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Supplemental information

Metformin alleviates hepatic iron overload and ferroptosis through AMPK-ferroportin pathway in HFD-induced NAFLD

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Supplemental information

Table S1. qPCR primers for mRNA validation (Related to STAR★METHODS)

Genes	Primer Sequence 5’ to 3’
GPX4-qPCR-F	GAC ATC GAC GGG CAC AT
GPX4-qPCR-R	ATC CGC AAA CCA CAC TCA
FPN-qPCR-F	CTC TGT CAG CCT GCT GTT TG
FPN-qPCR-R	TCA GGA TTT GGG GCC AAG ATG
GAPDH-qPCR-F	ACG GAT TTG GTC GTA TTGG
GAPDH-qPCR-R	TCC CGT TCT CAG CCT TG

F: Forward; R: Reverse

Abbreviations: GPX4, Glutathione Peroxidase 4; FPN, ferroportin; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

Supplemental figures and legends

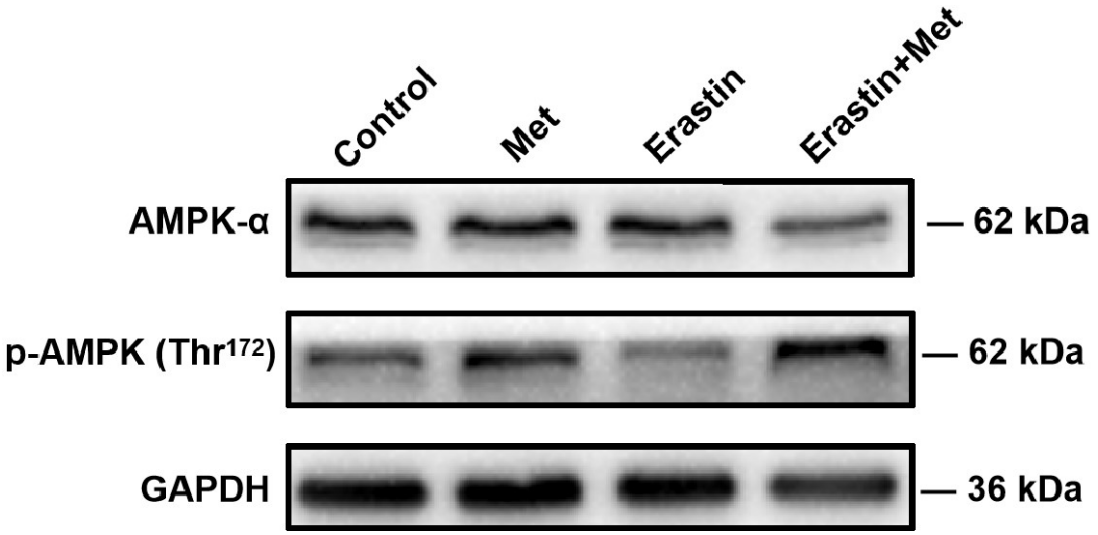

Figure S1. The effect of metformin on AMPKα, P-AMPKα protein in erastin-treated WRL68 cells. Related to Figure 3

Protein expressions of AMPKα, and P-AMPKα assessed by western blotting in WRL68 cells treated with erastin (20μM) in the absence or presence of metformin (2.5mM).

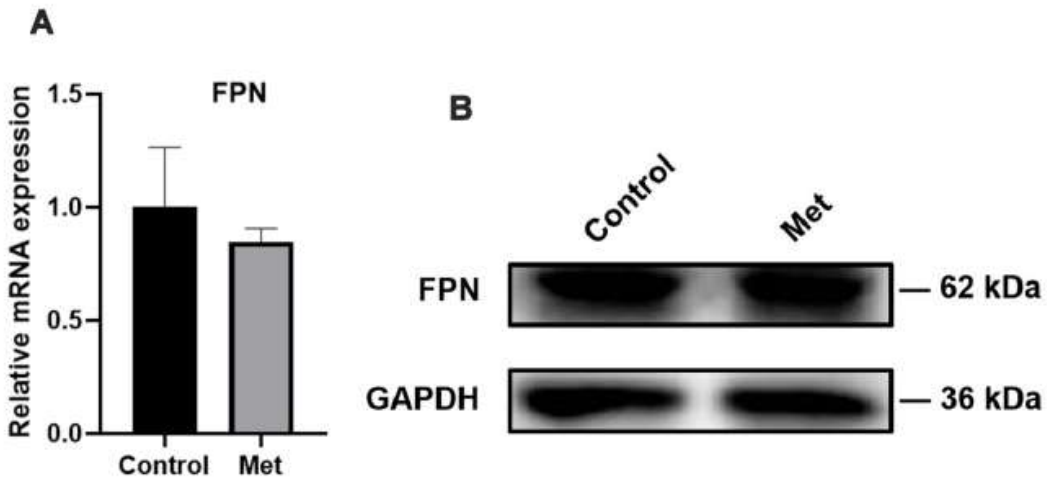

Figure S2. The effect of metformin on FPN expression in WRL68 cells. Related to Figure 5

(A) Effects of metformin on FPN mRNA expression in WRL68 cells. The data were expressed as mean ± SEM (n=3 in each group). The comparisons between two groups were assessed by Student's t tests.

(B) Effects of metformin on FPN protein expression in WRL68 cells. The expression of FPN protein was assessed by western blotting, GAPDH was used as the loading control.

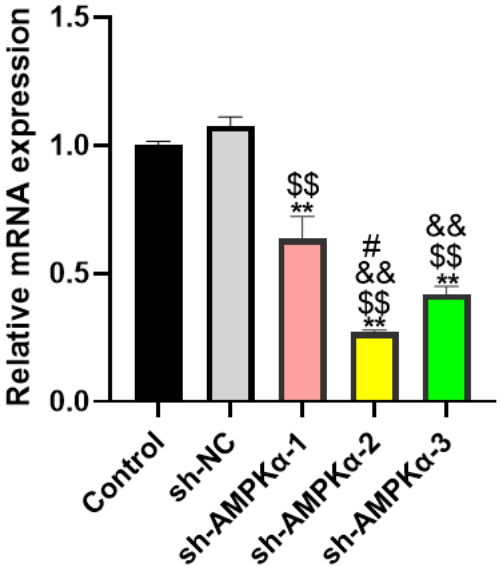

Figure S3. Silencing efficiency of sh-AMPKα constructs in WRL68 cells. Related to Figure 5

Short hairpin RNAs (shRNA) for transient silencing of AMPKα gene (sh-AMPKα) or non-targeting control shRNA (sh-NC) were constructed. The silencing efficiency was determined by RT-PCR. All the data are presented as mean ± SEM (n=3 in each group). Differences among groups were determined by one-way ANOVA analysis. *p<0.05, **p<0.01, *vs.* control group; $^{\$}p$<0.05, $^{\$\$}p$<0.01, *vs.* sh-NC group; $^{\&}p$<0.05, $^{\&\&}p$<0.01, *vs.* sh-AMPKα-1 group; $^{\#}p$<0.05, $^{\#\#}p$< 0.01, *vs.* sh-AMPKα-3 group. The one with the highest silencing efficiency (sh-AMPKα-2) was selected for the experiment.