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Supplemental information

Robust small molecule-aided cardiac reprogramming systems selective to cardiac fibroblasts

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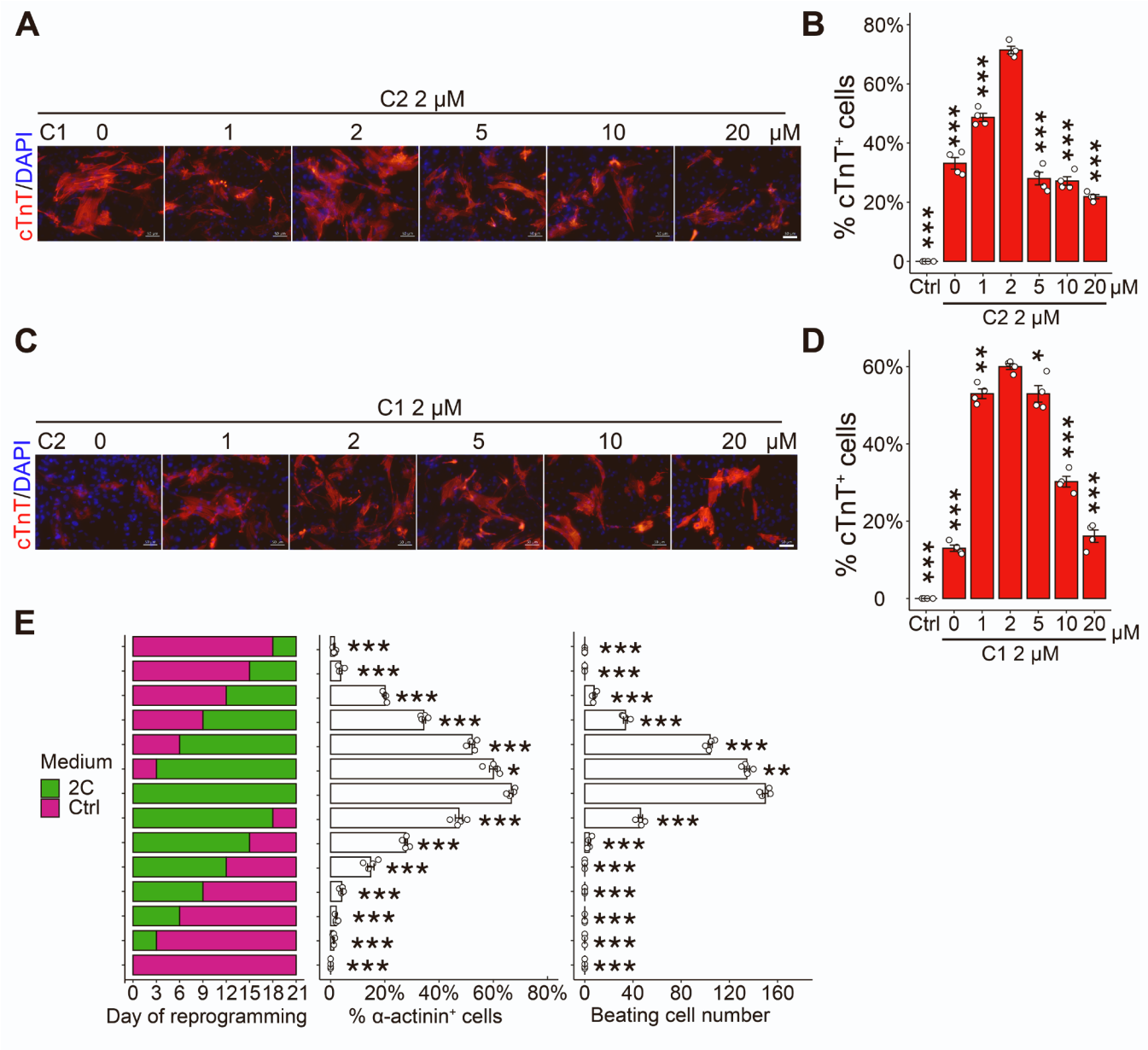

Figure S1. Dosage and timing optimization for 2C, related to Figure 1

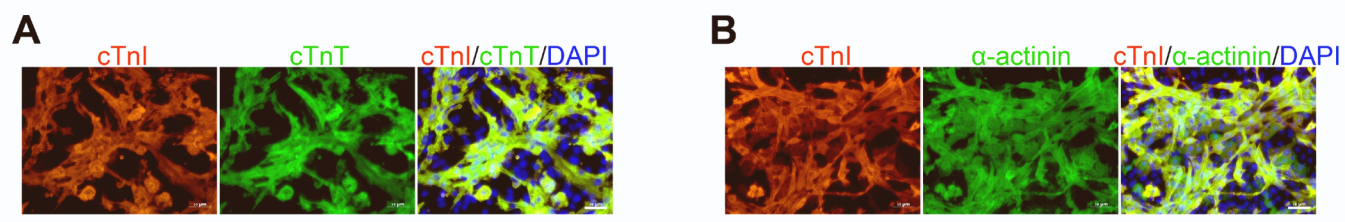

Figure S2. MT+2C induced iCMs co-express multiple cardiomyocyte-specific markers, related to Figure 1

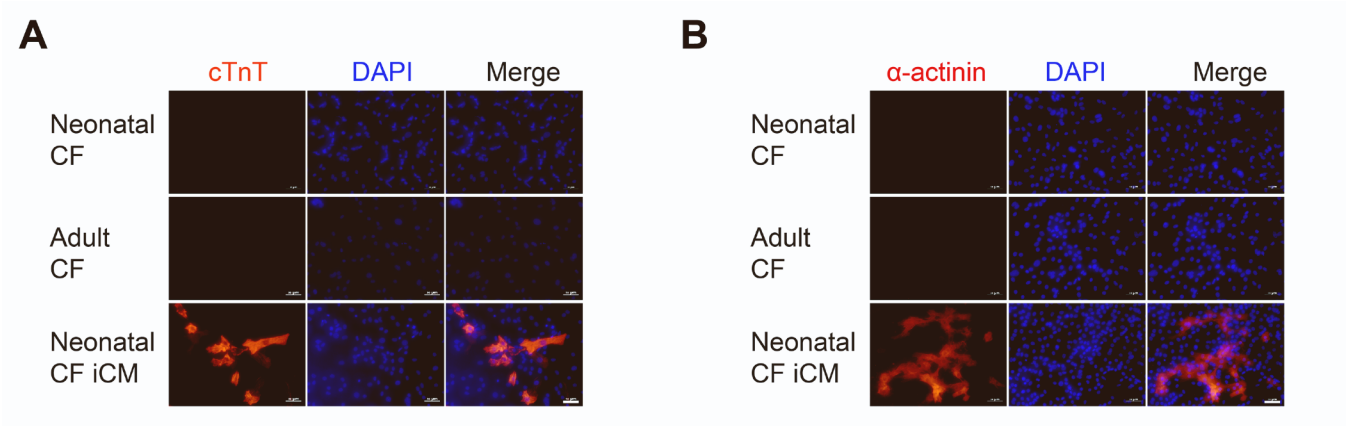

Figure S3 Confirmation of mouse cardiac fibroblasts, related to Figure 2

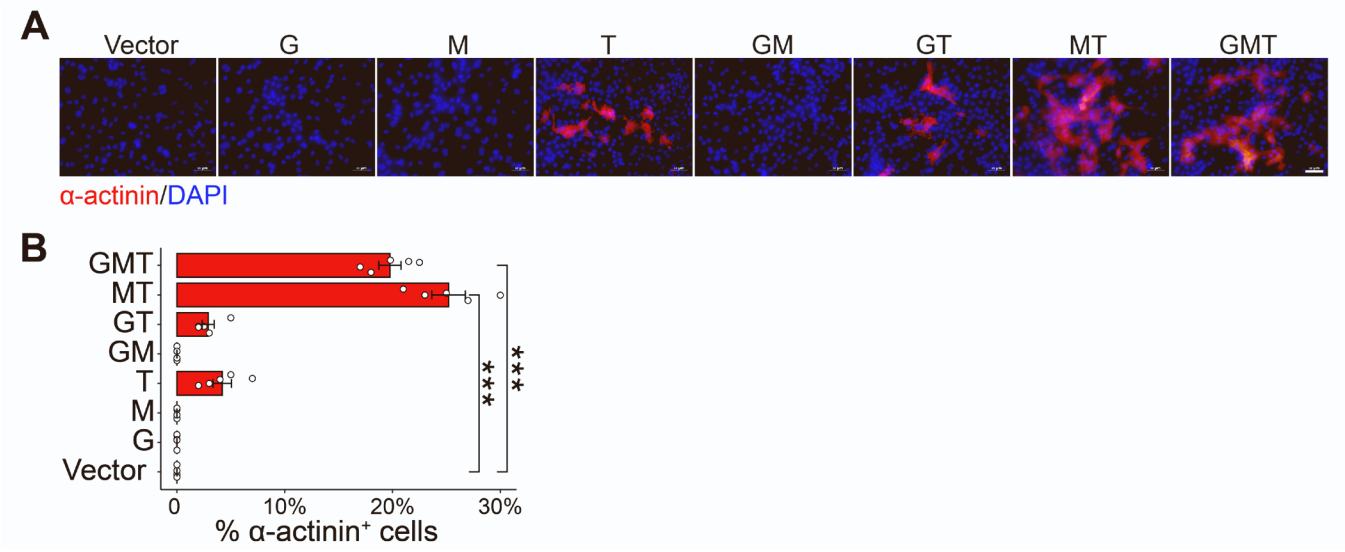

Figure S4. Different combinations of transcription factors reveal MT enables cardiac reprogramming in presence of 2C, related to Figure 2

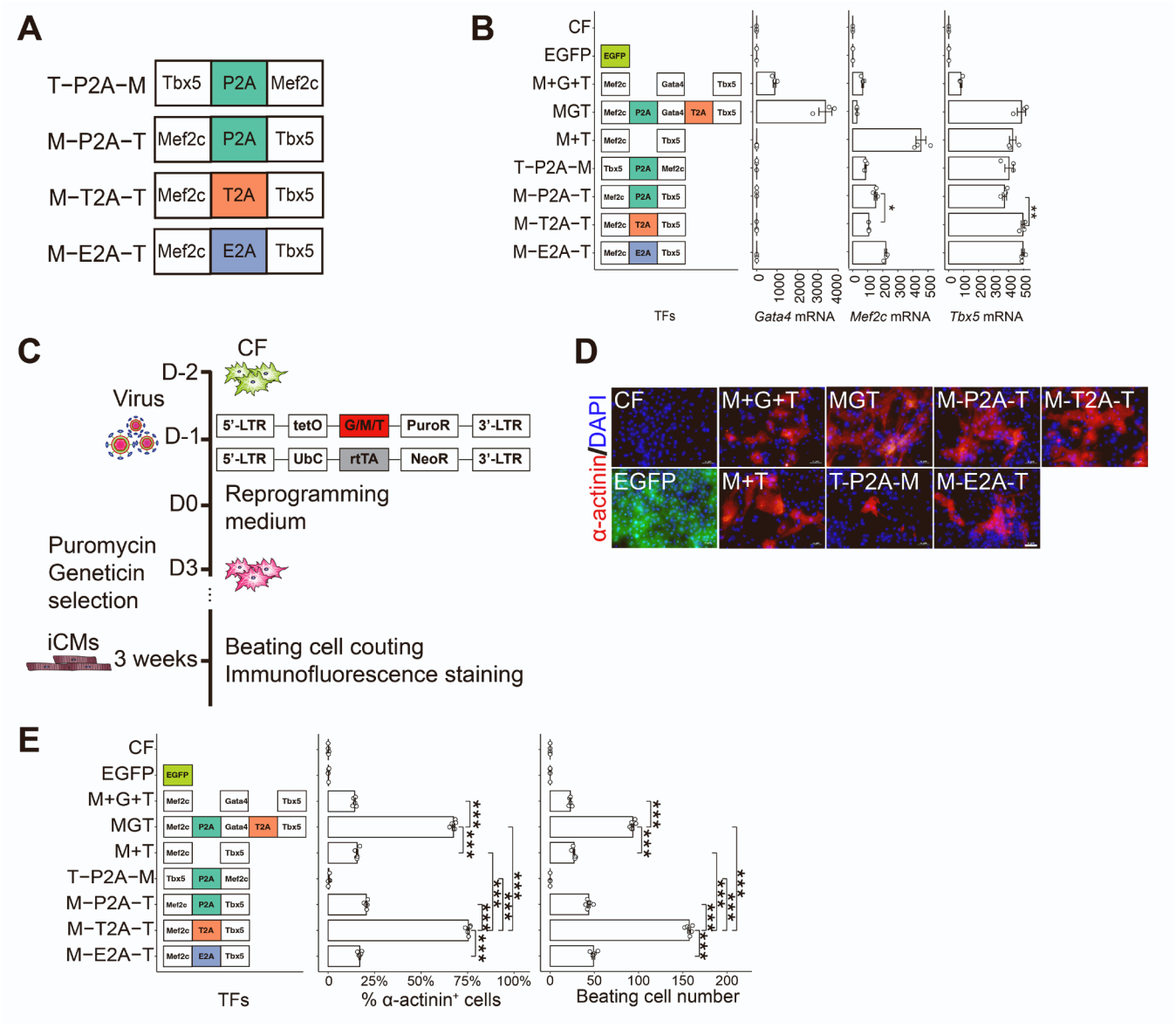

Figure S5 Optimized stoichiometry of Mef2c and Tbx5 results in higher efficiency, related to Figure 2

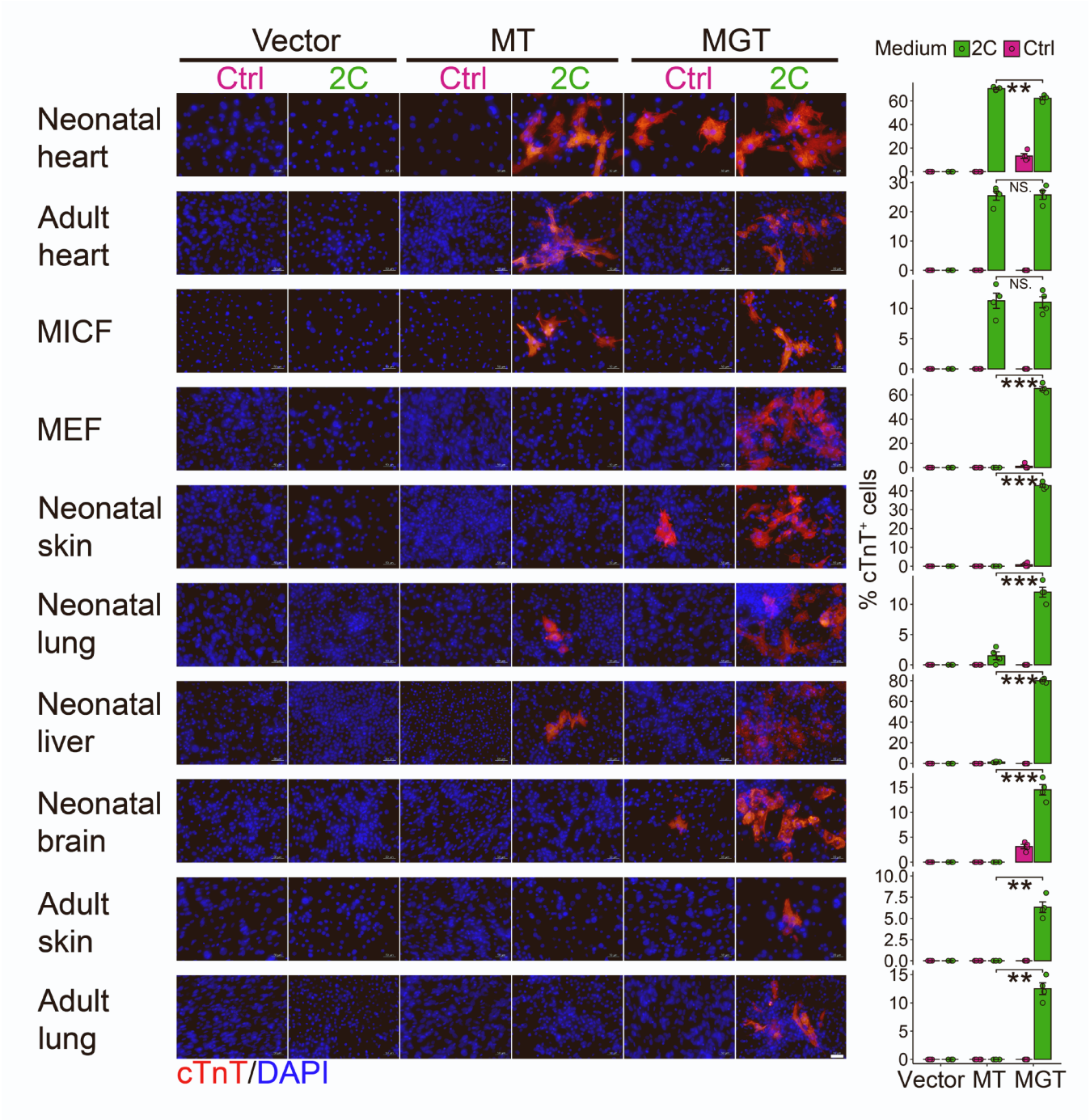

Figure S6 MT+2C selectively reprogram fibroblasts derived heart into iCMs, related to Figure 2

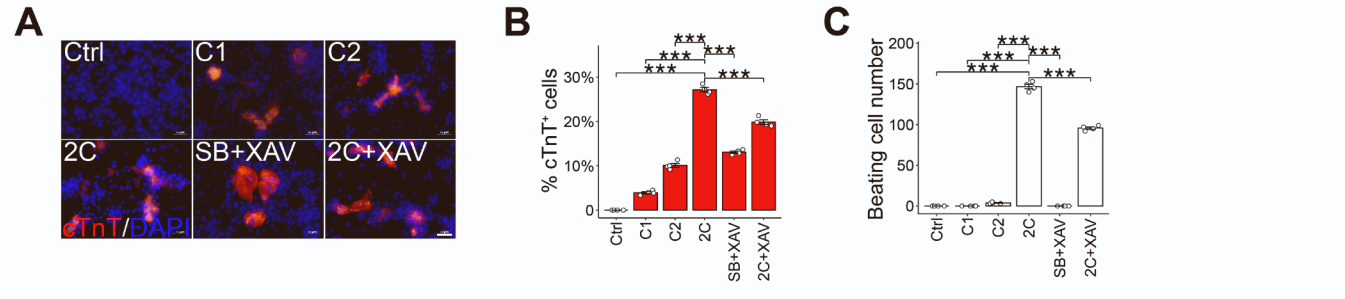

Figure S7. Both C1 and C2 are essential for substituting Gata4, related to Figure 2

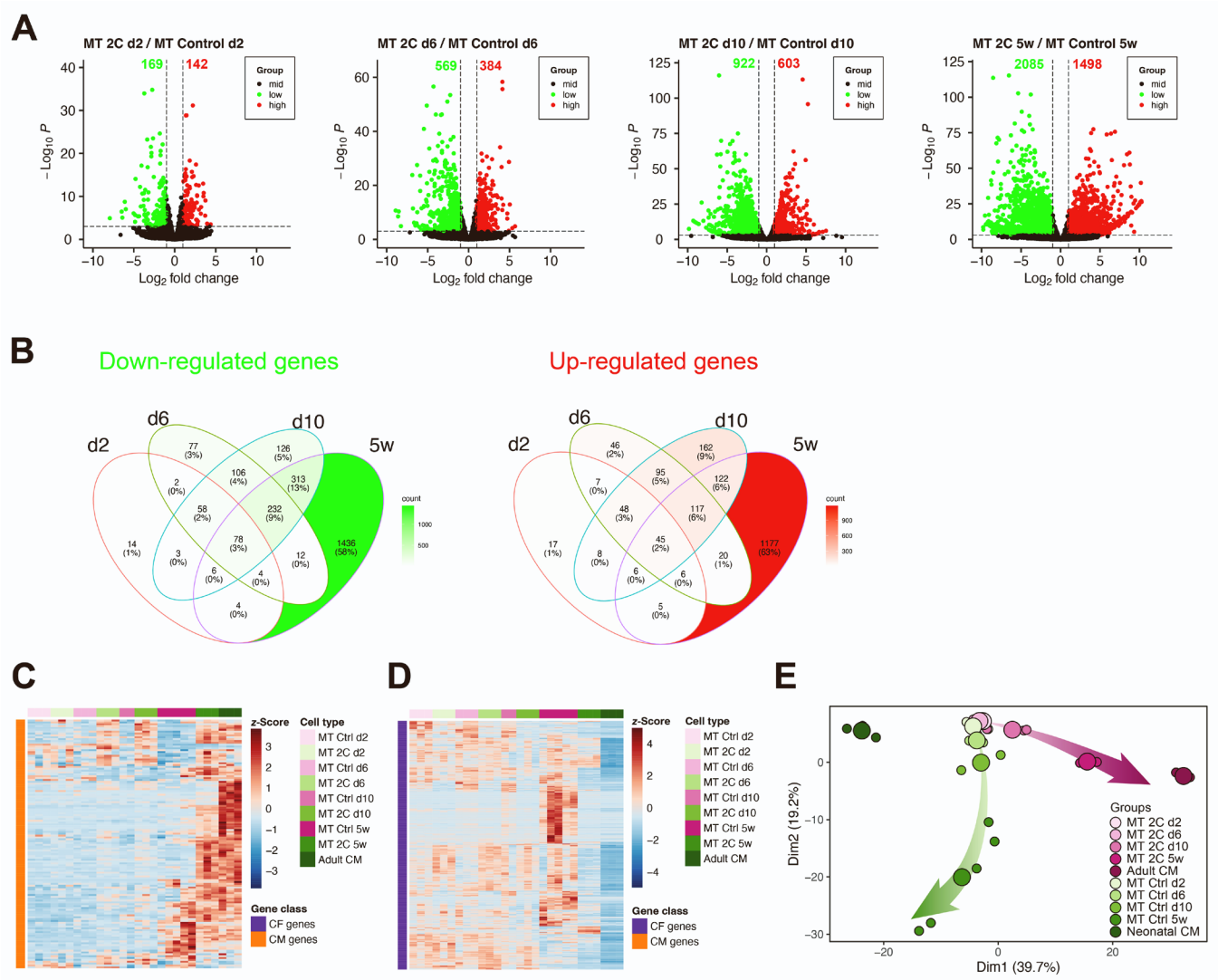

Figure S8. 2C enhances cardiac reprogramming progressively, related to Figure 3

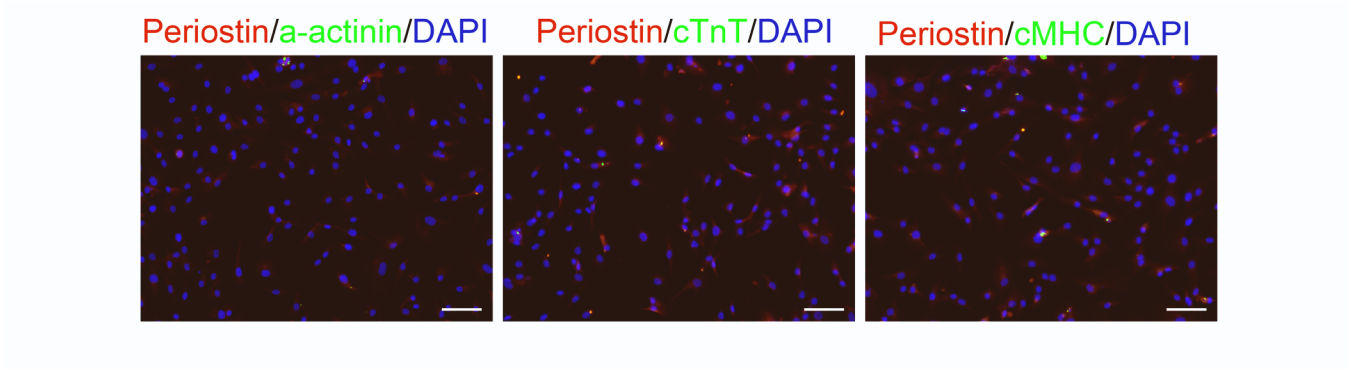

Figure S9. Confirmation of human cardiac fibroblasts, related to Figure 4

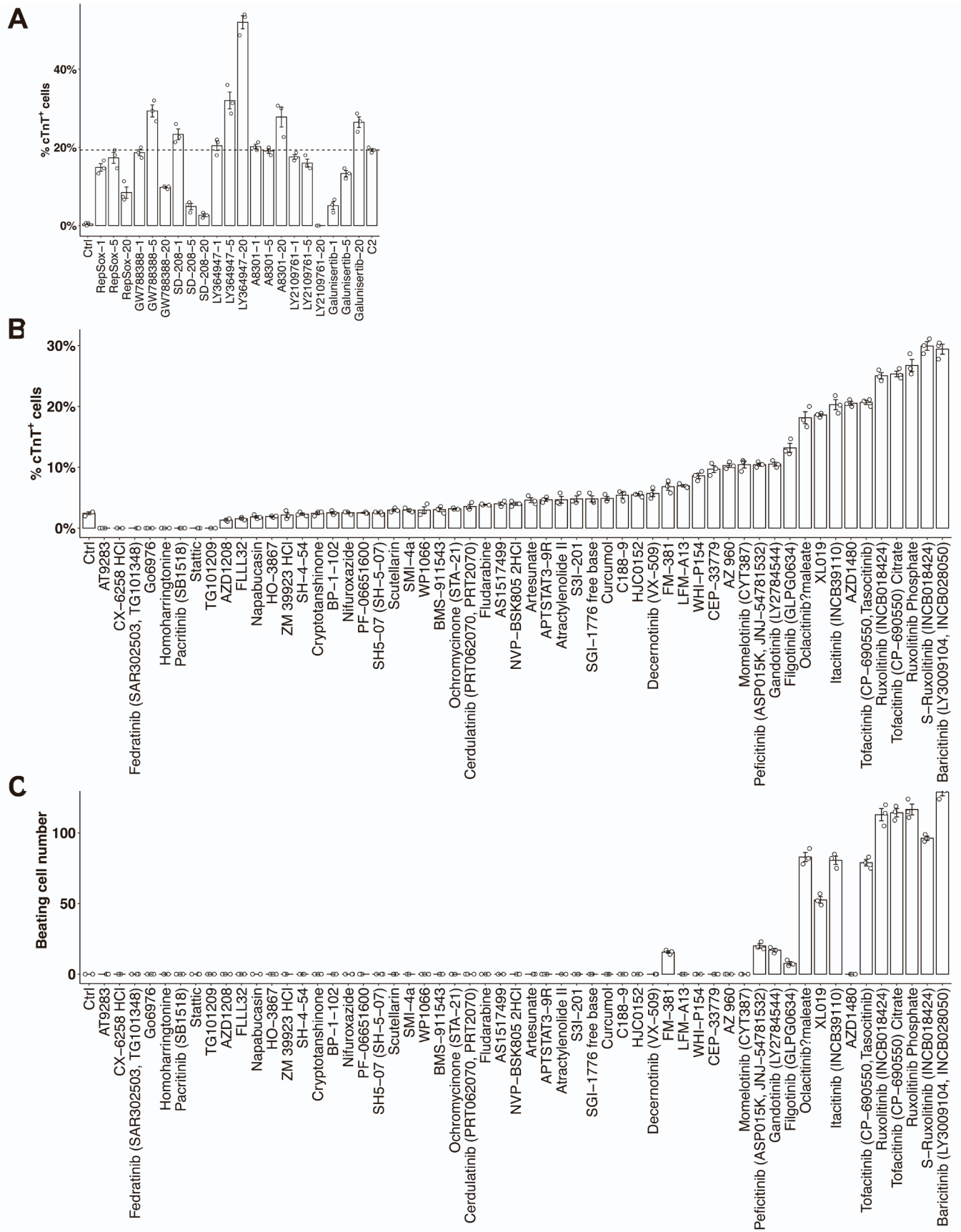

Figure S10. All TGF beta inhibitors tested works as C1, whereas only a few pan-Jak inhitors works as C2, related to Figure 7

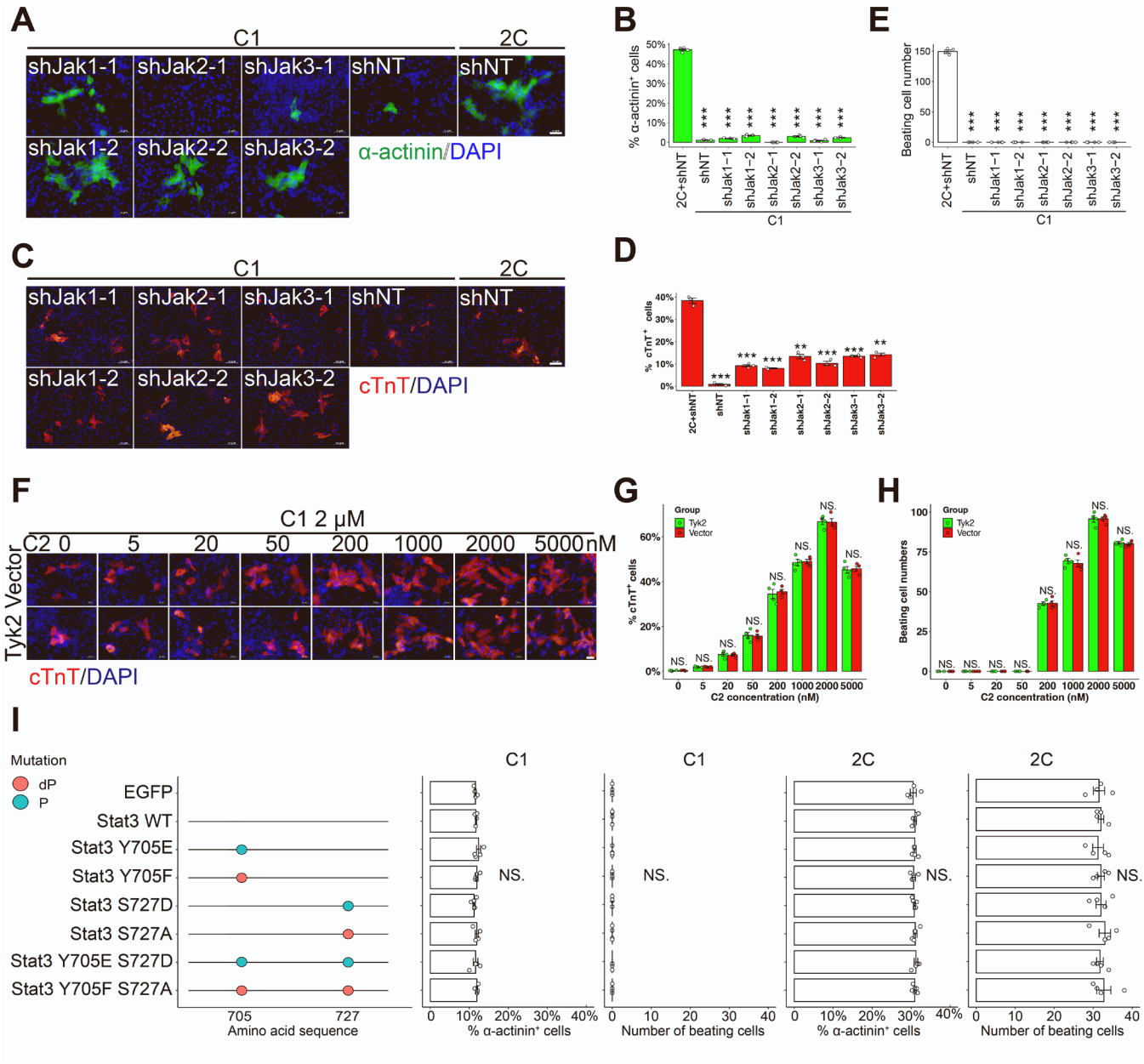

Figure S11. C2 enhances cardiac reprogramming independent of inhibiting the canonical Jak-Stat signal pathway, related to Figure 7

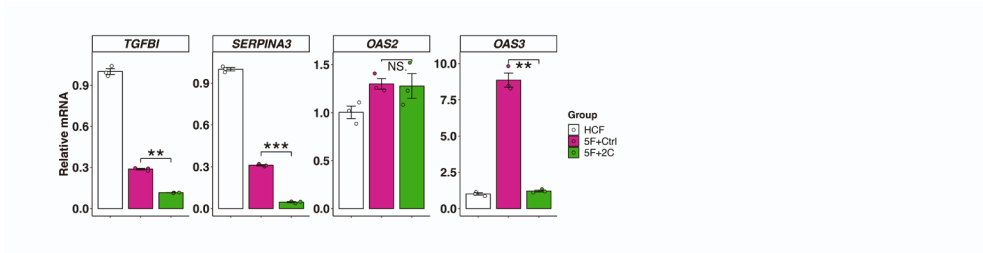

Figure S12. TGFBI, SERPINA3, and OAS3 are significantly down-regulated in 2C-treated hiCMs.

Supplemental figures legend

1. Figure S1. Dosage and timing optimization for 2C, related to Figure 1

(A-B) Representative immunocytochemistry images (A) and quantification (B) of cTnT+ cells 2 weeks after transduction with GMT and supplemented with C1 at different concentration. C2 was supplemented at a concentration of 2 μM. n = 4 independent experiments. Scale bars, 50 μm.
(C-D) Representative immunocytochemistry images (C) and quantification (D) of cTnT+ cells 2 weeks after transduction with GMT and supplemented with C2 at different concentrations. C1 was supplemented at a concentration of 2 μM. n = 4 independent experiments. Scale bars, 50 μm.
(E) Quantification of the percentage of α-actinin+ cells and the number of beating cells with different durations of 2C treatment. n = 4 independent experiments. All data are presented as the means ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001 versus the relevant control. NS., not significant.

2. Figure S2. MT+2C induced iCMs co-express multiple cardiomyocyte-specific markers, related to Figure 1

(A) MT+2C induced iCMs co-expressed cTnI and cTnT. Scale bars, 50 μm.
(B) MT+2C induced iCMs co-expressed cTnI and α-actinin. Scale bars, 50 μm.

3. Figure S3 Confirmation of mouse cardiac fibroblasts, related to Figure 2

(A-B) Neonatal mouse cardiac fibroblasts and adult mouse cardiac fibroblasts were validated with cTnT (A) and α-actinin (B) to exclude any contamination with cardiomyocytes.

4. Figure S4. Different combinations of transcription factors reveal MT enables cardiac reprogramming in presence of 2C, related to Figure 2

(A) Representative immunocytochemistry images of α-actinin+ cells 3 weeks after transduction with different combinations of transcription factors, supplemented with 2C medium. Scale bars, 50 μm.
(B) Quantification of α-actinin+ cells 3 weeks after transduction with different combinations of transcription factors, supplemented with 2C medium. n = 5 independent experiments. All data are presented as the means ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001 versus the relevant control. NS., not significant.

5. Figure S5 Optimized stoichiometry of Mef2c and Tbx5 results in higher efficiency, related to Figure 2

(A) Schematic of bi-cistronic constructs with different 2A sequences.
(B) Relative expression of Gata4, Mef2c, and Tbx5 in different bi-cistronic constructs, determined by qPCR. n = 3 independent experiments.
(C) Workflow of stoichiometry optimization of Mef2c and Tbx5.
(D-E) Representative immunocytochemistry images (D) and quantification of α-actinin+ cells and the number of spontaneous beating cells (E) 3 weeks after transduction with the indicated constructs in cardiac fibroblasts. n = 5 independent experiments. Scale bars, 50 μm. All data are presented as the means ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001 versus the relevant control. NS., not significant.

6. Figure S6 MT+2C selectively reprogram fibroblasts derived from heart into iCMs, related to Figure 2

Representative immunocytochemistry images and quantification of cTnT+ cells 3 weeks post transduction with MT (M-T2A-T) or MGT (M-P2A-G-T2A-T), in combinations with Ctrl or 2C treatment of various fibroblasts derived from indicated organs/tissues. n = 5 independent experiments. Scale bars, 50 μm. MICF, adult mouse cardiac fibroblasts isolated from mice treated with myocardial infarction. MEF, mouse embryonic fibroblasts.

7. Figure S7. Both C1 and C2 are essential for substituting Gata4, related to Figure 2

(A-C) Representative immunocytochemistry images (A), quantification of cTnT+ cells (B) and quantification of the number of spontaneous beating cells (C) 3 weeks after transduction with MT, in combination with Ctrl (DMSO), C1, C2, 2C, SB+XAV (SB43542+XAV939), or 2C+XAV (SB431542+Baricitinib+XAV939). Scale bars, 50 μm. n = 4 independent experiments. All data are

presented as the means ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001 versus the relevant control. NS., not significant.

8. Figure S8. 2C enhances cardiac reprogramming progressively, related to Figure 3

(A) Volcano map of differentially expressed genes (DEGs) between MT+2C and MT+Ctrl treatment of cardiac fibroblasts on d2, d6, d10, and 5 weeks after induction. n = 2-3 independent experiments.
(B) Venn diagram of down-regulated genes and up-regulated genes in (A).
(C) Relative expression of cardiomyocyte (CM) related genes in cultured cells with MT+Ctrl and MT+2C treatment d2, d6, d10, and 5 weeks after induction. n = 2-3 independent experiments.
(D) Relative expression of a set of cardiac fibroblast (CF) related genes in cultured cells with MT+Ctrl and MT+2C treatment d2, d6, d10, and 5 weeks after induction. n = 2-3 independent experiments.
(E) Principal component analysis (PCA) of DEGs between 2C and control (Ctrl) treatment of MT-transduced cardiac fibroblasts on d2, d6, d10, and 5 weeks after induction. n = 2-3 independent experiments.

9. Figure S9. Confirmation of human cardiac fibroblasts, related to Figure 4

Human cardiac fibroblasts (HCF) were confirmed by the expression of Periostin and without expression of a-actinin, cTnT or cMHC. Scale bars, 100 μm.

10. Figure S10. All TGF beta inhibitors tested works as C1, whereas only a few pan-Jak inhibitors work as C2, related to Figure 7

(A) Quantification for cTnT+ cells 3 weeks after transduction with MT, in combination with C2 and indicated TGF beta inhibitors at different concentrations. n = 3 independent experiments.
(B) Quantification for cTnT+ cells 3 weeks after transduction with MT, in combination with C1 and indicated Jak inhibitors. n = 3 independent experiments.
(C) Quantification of the number of spontaneous beating cells 3 weeks after transduction with MT, in combination with C1 and indicated Jak inhibitors. n = 3 independent experiments.

11. Figure S11. C2 enhances cardiac reprogramming independent of inhibiting the canonical Jak-Stat signal pathway, related to Figure 7

(A-B) Representative immunocytochemistry images (A) and quantification of α-actinin+ cells (B) 3 weeks after transduction with MT, supplemented with C1 treatment. n = 4 independent experiments. Scale bars, 50 μm.
(C-D) Representative immunocytochemistry images (C) and quantification of cTnT+ cells (D) 3 weeks after transduction with MT, supplemented with C1 treatment. n = 3 independent experiments. Scale bars, 50 μm.
(E) Quantification of the number of spontaneous beating cells 3 weeks after transduction with MT, supplemented with C1 treatment. n = 4 independent experiments.
(F-H) Representative immunocytochemistry images (F) , quantification of cTnT+ cells (G) and spontaneous beating cells (H) 3 weeks after transduction with MT, supplemented with C1 treatment and C2 at indicated concentrations. n = 4 independent experiments. Scale bars, 50 μm.
(I) Quantification of the percentage of α-actinin+ cells and the number of spontaneous beating cells 3 week after transduction with MT and indicated EGFP or Stat3 mutants, cultured in C1 or 2C medium as indicated in the figure. P and dP represent mutants at indicated amino acids to simulate the phosphorylated and dephosphorylated states of Stat3. n = 4 independent experiments. All data are presented as the means ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001 versus the relevant control. NS., not significant.

12. Figure S12. TGFBI, SERPINA3, and OAS3 are significantly down-regulated in 2C-treated hiCMs, related to Figure 7

Relative expression of TGFBI, SERPINA3, OAS2, and OAS3 in human cardiac fibroblasts control and human cardiac fibroblasts treated with 5F or 5F+2C, determined by qPCR. n = 3 independent experiments.

Supplemental table legend

1. Table S1. List of primers, related to STAR Methods.

Table S1. List of Primers for qPCR			
Species	**Gene Name**	**Forward Primer**	**Reverse Primer**
M. musculus	*Gapdh*	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
M. musculus	*Myh6*	GCCCAGTACCTCCGAAAGTC	GCCTTAACATACTCCTCCTTGTC
M. musculus	*Tnnt2*	CAGAGGAGGCCAACGTAGAAG	CTCCATCGGGGATCTTGGGT
M. musculus	*Actc1*	CTGGATTCTGGCGATGGTGTA	CGGACAATTTCACGTTCAGCA
M. musculus	*Ryr2*	ATGGCTTTAAGGCACAGCG	CAGAGCCCGAATCATCCAGC
M. musculus	*Nppa*	GCTTCCAGGCCATATTGGAG	GGGGGCATGACCTCATCTT
M. musculus	*Gja1*	ACAGCGGTTGAGTCAGCTTG	GAGAGATGGGGAAGGACTTGT
M. musculus	*Gata4*	CCCTACCCAGCCTACATGG	ACATATCGAGATTGGGGTGTCT
M. musculus	*Mef2c*	ATGCCATCAGTGAATCAAAGGAT	GTGGTACGGTCTCCCAACT
M. musculus	*Tbx5*	ATGGCCGATACAGATGAGGG	TTCGTGGAACTTCAGCCACAG
H. sapiens	*GAPDH*	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATGG
H. sapiens	*MYH6*	GCCCTTTGACATTCGCACTG	GGTTTCAGCAATGACCTTGCC
H. sapiens	*ACTN2*	CAAACCTGACCGGGGAAAAAT	CTGAATAGCAAAGCGAAGGATGA
H. sapiens	*TNNT2*	GGAGGAGTCCAAACCAAAGCC	TCAAAGTCCACTCTCTCTCCATC
H. sapiens	*ACTC1*	TCCCATCGAGCATGGTATCAT	GGTACGGCCAGAAGCATACA
H. sapiens	*COL1A1*	GAGGGCCAAGACGAAGACATC	CAGATCACGTCATCGCACAAC