

Supplementary Information

Chemical-induced cardiac reprogramming *in vivo*

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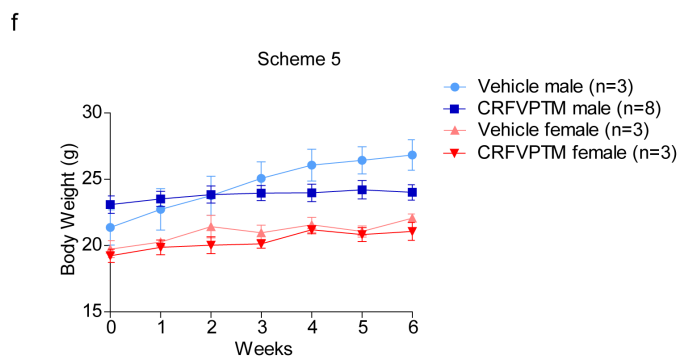
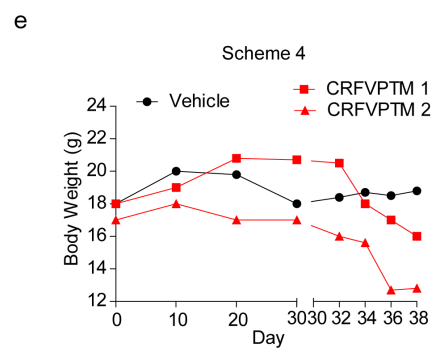
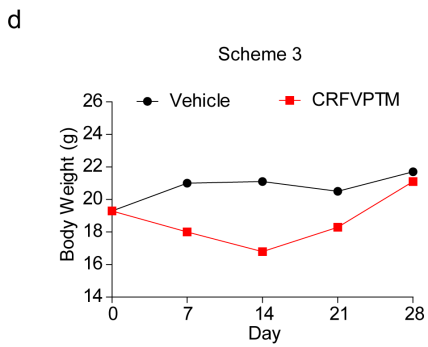
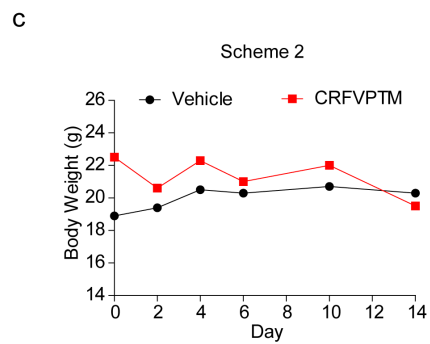
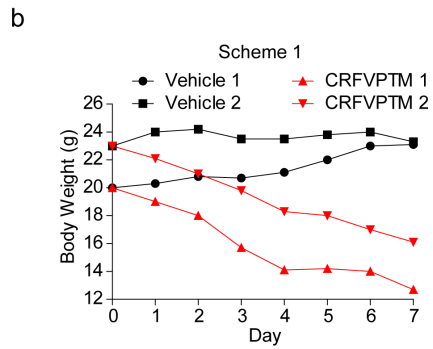
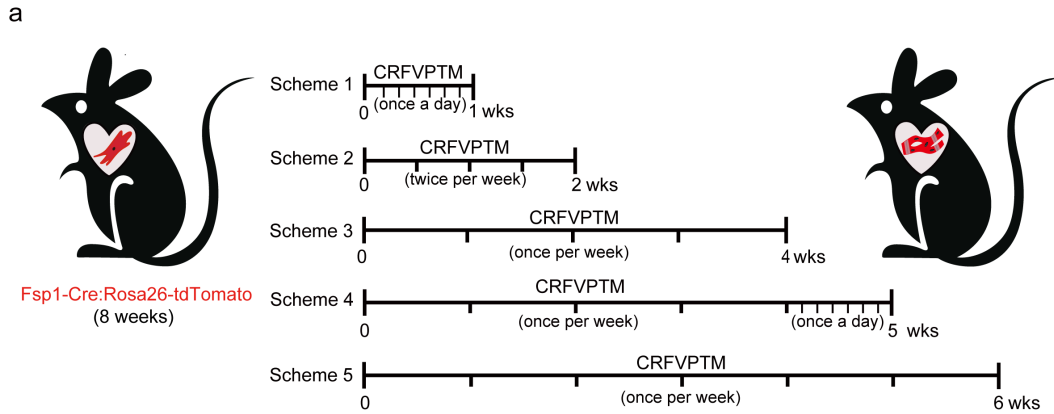
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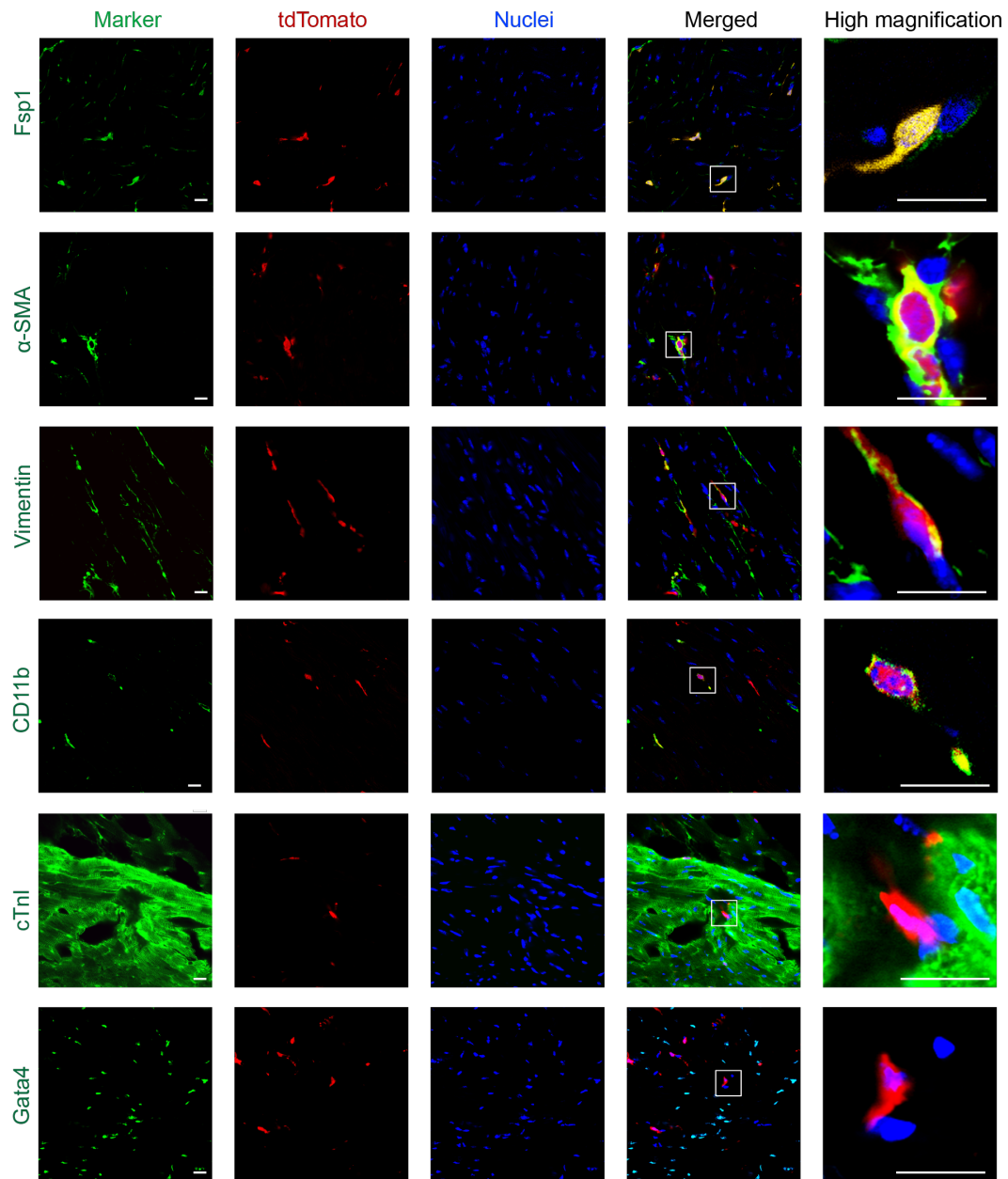
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Figure S1. Schemes of drug treatment to induce *in vivo* cardiac reprogramming in mice



(a) Schemes of drug treatment to induce *in vivo* cardiac reprogramming in *Fsp1-cre:R26R^{tdTomato}* mice. Scheme 1, mice were given CRFVPTM once a day for a week; Scheme 2, mice were given CRFVPTM twice a week for 2 weeks; Scheme 3, mice were given CRFVPTM once a week for 4 weeks; Scheme 4, mice were given CRFVPTM once a week for 4 weeks, then once a day for another week; Scheme 5, mice were given CRFVPTM once a week for 6 weeks. (b-f) Body weight of the *Fsp1-cre:R26R^{tdTomato}* mice treated with CRFVPTM or vehicle as illustrated in Scheme 1 to 5.

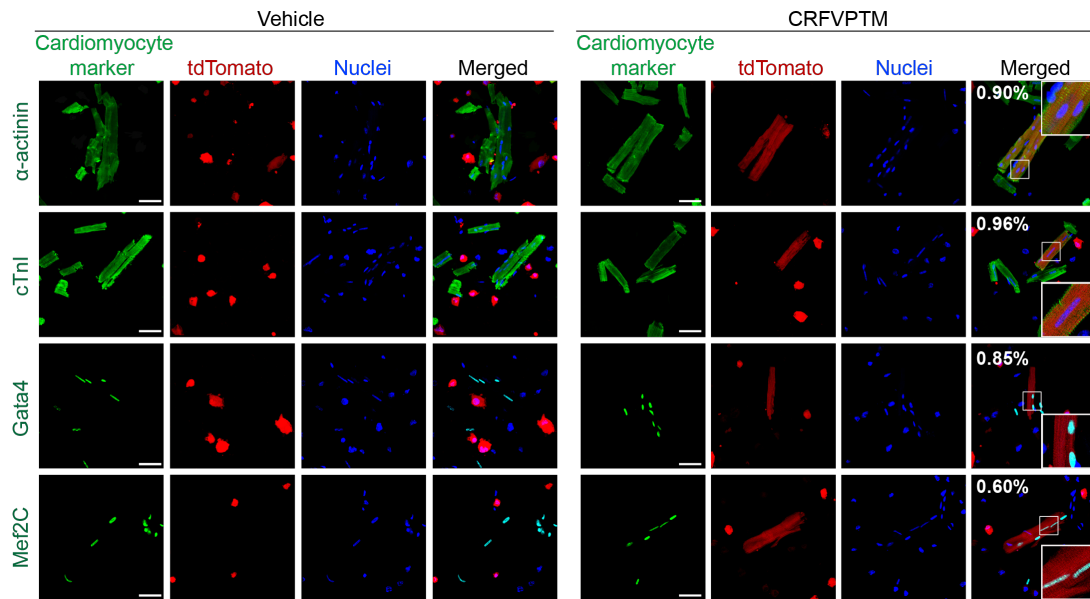
Figure S2. Characterization of tdTomato⁺ cells in the heart of *Fsp1-cre:R26^{RtdTomato}* mouse



Immunofluorescence staining of Fsp1, α-SMA, Vimentin, CD11b, cTnI and Gata4 in the cryosections of the hearts from *Fsp1-cre:R26^{RtdTomato}* mice before drug treatment.

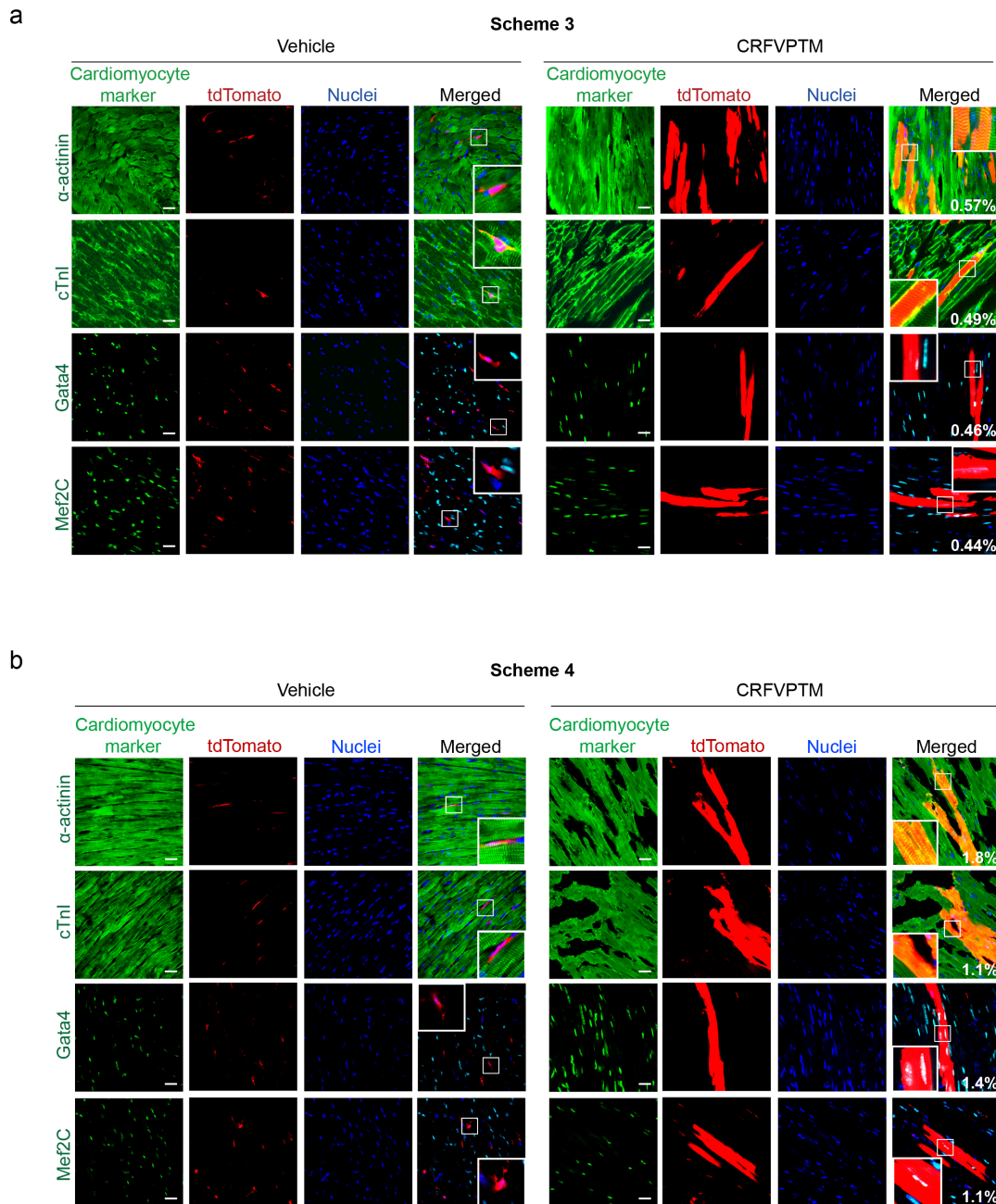
Nuclei were stained with hoechst. Scale bar, 20 μm.

Figure S3. Characterization of tdTomato⁺-CICMs in single layer culture



Hearts from the vehicle or CRFVPTM-treated *Fsp1-cre:R26R^{tdTomato}* mice (Scheme 5) were digested and the cells were cultured in single layer. Cells were then subjected to immunofluorescence staining of cardiac markers, including α -actinin, c-TnI, Gata4 and Mef2C. The numbers represent the percent of tdTomato⁺ cells expressing various cardiac markers (n=3 for each group, more than 2,000 tdTomato⁺ cells were counted from random fields from each mouse). Nuclei were stained with Hoechst. Scale bar, 50 μ m.

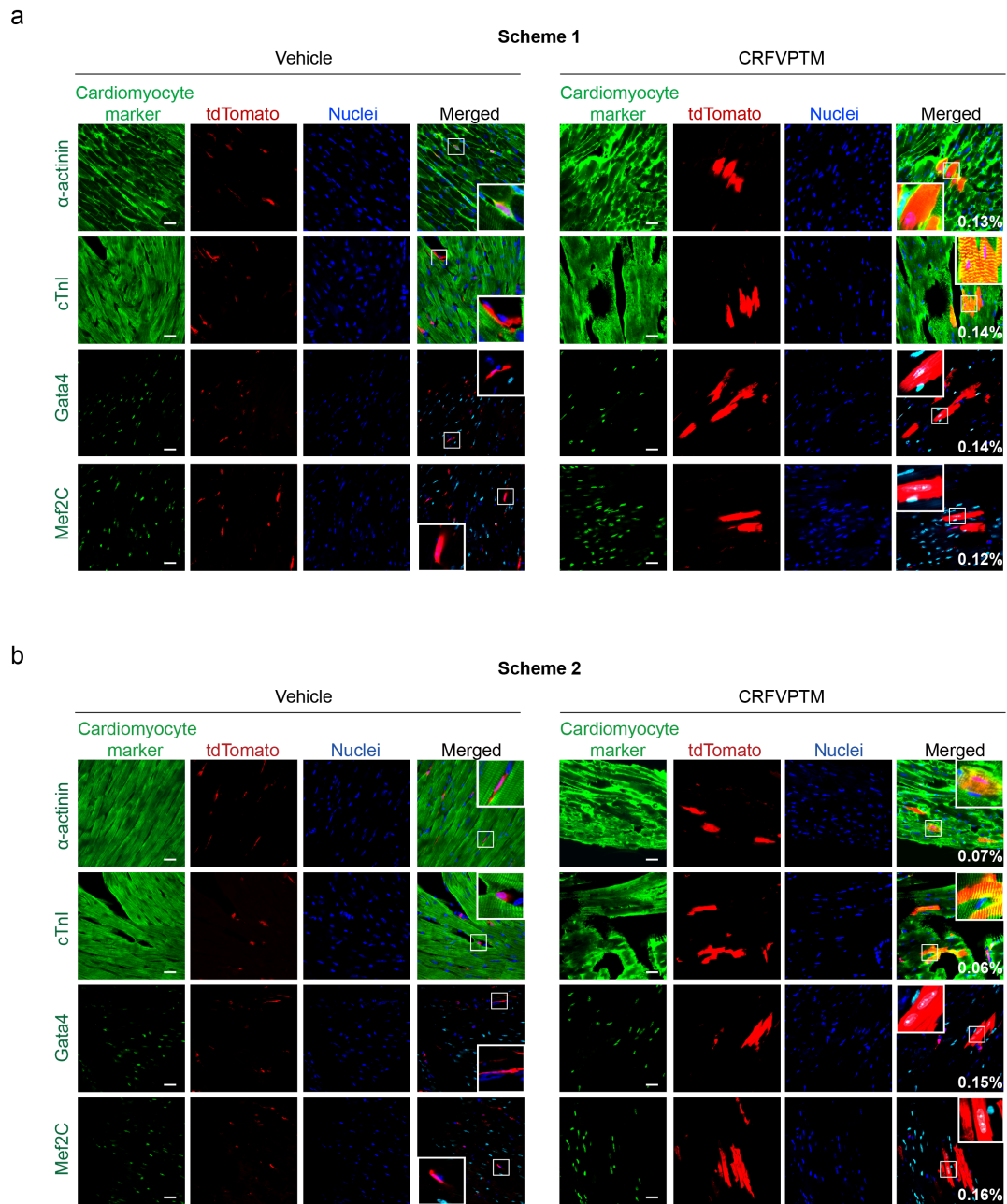
Figure S4. Cardiac reprogramming in mice from Scheme 3 and 4



(a and b) Immunofluorescence staining of cardiac markers, including α -actinin, c-TnI, Gata4 and Mef2C in cryosections of hearts from *Fsp1-cre:R26R^{tdTomato}* mice treated with CRFVPTM or vehicle as illustrated in Scheme 3 (a) and 4 (b). The numbers in the merged images of the drug-treatment group represent the percent of tdTomato⁺ cells

expressing various cardiac markers (5 sections from each mouse were analyzed). Nuclei were stained with Hoechst. Scale bar, 20 μm .

Figure S5. Cardiac reprogramming in mice from Scheme 1 and 2

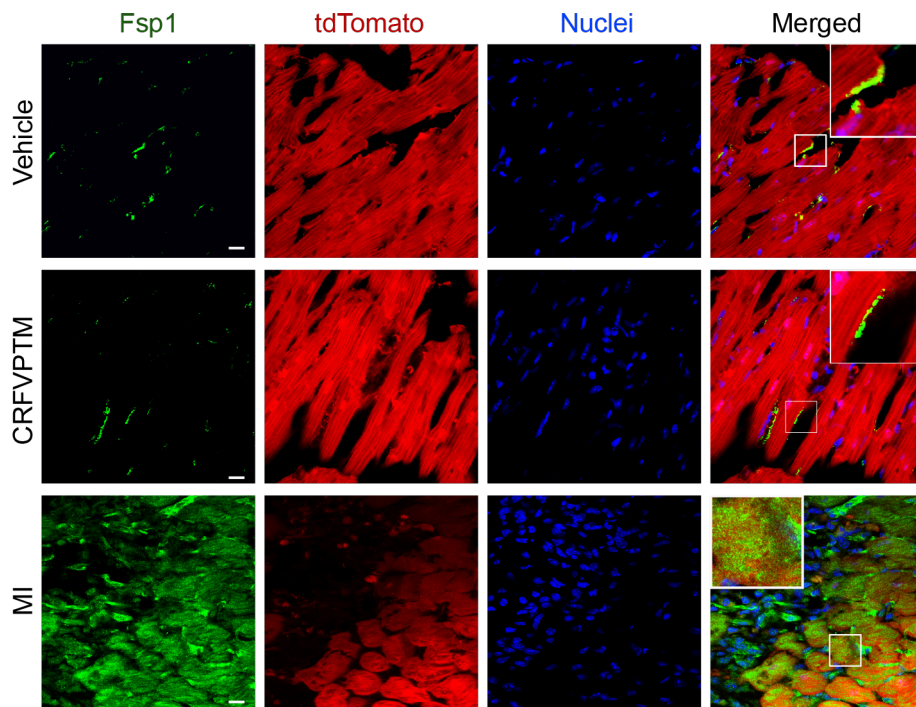


(a and b) Immunofluorescence staining of cardiac markers, including α -actinin, c-Tnl, Gata4 and Mef2C in cryosections of hearts from *Fsp1-cre:R26R^{tdTomato}* mice treated with CRFVPTM or vehicle as illustrated in Scheme 1 (a) and 2 (b). The numbers in the merged images of the drug-treatment group represent the percent of tdTomato⁺ cells

expressing various cardiac markers (5 sections from each mouse were analyzed).

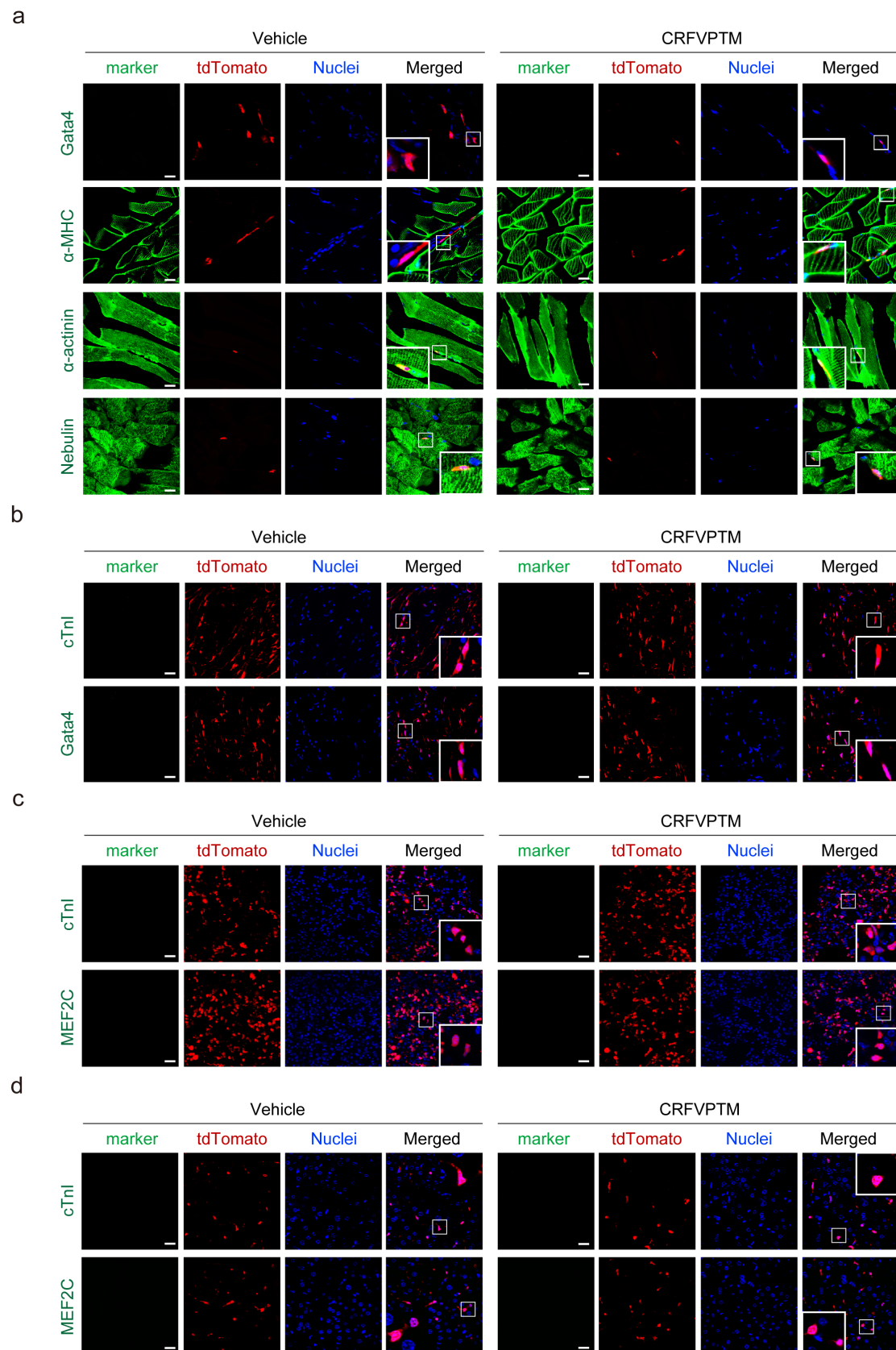
Nuclei were stained with Hoechst. Scale bar, 20 μm .

Figure S6. Staining of Fsp1 in the hearts of α -MHC-cre:R26R^{tdTomato} mice subjected to CRFVPTM-treatment or MI



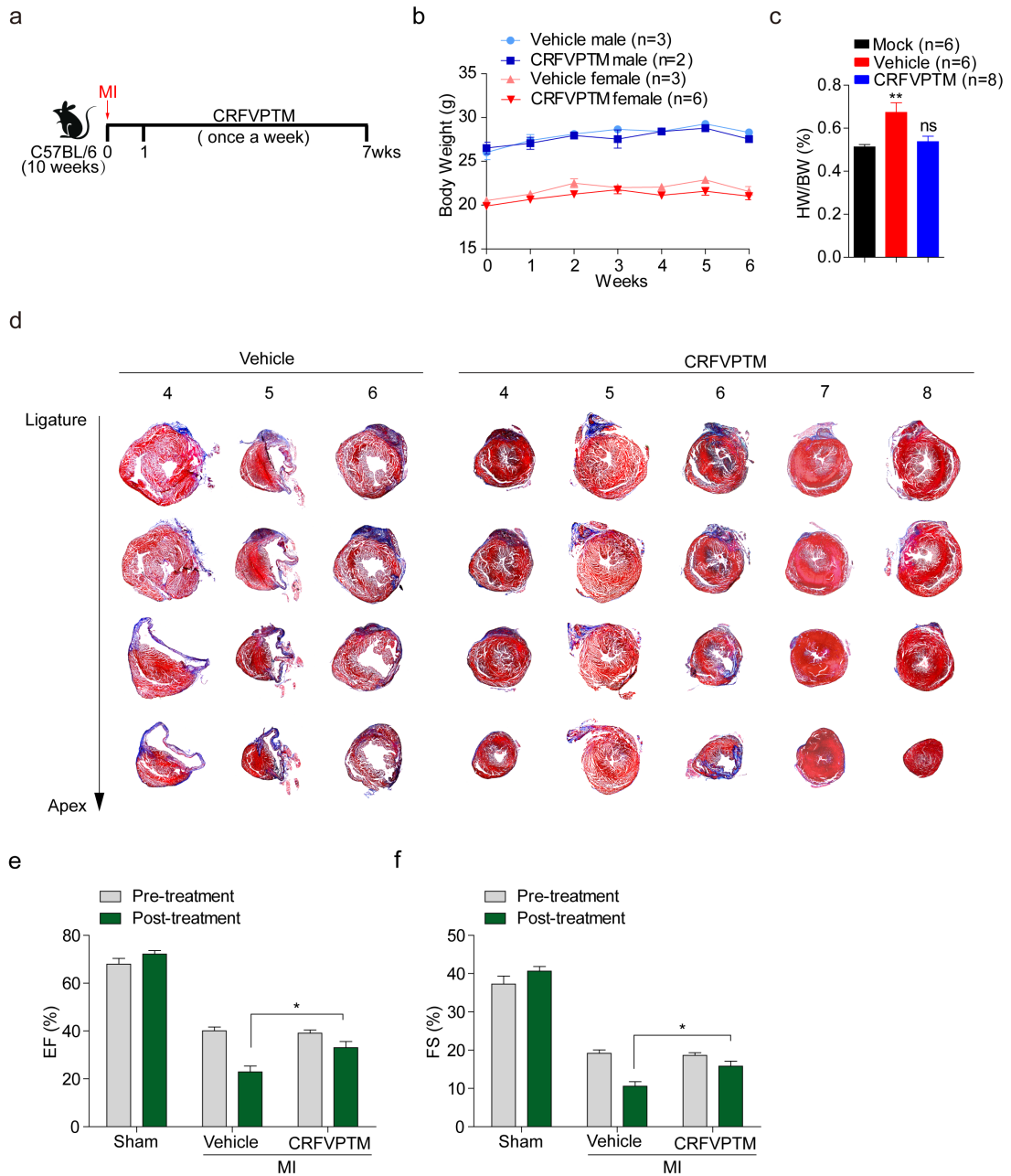
α -MHC-cre:R26R^{tdTomato} mice were treated with vehicle or CRFVPTM as illustrated in Scheme 5 (upper and middle panels), or subjected to myocardial infarction by coronary artery ligation (bottom panels). Fsp1 expression was detected in the cryosections of the hearts. Representative images are presented (5 sections from each mouse were analyzed, n=3 for each group). Nuclei were stained with Hoechst. Scale bar, 20 μ m.

Figure S7. Staining of cardiac markers in the skeletal muscle, tail tip, lung and liver of *Fsp1-cre:R26R^{tdTomato}* mice from Scheme 5



(a) Immunofluorescence staining of Gata4, α -MHC, α -actinin and nebulin in the cryosections of the tibialis anterior muscle isolated from *Fsp1-cre:R26R^{tdTomato}* mice treated with CRFVPTM or vehicle for 6 weeks (Scheme 5). Nuclei were stained with Hoechst. Scale bar, 20 μ m. (b-d) Immunofluorescence staining of cTnI, Gata4, or MEF2C and in the cryosections of the tail tip (b), lung (c) and liver (d) isolated from *Fsp1-cre:R26R^{tdTomato}* mice treated with CRFVPTM or vehicle for 6 weeks (Scheme 5). Nuclei were stained with Hoechst. Scale bar, 20 μ m.

Figure S8. Characterization of MI mice after chemical cocktail treatment



(a) Schematic drawing of the experimental procedure. Myocardial infarction (MI) was induced by LAD ligation. One week following MI, mice were dosed with CRFVPTM once a week for 6 weeks (Scheme 5). (b) Body weight of the MI mice treated with CRFVPTM or vehicle. (c) Quantification of heart weight (HW) normalized to body weight (BW) of the MI mice treated with CRFVPTM or vehicle once a week for 6

weeks. Data are presented as means \pm SEM. $^{**}P < 0.01$ versus mock group. (d) Images of Masson trichrome staining of the heart sections (as presented in Figure 1e) from the MI mice treated with CRFVPTM (mice number 4-8) or vehicle (mice number 4-6) once a week for 6 weeks. (e, f) Evaluation of cardiac functions with echocardiography. Mice were subjected sham operation or MI by LAD ligation. Sham operated mice received CRFVPTM once a week for 6 weeks. MI mice were treated with vehicle and CRFVPTM once a week for 6 weeks. Ejection fraction (EF, e) and fractional shortening (FS, f) of pre- and post-treated mice were presented. Pre-treatment refers to the 2 or 3 days after LAD ligation while post-treatment refers to 1 weeks after completion of the drug treatment. Data are presented as means \pm SEM (n=8 for sham groups, n=9 for MI vehicle group, n=10 for MI drug-treated group). $^{*}P < 0.05$ (Student's t-test).

Materials and Methods

Mouse line

The *Fsp1*-Cre mice (The Jackson Laboratory) that express Cre recombinase under the control of the *fibroblast specific protein-1* (*Fsp1* or *S100A4*) promoter were mated with the R26R^{tdTomato} mice (The Jackson Laboratory), in which the expression of tdTomato is terminated by a loxP-flanked STOP cassette. The progeny mice (*Fsp1*-Cre:R26R^{tdTomato}) with specific expression of the red fluorescent protein tdTomato in the fibroblasts were used for lineage tracing experiments¹⁻³. The α -MHC-Cre mice (The Jackson Laboratory) that express Cre recombinase under the control of the cardiac-specific *alpha myosin-heavy chain* (α -MHC-Cre or *Myh6*) promoter were mated with the R26R^{tdTomato} mice too. The progeny mice (α -MHC-Cre:R26R^{tdTomato}) with specific expression of the red fluorescent protein tdTomato in the cardiomyocytes^{4,5}. All experiment procedures for the use and the care of the animals complied with international guidelines for the care and use of laboratory animals and were approved by the Animal Ethics Committee of Shanghai Institute of Materia Medica.

Drug treatment

CRFTM, including CHIR99021 (C, 14 mg/kg), Repsox (R, 8.6 mg/kg), Forskolin (F, 61.6 mg/kg), TTNPB (T, 1 mg/kg) and Rolipram (M, 2.5 mg/kg), were mixed in 0.5% CMC-Na (sodium carboxymethyl cellulose)/saline. VP, including VPA (V, 250 mg/kg) and Parnate (P, 2.7 mg/kg), were dissolved in saline. Mice were treated with C6FTR by oral gavage and VP by intraperitoneal injection with intervals illustrated in Figure S1. The vehicle group only received 0.5% CMC-Na/saline by oral gavage and

saline by intraperitoneal injection.

Immunofluorescent staining

Hearts were removed and perfused via aortic cannulation with PBS with a constant flow of 1 ml/min to ensure the removing of all blood cells. Hearts, skeletal muscles and other tissue were fixed in 4% PFA at 4°C overnight and incubated in 20% sucrose at 4°C overnight. Tissue were embedded in OCT medium, frozen at -80 °C and sections with 10-15 µm thickness were prepared. After permeabilization with 1% Triton X-100 for 30 minutes, cryosections were treated with 5% BSA for 1-2 h, and then incubated with various primary antibodies at 4°C overnight. After thorough washing, secondary antibodies conjugated with Alexa Fluor 555, Alexa Fluor 488 and Alexa Fluor 647 were used. Nuclei were visualized with Hoechst 33342 (10 µg/ml). Images were captured with an Olympus FV10i confocal microscope. Antibodies used in this study are as following: Gata4 (SAB4501129, Sigma), c-TnI (ab47003, Abcam), α -Actinin (A7811, Sigma), Mef2c (5030S, CST), Connexin 43 (610061, BD Biosciences), N-cadherin (ab76057, Abcam), α -MHC (ab50967, Abcam), Nebulin (N9891, Sigma), Fsp1 (ABF32, Merckmillipore), α -SMA (ab2547, Abcam), Vimentin (ab8978, Abcam) , CD11b (ab184308, Abcam) .

Isolation of adult cardiomyocytes

Adult mice were anaesthetized with 10% chloral hydrate. Hearts were removed and perfused retrogradely via aortic cannulation with cardiomyocyte isolation buffer (CIB, containing 137 mM NaCl, 2.7 mM KCl, 1.05 mM MgSO₄·7H₂O, 0.42 mM NaH₂PO₄·2H₂O, 11.9 mM NaHCO₃, 5.6 mM glucose, 0.6 mg/mL Taurine and 1 mg/ml

2,3-Butanedione monoxime, pH=7.4) with a constant flow of 0.5 ml / min at 37°C by peristaltic pump to remove blood cells. Hearts were then perfused with perfusion buffer (CIB supplemented with 0.4 mM EGTA). After 3 min, hearts were digested with digestion buffer (CIB supplemented with 0.06 mg/ml Protease XIV, 0.36 mg/ml Collagenase II, 0.48 mg/ml Collagenase IV and 300 nM CaCl₂) with a constant flow of 1 ml/min at 37°C till the flow out of the cardiomyocytes. Hearts were then washed with growth medium (DMEM containing 10% FBS) to terminate the digestion, followed by perfusion with CIB to collect cardiomyocytes by gently squeezing of the hearts.

Patch clamp recording

Patch clamp recording was performed in a temperature-controlled room at approximately 25°C. The Giga-Ohm seal was achieved and the action potentials (APs) were recorded under the current-clamp at zero applied current (Axopatch-200B amplifier). The pipette solution contained 145 mM KCl, 1 mM MgCl₂, 5 mM EGTA, 10 mM HEPES and 10 mM Na₂ATP (pH=7.3 with KOH). Extracellular solution contained 140 mM NaCl, 3 mM KCl, 2 mM CaCl₂, 1.5 mM MgCl₂, 10 mM HEPES and 10 mM glucose (pH=7.4 with NaOH). Signals were filtered at 1 kHz, and digitized using a DigiData 1440 with pClamp9.2 software (Molecular Devices).

Mouse MI model

Permanent ligation of the left anterior descending coronary artery (LAD) at the left ventricle was performed in 10-wk-old C57BL/6 mice. The mice were anesthetized with 2.4% isoflurane/97.6% oxygen and placed in a supine position. Thoracotomy was performed at the third intercostal space, and self-retaining micro-retractors were placed

to separate the third and fourth rib to visualize the LAD. The LAD was surgically ligated at 1.5 mm distal to the left atrial appendage without tearing the pericardial sac. After LAD ligation, the retractors were removed and the chest was closed.

Measurement of scar area

The fibrotic scar formation in the hearts with MI was visualized with trichrome staining of the cryosections with a Masson's trichrome staining kit (Sigma, HT15-1) according to the manufacturer's instruction. The scar size was measured with ImagePro software (scar area (blue), healthy area (red)) on transections spanning four levels running from the ligation site to the apex of the heart (about 0.5 mm between adjacent levels, with the first level starting right below the ligation). From each level, 4 slides of heart tissue were measured (a total of 16 sections from each heart).

Echocardiography

All mice were lightly anesthetized by 1.5-3% isoflurane inhalation. Heart function was evaluated by transthoracic echocardiography using a Vevo 2100 instrument and data was analyzed with Vevo 2100 software package. Echocardiography performed 2-3 days post MI demonstrated a significantly reduction in ejection fraction before drug treatment. Echocardiography was then performed 1 week after completion of the drug treatment.

Statistical Analysis

Values are reported as the Means \pm SEM. P values were calculated by Student's t-test, P < 0.05 was considered statistically significant. All graphs were plotted with GraphPad Prism software.

References

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