

**Table. S1 Primary antibodies used in the western blot assay.**

**Table. S2 The primers used in Real-time PCR.**

**Fig. S1 PRDX2 overexpression alleviated CMEC dysfunction after HG/FFA injury by mitigating ferroptosis.**

(A) Time course of PRDX2 expression in left ventricle and primary CMECs in db/m and db/db mice. (B-C) Quantification of PRDX2 expression in left ventricle and primary CMECs. (D) Cell viability was measured by the CCK-8 assay. CMECs were exposed to HG/FFA condition for 72 h and treated with Ferrostain-1 (10  $\mu$ M). Fer-1: Ferrostain-1. E-J Cultured CMECs were transfected with ADV-PRDX2 (10 MOI) for 48 h and, with or without Erastin treatment (2.5  $\mu$ M, 24 h). (E) The efficiency of ADV-PRDX2 transfection was detected by western blot assay. (F) Representative images of the transwell assay and quantified number of migrated cells. Scale bar = 200  $\mu$ m. (G) Representative images of the tube formation assay and the quantified number of branch points. Scale bar = 200  $\mu$ m. (H) eNOS phosphorylation at Ser<sup>1177</sup> was detected by western blot analysis and quantified. (I) NO content in culture medium. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , significantly different as indicated. ns, not significant. Three to six biological replicates were performed, and the results are indicated in scatter plots.

**Fig. S2 Endothelial-specific overexpression of PRDX2 protected the cardiac microcirculation against ferroptosis.**

Four-week-old male db/db mice were transfected with AAV9-PRDX2 or AAV9-NC for 24 weeks. (A) AAV9 transfection efficiency in the cardiac microcirculation was detected by immunofluorescence staining of Flag (green) and CD31 (red). Scale bars: 40  $\mu$ m. (B) The specificity and transfection efficiency of AAV9-PRDX2 in primary CMECs was detected by western blot assay. (C) The protein expression of GPX4 and ACSL4 in primary CMECs was detected by western blot assay. (D) Representative immunofluorescence images of ferrous iron (red) and lipid peroxidation (green) in primary CMECs. (E) The fasting blood glucose levels in each experimental group. (F) The free fatty acid levels in the serum of mice in each experimental group. (G) Serum BNP levels in each experimental group. (H) The relative expression of ANP mRNA in left ventricle. LPO: lipid peroxidation. Scale bar = 15  $\mu$ m. ns: not significant. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , significantly different as indicated. ns, not significant. Four biological replicates were performed, and the results are indicated in scatter plots.

**Fig. S3 ISO treatment improved endothelial function by upregulating PRDX2.**

Cultured CMECs were transfected with ADV-shPRDX2 (15 MOI) for 48 h and then subjected to HG/FFA condition for 72 h with or without ISO treatment (10  $\mu$ M). (A) The transfection efficiency of ADV-shPRDX2 was detected by western blot assay. (B) Representative images of the transwell assay and the quantified number of migrated cells. Scale bar = 200  $\mu$ m. (C) Representative images of the tube formation assay and the quantified number of branch points. Scale bar = 200  $\mu$ m. (D) eNOS phosphorylation at Ser<sup>1177</sup> was detected by western blot assay and quantified. (E) NO content in culture medium. ns: not significant. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , significantly different as indicated. Four to six biological replicates were performed, and the results are indicated in scatter plots.

**Fig. S4 PRDX2 overexpression and ISO treatment improved mitochondrial dynamics and function.**

Cultured CMECs were subjected to HG/FFA condition for 72 h and treated with ISO treatment (10

$\mu\text{M}$ ) or PRDX2 overexpression (10 MOI). (A) Representative images of mitochondrial morphology (red) and quantitative analysis of mitochondrial length. Scale bar = 10  $\mu\text{m}$ . (B) Mitochondrial membrane potential in CMECs. MMP: Mitochondrial membrane potential. (C) Representative images of mitochondrial ultrastructure in CMECs under a transmission electron microscope and quantitative analysis of mitochondrial length to width ratio. (D) The protein expression of Nrf1 and TFAM in CMECs was detected by western blot assay. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , significantly different as indicated. ns, not significant. Four to six biological replicates were performed, and the results are indicated in scatter plots.

**Fig. S5 Manipulation of MFN1 and FIS1 expression affects ferroptosis in CMECs under HG/FFA condition.** (A) Co-IP assays among PRDX2-Flag and Drp1, FIS1. (B) Co-IP assays among PRDX2-Flag and MFN1. (C) Co-IP analysis of MFN2 and Flag-tagged PRDX2 in CMECs. (D-E) The transfection efficiency of ADV-MFN2 and ADV-MFN1 was detected by western blot assay. (F) Cell viability was measured by the CCK-8 assay. (G) Quantification of ROS intensity. (H) Quantification of lipid peroxidation. (I) Quantification of ferrous iron intensity. (J) The transfection efficiency of ADV-FIS1 was detected by western blot assay. (K) Cell viability was measured by the CCK-8 assay. (L) Quantification of ROS intensity. (M) Quantification of lipid peroxidation. (N) Quantification of ferrous iron intensity. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , significantly different as indicated. Four to six biological replicates were performed, and the results are indicated in scatter plots.

**Fig. S6 Mdivi-1 and MitoTEMPO improved mitochondrial dynamics and dysfunction caused by erastin.**

Cultured CMECs were exposed to erastin (2.5  $\mu\text{M}$ ) for 24 h and treated with Mdivi-1 (10  $\mu\text{M}$ ) or mitoTEMPO (10  $\mu\text{M}$ ). (A-B) Representative images of mitochondrial morphology (red) and quantitative analysis of mitochondrial length. Scale bar = 10  $\mu\text{m}$ . (C) MMP was measured in CMECs. (D) Cell viability was assessed by the CCK8 assay. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , significantly different as indicated. Four to six biological replicates were performed, and the results are indicated in scatter plots.

**Fig. S7 MFN2 improved mitochondrial function via the inhibition of mitoACSL4.**

Cultured CMECs were transfected with mitoACSL4 (15 MOI) and/or MFN2 (10 MOI) for 48 h and subjected to HG/FFA condition for 72 h. (A) Representative immunofluorescence images of Flag-mitoACSL4 (green) and COX IV (red). Scale bar = 15  $\mu\text{m}$ . (B) The expression of ACSL4 on mitochondria was evaluated by western blot analysis. (C) The expression of cytoplasmic ACSL4 was evaluated by western blot analysis. (D) Cell viability was measured by the CCK-8 assay. (E) Mitochondrial membrane potential in CMECs. MMP: Mitochondrial membrane potential. (F) Representative images of mitochondrial morphology (red) and quantitative analysis of mitochondrial length. Scale bar = 25  $\mu\text{m}$ . (G) Representative images and quantitative analysis of mitochondrial ROS (red). mitoROS: mitochondrial ROS. Scale bar = 25  $\mu\text{m}$ . \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , significantly different as indicated. ns, not significant. Four to twelve biological replicates were performed, and the results are indicated in scatter plots.

**Fig. S8 ISO treatment improved endothelial function by suppressing mitoACSL4.**

Cultured CMECs were transfected with mitoACSL4 (15 MOI) for 48 h and then subjected to HG/FFA condition for 72 h, with or without ISO treatment (10  $\mu\text{M}$ ). (A) The expression of mitochondrial ACSL4 was evaluated by western blot analysis. (B-C) Representative images of

mitochondrial morphology (red) and quantitative analysis of mitochondrial length. Scale bar = 10  $\mu$ m. **(D)** Quantitative analysis of mitochondrial ROS. **(E)** Mitochondrial membrane potential in CMECs. MMP: Mitochondrial membrane potential. **(F)** Representative images of the transwell assay and quantified number of migrated cells. Scale bar = 200  $\mu$ m. **(G)** Representative images of the tube formation assay and the quantified number of branch points. Scale bar = 200  $\mu$ m. **(H)** eNOS phosphorylation at Ser<sup>1177</sup> was detected by western blot assay and quantified. **(I)** NO content in culture medium. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , significantly different as indicated. ns, not significant. Four to six biological replicates were performed, and the results are indicated in scatter plots.

**Fig. S9 ISO treatment alleviated mitochondrial-derived ferroptosis in the cardiac microcirculation after long-term diabetes mellitus.**

Four-week-old male db/db mice were transfected with AAV9-mitoACSL4 or AAV9-NC for 24 weeks, with or without ISO treatment (25 mg/kg/d). **(A)** The expression of PRDX2, MFN2 and ACSL4 in primary CMECs was measured by western blot assay. **(B)** Representative immunoblots and quantitative analysis of ACSL4 expression in mitochondria. 3-6 hearts in one sample, two samples in each group. **(C)** AAV9 transfection efficiency in the cardiac microcirculation was detected by immunofluorescence staining of Flag (green) and CD31 (red). Scale bars: 40  $\mu$ m. **(D)** Representative immunofluorescence images and the quantification of mitochondrial ferrous iron (green). [Fe<sup>2+</sup>]<sub>m</sub>: mitochondrial ferrous iron. Scale bar = 15  $\mu$ m. **(E)** Representative immunofluorescence images and the quantification of mitochondrial lipid peroxidation (green). **(F)** The fasting blood glucose levels in each experimental group. **(G)** The free fatty acid levels in the serum of mice in each experimental group. **(H)** Serum BNP levels in each experimental group. **(I)** The relative expression of ANP mRNA in left ventricle. mitoLPO: mitochondrial lipid peroxidation. Scale bar = 15  $\mu$ m. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , significantly different as indicated. ns, not significant. Two to four biological replicates were performed, and the results are indicated in scatter plots.