

Biphasic Protective Effects of Macrophage Migration Inhibitory Factor in Ischemia/Reperfusion-Induced Acute Kidney Injury

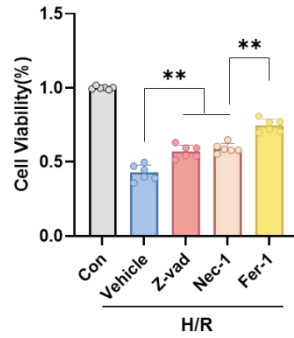
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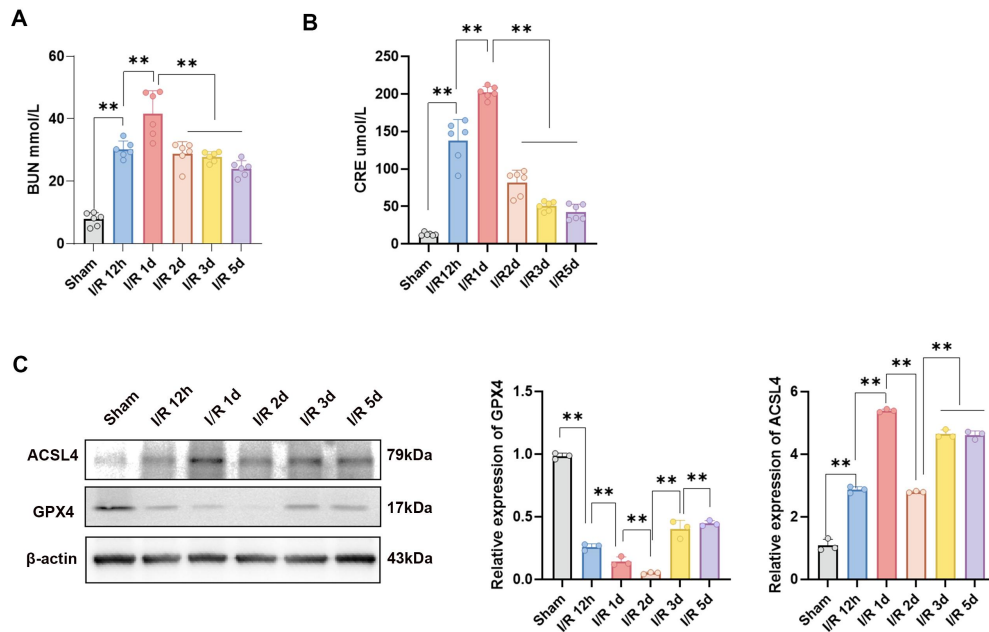
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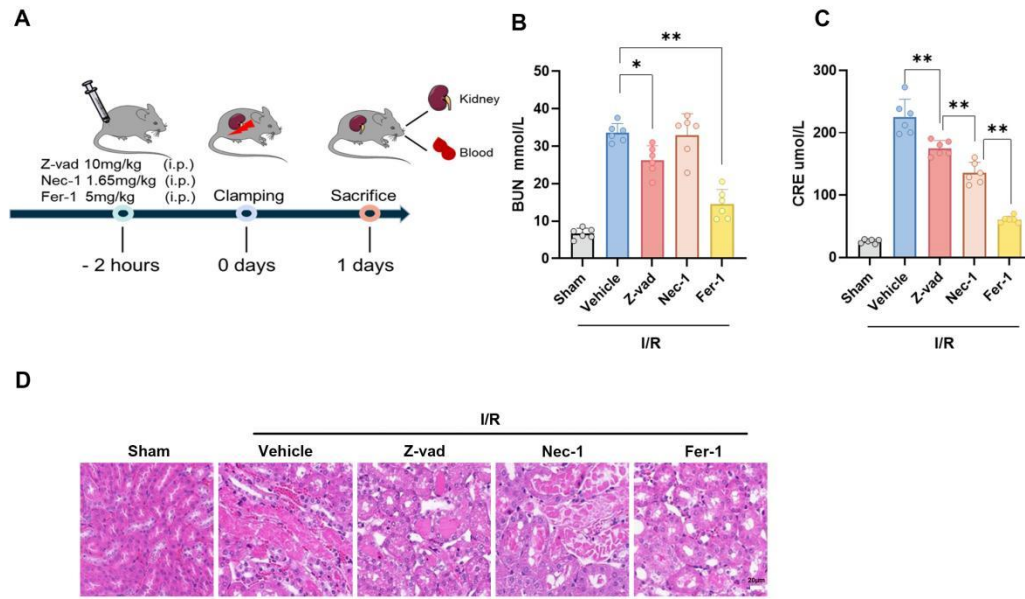


Supplementary Figure 1. Ferroptosis was dominantly inhibited by Fer-1 in HK-2 cells after H/R treatment. Cell viability of HK-2 cells was significantly improved by pretreatment with ferroptosis inhibitor Fer-1 for 2h, compared with Z-vad and Nec-1 after H/R treatment. Data are presented as means $\pm$ SD. Statistical analysis: one-way ANOVA with Tukey's test for cell viability (n=6 per group). * indicates $p<0.05$, ** indicates $p<0.001$.



Supplementary Figure 2. Ferroptosis was most severe in the mouse kidneys after I/R for 1d. (A) BUN levels of mice in I/R 1d group (n=6). (B) CRE levels of mice in I/R 1d group (n=6). (C) Western blot results of GPX4 and ACSL4 in mice exposed to different I/R times (n=3). Data are presented as means $\pm$ SD. Statistical analysis: one-way ANOVA with Tukey's test for BUN, CRE, and Western blots (n=3–6 per group). * indicates $p<0.05$, ** indicates

$p < 0.001$.



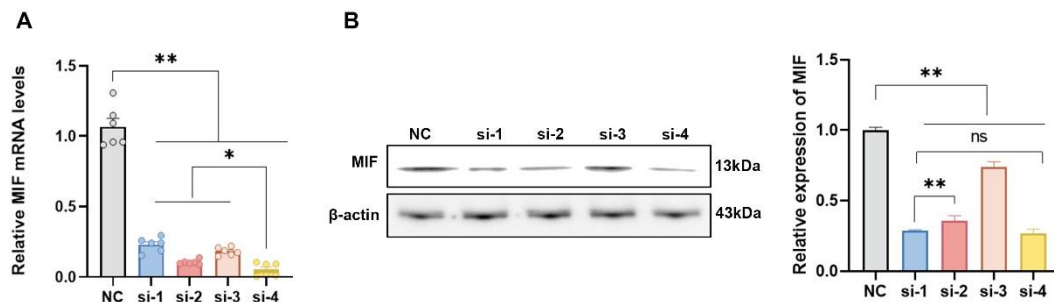
Supplementary Figure 3. Ferroptosis was the dominant cell death in I/R-AKI. (A)

Flow diagram of time points for inhibitor pretreatment and sample collection in mice.

(B) BUN levels of mice after pretreatment with Z-vad, Nec-1, and Fer-1, respectively (n=6).

(C) CRE levels of mice after pretreatment of Z-vad, Nec-1, and Fer-1, respectively (n=6).

(D) Representative HE staining images of mouse kidneys after pretreatment of Z-vad, Nec-1, and Fer-1, respectively (n=3). Data are presented as means $\pm$ SD. Statistical analysis: one-way ANOVA with Tukey's test for BUN and CRE (n=6 per group). * indicates $p < 0.05$, ** indicates $p < 0.001$.

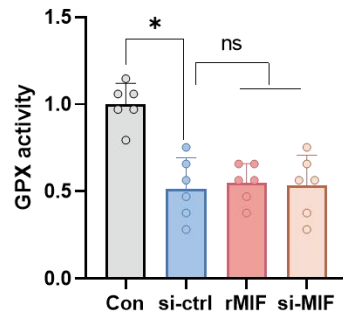


Supplementary Figure 4. Downregulation via si-RNA and verification of MIF in vitro. (A)

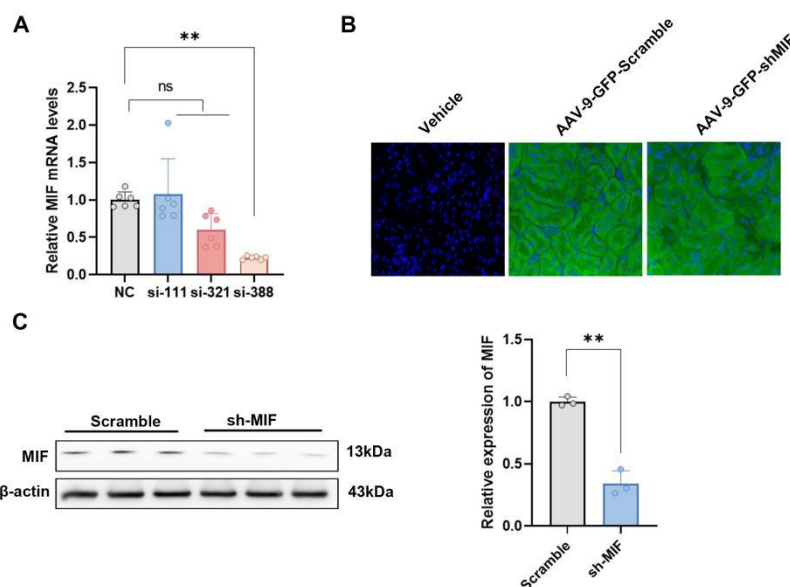
MIF mRNA levels of HK-2 cells after different si-RNA transfection for 24h (n=6).

(B) MIF protein levels of HK-2 cells after different si-RNA transfection for 24h (n=6).

24h (n=3). Data are presented as means $\pm$ SD. Statistical analysis: one-way ANOVA with Tukey's test for RT-PCR and Western blots (n=3–6 per group). * indicates $p<0.05$, ** indicates $p<0.001$.

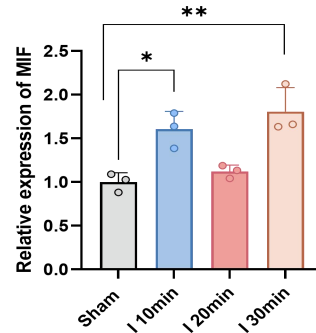


Supplementary Figure 5. The activity of GPX4 was detected with MIF upregulated or downregulated. GPX4 activity of HK-2 cells in different groups after upregulation and downregulation of MIF (n=6). Data are presented as means $\pm$ SD. Statistical analysis: one-way ANOVA with Tukey's test for GPX activity (n=6 per group). * indicates $p<0.05$, ** indicates $p<0.001$.

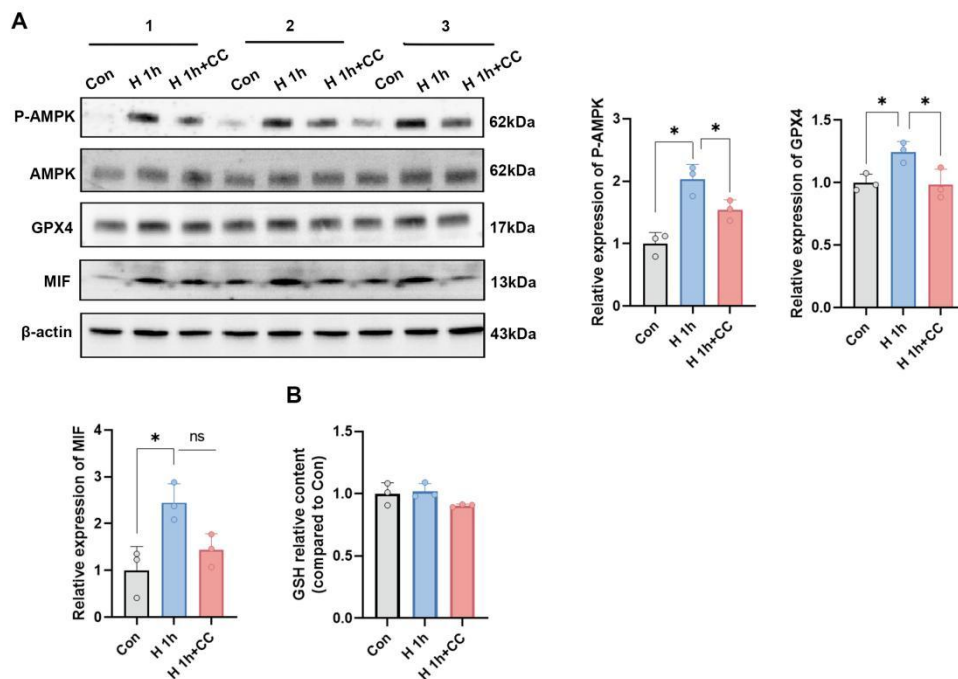


Supplementary Figure 6. Determination of AAV infectivity and downregulation of MIF in the kidney. (A) MIF mRNA levels of TCMK-1 cells after different si-RNA transfection for 24h (n=6). (B) Representative GFP immunofluorescence images of the kidney after AAV in situ injection for one month (n=3). (C) MIF protein levels of the kidney after AAV in situ injection for one month (n=6). Data are presented as

means $\pm$ SD. Statistical analysis: one-way ANOVA with Tukey's test for RT-PCR and Western blots (n=3–6 per group). * indicates $p<0.05$, ** indicates $p<0.001$.

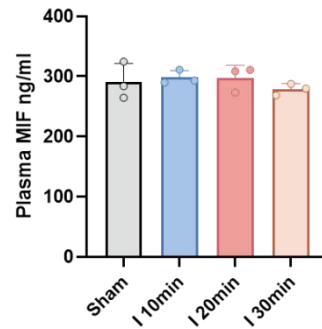


Supplementary Figure 7. Relative quantification of MIF compared with internal reference after ischemia in the kidney (n=3). Data are presented as means $\pm$ SD. Statistical analysis: one-way ANOVA with Tukey's test for Western blots (n=3 per group). * indicates $p<0.05$, ** indicates $p<0.001$.



Supplementary Figure 8. Inhibitor Compound C mitigated the phosphorylation of AMPK induced by MIF. (A) Western blot results of P-AMPK, GPX4, and MIF after the applications of Compound C (CC) and hypoxia for 1 hour (n=3). (B) GSH content of HK-2 in different groups after the pretreatment of CC and hypoxia for 1

hour (n=3). Data are presented as means $\pm$ SD. Statistical analysis: one-way ANOVA with Tukey's test for GSH and Western blots (n=3 per group). * indicates $p<0.05$, ** indicates $p<0.001$.



Supplementary Figure 9. Plasma concentration of MIF in ischemia model.

Circulating level of MIF of ischemia mouse model for 10min, 20min and 30min, respectively. Data are presented as means $\pm$ SD. Statistical analysis: one way ANOVA with Tukey's test for Elisa (n=3 per group). * indicates $p<0.05$, ** indicates $p<0.001$.