSL1, HAND1,SMAD6*	0.09310987	*FOSL1, HAND2,MESP1*	0.08364701
HAND2, TBX20,ZNF281	0.101010101	*ESRRG, MITF,SMAD6*	0.09310987	*FHL2, HAND1,NFYB*	0.08343763
NFYB, NKX2.5,NR2F1	0.100704935	*ETS2, NFYB,ASCL1*	0.092936803	*ETS2, MESP1,ZNF281*	0.083333333
AKT1, HAND2,NR2F1	0.100401606	*HAND2, HEY2,ZFPM2*	0.092850511	*ID1, IRX5,NR2F1*	0.083229297
HEY2, NR2F1,SMARCD3	0.100401606	*FOSL1, TBX5,ASCL1*	0.092764378	*FHL2, ID1,TCF21*	0.083194676
HAND1, MESP1,NKX2.5	0.100401606	*GATA6, ZFPM2,ZNF281*	0.092592593	*ID1, SOX9,TBX5*	0.08291874
FOSL1, MITF,SOX9	0.100401606	*ID1, MITF,NR2F1*	0.092592593	*ETS2, MITF,SMAD6*	0.08291874
ESRRA, ID1,SMARCD3	0.099954695	*GATA4, PBX1,ZFPM2*	0.092592593	*GATA4, ZFPM2,ASCL1*	0.08291874
ESRRG, IRX5,SRF	0.099950025	*ID1, MITF,ASCL1*	0.092336103	*NFYB, TBX20,ZNF281*	0.08291874
ESRRG, ID1,IRX5	0.099800399	*MITF, NKX2.5,ASCL1*	0.092336103	*GATA4, SOX9,ASCL1*	0.082781457
ESRRG, HAND1,ASCL1	0.099800399	*ID1, ZNF281,ASCL1*	0.092250923	*ESRRA, ESRRG,FOSL1*	0.082712986
GATA6, HEY2,MITF	0.099800399	*ESRRG, SMARCD3,SOX9*	0.091827365	*FOSL1, SMARCD3,SOX9*	0.082712986
ETS2, ID1,ZNF281	0.099502488	*GATA6, ID1,TBX20*	0.091324201	*MESP1, NKX2.5,ASCL1*	0.082508251
HAND2, SMARCD3,SNAI2	0.09905894	*HAND2, MITF,TBX5*	0.091074681	*ETS2, MITF,SNAI2*	0.082508251
IRX5, SRF,ASCL1	0.098911968	*GATA6, NKX2.5,ASCL1*	0.091074681	*MITF, NFYB,TCF21*	0.082508251
GATA6, ZNF281,ASCL1	0.098911968	*ESRRA, HEY2,ASCL1*	0.090909091	*NR2F1, SMARCD3,ZNF281*	0.082236842
AKT1, GATA4,NKX2.5	0.098911968	*HAND1, PBX1,TBX5*	0.090826521	*SMARCD3, SRF,ZNF281*	0.082236842
ETS2, NFYB,TCF21	0.098911968	*AKT1, FHL2,NKX2.5*	0.09057971	*HAND2, NKX2.5,SNAI2*	0.082101806
FOSL1, MITF,SMAD6	0.098425197	*GATA4, MESP1,TCF21*	0.090334237	*HAND2, TCF21,ASCL1*	0.081699346
ETS2, MEF2C,TCF21	0.098039216	*ID1, NR2F1,TCF21*	0.090334237	*ESRRG, NFYB,ASCL1*	0.081699346
MESP1, PBX1,ASCL1	0.097895252	*FOSL1, HEY2,MYOCD*	0.08984726	*ESRRA, SMARCD3,ASCL1*	0.081300813
AKT1, MITF,SRF	0.097847358	*ESRRA, ESRRG,TBX5*	0.089245872	*FOSL1, SMAD6,ZFPM2*	0.081300813
ESRRA, FHL2,HAND2	0.097751711	*GATA4, MITF,ASCL1*	0.088967972	*ID1, MEF2C,TCF21*	0.081300813
ETS2, ID1,ASCL1	0.097276265	*HAND2, NKX2.5,SOX9*	0.088888889	*HAND1, ID1,MITF*	0.081201786
HEY2, NKX2.5,TCF21	0.09718173	*IRX5, NFYB,SOX9*	0.088888889	*HEY2, MITF,SNAI2*	0.081103001
GATA4, GATA6,HAND1	0.096899225	*HEY2, IRX5,TBX20*	0.088652482	*HAND1, SOX9,TBX20*	0.08071025
ETS2, ZNF281,ASCL1	0.096618357	*GATA4, NKX2.5,PBX1*	0.08841733	*ID1, SRF,ZFPM2*	0.08071025
GATA6, HAND1,TCF21	0.096478534	*SNAI2, ZNF281,ASCL1*	0.088183422	*ESRRG, NR2F1,TCF21*	0.080321285
GATA6, SMARCD3,ASCL1	0.096153846	*ESRRG, SNAI2,ZNF281*	0.087950748	*MESP1, SNAI2,ASCL1*	0.080321285
MEF2C, SRF,ZFPM2	0.096061479	*AKT1, ESRRA,MITF*	0.087565674	*NKX2.5, SMARCD3,ZFPM2*	0.08
AKT1, ESRRG,ZFPM2	0.096061479	*AKT1, MITF,TBX5*	0.087412587	*HAND2, ZFPM2,ZNF281*	0.079617834
ESRRA, PBX1,SNAI2	0.096061479	*FHL2, MITF,NFYB*	0.087412587	*HAND1, HAND2,ID1*	0.079554495
ESRRG, GATA4,TBX20	0.096061479	*MEF2C, NR2F1,ZFPM2*	0.087183958	*GATA6, MITF,NFYB*	0.079491256
HAND1, SMAD6,SOX9	0.095785441	*ETS2, SMARCD3,TCF21*	0.086805556	*ID1, NFYB,TCF21*	0.079365079
AKT1, MITF,SMARCD3	0.095785441	*ID1, MESP1,NKX2.5*	0.086805556	*GATA6, MESP1,SNAI2*	0.079365079
FOSL1, SMAD6,ZNF281	0.095785441	*ESRRA, FOSL1,MITF*	0.086655113	*MITF, NR2F1,ASCL1*	0.079176564
ID1, NFYB,NR2F1	0.09569378	*ESRRG, GATA4,HEY2*	0.086355786	*ETS2, MITF,TBX5*	0.078864353
ESRRA, FOSL1,IRX5	0.095510984	*GATA6, IRX5,SNAI2*	0.086132644	*ESRRG, SMARCD3,ASCL1*	0.078616352
HAND1, NKX2.5,TBX20	0.095510984	*HAND1, SOX9,TCF21*	0.08605852	*ETS2, FOSL1,ASCL1*	0.077279753
GATA6, ID1,ZFPM2	0.095510984	*ETS2, PBX1,ASCL1*	0.08605852	*ESRRG, FOSL1,PBX1*	0.076982294
HAND1, HEY2,ASCL1	0.095238095	*PBX1, SNAI2,ZFPM2*	0.085910653	*NKX2.5, PBX1,ASCL1*	0.076982294
AKT1, FOSL1,MITF	0.095057034	*SMARCD3, SRF,ASCL1*	0.085910653	*IRX5, SRF,TCF21*	0.076628352
NFYB, SOX9,ZNF281	0.09487666	*GATA4, IRX5,MITF*	0.085763293	*FOSL1, SOX9,TBX20*	0.076628352
ESRRG, MESP1,ASCL1	0.09487666	*ETS2, FHL2,ZFPM2*	0.085360649	*MEF2C, MITF,SOX9*	0.076628352
GATA4, HAND2,ASCL1	0.09487666	*HEY2, SMARCD3,ASCL1*	0.084709869	*ID1, TBX5,TCF21*	0.076277651
MESP1, NR2F1,SNAI2	0.09469697	*ESRRG, GATA4,TCF21*	0.084602369	*PBX1, TBX5,ASCL1*	0.076103501
SMAD6, ZFPM2,ASCL1	0.09469697	*FHL2, FOSL1,ZNF281*	0.084602369	*NR2F1, TBX5,ASCL1*	0.075930144
ETS2, MITF,SOX9	0.09469697	*NKX2.5, NR2F1,ASCL1*	0.084602369	*MITF, SOX9,TBX5*	0.07558579
ESRRA, TBX5,ASCL1	0.094517958	*ID1, IRX5,ZFPM2*	0.084290675	*HEY2, SMAD6,TCF21*	0.075528701
FOSL1, ID1,MITF	0.093984962	*ESRRA, MITF,TBX20*	0.084175084	*ID1, TCF21,ZNF281*	0.075528701
SMARCD3, TBX5,ASCL1	0.093632959	*ID1, NR2F1,ZFPM2*	0.083963056	*ESRRG, MESP1,ZFPM2*	0.075414781

Table S7. List of all 4,960 combinations tested and their respective reprogramming efficiency (MYH6^{+}TNNT2^{+})

TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells
HAND1, IRX5,ZNF281	0.075244545	*GATA4, SMARCD3,ZFPM2*	0.062893082	*MESP1, MITF,ZNF281*	0.051652893
MITF, NR2F1,PBX1	0.075075075	*AKT1, NR2F1,SRF*	0.062774639	*NKX2.5, SNAI2,ZFPM2*	0.051652893
GATA6, NR2F1,ZNF281	0.075075075	*IRX5, MESP1,SOX9*	0.062656642	*ID1, NR2F1,SRF*	0.051599587
ESRRG, MESP1,SNAI2	0.075075075	*MITF, PBX1,SRF*	0.062656642	*MEF2C, TBX5,ZNF281*	0.051282051
FOSL1, HAND2,ID1	0.07423905	*FHL2, GATA6,TCF21*	0.062480475	*FOSL1, IRX5,TBX5*	0.051203277
ESRRA, ETS2,ASCL1	0.074183976	*MITF, NR2F1,SMAD6*	0.062189055	*HEY2, MITF,SMAD6*	0.050813008
NKX2.5, NR2F1,TCF21	0.073313783	*AKT1, SOX9,ASCL1*	0.061652281	*IRX5, TCF21,ASCL1*	0.050798036
ESRRA, MITF,SOX9	0.073099415	*HAND2, NKX2.5,TBX20*	0.061614295	*FOSL1, NKX2.5,PBX1*	0.050735667
GATA6, IRX5,ZNF281	0.073099415	*FOSL1, HAND2,SOX9*	0.06127451	*ESRRA, FHL2,PBX1*	0.050581689
AKT1, MEF2C,ZNF281	0.073099415	*SRF, TBX5,ZNF281*	0.061050061	*ESRRG, NFYB,SMARCD3*	0.050428643
FHL2, MITF,TBX20	0.072780204	*FOSL1, TCF21,ZNF281*	0.061050061	*GATA6, HAND2,ZNF281*	0.050403226
ESRRG, HEY2,SNAI2	0.072463768	*FHL2, HEY2,NR2F1*	0.060827251	*ESRRA, ESRRG,NR2F1*	0.050200803
NFYB, NKX2.5,ZFPM2	0.072463768	*NKX2.5, TBX20,ASCL1*	0.060827251	*MITF, SNAI2,SRF*	0.050125313
HAND1, NR2F1,SOX9	0.07230658	*SOX9, TBX20,ZFPM2*	0.060313631	*MEF2C, TCF21,ZNF281*	0.049751244
ESRRA, HAND1,SOX9	0.07230658	*ESRRG, ID1,NFYB*	0.060168472	*HEY2, MITF,NR2F1*	0.049751244
ETS2, GATA6,ZFPM2	0.07183908	*ESRRG, HEY2,TCF21*	0.059952038	*SOX9, ZNF281,ASCL1*	0.049652433
GATA6, MESP1,ZFPM2	0.071377587	*HEY2, PBX1,ASCL1*	0.059630292	*MITF, SOX9,ZNF281*	0.04950495
PBX1, SMAD6,ASCL1	0.071377587	*HAND2, PBX1,SNAI2*	0.05899705	*MESP1, SMARCD3,TCF21*	0.049261084
SMARCD3, TCF21,ZFPM2	0.070721358	*MITF, NKX2.5,SMAD6*	0.058173357	*SMAD6, SMARCD3,ASCL1*	0.049236829
NKX2.5, ZNF281,MYOCD	0.070323488	*ESRRG, IRX5,ZFPM2*	0.057273769	*MITF, NFYB,PBX1*	0.049019608
MESP1, NFYB,SMAD6	0.070175439	*NFYB, TBX5,ASCL1*	0.057077626	*AKT1, FHL2,NR2F1*	0.048875855
MEF2C, MITF,NFYB	0.070175439	*HEY2, NKX2.5,ZFPM2*	0.056980057	*HEY2, MESP1,ZFPM2*	0.048804295
HEY2, TBX20,ZFPM2	0.069851853	*NR2F1, SNAI2,TCF21*	0.056753689	*ESRRG, ID1,SMARCD3*	0.048520136
IRX5, SMARCD3,ZNF281	0.069783671	*ESRRA, NFYB,ZFPM2*	0.056306306	*MESP1, ZNF281,MYOCD*	0.048402711
MITF, SMAD6,TBX5	0.069735007	*HAND2, MITF,SRF*	0.056211355	*PBX1, SMARCD3,TCF21*	0.048204387
HEY2, PBX1,TCF21	0.069735007	*MEF2C, MITF,TBX5*	0.056116723	*ESRRG, SRF,ZFPM2*	0.048169557
FOSL1, HAND2,NKX2.5	0.069444444	*ESRRG, ID1,NR2F1*	0.056022409	*FHL2, HAND2,MESP1*	0.04803074
ESRRG, SNAI2,TBX20	0.069444444	*HAND2, PBX1,SMARCD3*	0.056022409	*HAND2, SMAD6,SNAI2*	0.047824008
ETS2, PBX1,ZFPM2	0.069444444	*HAND1, HAND2,MITF*	0.05574136	*MITF, SMAD6,TBX20*	0.047348485
ESRRA, SMAD6,ZFPM2	0.068870523	*ID1, NKX2.5,ASCL1*	0.05574136	*AKT1, ESRRA,ID1*	0.047147572
FOSL1, TBX20,ZNF281	0.068728522	*HAND2, MEF2C,TCF21*	0.05574136	*NKX2.5, TCF21,MYOCD*	0.046948357
NFYB, ZFPM2,ASCL1	0.068681319	*AKT1, SMARCD3,ZFPM2*	0.055694793	*AKT1, MEF2C,NFYB*	0.046750818
HAND1, NKX2.5,NR2F1	0.068587106	*MESP1, NFYB,ASCL1*	0.055555556	*ESRRG, NR2F1,SNAI2*	0.046750818
FOSL1, ID1,TBX5	0.068587106	*AKT1, NR2F1,PBX1*	0.05527916	*HAND1, PBX1,TCF21*	0.046511628
MEF2C, MITF,PBX1	0.068306011	*MESP1, ZFPM2,ASCL1*	0.055248619	*ESRRA, ESRRG,SOX9*	0.046490005
ESRRA, GATA4,PBX1	0.067476383	*SMAD6, TBX20,TCF21*	0.054945055	*IRX5, ZNF281,MYOCD*	0.046425255
ID1, TBX5,ASCL1	0.067476383	*GATA6, SMAD6,TBX20*	0.054914882	*ESRRA, GATA6,HAND1*	0.046296296
MEF2C, TBX20,ZFPM2	0.067204301	*NFYB, SRF,ZNF281*	0.054764513	*ESRRA, ETS2,MYOCD*	0.045787546
MITF, TBX20,ZFPM2	0.067139734	*PBX1, SOX9,ZNF281*	0.054585153	*FHL2, ZNF281,ASCL1*	0.045787546
MITF, ZFPM2,ASCL1	0.067114094	*NFYB, TBX20,ZFPM2*	0.054377379	*GATA6, MEF2C,ZFPM2*	0.045537341
HEY2, MESP1,ASCL1	0.066800267	*AKT1, ESRRG,IRX5*	0.053850296	*SOX9, ZFPM2,MYOCD*	0.045454545
GATA4, HEY2,ZNF281	0.066600067	*IRX5, MEF2C,TCF21*	0.053418803	*AKT1, ESRRG,FHL2*	0.045413261
ESRRG, NR2F1,ZFPM2	0.066401062	*GATA6, SNAI2,ZNF281*	0.052910053	*NFYB, PBX1,ZFPM2*	0.045248869
MITF, NKX2.5,SOX9	0.065487885	*AKT1, MITF,ZNF281*	0.052910053	*AKT1, NR2F1,TBX20*	0.045228403
ID1, SNAI2,ZFPM2	0.065359477	*ESRRG, HEY2,SMARCD3*	0.052826202	*MEF2C, TCF21,ZFPM2*	0.045167118
HEY2, TBX5,TCF21	0.064536947	*FOSL1, NFYB,ZFPM2*	0.052742616	*ESRRG, SMARCD3,ZNF281*	0.045045045
GATA4, MESP1,ZNF281	0.064474533	*ETS2, NR2F1,MYOCD*	0.052576236	*ETS2, MESP1,ZFPM2*	0.04492363
HAND2, ID1,ASCL1	0.064226076	*FHL2, TBX5,ZFPM2*	0.052493438	*NKX2.5, NR2F1,TBX20*	0.044863167
HEY2, IRX5,ASCL1	0.064102564	*NR2F1, ZFPM2,MYOCD*	0.052301255	*MEF2C, TBX5,TCF21*	0.044802867
HEY2, MESP1,TCF21	0.06385696	*MITF, SRF,ZNF281*	0.052137643	*AKT1, ESRRA,SOX9*	0.044742729
ESRRG, MEF2C,NR2F1	0.063734863	*ESRRG, GATA4,PBX1*	0.05200208	*AKT1, MEF2C,TCF21*	0.044385264
ESRRG, IRX5,NR2F1	0.063613232	*MITF, NR2F1,SOX9*	0.05192108	*HEY2, IRX5,SOX9*	0.044267375
HEY2, MITF,SMARCD3	0.063131313	*GATA6, PBX1,TCF21*	0.051813472	*HAND2, NFYB,SNAI2*	0.044208665
ESRRA, HAND1,SMARCD3	0.062893082	*ID1, TBX20,TCF21*	0.051679587	*MEF2C, PBX1,ZNF281*	0.043975374

Table S7. List of all 4,960 combinations tested and their respective reprogramming efficiency (MYH6^{+}TNNT2^{+})

TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells
AKT1, NKX2.5,TBX20	0.043572985	*GATA4, HAND1,TCF21*	0.029394474	*FOSL1, SNAI2,ZNF281*	0
AKT1, NR2F1,SOX9	0.043516101	*HEY2, NKX2.5,PBX1*	0.029239766	*PBX1, SRF,TCF21*	0
HAND2, MITF,ZFPM2	0.04302926	*IRX5, ZFPM2,ASCL1*	0.028176951	*PBX1, ZFPM2,ZNF281*	0
ESRRA, ETS2,SMARCD3	0.042900043	*ID1, NKX2.5,TCF21*	0.02761668	*HAND1, MITF,NKX2.5*	0
MITF, SMAD6,SOX9	0.042789902	*GATA4, TBX5,TCF21*	0.026968716	*ESRRA, SMARCD3,TCF21*	0
AKT1, SOX9,TBX5	0.042735043	*NFYB, SMAD6,ZFPM2*	0.026946915	*ETS2, GATA4,ZNF281*	0
ETS2, GATA6,ZNF281	0.042462845	*MEF2C, SNAI2,TCF21*	0.02558199	*MESP1, SNAI2,ZFPM2*	0
ETS2, SMARCD3,ZFPM2	0.042444822	*ETS2, ZFPM2,MYOCD*	0.025464731	*ETS2, SMARCD3,ZNF281*	0
NKX2.5, SMARCD3,ZNF281	0.042408821	*ID1, PBX1,ZFPM2*	0.025406504	*MESP1, TBX5,ZFPM2*	0
GATA4, TCF21,MYOCD	0.041736227	*HEY2, ID1,TBX5*	0.02513826	*MITF, PBX1,ZNF281*	0
GATA6, ID1,ZNF281	0.041666667	*MESP1, PBX1,ZFPM2*	0.024437928	*ESRRG, NFYB,ZNF281*	0
ID1, SRF,ZNF281	0.041631973	*PBX1, TBX20,TCF21*	0.023758612	*ESRRG, HEY2,MEF2C*	0
ETS2, GATA6,TCF21	0.041511	*NKX2.5, TBX20,ZFPM2*	0.022925264	*FHL2, ID1,ZFPM2*	0
FOSL1, HEY2,NKX2.5	0.041511	*ESRRG, HAND2,IRX5*	0	*AKT1, TBX5,ASCL1*	0
MESP1, TBX20,TCF21	0.041254125	*HEY2, ID1,NR2F1*	0	*HAND1, MITF,SRF*	0
ID1, NR2F1,PBX1	0.041203131	*IRX5, SNAI2,TCF21*	0	*AKT1, MITF,ZFPM2*	0
MITF, NFYB,SMARCD3	0.041101521	*NKX2.5, TCF21,ASCL1*	0	*HAND2, TBX5,ZFPM2*	0
ESRRA, FHL2,TBX5	0.040257649	*AKT1, GATA6,ZFPM2*	0	*PBX1, SMAD6,ZFPM2*	0
MEF2C, NKX2.5,ZFPM2	0.039968026	*NR2F1, SMARCD3,ZFPM2*	0	*AKT1, FOSL1,HAND2*	0
AKT1, ESRRG,NR2F1	0.039872408	*AKT1, ETS2,ASCL1*	0	*HEY2, ID1,ZNF281*	0
ESRRA, GATA6,SNAI2	0.039872408	*ESRRG, SRF,ZNF281*	0	*PBX1, SRF,ZFPM2*	0
HAND2, SNAI2,ZFPM2	0.039494471	*NR2F1, TBX20,ZFPM2*	0	*AKT1, SOX9,TCF21*	0
HEY2, IRX5,NFYB	0.039401103	*GATA6, PBX1,SOX9*	0	*AKT1, GATA4,MITF*	0
ESRRA, FOSL1,TBX5	0.039077765	*AKT1, FHL2,ASCL1*	0	*ESRRG, TBX20,TCF21*	0
AKT1, SMARCD3,TBX20	0.039032006	*ESRRG, ZFPM2,ASCL1*	0	*MESP1, NR2F1,ZNF281*	0
HAND1, NKX2.5,ASCL1	0.038986355	*PBX1, TCF21,ASCL1*	0	*AKT1, GATA6,MITF*	0
ID1, SRF,TCF21	0.03875969	*ETS2, GATA4,ZFPM2*	0	*ESRRG, ZNF281,ASCL1*	0
ESRRA, GATA4,GATA6	0.038491147	*MESP1, NR2F1,TCF21*	0	*ID1, SNAI2,ZNF281*	0
GATA6, TBX5,TCF21	0.038402458	*AKT1, TBX5,ZNF281*	0	*MESP1, SNAI2,ZNF281*	0
ID1, NKX2.5,SNAI2	0.03805175	*ETS2, HAND2,ZNF281*	0	*AKT1, HAND1,MITF*	0
FHL2, GATA4,IRX5	0.037792895	*MEF2C, SOX9,ZFPM2*	0	*ID1, TBX5,ZNF281*	0
HEY2, NR2F1,SRF	0.037750094	*MESP1, SNAI2,TCF21*	0	*MESP1, TBX5,ZNF281*	0
FHL2, HAND1,ZFPM2	0.037664783	*NFYB, NR2F1,TBX20*	0	*MITF, TBX20,TCF21*	0
NR2F1, PBX1,SMARCD3	0.037495313	*ETS2, NKX2.5,ZFPM2*	0	*ESRRA, NKX2.5,ZFPM2*	0
GATA4, SRF,ZNF281	0.037202381	*AKT1, HAND1,ASCL1*	0	*IRX5, SNAI2,ZNF281*	0
MITF, SOX9,TCF21	0.036683786	*HAND2, NR2F1,TCF21*	0	*NR2F1, SOX9,MYOCD*	0
FOSL1, NKX2.5,SOX9	0.036630037	*AKT1, HAND2,ASCL1*	0	*AKT1, ID1,MITF*	0
IRX5, TBX20,ZFPM2	0.036350418	*HAND2, SNAI2,TCF21*	0	*ESRRA, TBX20,ZFPM2*	0
ESRRA, GATA6,SMARCD3	0.036075036	*AKT1, HEY2,ASCL1*	0	*NFYB, SRF,TCF21*	0
MITF, TCF21,ZNF281	0.035561878	*FHL2, MESP1,ZFPM2*	0	*ESRRG, HAND2,TCF21*	0
FOSL1, GATA4,ZNF281	0.034831069	*HEY2, ID1,ZFPM2*	0	*NFYB, ZFPM2,ZNF281*	0
HAND2, SMARCD3,ZFPM2	0.034435262	*FHL2, MITF,ZNF281*	0	*AKT1, MESP1,MITF*	0
AKT1, MEF2C,ZFPM2	0.034048349	*NFYB, TCF21,MYOCD*	0	*HAND2, PBX1,ASCL1*	0
ESRRA, GATA6,NFYB	0.033875339	*HAND1, NR2F1,SNAI2*	0	*NKX2.5, SMAD6,ZNF281*	0
FHL2, SOX9,ZFPM2	0.033433634	*FHL2, PBX1,ZFPM2*	0	*AKT1, ESRRA,ZNF281*	0
HAND2, SOX9,ZFPM2	0.033068783	*NKX2.5, SMAD6,TCF21*	0	*FOSL1, PBX1,TCF21*	0
AKT1, ZFPM2,ASCL1	0.032144005	*HEY2, SOX9,ZNF281*	0	*HAND2, TBX5,ZNF281*	0
HEY2, NKX2.5,SOX9	0.03208213	*FHL2, TCF21,ZNF281*	0	*NKX2.5, SRF,ZNF281*	0
ETS2, TBX5,TCF21	0.03192848	*HAND2, HEY2,NR2F1*	0	*AKT1, MITF,NFYB*	0
HAND2, SMAD6,ZFPM2	0.031152648	*FOSL1, HEY2,ZNF281*	0	*AKT1, ID1,TCF21*	0
ESRRG, MESP1,SOX9	0.031065548	*NR2F1, TBX20,ZNF281*	0	*HEY2, NFYB,TCF21*	0
GATA6, NR2F1,TCF21	0.030358227	*AKT1, NKX2.5,ASCL1*	0	*AKT1, MESP1,ZFPM2*	0
GATA4, ZNF281,ASCL1	0.03030303	*HAND1, MESP1,MITF*	0	*ESRRG, TBX20,ZFPM2*	0
HEY2, TCF21,ZFPM2	0.029550827	*AKT1, NR2F1,ASCL1*	0	*NR2F1, TBX5,TCF21*	0

Table S7. List of all 4,960 combinations tested and their respective reprogramming efficiency (MYH6^{+}TNNT2^{+})

TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells
PBX1, SMAD6,ZNF281	0	*HEY2, NFYB,ZNF281*	0	*HAND2, PBX1,ZFPM2*	0
ETS2, NR2F1,TCF21	0	*ESRRA, FOSL1,SOX9*	0	*ESRRA, MESP1,MITF*	0
PBX1, SRF,ZNF281	0	*FHL2, FOSL1,IRX5*	0	*SMAD6, TCF21,ZNF281*	0
AKT1, MITF,SNAI2	0	*ESRRA, SNAI2,ASCL1*	0	*ESRRA, HEY2,ZFPM2*	0
HAND2, HEY2,MITF	0	*FOSL1, MESP1,ZNF281*	0	*GATA4, MEF2C,ZNF281*	0
ESRRA, ETS2,ZFPM2	0	*HEY2, SMAD6,ZNF281*	0	*ETS2, NFYB,NR2F1*	0
ESRRG, HEY2,MESP1	0	*SMARCD3, SOX9,ZFPM2*	0	*SNAI2, TBX20,ZFPM2*	0
FOSL1, MESP1,NKX2.5	0	*ESRRA, SOX9,ASCL1*	0	*ESRRA, MITF,NKX2.5*	0
AKT1, MITF,SOX9	0	*ESRRA, HEY2,TCF21*	0	*HEY2, MITF,SOX9*	0
HAND2, ID1,MITF	0	*HEY2, SRF,ZNF281*	0	*FOSL1, IRX5,SMAD6*	0
FHL2, FOSL1,ZFPM2	0	*SMARCD3, TCF21,ZNF281*	0	*GATA4, TBX5,ZFPM2*	0
ESRRA, NKX2.5,ZNF281	0	*ESRRA, SRF,ASCL1*	0	*ID1, MESP1,ZNF281*	0
FHL2, HAND1,ZNF281	0	*ESRRA, MEF2C,ZFPM2*	0	*SRF, TCF21,ZNF281*	0
SMARCD3, SNAI2,ZNF281	0	*FOSL1, SOX9,ZNF281*	0	*AKT1, ESRRA,GATA4*	0
HAND2, MEF2C,MITF	0	*HEY2, ZFPM2,ASCL1*	0	*FOSL1, MEF2C,MESP1*	0
FHL2, SMAD6,TCF21	0	*SNAI2, ZNF281,MYOCD*	0	*ESRRA, MITF,PBX1*	0
SNAI2, SRF,TCF21	0	*HAND1, HAND2,IRX5*	0	*ID1, PBX1,ZNF281*	0
FHL2, SRF,TCF21	0	*ESRRA, TBX20,ASCL1*	0	*TBX20, TCF21,ASCL1*	0
HEY2, SRF,ZFPM2	0	*HEY2, SNAI2,ASCL1*	0	*ESRRA, FOSL1,GATA6*	0
SOX9, TBX5,ZNF281	0	*ETS2, FHL2,TCF21*	0	*ESRRA, MITF,SMAD6*	0
HAND2, MITF,NKX2.5	0	*ID1, MESP1,ZFPM2*	0	*ESRRG, ETS2,ZNF281*	0
FOSL1, ID1,TCF21	0	*SOX9, TCF21,ZNF281*	0	*ID1, SOX9,ZNF281*	0
ESRRA, ID1,IRX5	0	*HEY2, SOX9,ASCL1*	0	*SMAD6, SOX9,ZNF281*	0
ID1, MESP1,TCF21	0	*ID1, MITF,ZNF281*	0	*ESRRG, MEF2C,TCF21*	0
TBX20, TBX5,ZNF281	0	*ETS2, HEY2,ZNF281*	0	*ESRRA, GATA4,SMAD6*	0
FOSL1, MITF,ZNF281	0	*ID1, SOX9,ZFPM2*	0	*GATA6, SRF,TCF21*	0
TBX5, ZFPM2,ZNF281	0	*SMAD6, SOX9,TCF21*	0	*IRX5, MESP1,ZNF281*	0
ID1, PBX1,TCF21	0	*NKX2.5, PBX1,SOX9*	0	*HEY2, NKX2.5,TBX5*	0
AKT1, HAND2,NKX2.5	0	*ESRRG, GATA4,ZNF281*	0	*ID1, MESP1,ASCL1*	0
ESRRG, TBX20,ZNF281	0	*ETS2, SNAI2,ZNF281*	0	*IRX5, PBX1,ZNF281*	0
ID1, TCF21,ZFPM2	0	*SMAD6, TCF21,ZFPM2*	0	*SMARCD3, ZFPM2,ZNF281*	0
ESRRA, ID1,ASCL1	0	*HEY2, ID1,MITF*	0	*ESRRA, MITF,SRF*	0
AKT1, SOX9,ZNF281	0	*SMARCD3, SOX9,ZNF281*	0	*AKT1, NFYB,ZFPM2*	0
IRX5, MITF,ZFPM2	0	*HEY2, IRX5,MITF*	0	*HAND1, MESP1,TCF21*	0
ETS2, MEF2C,ZNF281	0	*IRX5, MITF,ZNF281*	0	*HAND1, MITF,ZFPM2*	0
ESRRA, ETS2,ZNF281	0	*SMARCD3, TCF21,MYOCD*	0	*SOX9, SRF,TCF21*	0
SMAD6, SNAI2,ZNF281	0	*AKT1, ESRRG,TCF21*	0	*ESRRG, PBX1,SMARCD3*	0
ESRRA, MEF2C,TCF21	0	*ESRRA, HAND1,MITF*	0	*ID1, IRX5,MESP1*	0
IRX5, TCF21,ZFPM2	0	*HEY2, MESP1,MITF*	0	*HAND2, PBX1,ZNF281*	0
SMAD6, TBX5,ZNF281	0	*IRX5, SOX9,ZFPM2*	0	*SOX9, ZFPM2,ZNF281*	0
HEY2, MEF2C,ASCL1	0	*ESRRA, HAND2,MITF*	0	*HAND2, SOX9,ZNF281*	0
ESRRG, HAND2,ZNF281	0	*SOX9, TCF21,MYOCD*	0	*ESRRG, TBX20,TBX5*	0
FHL2, NFYB,ZFPM2	0	*ESRRA, HEY2,MITF*	0	*ID1, NR2F1,ASCL1*	0
FHL2, SMAD6,ZFPM2	0	*FHL2, ZFPM2,ASCL1*	0	*ESRRA, FHL2,TCF21*	0
HAND2, PBX1,TCF21	0	*MEF2C, NFYB,ZNF281*	0	*HAND1, TCF21,ZFPM2*	0
FOSL1, ID1,NKX2.5	0	*SRF, TCF21,ZFPM2*	0	*HEY2, IRX5,ZNF281*	0
ESRRA, NR2F1,ASCL1	0	*ESRRA, ID1,MITF*	0	*TCF21, ZFPM2,ZNF281*	0
SOX9, TCF21,ZFPM2	0	*HEY2, MITF,NKX2.5*	0	*ID1, SMAD6,ASCL1*	0
FOSL1, IRX5,PBX1	0	*AKT1, SMAD6,TCF21*	0	*ESRRA, HEY2,ZNF281*	0
ESRRA, FHL2,ID1	0	*TBX20, TCF21,MYOCD*	0	*HEY2, SMARCD3,TCF21*	0
GATA6, MESP1,NFYB	0	*TBX5, ZNF281,ASCL1*	0	*SMARCD3, SRF,ZFPM2*	0
ESRRA, SMAD6,ASCL1	0	*ESRRA, MEF2C,MITF*	0	*FOSL1, TBX5,TCF21*	0
HEY2, NKX2.5,ASCL1	0	*HEY2, MITF,PBX1*	0	*ESRRA, TBX5,ZNF281*	0
HEY2, NR2F1,ASCL1	0	*GATA4, GATA6,TCF21*	0	*GATA4, GATA6,ZFPM2*	0

Table S7. List of all 4,960 combinations tested and their respective reprogramming efficiency (MYH6^{+}TNNT2^{+})

TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells
SOX9, SRF,ZFPM2	0	ESRRG, FOSL1,MITF	0	ID1, NFYB,ZNF281	0
SRF, TCF21,ASCL1	0	IRX5, MITF,ASCL1	0	SOX9, TBX20,ZNF281	0
GATA4, SOX9,TBX20	0	GATA6, SMAD6,ZNF281	0	ESRRG, GATA6,ZNF281	0
HAND2, IRX5,TBX20	0	IRX5, NFYB,ASCL1	0	ID1, SMAD6,ZNF281	0
GATA4, SNAI2,ZNF281	0	ESRRA, PBX1,TCF21	0	ID1, ZFPM2,ASCL1	0
ESRRG, MEF2C,ASCL1	0	SMAD6, SRF,ZNF281	0	TBX5, TCF21,ZFPM2	0
ID1, IRX5,MITF	0	HEY2, NKX2.5,ZNF281	0	AKT1, GATA4,ZFPM2	0
AKT1, ESRRG,ZNF281	0	ESRRG, HAND2,MITF	0	IRX5, NFYB,ZNF281	0
FOSL1, HAND1,PBX1	0	ESRRA, TCF21,ZFPM2	0	ZFPM2, ZNF281,MYOCD	0
ID1, MEF2C,MITF	0	HAND1, ID1,ZFPM2	0	AKT1, HAND2,ZNF281	0
AKT1, IRX5,TCF21	0	SMARCD3, TBX20,TCF21	0	IRX5, SMAD6,ZNF281	0
GATA6, NFYB,ZFPM2	0	ESRRG, FHL2,TCF21	0	SMAD6, TBX20,ZFPM2	0
SMAD6, ZFPM2,ZNF281	0	HEY2, TBX20,ZNF281	0	AKT1, NKX2.5,ZFPM2	0
AKT1, SMAD6,ZNF281	0	SMARCD3, ZNF281,ASCL1	0	SNAI2, SOX9,ZFPM2	0
ESRRG, HAND1,HEY2	0	ESRRG, ID1,MITF	0	SRF, TBX20,ZFPM2	0
AKT1, SRF,ZNF281	0	ESRRG, GATA6,ZFPM2	0	ETS2, MEF2C,ASCL1	0
SMARCD3, ZFPM2,ASCL1	0	SNAI2, TBX5,ZNF281	0	ESRRA, HAND1,TCF21	0
HEY2, ID1,MESP1	0	IRX5, SMARCD3,ASCL1	0	TBX5, TCF21,ZNF281	0
SNAI2, TBX5,TCF21	0	ESRRG, HEY2,ZNF281	0	ESRRA, ID1,ZFPM2	0
ESRRG, NR2F1,ASCL1	0	SOX9, TBX20,TCF21	0	ZFPM2, ZNF281,ASCL1	0
ESRRA, FHL2,ZFPM2	0	IRX5, SNAI2,ASCL1	0	ESRRA, MESP1,ZNF281	0
SOX9, SRF,ZNF281	0	GATA6, HEY2,NKX2.5	0	HEY2, MEF2C,ZFPM2	0
ESRRA, FOSL1,HAND1	0	ESRRG, MESP1,MITF	0	SMAD6, TBX20,ZNF281	0
ESRRG, HEY2,NR2F1	0	SRF, ZFPM2,ASCL1	0	HAND1, NFYB,TCF21	0
ESRRG, PBX1,ASCL1	0	TBX20, ZNF281,ASCL1	0	SMARCD3, SNAI2,TCF21	0
ESRRA, GATA6,ZNF281	0	IRX5, TBX20,ASCL1	0	HAND2, SNAI2,TBX5	0
HAND1, MITF,ZNF281	0	AKT1, IRX5,ZNF281	0	ESRRA, SOX9,ZNF281	0
SOX9, ZFPM2,ASCL1	0	HEY2, ID1,NFYB	0	SMARCD3, TBX5,TCF21	0
ESRRA, FOSL1,TBX20	0	AKT1, NKX2.5,TCF21	0	SNAI2, SOX9,ZNF281	0
ESRRG, ID1,PBX1	0	GATA4, MITF,ZFPM2	0	ETS2, NR2F1,ASCL1	0
ESRRG, SMAD6,ASCL1	0	IRX5, SRF,ZFPM2	0	HAND1, ZFPM2,ZNF281	0
ID1, MITF,SMAD6	0	ESRRG, HAND2,PBX1	0	MEF2C, SRF,ASCL1	0
HAND1, PBX1,ZFPM2	0	ESRRG, MITF,NR2F1	0	HAND2, NFYB,ZFPM2	0
ID1, ZFPM2,ZNF281	0	IRX5, MEF2C,MITF	0	SOX9, TBX5,TCF21	0
ESRRA, GATA6,NR2F1	0	AKT1, SMARCD3,TCF21	0	FOSL1, TCF21,ZFPM2	0
ESRRG, SNAI2,ASCL1	0	GATA4, PBX1,TCF21	0	ESRRG, IRX5,SOX9	0
ID1, MITF,SNAI2	0	ESRRA, FOSL1,HAND2	0	MEF2C, TBX5,ASCL1	0
ESRRG, HEY2,ZFPM2	0	IRX5, MESP1,MITF	0	TBX20, TBX5,TCF21	0
HAND1, TCF21,ZNF281	0	AKT1, TBX20,TCF21	0	MEF2C, MESP1,MITF	0
TCF21, ZFPM2,ASCL1	0	GATA4, SOX9,TCF21	0	AKT1, ETS2,ZFPM2	0
ESRRA, HAND1,NFYB	0	AKT1, ZNF281,ASCL1	0	GATA4, ID1,ZFPM2	0
ID1, MITF,SOX9	0	MEF2C, SMARCD3,ZNF281	0	HAND1, HAND2,NKX2.5	0
HAND2, ID1,ZFPM2	0	SRF, ZNF281,MYOCD	0	ETS2, SOX9,ASCL1	0
HAND2, MESP1,ZNF281	0	ESRRG, IRX5,SNAI2	0	AKT1, GATA4,ZNF281	0
FOSL1, TBX5,ZFPM2	0	MESP1, SMAD6,SOX9	0	AKT1, NKX2.5,ZNF281	0
IRX5, ZFPM2,ZNF281	0	IRX5, MITF,NFYB	0	SMARCD3, SNAI2,ZFPM2	0
AKT1, IRX5,ZFPM2	0	GATA6, IRX5,ZFPM2	0	ESRRG, PBX1,SRF	0
GATA4, GATA6,ZNF281	0	HAND1, ID1,ZNF281	0	AKT1, SMARCD3,ZNF281	0
SNAI2, TBX5,ZFPM2	0	SMARCD3, TBX20,ZNF281	0	GATA4, SOX9,ZNF281	0
AKT1, ZNF281,MYOCD	0	HAND1, TCF21,ASCL1	0	SMARCD3, TBX5,ZFPM2	0
GATA4, MITF,TCF21	0	ESRRA, TCF21,ZNF281	0	ETS2, TBX5,ASCL1	0
MEF2C, SMARCD3,ZFPM2	0	HAND2, NFYB,TCF21	0	MEF2C, MITF,NR2F1	0
NKX2.5, SNAI2,SOX9	0	ID1, IRX5,TCF21	0	AKT1, TBX20,ZNF281	0
ESRRG, FHL2,MITF	0	SNAI2, TCF21,ZFPM2	0	HEY2, NR2F1,ZFPM2	0

Table S7. List of all 4,960 combinations tested and their respective reprogramming efficiency (MYH6^{+}TNNT2^{+})

TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells
SNAI2, TCF21,ASCL1	0	*GATA4, SMAD6,TCF21*	0	*FHL2, IRX5,ZFPM2*	0
SOX9, TBX5,ZFPM2	0	*FHL2, NFYB,SOX9*	0	*MITF, NKX2.5,NR2F1*	0
MEF2C, MITF,SMAD6	0	*ESRRA, FOSL1,ZNF281*	0	*FHL2, ZNF281,MYOCD*	0
ESRRA, HAND1,ZFPM2	0	*GATA4, SRF,TCF21*	0	*GATA6, NR2F1,ZFPM2*	0
TBX20, TBX5,ZFPM2	0	*MESP1, MITF,SNAI2*	0	*FHL2, MITF,NR2F1*	0
MEF2C, MITF,SMARCD3	0	*MESP1, MITF,SOX9*	0	*FOSL1, HAND1,ZNF281*	0
ESRRA, ID1,ZNF281	0	*MESP1, SOX9,TCF21*	0	*GATA6, SNAI2,ZFPM2*	0
TBX5, TCF21,ASCL1	0	*FHL2, IRX5,ASCL1*	0	*MEF2C, ZNF281,ASCL1*	0
ESRRA, TCF21,ASCL1	0	*MESP1, MITF,SRF*	0	*FOSL1, NFYB,TCF21*	0
ETS2, HAND1,MITF	0	*HAND1, HAND2,TCF21*	0	*GATA6, IRX5,SRF*	0
ESRRG, ID1,TCF21	0	*MESP1, TCF21,ZFPM2*	0	*HAND2, SOX9,TBX20*	0
ID1, SMARCD3,TCF21	0	*MITF, NR2F1,ZFPM2*	0	*HAND1, HAND2,ZNF281*	0
AKT1, IRX5,TBX20	0	*MITF, SMARCD3,TCF21*	0	*MESP1, SOX9,ZNF281*	0
ETS2, HEY2,MITF	0	*ESRRG, HAND1,ZFPM2*	0	*FOSL1, SRF,TCF21*	0
MEF2C, MITF,TBX20	0	*FOSL1, HAND1,SOX9*	0	*HAND1, NKX2.5,ZFPM2*	0
HAND1, SRF,ZFPM2	0	*HAND2, NFYB,TBX20*	0	*ESRRG, HAND2,SOX9*	0
AKT1, ETS2,ZNF281	0	*MITF, NFYB,ASCL1*	0	*ESRRG, SNAI2,ZFPM2*	0
ESRRG, FOSL1,SOX9	0	*FOSL1, TCF21,ASCL1*	0	*MITF, NKX2.5,TCF21*	0
FOSL1, HAND1,SNAI2	0	*AKT1, GATA6,TCF21*	0	*ESRRA, FOSL1,MEF2C*	0
ETS2, MESP1,MITF	0	*GATA4, NFYB,ZFPM2*	0	*ESRRG, TBX5,ZFPM2*	0
ESRRA, FOSL1,ZFPM2	0	*NFYB, SMAD6,ZNF281*	0	*HAND1, TBX20,ZFPM2*	0
GATA4, HAND1,ZFPM2	0	*GATA4, SMAD6,ZFPM2*	0	*MITF, SMARCD3,ZNF281*	0
ESRRA, HAND1,ZNF281	0	*ESRRG, HAND1,MESP1*	0	*FOSL1, NKX2.5,SMAD6*	0
GATA4, ID1,ZNF281	0	*GATA6, IRX5,SOX9*	0	*FHL2, MITF,SRF*	0
FOSL1, ID1,SNAI2	0	*MITF, SMAD6,ASCL1*	0	*ETS2, FHL2,ZNF281*	0
ESRRA, NFYB,TCF21	0	*AKT1, NR2F1,ZFPM2*	0	*ESRRG, IRX5,TBX5*	0
MESP1, SMAD6,ASCL1	0	*MITF, SMARCD3,ASCL1*	0	*ETS2, MESP1,TCF21*	0
ESRRA, SMAD6,TCF21	0	*AKT1, SNAI2,ZFPM2*	0	*NFYB, NKX2.5,TCF21*	0
MESP1, SMARCD3,ASCL1	0	*GATA6, HEY2,TCF21*	0	*MITF, NKX2.5,SMARCD3*	0
ESRRA, SRF,TCF21	0	*MITF, SNAI2,ASCL1*	0	*NFYB, SMARCD3,TCF21*	0
MESP1, MITF,TCF21	0	*AKT1, TBX5,ZFPM2*	0	*NFYB, TBX20,TCF21*	0
ESRRA, ZFPM2,ZNF281	0	*ESRRG, ID1,SOX9*	0	*ETS2, SOX9,TCF21*	0
MITF, NR2F1,TCF21	0	*MITF, SOX9,ASCL1*	0	*NFYB, ZNF281,ASCL1*	0
ESRRG, HAND1,TCF21	0	*ESRRA, ESRRG,ZNF281*	0	*ETS2, TCF21,ZFPM2*	0
MESP1, SRF,ASCL1	0	*MITF, SRF,ASCL1*	0	*FOSL1, HAND2,ASCL1*	0
ESRRG, ID1,ZFPM2	0	*NR2F1, TBX5,ZNF281*	0	*NKX2.5, TBX20,ZNF281*	0
HAND1, NFYB,ZNF281	0	*MITF, TBX20,ASCL1*	0	*FHL2, HAND2,ZFPM2*	0
MESP1, SOX9,TBX5	0	*HAND1, HAND2,ZFPM2*	0	*GATA6, TBX5,ZNF281*	0
HAND1, SMAD6,ZNF281	0	*FHL2, FOSL1,MITF*	0	*NR2F1, PBX1,TCF21*	0
ESRRG, PBX1,ZNF281	0	*HAND1, NKX2.5,TCF21*	0	*MITF, NR2F1,SNAI2*	0
NFYB, SRF,ZFPM2	0	*MITF, NR2F1,MYOCD*	0	*HAND1, SMARCD3,ZNF281*	0
AKT1, HEY2,TCF21	0	*ESRRG, HAND1,ZNF281*	0	*NR2F1, TCF21,ZFPM2*	0
HAND1, ZFPM2,ASCL1	0	*HAND1, TBX20,TCF21*	0	*FOSL1, MEF2C,ASCL1*	0
MESP1, MITF,NFYB	0	*FHL2, HAND1,MITF*	0	*FHL2, SMARCD3,TCF21*	0
ESRRG, SNAI2,SOX9	0	*MESP1, SOX9,ZFPM2*	0	*PBX1, SMARCD3,ZNF281*	0
NR2F1, SNAI2,ZFPM2	0	*ESRRG, SMAD6,TCF21*	0	*FOSL1, MESP1,ASCL1*	0
MESP1, MITF,NR2F1	0	*FHL2, HEY2,MITF*	0	*FOSL1, ZFPM2,ASCL1*	0
GATA4, HAND1,ZNF281	0	*GATA4, NFYB,ZNF281*	0	*SMAD6, SMARCD3,TCF21*	0
NR2F1, TBX5,ZFPM2	0	*MITF, SMARCD3,ZFPM2*	0	*GATA4, HAND2,ZFPM2*	0
ID1, NKX2.5,SMARCD3	0	*ETS2, MITF,TCF21*	0	*FOSL1, NKX2.5,ASCL1*	0
MESP1, MITF,PBX1	0	*AKT1, NR2F1,SMAD6*	0	*GATA4, NKX2.5,TCF21*	0
AKT1, TBX5,TCF21	0	*FHL2, HAND2,TCF21*	0	*FOSL1, NR2F1,ASCL1*	0
GATA4, NFYB,TCF21	0	*GATA6, HEY2,ZFPM2*	0	*GATA4, SMARCD3,TCF21*	0
AKT1, HAND2,SOX9	0	*MITF, NFYB,NR2F1*	0	*MITF, TBX5,TCF21*	0

Table S7. List of all 4,960 combinations tested and their respective reprogramming efficiency (MYH6^{+}TNNT2^{+})

TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells
ESRRG, TBX5,ZNF281	0	*FOSL1, MITF,TBX20*	0	*GATA4, NR2F1,TCF21*	0
GATA4, TBX20,TCF21	0	*MITF, SRF,TBX5*	0	*GATA4, ID1,MITF*	0
MITF, SNAI2,SOX9	0	*FOSL1, IRX5,ZFPM2*	0	*GATA4, SNAI2,TCF21*	0
MITF, NFYB,SRF	0	*FOSL1, MITF,TBX5*	0	*PBX1, TBX5,ZNF281*	0
HAND2, SOX9,TBX5	0	*MITF, TBX20,TBX5*	0	*MESP1, SMARCD3,SRF*	0
MITF, NKX2.5,SRF	0	*ESRRG, SOX9,TCF21*	0	*GATA4, MEF2C,MITF*	0
ETS2, MITF,ZNF281	0	*ESRRG, TCF21,ZFPM2*	0	*FOSL1, NKX2.5,ZFPM2*	0
MITF, NR2F1,SRF	0	*GATA6, MITF,ZNF281*	0	*GATA6, HAND1,ZFPM2*	0
ESRRG, HAND2,SRF	0	*AKT1, ESRRG,GATA4*	0	*GATA4, MESP1,MITF*	0
ETS2, SOX9,ZFPM2	0	*GATA6, PBX1,ZFPM2*	0	*NFYB, NR2F1,ZFPM2*	0
NR2F1, TCF21,ZNF281	0	*MITF, NFYB,ZFPM2*	0	*FOSL1, TBX20,ZFPM2*	0
GATA6, NKX2.5,PBX1	0	*ESRRA, GATA4,ID1*	0	*GATA6, TCF21,ASCL1*	0
HEY2, MEF2C,NR2F1	0	*ESRRG, ID1,TBX5*	0	*NFYB, SNAI2,ZFPM2*	0
MESP1, NKX2.5,SMAD6	0	*ETS2, HAND1,ZFPM2*	0	*ESRRA, HAND2,SOX9*	0
SMAD6, SMARCD3,ZFPM2	0	*GATA6, SOX9,ZFPM2*	0	*NFYB, TBX5,ZFPM2*	0
FHL2, GATA4,ZFPM2	0	*MITF, TBX5,ZNF281*	0	*HAND2, MESP1,TCF21*	0
MESP1, NFYB,TCF21	0	*GATA6, TCF21,ZNF281*	0	*NR2F1, SMAD6,ZFPM2*	0
GATA4, NKX2.5,ZFPM2	0	*NKX2.5, NR2F1,ZFPM2*	0	*NR2F1, SRF,ZFPM2*	0
MITF, SOX9,SRF	0	*ESRRA, GATA6,TBX20*	0	*AKT1, HAND1,IRX5*	0
MESP1, SRF,TCF21	0	*GATA4, ID1,ASCL1*	0	*GATA4, MITF,PBX1*	0
ESRRG, NFYB,SOX9	0	*NFYB, SMAD6,ASCL1*	0	*PBX1, SNAI2,ASCL1*	0
FOSL1, HAND2,MITF	0	*HAND1, HEY2,ZFPM2*	0	*HAND2, NKX2.5,TCF21*	0
FOSL1, IRX5,TCF21	0	*HAND1, MEF2C,ZNF281*	0	*MESP1, NKX2.5,TCF21*	0
GATA6, MITF,ZFPM2	0	*NKX2.5, TBX5,ZFPM2*	0	*NR2F1, SMAD6,SNAI2*	0
MITF, NKX2.5,ZNF281	0	*ESRRG, NR2F1,SMARCD3*	0	*HAND2, ZNF281,ASCL1*	0
MITF, PBX1,TBX20	0	*ID1, IRX5,SOX9*	0	*FHL2, NR2F1,TBX20*	0
FOSL1, NFYB,ZNF281	0	*HAND1, SNAI2,ZFPM2*	0	*GATA4, MITF,SOX9*	0
MITF, TBX5,ZFPM2	0	*HAND1, TBX5,ZFPM2*	0	*HEY2, NR2F1,ZNF281*	0
FOSL1, IRX5,MITF	0	*PBX1, TBX5,ZFPM2*	0	*MESP1, ZNF281,ASCL1*	0
GATA6, SOX9,TCF21	0	*FHL2, IRX5,MESP1*	0	*FHL2, HEY2,ZFPM2*	0
NFYB, NKX2.5,ZNF281	0	*HAND2, HEY2,ZNF281*	0	*MITF, SMAD6,ZNF281*	0
MITF, SOX9,TBX20	0	*MESP1, NFYB,ZNF281*	0	*FHL2, NR2F1,ZFPM2*	0
ETS2, ID1,ZFPM2	0	*NKX2.5, SOX9,TBX5*	0	*ID1, NKX2.5,ZFPM2*	0
FOSL1, MITF,NFYB	0	*MESP1, SMAD6,ZNF281*	0	*SMAD6, SOX9,ASCL1*	0
HAND1, NR2F1,TCF21	0	*MESP1, SRF,ZNF281*	0	*FHL2, SNAI2,ZFPM2*	0
NKX2.5, SNAI2,TCF21	0	*ESRRG, TCF21,ZNF281*	0	*FOSL1, HAND1,ID1*	0
MITF, NFYB,TBX5	0	*ETS2, HAND1,ZNF281*	0	*ID1, TBX20,ZFPM2*	0
ETS2, PBX1,ZNF281	0	*GATA4, SNAI2,ASCL1*	0	*GATA6, HEY2,ASCL1*	0
HAND1, SNAI2,TCF21	0	*NFYB, SNAI2,TCF21*	0	*NFYB, TBX5,ZNF281*	0
FOSL1, MITF,NR2F1	0	*NKX2.5, SOX9,ASCL1*	0	*FOSL1, NKX2.5,ZNF281*	0
MITF, NKX2.5,TBX5	0	*ETS2, SMAD6,TCF21*	0	*NKX2.5, SOX9,TCF21*	0
MITF, NR2F1,TBX5	0	*HAND1, HEY2,ZNF281*	0	*FOSL1, IRX5,MEF2C*	0
FHL2, GATA4,ZNF281	0	*GATA4, SRF,ASCL1*	0	*SMARCD3, SNAI2,ASCL1*	0
NR2F1, TCF21,MYOCD	0	*NKX2.5, NR2F1,ZNF281*	0	*MEF2C, NR2F1,ZNF281*	0
FOSL1, HAND1,HAND2	0	*AKT1, SOX9,TBX20*	0	*NKX2.5, TCF21,ZFPM2*	0
FHL2, NKX2.5,ZNF281	0	*NKX2.5, SNAI2,ZNF281*	0	*ESRRG, TCF21,ASCL1*	0
HAND2, NKX2.5,NR2F1	0	*GATA4, GATA6,MITF*	0	*NR2F1, SMAD6,ZNF281*	0
FOSL1, MITF,SNAI2	0	*NR2F1, MYOCD,ASCL1*	0	*AKT1, ESRRA,NKX2.5*	0
GATA6, HEY2,SOX9	0	*GATA4, HAND1,MITF*	0	*HEY2, IRX5,NR2F1*	0
MITF, SNAI2,TBX5	0	*NR2F1, PBX1,ASCL1*	0	*GATA6, MITF,ASCL1*	0
FHL2, TBX20,ZNF281	0	*FHL2, NR2F1,TCF21*	0	*ETS2, IRX5,TCF21*	0
SMAD6, SMARCD3,ZNF281	0	*GATA4, HEY2,TCF21*	0	*NR2F1, SRF,ZNF281*	0
FOSL1, MITF,SRF	0	*FHL2, SNAI2,TCF21*	0	*HEY2, MEF2C,SMARCD3*	0
GATA4, TBX20,ZNF281	0	*ESRRG, HAND2,TBX5*	0	*SMARCD3, TBX20,ASCL1*	0

Table S7. List of all 4,960 combinations tested and their respective reprogramming efficiency (MYH6^{+}TNNT2^{+})

TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells	TF trio combination	%MYH6+TNNT2+ cells
ETS2, NFYB,ZNF281	0	*GATA6, MITF,SMAD6*	0		
HAND2, NKX2.5,ZFPM2	0	*ETS2, HAND2,ZFPM2*	0		
NR2F1, ZFPM2,ASCL1	0	*MEF2C, SOX9,TCF21*	0		
GATA6, NFYB,ASCL1	0	*NKX2.5, PBX1,ZNF281*	0		
ETS2, SMAD6,ZNF281	0	*HEY2, NKX2.5,SMARCD3*	0		
PBX1, SOX9,TCF21	0	*ETS2, NKX2.5,TCF21*	0		
ETS2, SRF,ZNF281	0	*HEY2, ID1,TCF21*	0		
PBX1, TCF21,ZFPM2	0	*HEY2, PBX1,SMAD6*	0		
GATA6, NR2F1,ASCL1	0	*GATA6, MITF,SOX9*	0		
SNAI2, SOX9,ASCL1	0	*NR2F1, SMARCD3,TCF21*	0		
HEY2, MITF,TCF21	0	*ESRRA, HAND1,TBX5*	0		
GATA6, PBX1,ASCL1	0	*HEY2, MITF,ZNF281*	0		
SNAI2, SRF,ASCL1	0	*NR2F1, TBX20,TCF21*	0		
FHL2, GATA6,ZFPM2	0	*ESRRA, HAND2,TBX20*	0		
ID1, NKX2.5,ZNF281	0	*HEY2, PBX1,ZFPM2*	0		
MESP1, SMARCD3,ZFPM2	0	*NR2F1, ZNF281,ASCL1*	0		
AKT1, FOSL1,SOX9	0	*NFYB, SOX9,TBX5*	0		
SNAI2, TBX20,ASCL1	0	*GATA6, MITF,TBX5*	0		
MESP1, TBX20,ZFPM2	0	*HEY2, SOX9,ZFPM2*	0		
SNAI2, TBX5,ASCL1	0	*ESRRG, SOX9,TBX5*	0		
ID1, TBX20,ZNF281	0	*HAND1, HAND2,ASCL1*	0		
MITF, SNAI2,TCF21	0	*ID1, MEF2C,ZFPM2*	0		
MITF, SRF,ZFPM2	0	*MITF, PBX1,TCF21*	0		
AKT1, HAND2,IRX5	0	*HAND1, ID1,ASCL1*	0		
IRX5, TBX20,ZNF281	0	*HAND1, IRX5,ASCL1*	0		
NKX2.5, PBX1,ZFPM2	0	*FHL2, TCF21,ZFPM2*	0		
ESRRA, NFYB,SOX9	0	*ID1, SNAI2,TCF21*	0		
ESRRG, SRF,TCF21	0	*MITF, TCF21,ASCL1*	0		
GATA6, TBX5,ASCL1	0	*NR2F1, SOX9,TBX20*	0		
ESRRG, ZFPM2,ZNF281	0	*FOSL1, GATA6,TCF21*	0		
NKX2.5, TCF21,ZNF281	0	*HAND1, MESP1,ASCL1*	0		
SRF, TBX20,ASCL1	0	*FOSL1, HEY2,ZFPM2*	0		
ETS2, HAND2,TCF21	0	*NFYB, SOX9,ZFPM2*	0		
HAND2, SMARCD3,ZNF281	0	*IRX5, NR2F1,TCF21*	0		
NR2F1, ZNF281,MYOCD	0	*GATA6, MEF2C,NKX2.5*	0		
GATA6, HAND2,MITF	0				
PBX1, TCF21,ZNF281	0				
GATA6, ID1,MITF	0				
TBX20, TBX5,ASCL1	0				
HEY2, MITF,ZFPM2	0				
GATA6, IRX5,MITF	0				
FHL2, MITF,TCF21	0				
MESP1, SMARCD3,ZNF281	0				
FOSL1, HEY2,TCF21	0				
HEY2, SOX9,TCF21	0				
MESP1, TBX20,ZNF281	0				
MITF, SNAI2,ZFPM2	0				
FOSL1, NR2F1,TCF21	0				
MEF2C, MESP1,TCF21	0				
MEF2C, MITF,ZFPM2	0				
GATA6, NFYB,NKX2.5	0				
GATA6, MITF,PBX1	0				
NFYB, TCF21,ZFPM2	0				
ESRRA, FOSL1,PBX1	0				

Table S7. List of all 4,960 combinations tested and their respective reprogramming efficiency (MYH6^{+}TNNT2^{+})

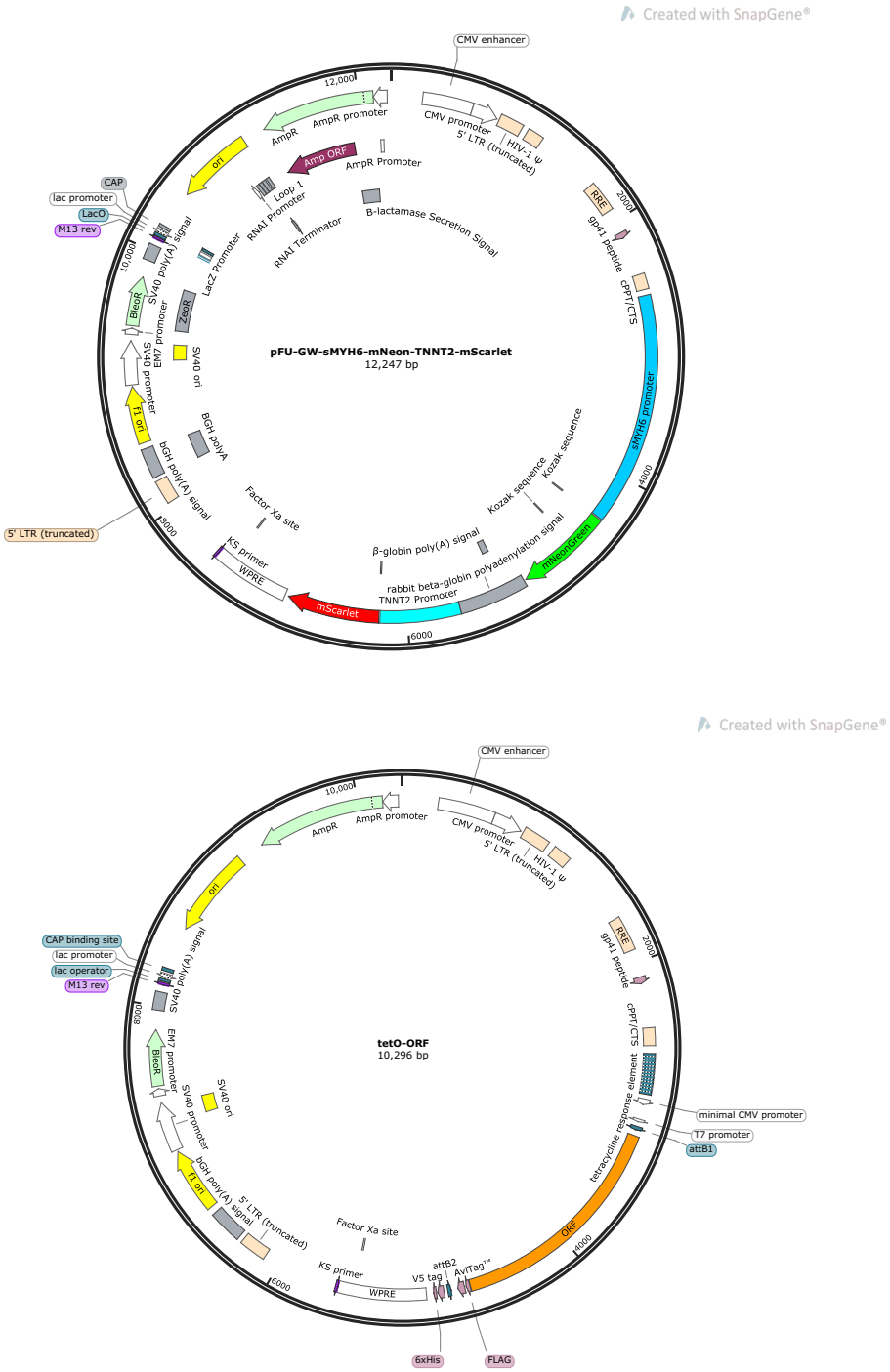

Figure S1. Plasmid maps. (A) Dual-fluorescent reporter plasmid pFU-GW-sMYH6-mNeon-TNNT2-mScarlet (Addgene #170712). (B) cDNA expression plasmid tetO-ORF.

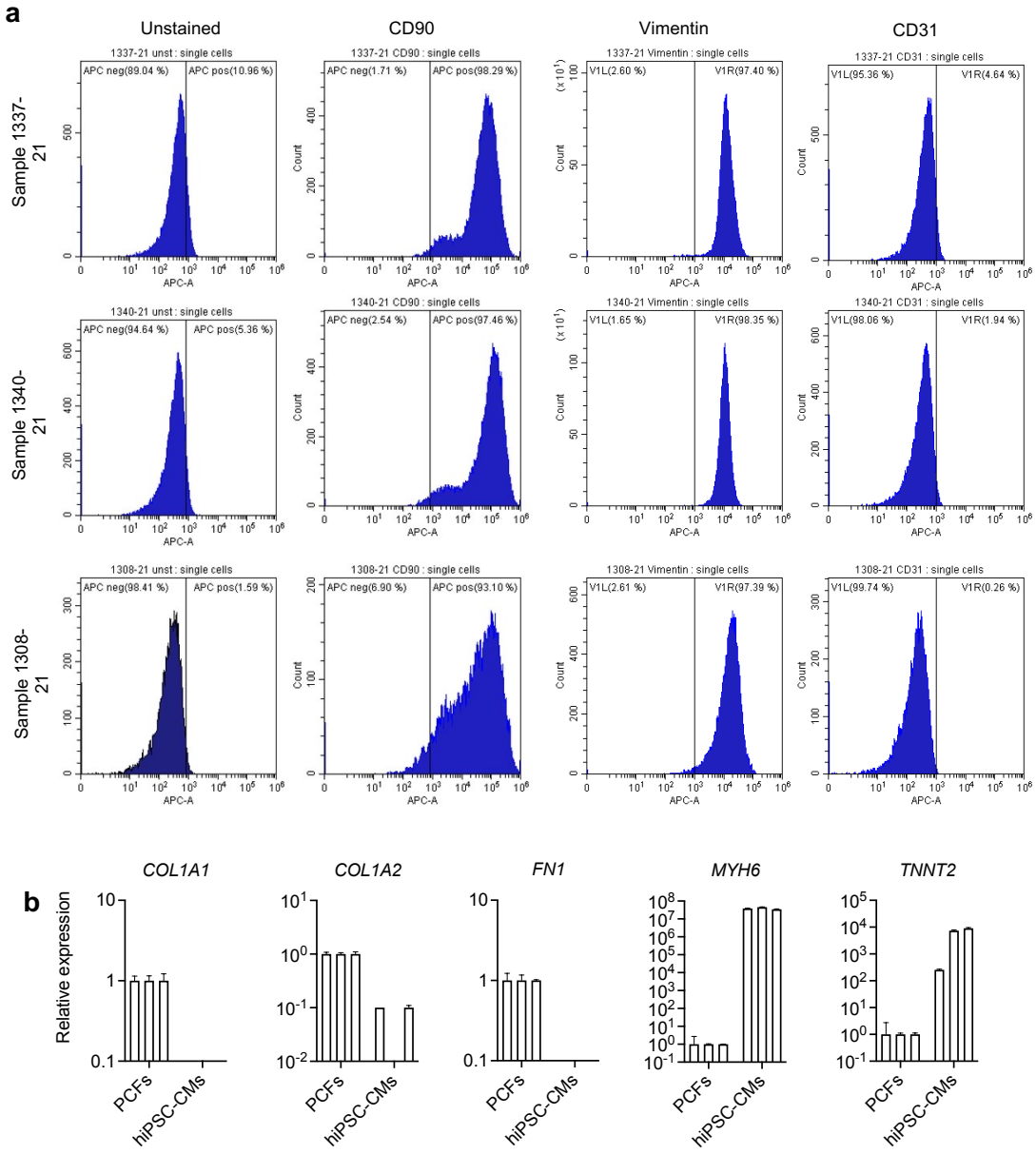

Figure S2. Outgrowth PCFs cells express fibroblast markers. (a) Cells stain positive for fibroblast markers CD90 and vimentin and negative for endothelial cell marker CD31 by flow cytometry (*n* = 3 samples). (b) Cells express fibroblast markers COL1A1, COL1A2, and FN1 and do not express cardiac markers MYH6 nor TNNT2 by qRT-PCR (*n* = 3 samples) .

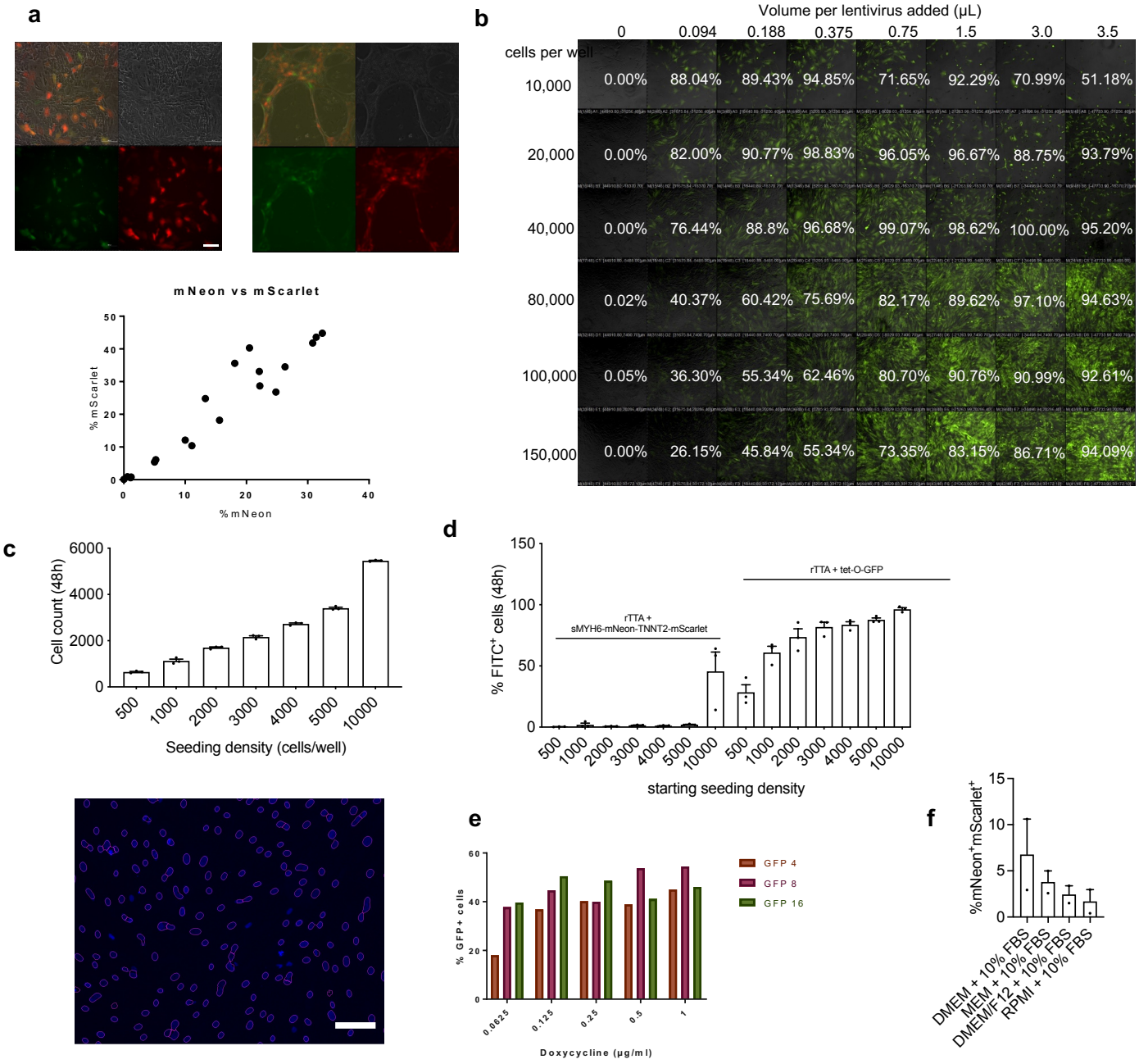

Figure S3. Lentiviral Transduction Optimization. (a) Validation of pFU-GW-sMYH6-mNeon-TNNT2-mScarlet reporter. hiPSC-CMs (d32) transduced with dual reporter (left) exhibited mNeon and mScarlet fluorescence 72 h post-transduction, and transduced hiPSCs differentiated into hiCMs exhibited mNeon and mScarlet fluorescence starting at differentiation d17 (right). mNeon and mScarlet fluorescence linearly correlate (bottom) (Scale bar = 100 µM) **(b)** Lentiviral transduction efficiency of primary cardiac fibroblasts with pFU-tet-O-GFP and rTTA. Cells were passaged to a Matrigel-coated 96-well plate at various seeding densities and transduced using Transdux Max with a range of lentiviral volumes dispensed by liquid handler. Cells were imaged and analyzed by flow cytometry at 72 h post-transduction. Percent positive GFP cells in white text. For direct reprogramming, MOI was scaled accordingly and 50nL of concentrated lentivirus was used to infect 3,000 cells per well in a 384-well plate, resulting in a transduction efficiency of >90%. **(c)** Validation of VALA gating algorithm. Increased seeding density corresponds with higher cell count (left). Representative gating image (right). Cells for transduction were seeded at 3,000 cells per well in a 384-well plate. Average pixel intensity within the nuclear gate is used to quantify percent positive cells using our fluorescent reporter (Scale bar = 100 µM). **(d)** Seeding density optimization. Cells were plated in 384-well plates and transduced with tet-O-GFP to assess transduction efficiency and with sMYH6-mNeon alone as a negative control. Percent positive cells were quantified at 48h post-transduction. Seeding cells at 3,000 cells per well resulted in >90% transduction efficiency and no fluorescence in reporter-transduced cells. (n=3 biological replicates) **(e)** Doxycycline dose optimization. 0.125 µg/mL doxycycline is sufficient to induce GFP expression at 72 h post-transduction and no toxicity is observed at concentrations up to 1 µg/mL. We used 0.25 µg/mL doxycycline for direct reprogramming. **(f)** Basal media selection. Cells were transduced with pFU-GW-sMYH6-mNeon-TNNT2-mScarlet, rTTA, and tet-O-MYOCD. Fluorescence was quantified on reprogramming day 6 (*n* = 2 biological replicates). DMEM+10% FBS was used going forward for direct reprogramming.

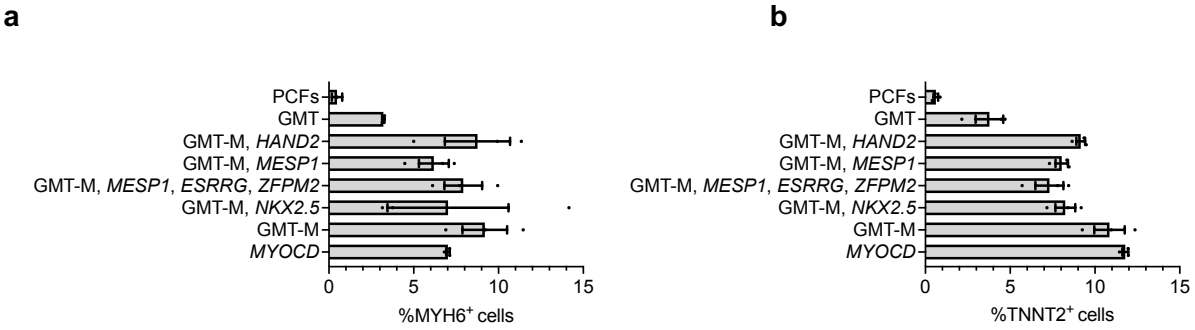

Figure S4. Published transcription factor combinations activate MYH6 and TNNT2 promoters at low levels. Quantification of **(a)** MYH6$^+$ and **(b)** TNNT2$^+$ cells as measured by pFU-GW-sMYH6-mScarlet-TNNT2-mNeon reporter fluorescence on reprogramming day 6 (n = 3 biological replicates). All cells were subject to reporter overexpression.

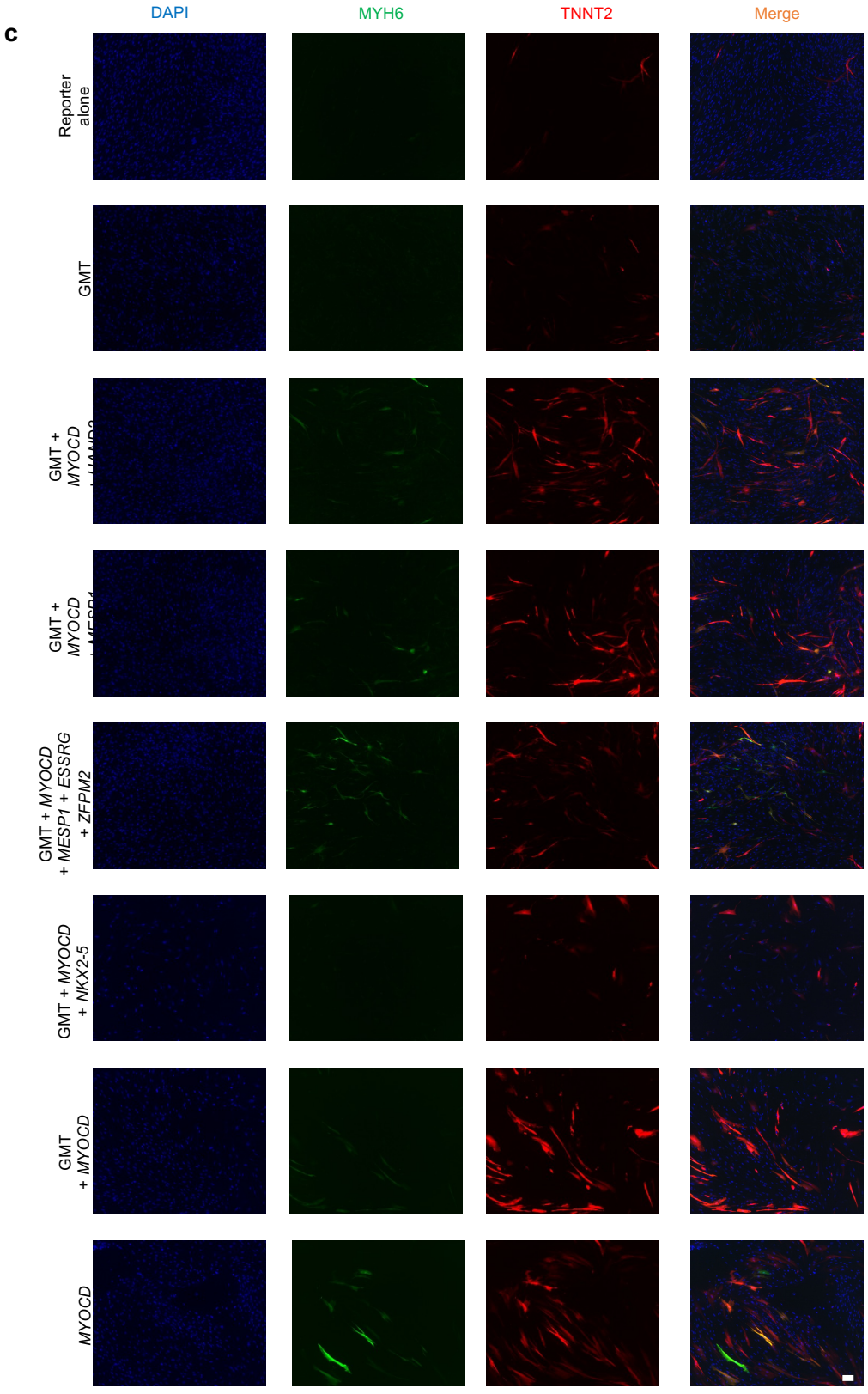

Figure S4 (continued). Published transcription factor combinations activate MYH6 and TNNT2 promoters at low levels. Quantification of **(c)** Representative images of primary cardiac fibroblasts transduced with sMYH6-mNeonGreen-TNNT2-mScarlet and reprogrammed using published factor combinations. Scale bar = 100 μM. Images acquired using high content image cytometry at reprogramming day 6 (KIC, VALA Sciences).

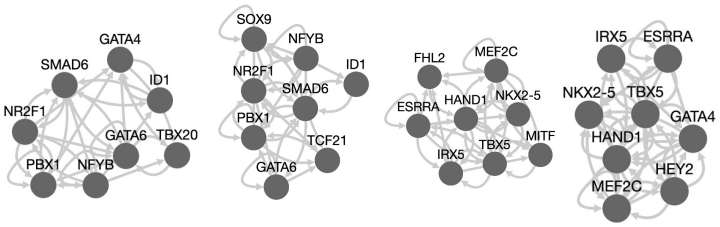

Figure S5. Reprogramming transcription factor networks predicted by Mogrify. Left to right: fibroblast to cardiac myocyte, cardiac fibroblast to cardiac myocyte, cardiac fibroblast to adult heart, and fibroblast to adult heart.

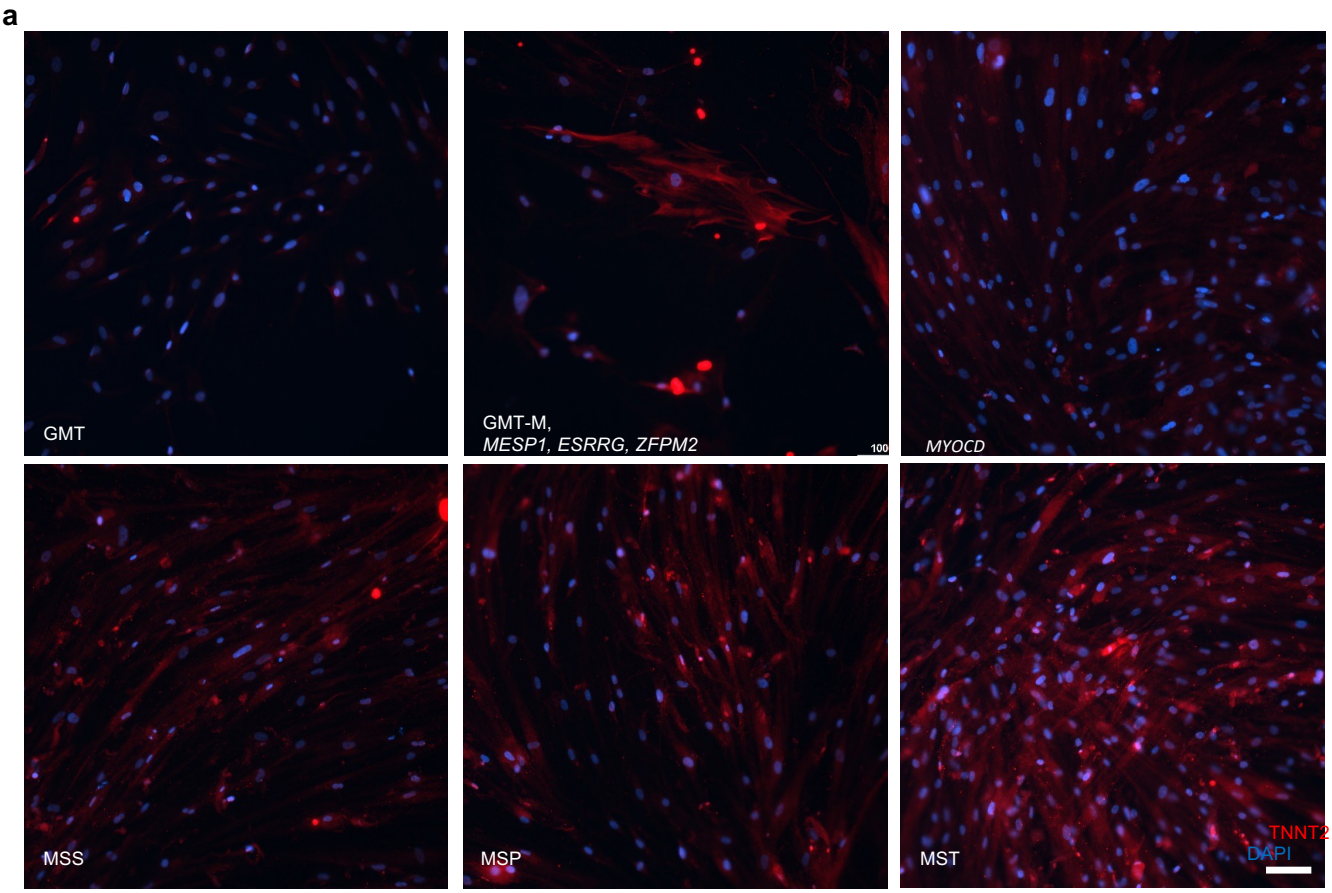

Figure S6. TNNT2 immunostaining of top 3 novel combinations; reprogramming day 60.

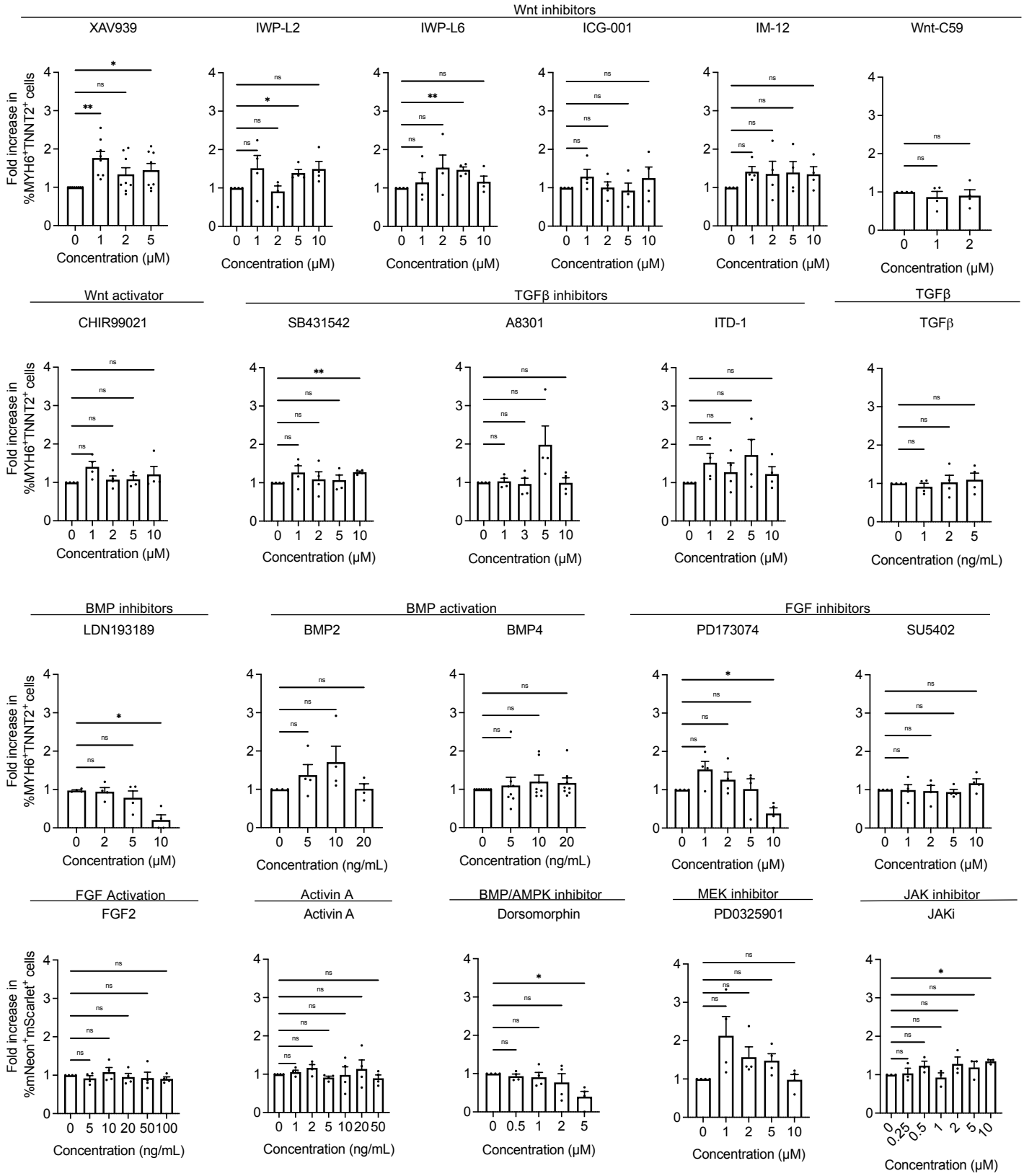

Figure S7. Small Molecule Screen. Cells were transduced with MST and exposed to small molecules starting on reprogramming day 3. Fluorescence was quantified on reprogramming day 6. (n = 40 wells analyzed for each condition from 3-4 biological replicates; mean of biological replicates plotted). P values were calculated by ANOVA followed by Fisher's LSD test. ns $P > 0.05$, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$, **** $P \leq 0.0001$.

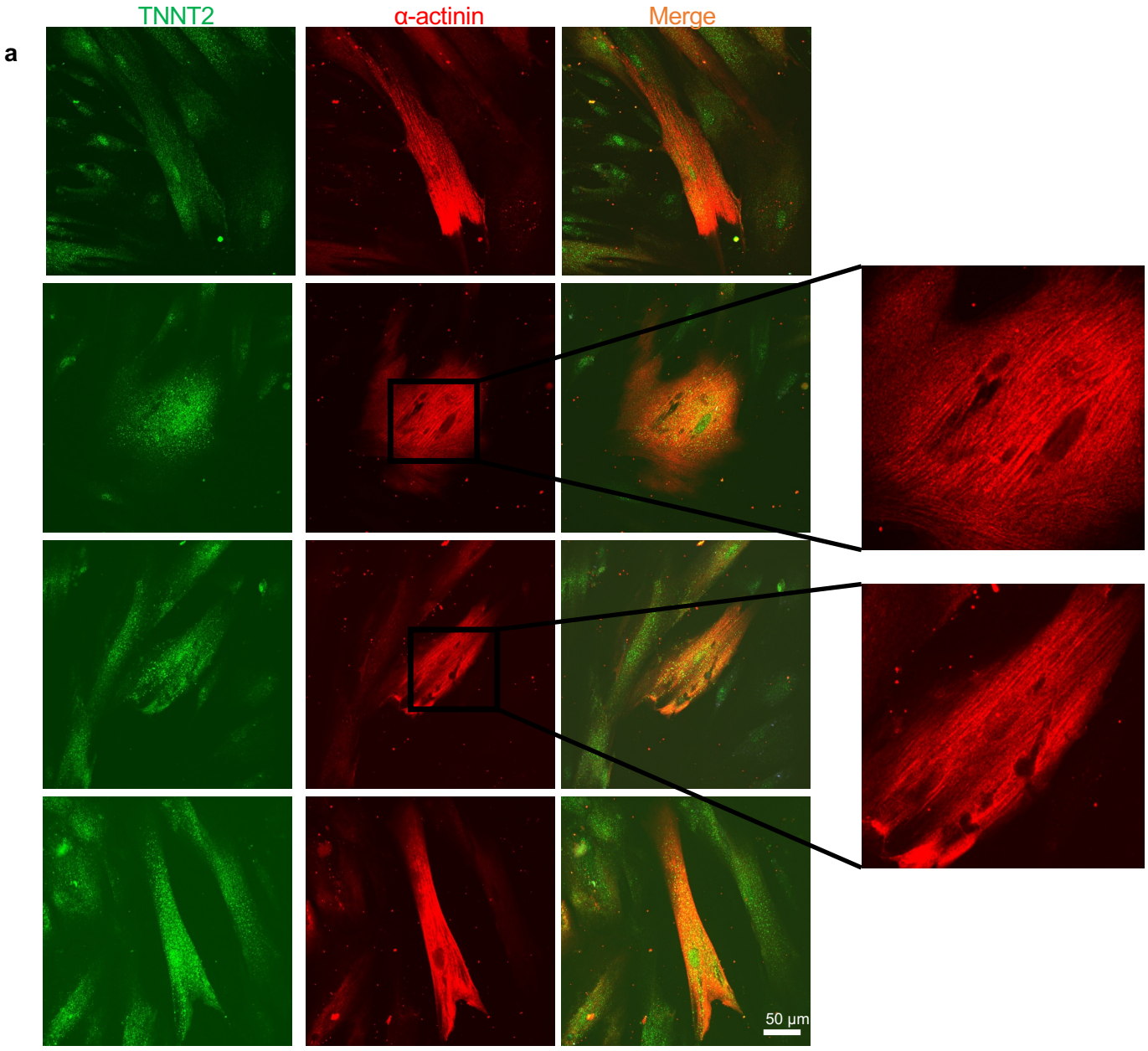

Figure S8. MST-derived cells express sarcomeric genes and proteins. (a) TNNT2 and α-actinin immunostaining on reprogramming day 30. D-BAITS (DMEM, BSA, Ascorbic acid, Insulin, Transferrin, Sodium selenite). MST (*MYOCD*, *SMAD6*, *TBX20*). **(b)** Comparative expression of cardiac sarcomeric genes in MST-reprogrammed hiCMs vs. primary cardiac fibroblasts. Transcripts per million for expression. Error bars: mean ± SEM.

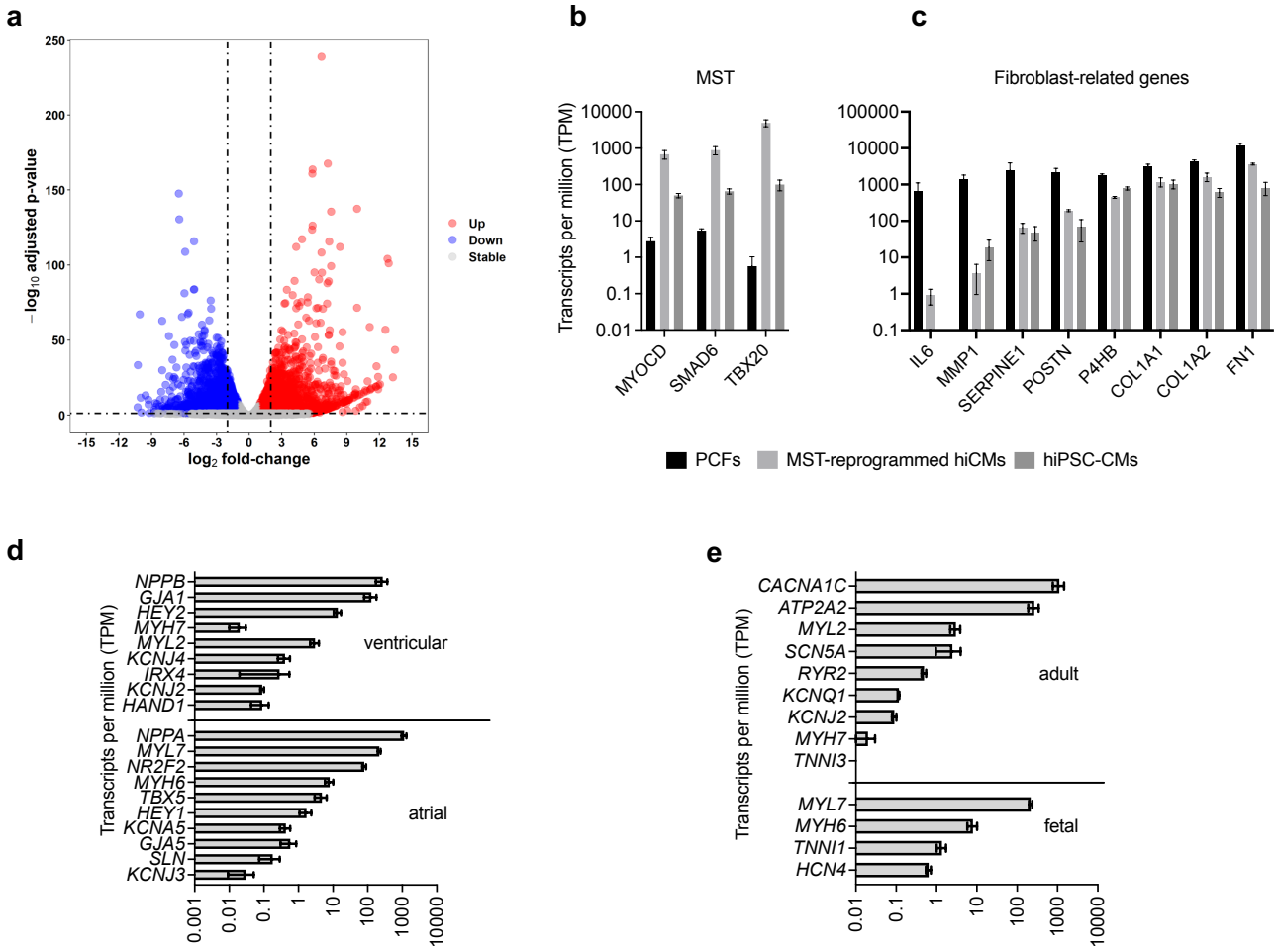

Figure S9. Transcriptional reprogramming of MST-derived hiCMs toward a cardiac state. (a) Volcano plot displaying 9,293 differentially expressed genes in MST-differentiated hiCMs relative to untransduced PCFs (FDR < 0.05). Comparative expression of cardiac genes in MST-reprogrammed hiCMs vs. primary cardiac fibroblasts. Transcripts per million for expression of **(b)** *MYOCD*, *SMAD6*, *TBX20* **(c)** fibroblast-related genes **(d)** atrial and ventricular genes **(e)** cardiac adult and fetal genes.