Supplemental Figure Legends

Figure S1. The capacity for reprogramming is impaired in activated cardiac fibroblasts after myocardial infarction.

(A and B) Immunostaining (A) and quantification (B) for cTnI+ cells on day 7 post-infection of QCFs and MFs with MGTMS. Scale bar: 200 μm, (n = 4, *** p < 0.001).

(C) mRNA expression of QCFs and MFs 7 days after MGTMS transduction as determined by qPCR, QCFs used as control (n = 4, * p < 0.05, ** p < 0.01, ns, non-significant).

(D) UMAP visualization of 10 candidate transcription factors (TFs) detected in data.

(E) UMAP visualization of cardiac fibroblast subpopulations separated according to experimental condition from published data[1]. (MI-D3: MI-day 3, MI-D7: MI-day 7).

(F) UMAP visualization of 10 candidate transcription factors (TFs) from published data.

Figure S2. A targeted TF knockdown screen identifies Meox1 as a cardiac reprogramming barrier.

(A) qPCR of Meox1 expression of MICFs infected with Meox1 shRNA or scramble control (shCtrl). Expression value in shCtrl-infected cells was set as 1 (n = 3, *** p < 0.001).

(B and C) Immunostaining (B) and quantification (C) for cTnI+ iCMs 14 days after MGT and shMeox1, or shCtrl transduction on MICFs. Scale bar: 100 μm (n = 4, *** p < 0.001).

(D) Quantification of spontaneously contracting iCMs with MGT viral infection 6 weeks after being reprogrammed from MICFs, corresponding to Movie S3 and Movie S4 (n = 4, ** p < 0.01).

(E and F) Immunostaining (E) and quantification (F) of cTnT+ iCMs 14 days after MGTM and vector control, or Meox1 overexpression on MICFs. Scale bar: 100 μm (n = 3, ** p < 0.01).

(G) qPCR of Meox1 expression of MICFs infected with Meox1 shRNA or co-infected with shMeox1 as well as a vector expressing an shRNA-resistant mutant or wild-type

Meox1 (n = 3, *** p < 0.001).

(H and I) Verification of shRNA target specificity. Immunostaining (H) and quantification (I) of cTnI+ iCMs from MICFs infected with scramble control or Meox1 shRNA alone or co-infected with shMeox1 and a vector expressing an shRNA-resistant mutant or wild-type Meox1. Data were collected 2 weeks post-induction. Scale bar: 200 μm (n = 3, *** p < 0.001).

(J) Volcano plot illustrating extensive transcriptomic changes in vector control or Meox1 overexpressing MICFs 6 days post-MGTM transduction. n = 2 biologically independent samples per group.

(K and L) GO analysis demonstrating biological processes (BP) and cellular components (CC) associated with genes down- (K) and up- (L) regulation after Meox1 overexpression related to figure S2 (J) as assessed using RNA-seq.

(M) Meox1 expression in CF as detected by bulk RNA-seq[2] from published data. CFs were isolated from non-infarcted neonatal (P1_CF) and adult (P56_CF) mouse hearts, as well as myocardial infarcted neonatal (MIP1_CF) and adult (MIP56_CF) mouse hearts at day 3 after MI surgery.

(N-Q) Immunostaining and quantification for cTnI+ iCMs 14 days after MGTM and shMeox1, or shCtrl transduction of QCFs (N) (O) and MFs (P) (Q). Scale bar: 200 μm (n = 3, ** p < 0.01, *** p < 0.001).

Figure S3. Meox1 transcriptionally activates and stabilizes a fibrosis-specific program of cardiac fibroblasts.

(A) mRNA expression of Meox1 in vector-control or mutant MEOX1^{Q219E} overexpression MICFs 2 weeks post-MGTM transduction (n = 3, *** p < 0.001).

(B) Cell quantity difference between shCtrl- or shMeox1-infected MICFs after MGTM transduction. Cells were counted by nuclear staining at 7 days and 14 days after induction (n > 3, ns, non-significant).

(C) Quantification of Ki67+ cells 2 weeks after MGTM and shMeox1, or shCtrl transduction of MICFs. The proportion of Ki67+ cells had no change after Meox1 knockdown (n = 3, ns, non-significant).

(D and E) Immunostaining (D) and quantification (E) of cTnI+ iCMs 7 days after MGT and shMeox1, or shCtrl transduction on MICFs. Scale bar: 100 μm (n = 3, *** p < 0.001).

(F and G) GO analysis illustrating biological processes associated with MEOX1 binding TSS +-5kb genes on CFs (F) or TGF-β1-treated CFs (G). TSS, transcription start site.

(H-K) Immunostaining and quantification of cTnI+ (H) (I) and cTnT+ (J) (K) iCMs 14 days after MGTM and vector control (Ctrl), wild-type Meox1, or Meox1 fusion protein in which the MyoD transactivation domain (TAD domain) (45, 46) was fused to the Meox1 C-terminus region (Moex1-TAD) of MICFs. Scale bar: 100 μm (n = 3, * p < 0.05, ns, non-significant).

(L) mRNA expression of Meox1 and CM marker genes in Meox1 or Moex1-TRD overexpression group relative to Ctrl in MICFs 2 weeks after MGTM transduction (n = 4, * p < 0.05, *** p < 0.001, ns, non-significant).

Figure S4. Meox1 knockdown together with Mef2c and Myocd expression is sufficient to induce cardiac reprogramming.

(A-C) Representative images of cTnI+ iCMs on shCtrl- or shMeox1-infected MICFs expressing different combinations of Gata4, Mef2c, Tbx5, and Myocd (Scale bar: 200 μm).

Figure S5. Recombinase-based lineage tracing demonstrates improved cardiac reprogramming efficiency under Meox1 knockdown.

(A) Strategy for labeling resident cardiomyocytes and non-myocytes by Tnni3-Dre;IR1;R26-iCre, non-myocytes were labeled by Doxycycline- (Dox) induced Cre-loxP recombination using R26-iCre (R26-rtTA;Tet-o-Cre).

(B) Schematic diagram illustrating experimental procedures. MI, myocardial infarction. TAM, tamoxifen. SR, SB431542 (4mg/kg/day), Ruxolitibib (1mg/kg/day).

(C) FU-tet-o-EGFP lentivirus injected into the border zone of an MI heart. Mice were sacrificed at 7 days post-MI. Immunostaining for tdTomato, EGFP, and ZsGreen in EGFP-overexpressing heart sections from a Tnni3-Dre;IR1; R26-iCre mouse. Different

pseudo-colors indicate second antibody signals separately: white, Alexa555 (anti-tdTomato); green, Alexa405 (anti-EGFP); red, Alexa647 (anti-ZsGreen). White box in the merged picture indicating the area of high magnification images shown in the right panel. Scale bar, 100 μm.

(D-F) Immunostaining of tdTomato, ZsGreen, and cTnI in MI (D), MGTM+shCtrl (E), and MGTM+shMeox1 (F) heart sections from Tnni3-Dre;IR1; R26-iCre mice. White boxes in the merged pictures indicate areas of high magnification images shown in the right panels. Scale bar, 100 μm.

(G) Quantitative analyses of ZsGreen+tdtomato-CM numbers per section. Data analyzed by unpaired T-test. n=5, independent biological replicates.

(H) Immunostaining of heart sections from Tnni3-Dre;IR1;Postn-MerCreMer MI mouse. ZsGreen+ MFs can be observed in uninjured area of hearts. Scale bar, 100 μm.

(I and J) Immunostaining (I) and Quantitative analyses (J) of ZsGreen+tdtomato+ fused CMs per heart sections from Tnni3-Dre;IR1;Postn-MerCreMer MI mouse. Scale bar, 100 μm. n = 5, independent biological replicates.

All data are presented as mean±SEM. Unpaired t-test. ns, no significance, $p>0.05$, *$P<0.05$, **$P<0.01$, ***$P<0.001$, ****$P<0.0001$ vs the relevant control.

Tables

Table S1: List of shRNA sequences used in shRNA library screening

Table S2: List of primers used in quantitative RT-PCR

Supplemental Movies

Supplemental Movie 1: MICFs infected MGTM and shCtrl induced beating iCMs after 6 weeks induction

Supplemental Movie 2: MICFs infected MGTM and shMeox1 induced beating iCMs after 6 weeks induction

Supplemental Movie 3: MICFs infected MGT and shCtrl induced beating iCMs after 6 weeks induction

Supplemental Movie 4: MICFs infected MGT and shMeox1 induced beating iCMs after 6 weeks induction

Reference

1. Farbehi, N. *et al.* Single-cell expression profiling reveals dynamic flux of cardiac stromal, vascular and immune cells in health and injury. *eLife.* **8**, (2019).
2. Quaife-Ryan, G. A. *et al.* Multicellular transcriptional analysis of mammalian heart regeneration. *Circulation*. **136**, 1123-1139 (2017).