

PEGylated nicotinamide mononucleotide targeted ferroptosis in acute kidney injury by suppressing FAR1 palmitoylation in an ACAA1-dependent manner

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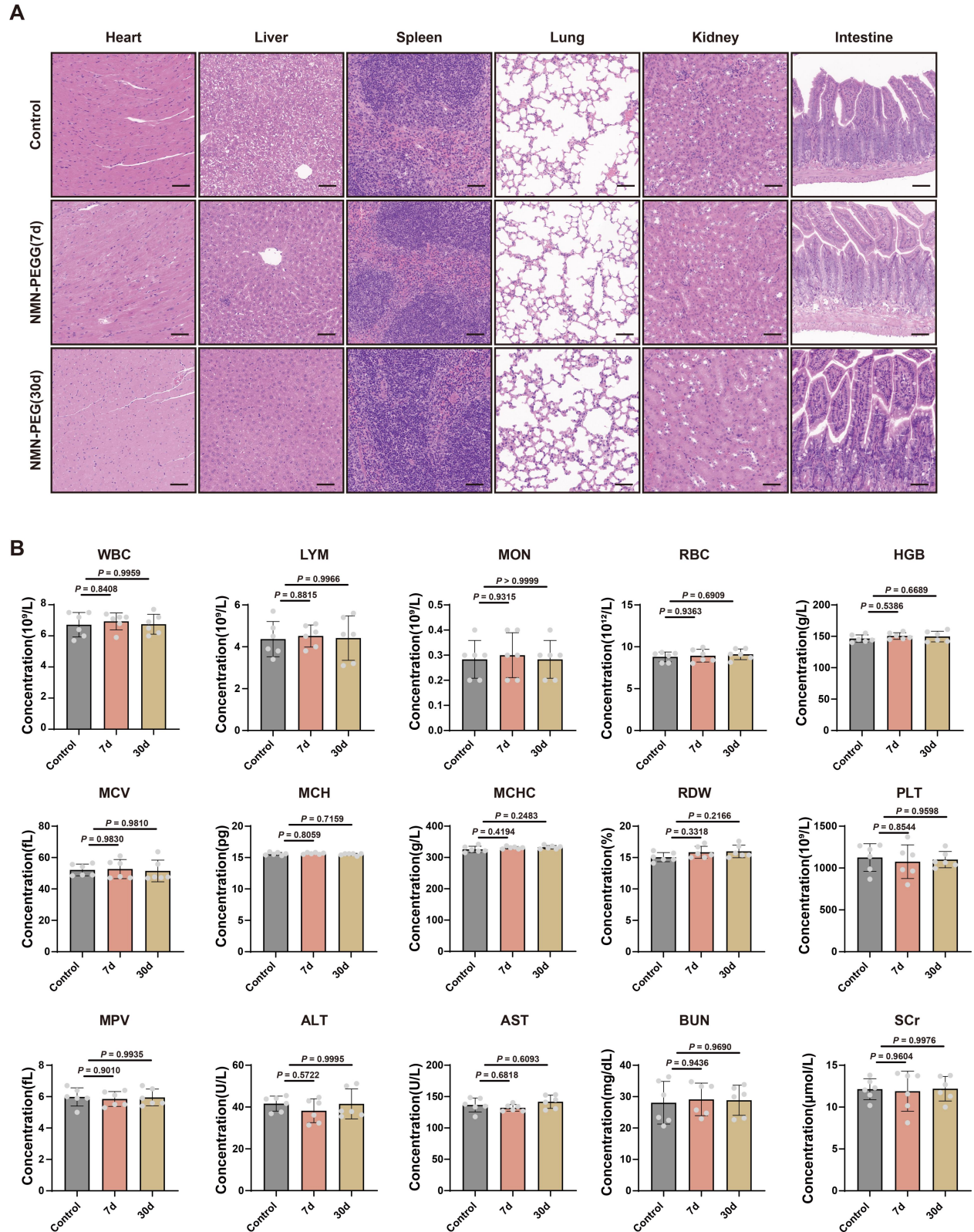


Fig S1. *In vivo* toxicity assessment of PEGylated-NMN. (A) H&E staining of major organs in mice on days 7 and 30 after PEGylated-NMN (20mg/kg) treatment for two consecutive days. Scale bar

= 50 μ m. (B) Routine blood examination, liver and renal function were measured from mice in (A) (n = 6). ns. no significant. WBC, white blood cell; LYM, lymphocyte; MON, monocyte count; RBC, red blood cell; HGB, hemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, MCH concentration; RDW, red cell distribution width; PLT, platelet; MPV, mean platelet volume; ALT, alanine transaminase; AST, aspartate transaminase; BUN, blood urea nitrogen; SCR, serum creatinine.

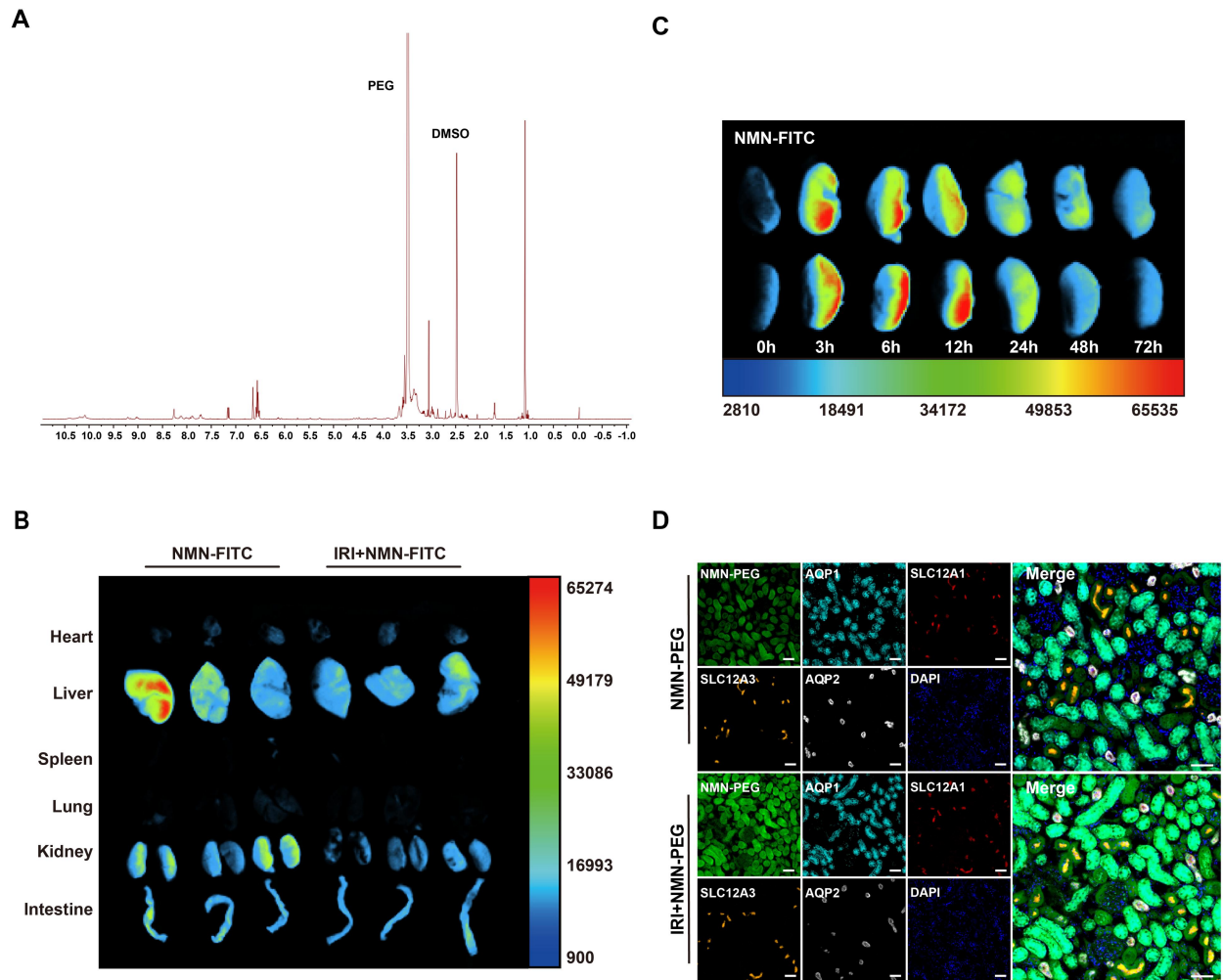


Fig S2. Preferential uptake of PEGylated NMN in the cortex of the kidney in AKI mouse models.

(A) NMR spectrum of NMN-PEG. (B) Imaging of major organs in mice after injection of FITC-labeled NMN. (C) Representative fluorescence images of the kidneys of mice at different time

points (3, 6, 12, 24, 48, and 72 h) after the injection of FITC-labeled NMN. (D) Representative fluorescent images of PEGylated NMN, AQP1, AQP2, SLC12A1 and SLC12A3 in the kidneys of NMN-PEG-treated control and AKI mice are also displayed. Scale bar: 20 μ m.

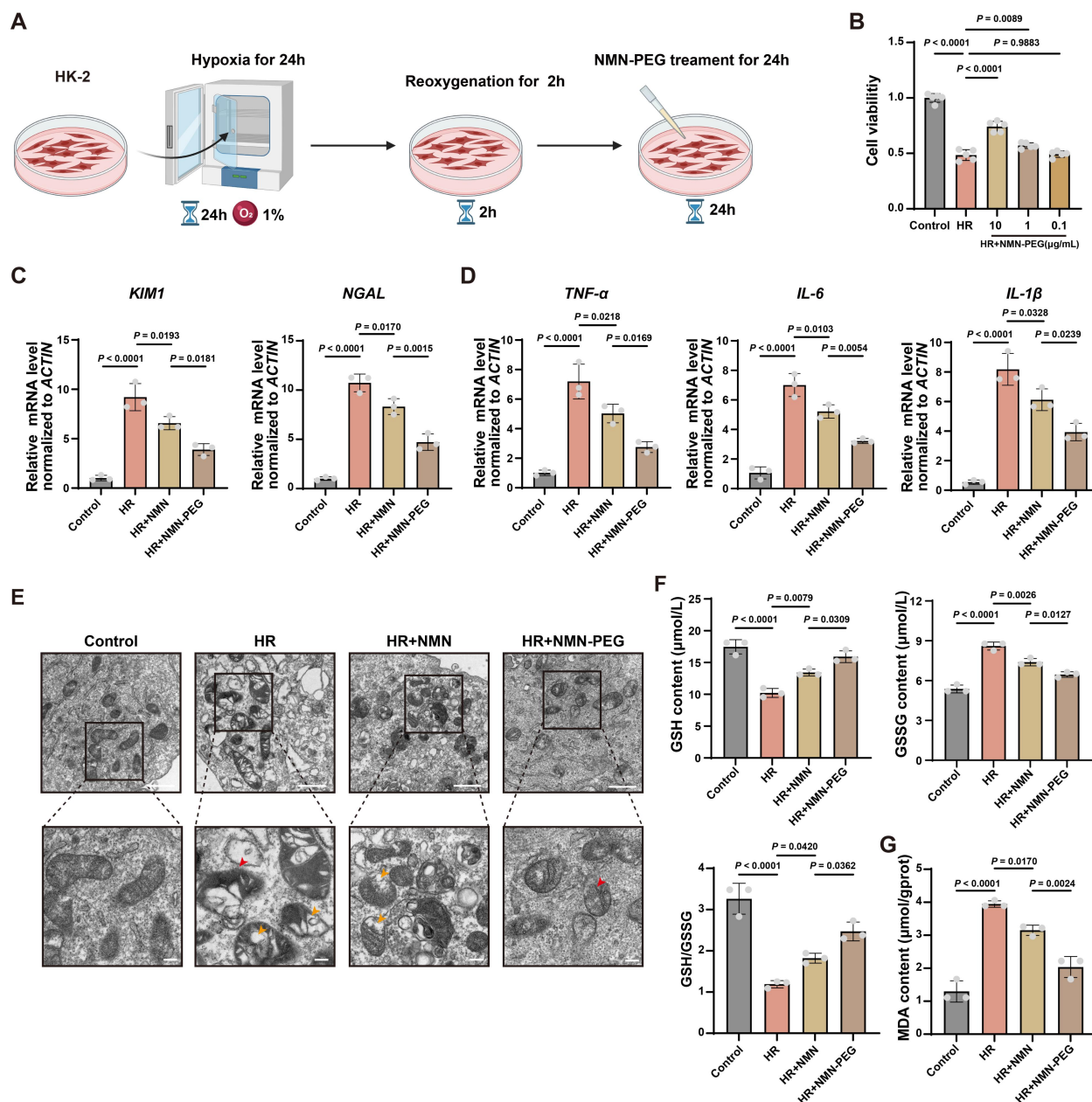
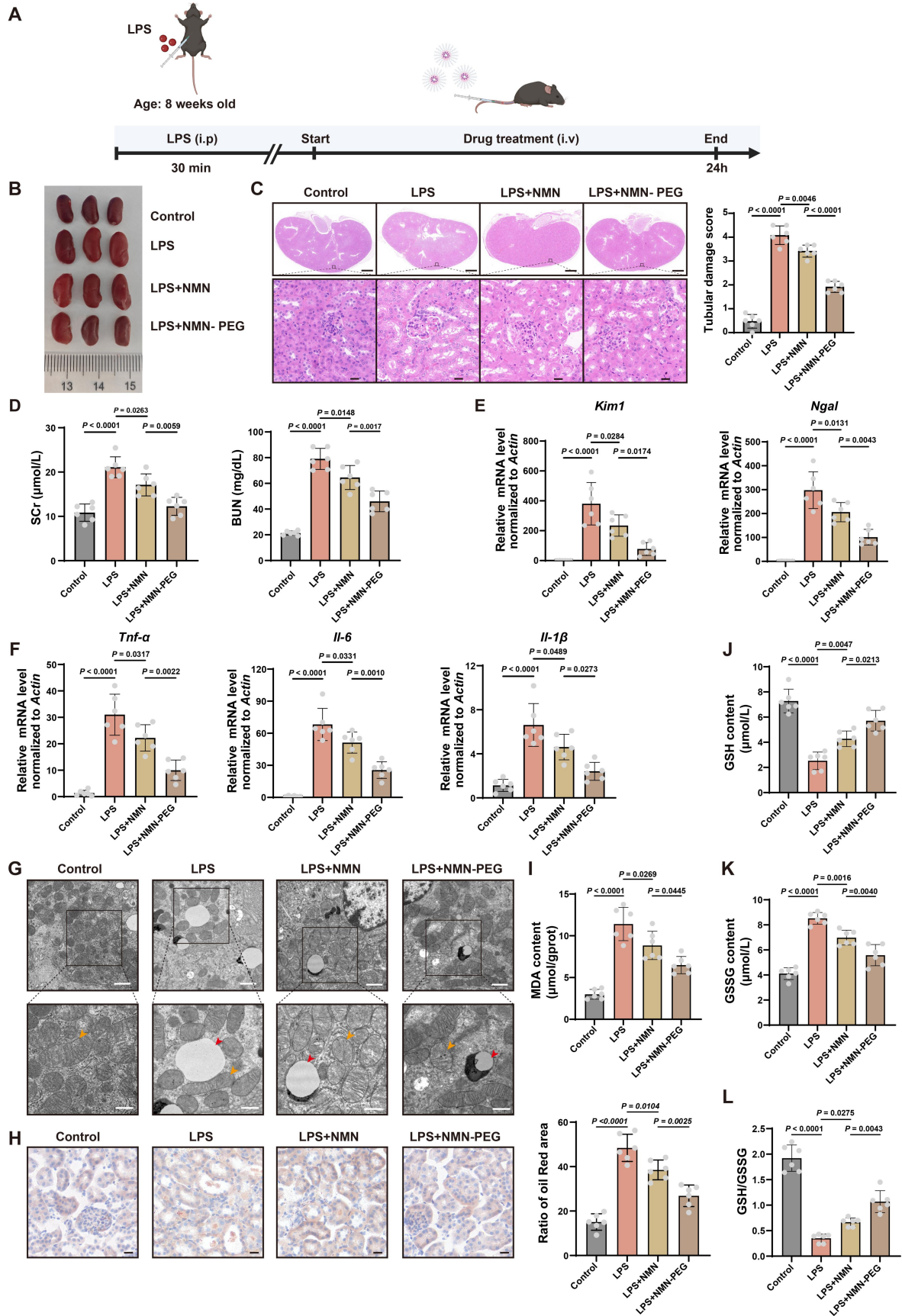


Fig S3. PEGylated-NMN alleviated HR induced ferroptosis *in vitro*. (A) Diagram of HR and PEGylated-NMN treatment in HK-2. (B) Effects of different concentrations of PEGylated-NMN

48 on the viability of HK-2 after HR (n = 6). (C, D) Relative mRNA expression of *KIMI*, *NGAL*,
49 *TNF- α* , *IL-6* and *IL-1 β* in HK-2 (n = 3). (E) Representative TEM images of HK-2 cells (left panel
50 scale bar = 1.0 μ m; right panel scale bar = 0.5 μ m). Mitochondrial outer membrane rupture or
51 mitochondrial crest injury are indicated by yellow arrows. Mitochondrial shrinkage is indicated by
52 red arrows. (F) GSH, GSSG and GSH/GSSG levels in HK-2 (n = 3). (G) MDA levels in HK-2 (n
53 = 3). The data are presented as mean $\pm$ SDs. HR, hypoxia-reoxygenation.

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56 **Fig S4.** PEGylated-NMN alleviated LPS-induced pathological changes and ferroptosis *in vivo*. (A)
57 Diagram of LPS and PEGylated-NMN treatment *in vivo*. (B) The macroscopic and H&E images
58 of the kidney. (C) Representative images of H&E staining (up panel scale bar = 1000 μ m; down
59 panel scale bar = 20 μ m). Tubular damage was scored according to H&E staining (n = 6). (D) The
60 serum levels of SCr and BUN from mice (n = 6). (E, F) Relative mRNA expression of *Kim1*, *Ngal*,
61 *Tnf- α* , *Il-6*, *Il-1 β* in kidney tissues (n = 6). (G) Representative TEM images of kidney mitochondria
62 from different groups of mice (upper scale bar: 1.0 μ m; lower scale bar: 0.5 μ m). Mitochondria
63 are indicated by yellow arrows. Lipid droplets are indicated by red arrows. (H) Representative Oil
64 Red O-staining images of kidney in different groups of mice (scale bar: 20 μ m). (I) The content of
65 MDA in the kidney tissues of the mice was analyzed (n = 6). (J–L) Renal expression of GSH and
66 GSSG and the ratio of GSH/GSSG in mice from different groups (n = 6). The data are presented
67 as mean $\pm$ SDs.

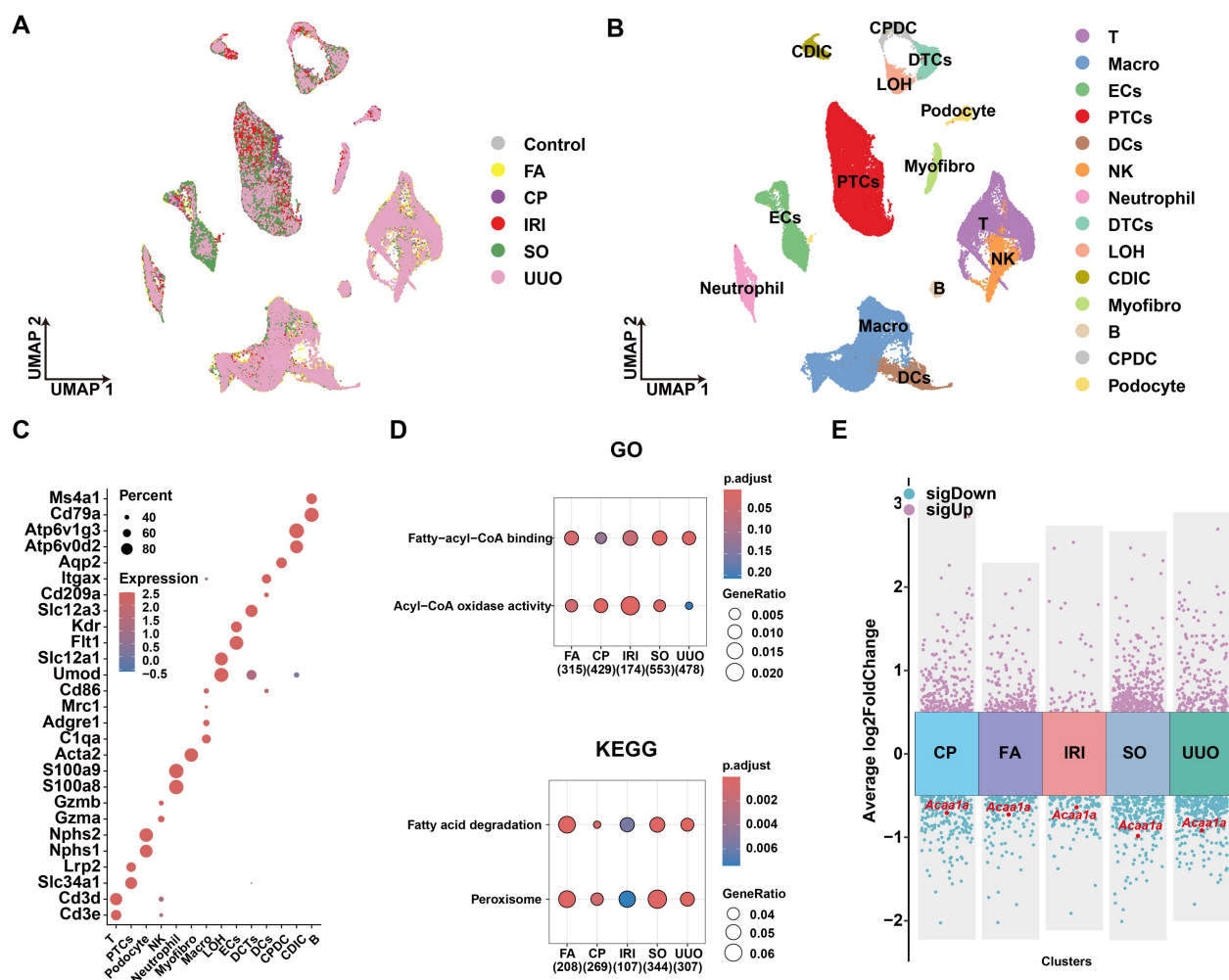


Fig S5. scRNA-seq identifies ACAA1 downregulation as a conserved metabolic node in AKI pathogenesis. (A) UMAP visualization of scRNA-seq data of the kidney derived from multiple AKI mice models. The previously published scRNA-seq data of the kidneys from five common AKI etiologies (CP: cisplatin, IRI: ischemia-reperfusion injury, UUO: unilateral ureteral obstruction, FA: folic acid, SO: sodium oxalate) were analyzed and visualized by UMAP. (B, C) Cell clustering of the scRNA-seq data by signature genes. The UMAP (B) and dot plot (C) analysis revealed 14 clusters of different cell types according to the signature genes by the scRNA-seq data. (D) The GO and KEGG analysis of the enriched pathways in different AKI mice models. Fatty acid metabolism and peroxisome function were enriched in all the AKI models. (E) Box plots of

78 renal DEGs in the five AKI models. ACAA1 was revealed to be significantly downregulated in all
79 the AKI models.

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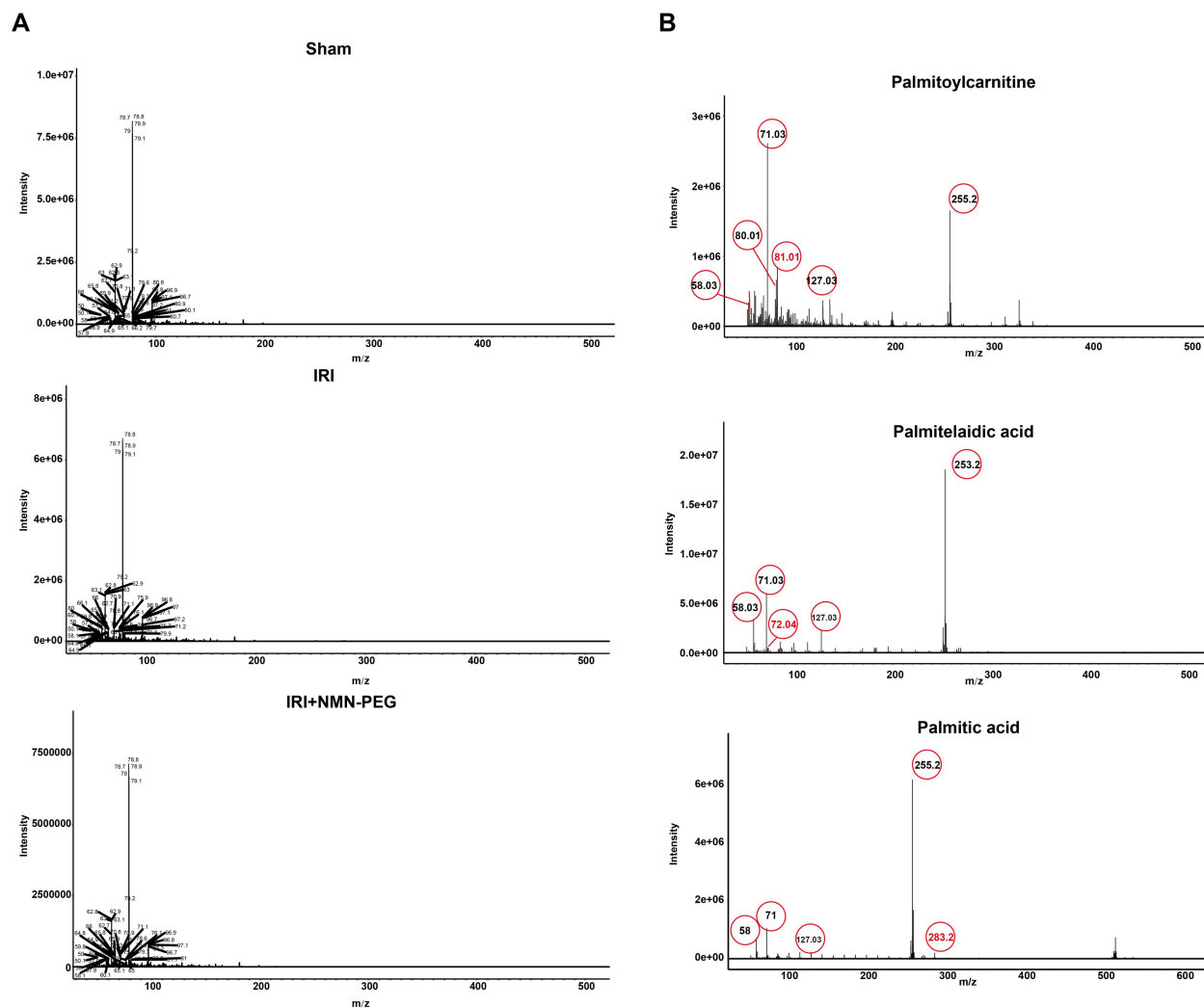


Fig S6. Intensity diagram of different ion species. (A) Intensity plot shows that ion species with zonation pattern stand out among all peaks. (B) Mass spectrum peaks of palmitoylcarnitine, palmitelaidic acid and myristic acid standard.

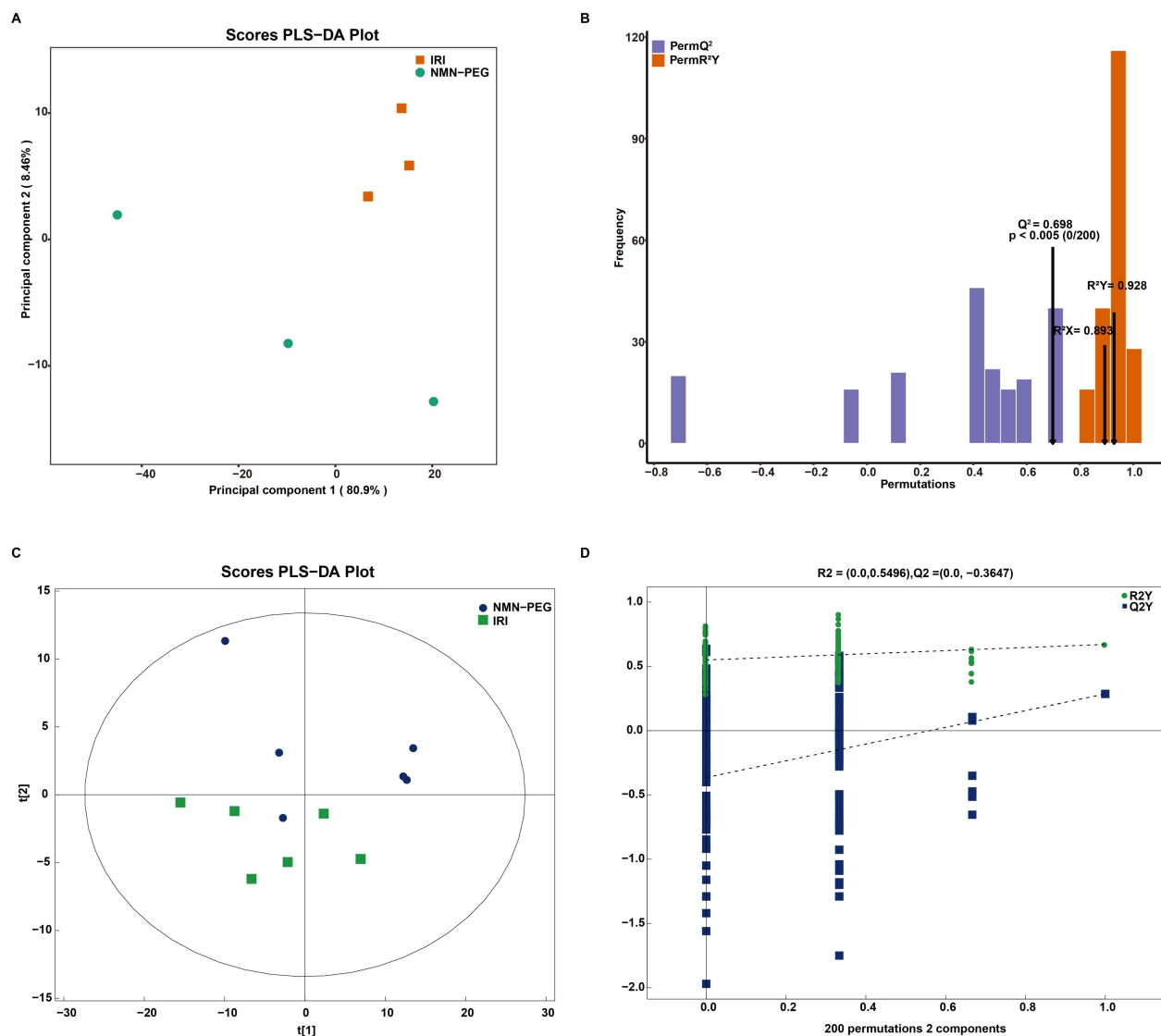


Fig S7. Principal component analysis of metabolomics. (A) The PLS-DA analysis of MALDI-MSI-based spatial metabolomics. (B) OPLS-DA permutation diagram of MALDI-MSI-based spatial metabolomics. (C) The PLS-DA analysis of targeted metabolomics of renal cortex. (D) Permutation diagram of targeted metabolomics of renal cortex.

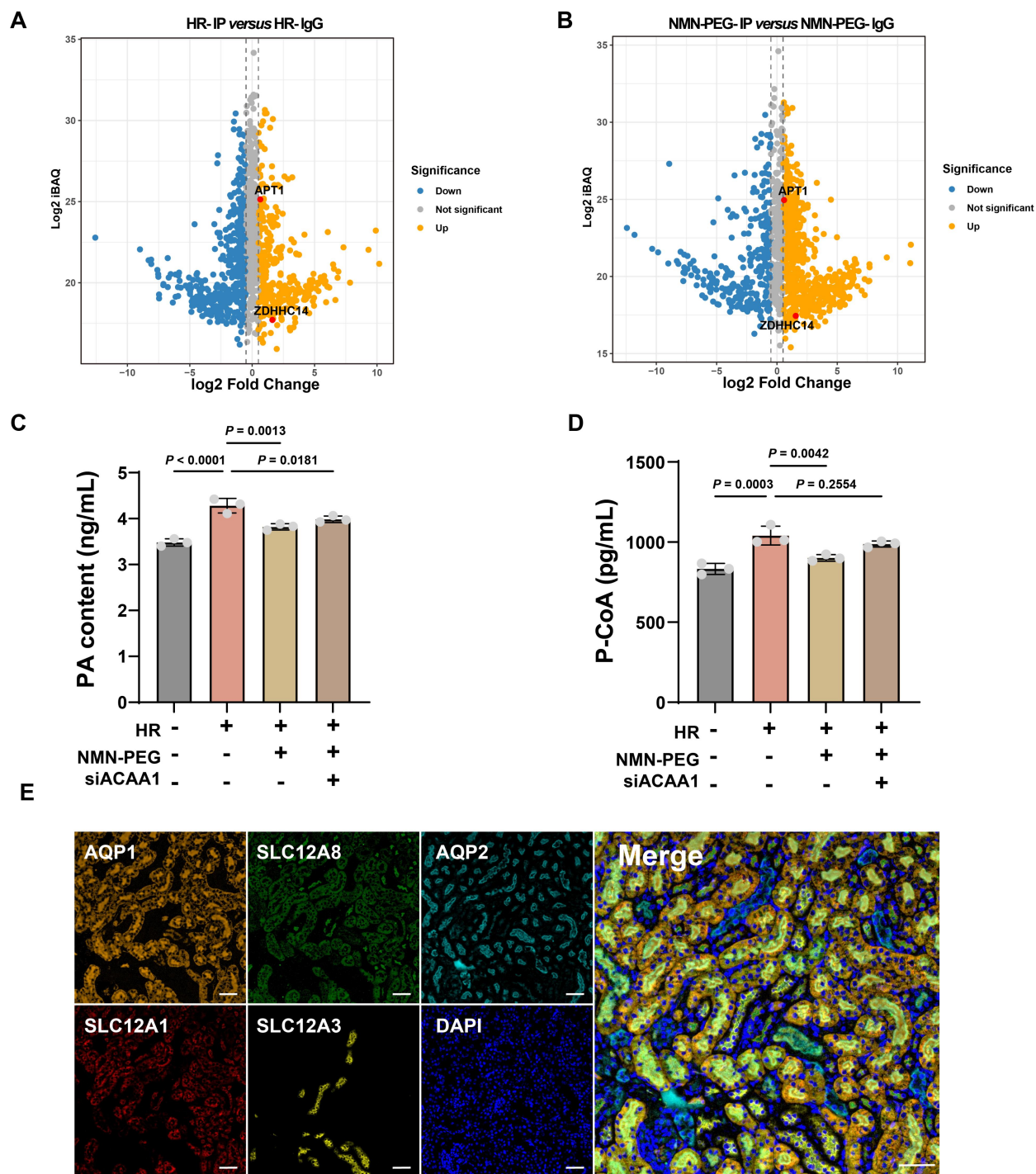


Fig S8. ACAA1 suppresses FAR1 palmitoylation by depleting its metabolic substrate, Palmitoyl-CoA. (A&B) Volcano plots from IP-MS analysis assessing the abundance of candidate palmitoylation enzymes. No significant changes in APT1 and ZDHHC14 levels were observed in HR group, or in NMN-PEG group. (C) Quantification of intracellular palmitic acid levels (n = 3).

98 (D) Quantification of intracellular Palmitoyl-CoA (P-CoA) levels (n = 3). (E) Representative
99 multi-fluorescent images of SLC12A8, AQP1, AQP2, SLC12A1 and SLC12A3 in the kidneys of
100 IRI-treated AKI mice. Scale bar: 20 μ m. The data are presented as mean $\pm$ SDs.

Table S1. The list of mouse primer sequences

Mouse Gene	Sequence
<i>F-Tnf-α</i>	GATCGGTCCCCAAAGGGATG
<i>R-Tnf-α</i>	CACTTGGTGGTTTGTGAGTGT
<i>F-Il-6</i>	TTCCTCTGGTCTTCTGGAGT
<i>R-Il-6</i>	TCTGTGACTCCAGCTTATCTCTTG
<i>F-Il-1β</i>	ATGCCACCTTTTGACAGTGATG
<i>R-Il-1β</i>	TGATGTGCTGCTGCGAGATT
<i>F-Ngal</i>	GGAACGTTTCACCCGCTTTG
<i>R-Ngal</i>	CCACACTCACCACCCATTCA
<i>F-Kim1</i>	GTCAGCATCTCTAAGCGTGG
<i>R-Kim1</i>	TCTTCAGCTCGGGAATGCAC
<i>F-β-actin</i>	CACCCTGTGCTGCTCACC
<i>R-β-actin</i>	GAAGGTCTCAAACATGATCTGGGT

104 **Table S2. The list of human primer sequences**

Human Gene	Sequence
<i>F-TNF-α</i>	GCCCATGTTGTAGCAAACCT
<i>R-TNF-α</i>	GGACCTGGGAGTAGATGAGGT
<i>F-IL-6</i>	AACTCCTTCTCCACAAGCGCC
<i>R-IL-6</i>	GAAGAGGTGAGTGGCTGTCTG
<i>F-IL-1β</i>	CCCTAAACAGATGAAGTGCTCCT
<i>R-IL-1β</i>	CTTGCTGTAGTGGTGGTCGG
<i>F-NGAL</i>	TCACCCTCTACGGGAGAACC
<i>R-NGAL</i>	GGTCGATTGGGACAGGGAAG
<i>F-KIM1</i>	GCCACTTCACCATCTTCACC
<i>R-KIM1</i>	ACGGTGTCATTCCCATCTGTT
<i>F-ACAA1</i>	GGCATGGCCTGTGGGATAA
<i>R-ACAA1</i>	TCTCAGCTTGGAAACAGCCC
<i>F-β-ACTIN</i>	AGGCACCAGGGCGTGA
<i>R-β-ACTIN</i>	CGATGGGGTACTTCAGGGTG

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