SUPPLEMENTAL MATERIAL
Supplemental Methods

In vitro migration assay

The macrophages were collected from the peritoneal cavity 3 days after intraperitoneal injection with 3% thioglycollate (BD Bioscience). Migration assay was performed using a transwell plate fitted with an 8-μm filter (Corning). The cells for Erdr1 knockdown assay were first transduced with empty vector or erythroid differentiation regulator 1 (Erdr1)-shRNA by adenovirus. Two days post-transduction $5 \times 10^4$ cells were plated in the upper well and the chemokine monocyte chemoattractant protein-1 (MCP-1) at various concentrations was added to the bottom well. Following overnight incubation at 37°C, the cells migrated to the bottom were fixed with 2% paraformaldehyde and the nuclei were stained with DAPI (Sigma) for quantification. The average number of cells was examined using high-content imaging analysis system (ImageXpress Micro XLS System, Molecular Devices). The migration ability of cells was calculated as a migration index in which the number of cells in the presence of chemokine was divided by the number of cells in the absence of chemokine.

In vitro apoptotic assay

To induce macrophage apoptosis, the cells ($5 \times 10^4$) were incubated with 1% O$_2$ for 24 and 72 hours. The hypoxia-induced cell apoptosis was evaluated using MTT assay according the manufacturer’s protocol (Invitrogen). Absorbance of samples was read at 540 nm.
**Immunohistochemistry and immunofluorescence microscopy**

The hearts were harvested and fixed with 4% paraformaldehyde and embedded in paraffin. For immunofluorescent staining of Sca-1, tissue sections were stained with rat anti-Sca-1 antibody (BD Biosciences). The plasma membrane was immunostained with wheat germ agglutinin (WGA, 5 μg/ml, Invitrogen). Appropriate secondary antibodies (Invitrogen) were used for visualization under a fluorescence microscope. Nuclei were stained with 4,6-diamidino-2-phenylindole (DAPI, 1 μg/ml; Sigma) for visualization.

**RNA extraction and gene expression analysis**

The macrophages for RNA extraction were collected from the peritoneal cavity of WT and EP2+/− mice 3 days after intraperitoneal injection with 3% thioglycollate (BD Bioscience). The sham-operated hearts or ischemic hearts were harvested at the designated time point. In ischemic hearts, only the ischemic region of the hearts were collected for extraction of total RNA using Trizol (Invitrogen). The extracted RNA was then reverse transcribed using the SuperScript III (Invitrogen) system according to the manufacturer’s protocol. The SYBR Green reagent (Maestrogen) was used for quantitative PCR according to the manufacturer’s protocol. The sequence-specific primers designed for quantitative PCR were as follows:

Erdr1: forward- GGGCGTGAATGGAAAGTCTA; reverse- CAGGCTTCTACCTTGTGGA

Selp: forward- CTTTGGTCCGAACACCACCTT; reverse- GAAGGTGCAGGTTGATCCAT
Gata2: forward- CCAGCAATCCAAAGAAGAGC; reverse- AGACTGGAGGAAGGGTGAT

Thbs1: forward- GGGCAGGAAGACTATGACA; reverse- CTCCCCGTTTTTGCTGTGT

IL-1α: forward- AAGCAACGGGAAGATTTCTGA; reverse- TGACAAACTTCTGCCTGACG

Tgf-β2: forward- CCGGAGGTG ATTTCCATCTA; reverse- GGACTGTCTGGAGCAAAAAGC

iNos: forward- GCATGAGCTGGTGTTTGG; reverse- AGCTGCTTTTGCAAGGATGT

TNF-α: forward- GCCTGTTCATTCCTGCTT; reverse- CACTTGGTGTTTGCTACGA

COX2: forward- GCTTCTTTGCCCAGCACCT; reverse- GACCAGGCACCCAGACCAAGA

IL-1β: forward- TGTCTTTCTCCTTGCTCTG; reverse- TGTGCTGGTGCTTCATTCAT

CD11b: forward- CACCAAAAACGTCAAGGAGAATAC; reverse- CTACCAGAGCCATCAATCAAG
Figure S1. Loss of EP2 receptor did not alter cardiac function in sham-operated animals but worsened cardiac function after injury

(A), Cardiac function of EP2 null (EP2−/−) mice was not significantly different from their WT and heterozygous (EP2+/−) knockout littermates when subjected to sham surgery. (B), Cardiac function of EP2−/− mice and their heterozygous and WT littermates was examined at 1 month post-MI. The experiments were performed with n ≥ 14 animals per genotype. Statistical analyses were performed using one-way ANOVA with Dunnette’s test. *, P = 0.0376, n.s = not significant.
Figure S2. Loss of EP2 resulted in reduced expression of inflammation modulators. At day 3 and day 7 post-MI, the expression of genes of interest in the injured hearts of EP2−/− mice was compared to that in the WT animals. Analysis was performed with 3 animals per time point for each genotype. Statistical analysis was performed using one-way ANOVA with Tukey’s test. *, $P = 0.0466$ and *, $P = 0.0292$ for TNF-α and IL-1β, respectively; **, $P = 0.0017$, ***, $P = 0.0007$, ****, $P < 0.0001$ and n.s., not significant vs. WT control at the same time point.
Figure S3. Immune cell number was not significantly altered in sham-operated animals.

Following sham operation, the number of immune cells was examined in excised heart tissue and the number of monocytes was analysed in various tissues. Representative flow cytometric results of F4/80+ macrophages and Gr1+ neutrophils are shown in (A). The numbers of F4/80+ macrophages (B, left panel) and Gr1+ neutrophils (B, right panel) relative to the weight of excised tissue from EP2−/− and WT mice were compared. Statistical analysis was performed using unpaired t test. n.s., not significant. (C) Representative flow cytometric analyses of CD115+/Ly6c+ monocytes, as indicated in the red boxes, are shown in various tissues from sham-operated animals. (D) The number of monocytes examined in different tissues of sham-operated WT and EP2−/− mice were quantified and statistically analysed using unpaired t test. n.s., not significant.
Figure S4. Macrophage marker expression peaked at day 3 post-injury.

The expression of pan-macrophage marker CD11b in the injured hearts of WT mice was examined at different time points after myocardial injury. Analysis was performed on 4 animals per time point. The gene expression was undetectable in 2 samples at day 7 post-injury. Statistical analyses were performed using one-way ANOVA with Dunnette’s test. ***, P = 0.0001 and **, P = 0.0072 and n.s., not significant vs. sham control.
Figure S5. EP2 signaling had little effect on polarization of M1 and M2 macrophage subpopulations.

The cardiomyocyte-depleted small cell population was analyzed for the F4/80⁺/CD206⁻ M1 and F4/80⁺/CD206⁺ M2 macrophage subpopulations at day 3 post-myocardial injury. Shown is a representative image of the gating of the M1 and M2 macrophage subpopulations (Left). Statistical analysis revealed that the M1 and M2 macrophage ratio was not significantly changed in WT (n=5) and EP2 null (EP2⁻/⁻, n=5) mice after injury (Right). Statistical analysis was performed using unpaired t test. n.s. = not significant.
In the injured myocardium, the macrophages in the cardiomyocyte-depleted small cell fraction were analyzed for expression of EP2 receptor. The results showed that EP2 expression was induced in response to heart injury.
Figure S7. EP2 deficiency had negligible effect on cell viability.

Macrophages isolated from WT and EP2 null (EP2−/−) mice were exposed to hypoxic conditions for induction of apoptosis. Cell viability was then analyzed at different time points following culturing under hypoxic conditions. The experiment was repeated 4 and 5 times for the 24 and 72 hour time point, respectively. Statistical analysis was performed using unpaired t test. n.s., not significant.
Figure S8. The shRNA-dependent gene knockdown effectively lowered expression of *Erdr1* gene in EP2 deficient macrophages.

Following viral transduction of *Erdr1* shRNA in the EP2 null (EP2<sup>−/−</sup>) macrophages, quantitative PCR was conducted to examine the expression level of the target gene. The cells transduced with vector alone were the control. Macrophages were isolated from 3 animals for viral transduction. Statistical analysis was performed using one-way ANOVA with Tukey’s test. ****, *P* < 0.0001.
Figure S9 Sca-1+ stem cells were observed at in the injured myocardium.

At day 3 post-infarction, the injured myocardium were stained for the stem cell marker Sca-1. Shown are representative images of Sca-1+ cells observed at the infarct region (A) and peri-infarct area (B) in the injured myocardium. Scale bars, 20 µm.