

SUPPLEMENTARY MATERIAL

S1.1 *Creation of MI and intracardiac injection of EPO analog*

Eight-week-old male C57BL6/J mice were anesthetized with 2% isoflurane inhalation. The chest was disinfected with 70% ethanol, a small hole was made at the fourth intercostal space and the heart was popped out of the chest. The left anterior descending (LAD) coronary artery was ligated at a site about 3 mm from its origin using a 6-0 silk suture to create permanent MI according to a novel procedure [1]. The chest was then closed. The whole surgery time needed was only about 30 s. No ventilation was used during the surgery. Almost all animals kept alive after the surgery.

In the *in vivo* study, we used darbepoetin alpha (a long-acting EPO analog) (Amgen, CA, USA) instead of EPO. For the convenience of description and consistency of terms, we indicated darbepoetin alpha as “EPO_{anlg}” in the following text for *in vivo* study. EPO (not EPO_{anlg}) was used in the *in vitro* cell study. Intracardiac (i.c.) injection of EPO_{anlg} (30 µg/kg) was performed after LAD ligation. EPO_{anlg} was dissolved with saline and injected into the border zone of MI at three points. The total injection volume was only 20 µL. Thin-wall needles (BD Precision Glide™ 30 G) were used to keep minimal injection damage. After injection, the heart was immediately placed back into the chest and the chest was closed by a suture. Same volume of PBS was injected in the control MI group (PBS group).

S1.2 *Masson's trichrome staining and determination of infarct size*

After *in vivo* experiments, mice were sacrificed and hearts were harvested. Hearts were fixed with 10% formaldehyde for 48 h, then were kept in 70% ethanol. The heart tissues were routinely developed and embedded in paraffin wax. Tissue sections of 5 µm were cut with a microtome and mounted on slides. Tissue slides were heated at 62 °C to avoid section detachment, then were deparaffinized and rehydrated just before staining. To perform Masson's trichrome staining, tissue slides were put in Bouin's solution at room temperature (RT) for overnight. Slides were then briefly rinsed in distilled water, dipped in working Weigert's iron solution for 5 min, and washed with running tap water for 5 min. Slides were then dipped in Beibrich Scarlet-Acid Fuchsin solution for 5 min and then rinsed in distilled water. Tissue sections were stained in phosphomolybdic/phosphotungstic acid solution for 10 min,

then were transferred to aniline blue solution and stained for 10 min, followed by rinse in distilled water. Tissue slides were then placed in 1% acetic acid solution for 1.5 min. After staining, tissue slides were dehydrated with xylene and coverslipped using polymount [2].

Myocardial infarct size and LV chamber area at the first, second and fourth week post MI was determined based on Masson's trichrome staining and calculated according to the methods we described previously [1]. The percentage of infarct area and LV chamber area was measured using Sigmascan software.

S1.3 Immunofluorescent staining

Immunofluorescent staining was used to identify CD31 of endothelial cells in the myocardium and EPO receptors in Lin⁻ Sca-1⁻ cells and Lin⁻ Sca-1⁺ stem cells (SCs) [3]. To stain CD31, mice hearts (two weeks post MI or sham) were dissected and flushed with PBS, then were embedded in OCT and stored at -20 °C. Hearts were sectioned and fixed in 4% formalin for 5 min, rinsed with PBS for 3 × 5 min, blocked with 10% goat serum in PBS for 1 h, then were incubated with CD31 antibody (1:50 in block solution) over night at 4 °C. Sections were then rinsed with PBS for 3 × 5 min and incubated with secondary antibody (dilution 1:300 in block solution, Invitrogen Alexa Fluor 555) for 1 h at RT avoid light. Sections were rinsed again with PBS and mounted with anti-fade mounting medium (Vector H-1200). Immunofluorescent (IF) images reflecting CD31-positive signals were taken under a fluorescent microscope.

To stain EPO receptors in the cells using IF method, the third passage of Lin⁻ Sca-1⁻ cells and Lin⁻ Sca-1⁺ SCs were seeded in 96-well plates. After 24 h adhesion, the culture medium was discarded. Cells were washed with PBS for 3 × 5 min. Cells were then fixed with 4% paraformaldehyde at RT for 15 min, and washed with PBS for 3 × 5 min. Cells were blocked with 1% TritonX-100/goat serum for 1 h, then were incubated with anti-EPO receptor antibody (Abcam, 1:100 in blocking solution) overnight at 4 °C, followed by PBS wash for 3 × 5 min. Cells were then incubated with FITC-labeled goat anti-rabbit IgG (1:100 in blocking solution) at RT for 1 h avoid light, and washed with PBS for 3 × 5 min. Nuclei were stained with DAPI solution for 20 min. After PBS wash, cell images were taken under a fluorescent microscope.

S1.4 Isolation and culture of $\text{Lin}^- \text{Sca-1}^+$ SCs

For cloning study, $\text{Lin}^- \text{Sca-1}^+$ SCs were isolated from the hearts of neonatal (1–3 days old) C57BL/6J mice. For other cellular experiments, $\text{Lin}^- \text{Sca-1}^+$ SCs were isolated from the hearts of adult (8-week old) male C57BL/6J mice [4].

To isolate $\text{Lin}^- \text{Sca-1}^+$ SCs from hearts of neonatal mice, mice were sacrificed by putting them into a nitrogen tank. Mice were then dipped in 18% ethanol and hearts were immediately harvested, rinsed in pre-cooled ADS buffer (4 °C). The hearts were cut into pieces and digested with enzymes (collagenase II and trypsin) for several times. Digestion was stopped and supernatant were collected. Cell suspension was centrifuged at 1 300 rpm for 7 min at 4 °C. Cell pellets were resuspended and filtered with 70 μm and 30 μm pre-sorting filters.

To isolate $\text{Lin}^- \text{Sca-1}^+$ SCs from hearts of adult mice, mice were heparinized and then were anesthetized with pentobarbital sodium. The chest was opened, and the heart was harvested and perfused on a Langendorff apparatus with Ca^{2+} -free Tyrode's solution for 4 min and then with collagenase II (265 U/mL in DME/F-12 medium) for 10 min. Heart tissue was collected to a dish when it became looser and bigger, and then was cut into small pieces inside a hood. Stop solution was added to the dish to cease the digestion. Cell suspension was centrifuged at 1300 rpm for 7 min at 4 °C. Cell pellets were resuspended and filtered with 70 μm and 30 μm pre-sorting filters.

Above two kinds (neonatal and adult) of filtered cells were centrifuged again at 1 300 rpm for 7 min at 4 °C. Cell pellets were resuspended, and cell suspension were incubated with biotin-antibody cocktail (10 $\mu\text{L}/10^7$ cells) at 4 °C for 10 min. Cell pellets were then incubated with anti-biotin microbeads (20 $\mu\text{L}/10^7$ cells) at 4 °C for 15 min. Cells were purified by the magnetic column and Lin^- cells were obtained. Lin^- cell suspension was incubated with anti-Sca-1-FITC (10 $\mu\text{L}/10^7$ cells) for 10 min at 4 °C, washed with PBS, and incubated with anti-FITC microbeads (20 $\mu\text{L}/10^7$ cells) for 15 min at 4 °C. Sca-1^+ SCs were collected by positive magnetic sorting. After sorting, the percentage of $\text{Lin}^- \text{Sca-1}^+$ SCs was $(85.03 \pm 5.28)\%$ which was significantly higher than the $\text{Lin}^- \text{Sca-1}^-$ SCs $(7.64 \pm 3.31)\%$ ($P < 0.001$, Fig. S1). Therefore, by magnetic sorting, high purity cardiac resident $\text{Lin}^- \text{Sca-1}^+$ SCs were obtained.

S1.5 Western blotting

Western Blotting was used to evaluate the expression levels of EPO receptor in Lin⁻ Sca-1⁻ cells and Lin⁻ Sca-1⁺ SCs, and p38, p-p38, Stat-5, p-Stat-5, Erk-1/2 and p-Erk-1/2 in Lin⁻ Sca-1⁺ SCs. Lin⁻ Sca-1⁻ cells and Lin⁻ Sca-1⁺ SCs isolated from adult mice were used. The third passages of Lin⁻ Sca-1⁻ and Lin⁻ Sca-1⁺ SCs (2 × 10⁵ cells/mL) were seeded in 6-well plates. After 24-h cell adhesion, EPO (0.5 μmol/L) was added to the wells in EPO group. At 10 min, 30 min, 1 h, 3 h and 12 h after treatment, cells were collected by 100 μL of lysate (cell lysate:PMSF:phosphatase inhibitor = 100:1:1) on ice. After centrifugation (13 000 rpm for 15 min at 4 °C), the supernatant was used for western blotting assay. SDS-PAGE separation gel (12%) was prepared, and agarose gel electrophoresis was performed. Proteins were transferred to the PVDF membranes, and the membranes were then incubated with primary antibodies against EPOR (1:500), p38 (1:1 000), p-p38 (1:1 000), Stat-5 (1:200), p-Stat-5 (1:500), Erk-1/2 (1:1 000), p-Erk-1/2 (1:1 000), β-actin (1:200) (in blocking solution) respectively overnight at 4 °C. Membranes were rinsed with TBST for 3 × 5 min, and incubated with secondary antibodies (horseradish enzyme labeled goat anti-mouse IgG, 1:1 000; horseradish enzyme labeled goat anti-rabbit IgG, 1:1 000) for 1 h at RT. Membranes were rinsed with TBST for 3 × 5 min. Finally, membranes were completely immersed in the luminescent working solution for 1 min at RT avoid light. Membranes were scanned to collect and analyze data.

S1.6 *In vivo* hemodynamic measurements

Mice were anesthetized with a 2% Avertin, and the right common carotid artery was isolated and cannulated with 1.4 French micromanometer (Millar Instruments, Houston, TX). External jugular vein was isolated for the administration of β-adrenergic receptor (βAR) agonist - isoproterenol (Iso). These parameters as well as maximal values of the instantaneous first derivative of LV pressure (+dP/dt_{max}, as a measure of cardiac contractility), minimum values of the instantaneous first derivative of LV pressure (-dP/dt_{min}, as a measure of cardiac relaxation), aortic systolic pressure (ASP) and heart rate (HR) were recorded at baseline and after administration of Iso (0.1 to 10 ng) by this catheter advanced into the LV cavity [1, 5].

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