Supplemental information

Small molecules enable cardiac reprogramming of mouse fibroblasts with a single factor, Oct4

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Supplemental Information Section contains: Four supplemental figures and legends, one supplemental table, two supplemental movie titles and legends, supplemental experimental procedures and Supplemental References. The titles of supplemental figures, table and movies are as follows:

Supplemental figure S1. Identification of small molecules that promote the direct conversion of MEFs into beating cardiomyocytes with OKS. (Related to Figure 1)

Supplemental figure S2. Tube-like beating cells generated by SCPF without Oct4 are not typical cardiomyocytes. (Related to Figure 1)

Supplemental figure S3. TTX sensitive sodium currents were measured in the induced cardiomyocytes. (Related to Figure 2)

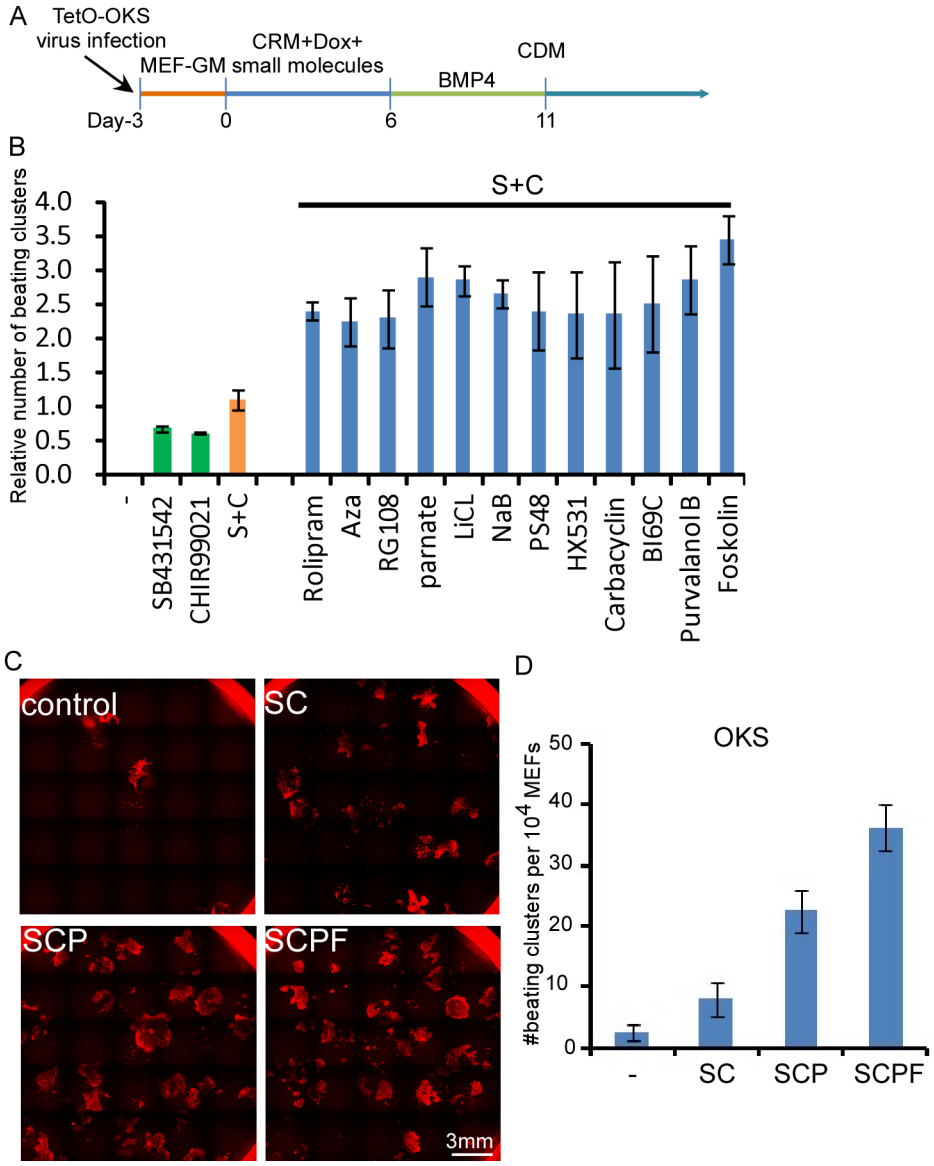
Supplemental figure S4. The effect of CHIR99021 is mainly dependent on the activation of Wnt signaling. (Related to Figure 1)

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

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

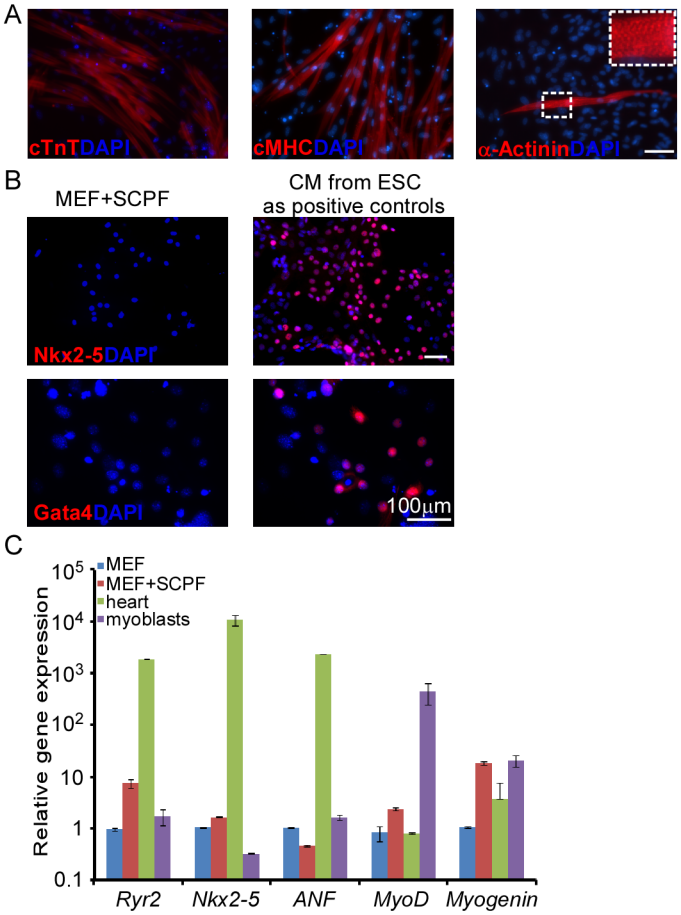
Supplemental movie S2. The same beating cluster was shown in Figure 4A and B. (Related to Figure 4)

Fig S1



Supplemental figure S1. Identification of small molecules that promote the direct conversion of MEFs into beating cardiomyocytes with OKS. (Related to Figure 1)
(A) The scheme of direct cardiac reprogramming with OKS and small molecules. Different media were used sequentially as described in Experimental Procedures. Small molecules were added to the CRM media from day 0 to day 6. (B) Primary hits that enhanced cardiac reprogramming under the basal condition containing CHIR99021 and SB431542(S+C). (C) immnostaining of cTnT on day 21, by whole-well imaging of cells grown in 12-well plates. S, SB431542; C, CHIR99021; P, parnate; and F, forskolin. Scale bar is 3 mm. (D) Quantitative analysis of the number of spontaneously beating clusters.

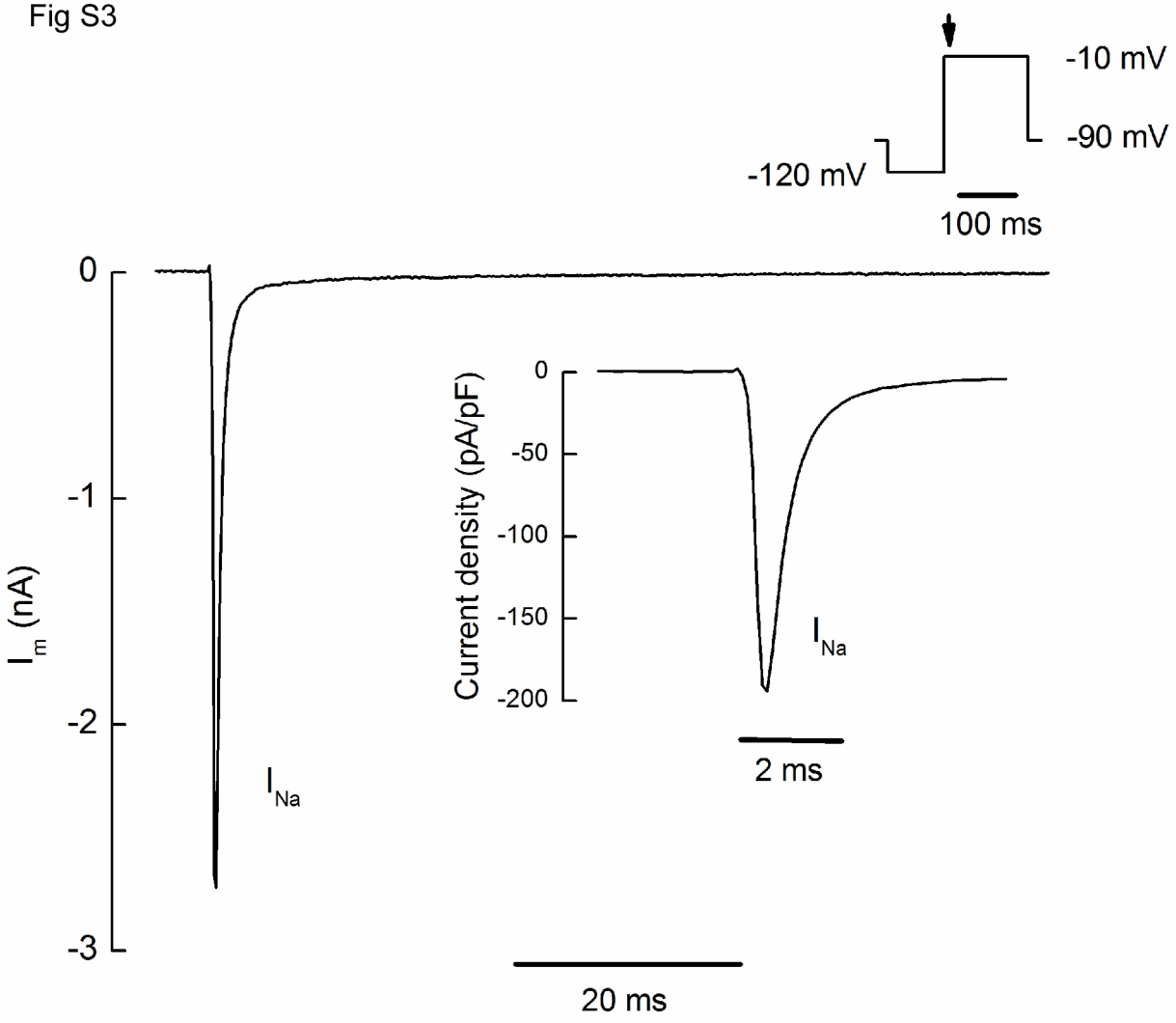
FigS2



Supplemental figure S2. Tube-like beating cells generated by SCPF without Oct4 are not typical cardiomyocytes. (Related to Figure 1)

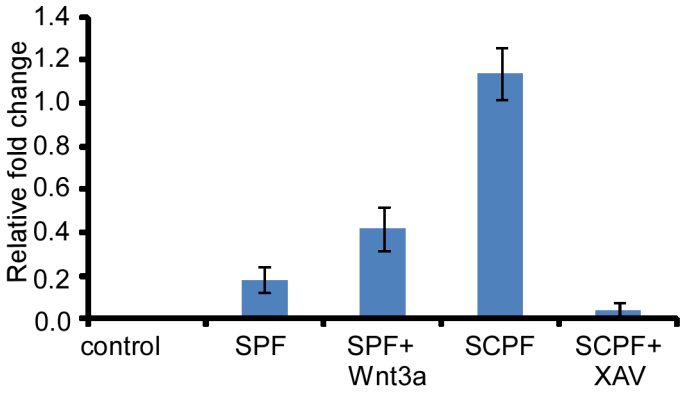
MEFs were treated with SCPF for 10days and then immunostained with cardiac structure protein markers, cTnT, cMHC, α-actinin (A) and cardiac transcription factors, Gata4 and Nkx2-5 (B). Cardiomyocytes derived from mESC were used as positive controls. (C) qPCR analysis of indicated gene markers in SCPF treated MEFs. mRNAs from neonatal hearts or skeletal muscle myoblasts were used as positive controls.

Fig S3



Supplemental figure S3. TTX sensitive sodium currents were measured in the induced cardiomyocytes. (Related to Figure 2)
Beating clusters were digested into single cells and replated onto matrigel gel coated glass coverslips at day 40. Shown is a typical INa difference current, evoked by the voltage protocol shown in the inset, measured by subtraction of the currents before and after application of 50μM tetrodotoxin (TTX).

Fig S4



Supplemental figure S4. The effect of CHIR99021 is mainly dependent on the activation of Wnt signaling.

TTFs were transduced with Oct4 and then seeded into 12well plates. Following the scheme shown in Figure 1A, different combinations of small molecules and 100 ng/ml Wnt3a were added in CRM till day 15. The number of beating clusters were counted at day 35 and normalized to the condition of SCPF treatment.