Metabolic Rewiring of Kynurenine Pathway during Hepatic Ischemia–Reperfusion Injury Exacerbates Liver Damage by Impairing NAD Homeostasis

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Supporting Information

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Supplementary Figure Legends

Supplementary Figure 1. Metabolic rewiring of the KP in the murine hepatic IR model. (A) Representative HE of the liver sections from mice subjected to 90-min of ischemia followed by different reperfusion durations. (B) Global sample distribution profiles and relationships analyzed by the hierarchical clustering. (C) Volcano plots indicating the differentially expressed metabolites (red, upregulated metabolites; blue, downregulated metabolites) in ischemia group relative to sham controls. The metabolites in KP were highlighted. The vertical dashed gray lines in the plot represent log2 normalized fold change equal to 1 and -1. The horizontal dashed gray line represents false discovery rate (FDR) equal to 0.05. (A-B) n = 4 mice in each group.

Supplementary Figure 2. Afmid and Kyat2 are dramatically upregulated in the post-ischemic liver. (A) Representative IHC staining of Kmo, Kyat1 and Kyat3 in the liver sections from mice subjected to 90-min of ischemia and subsequent reperfusion for the indicated durations. (B) Representative IHC of KYAT2 in the liver sections of individuals subjected to hepatic IR surgery.

Supplementary Figure 3. Upregulation of Kyat2 is related to eIF2α phosphorylation in the ischemic livers. (A) Relative mRNA expression of Afmid and Kyat2 in sham or ischemic livers. n = 4 mice per group. (B-C) The protein levels (B) and quantitative analysis (C) of Kyat2 and p-eIF2α in livers from mice subjected to sham, ischemia with 5 mg/kg ISRIB by i.p. or vehicle for 90-min. GAPDH served as a loading control. (D) The correlation of quantitative protein levels between Kyat2 and p-eIF2α. The correlations were evaluated with Spearman’s test. (E) Representative IHC staining of Kyat2 and p-eIF2α in livers from mice subjected to sham, ischemia with 5 mg/kg ISRIB by i.p. or vehicle for 90-min using serial section. (B-E) n = 3 mice per group. (A,C) Paired student’s t test was used. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. Data are presented as the mean ± SEM.

Supplementary Figure 4. Elevated oxidative stress in the livers subjected to hepatic IR surgery. (A) UPLC-MS/MS detection of a panel of oxidized fatty acids in ischemic livers and sham-operated controls. n = 4 mice per group. (B-D) Representative IHC staining of E06 (B), 4-HNE (C), and MDA (D) in sham and IR-treated livers. Paired student’s t test was used. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. Data are presented as the mean ± SEM.

Supplementary Figure 5. Kyat2 knockdown restores liver NAD and NADH levels and reduces lipid
peroxidation and inflammatory in the ischemic livers. (A) The short interfering RNAs (siRNAs) corresponding to three target sites of mouse Kyat2 coding region (indicated as siKyat2 #1, #2, #3, respectively) were designed and tested their targeting efficiency. The most two potent polymer #1 and #3 were mixed and used for in vivo experiments. (B) Cationic liposome-encapsulated siKyat2 and siCtrl were injected intravenously into the mice and hepatic IR was performed 48 hours later. The protein levels of Kyat2 in livers from siKyat2- or siCtrl-treated mice with 90-min ischemia were determined by immunoblotting. (C) Representative IHC staining of E06, MDA, 4-HNE in mice liver sections from (B). (D) Representative images of gross morphology of livers under IR treatment with siCtrl or siKyat2 injection for 24 h. (E) Serum ALT and AST levels in mice from (D). (F) Representative IHC staining of Ly6B and CD45 in mice liver tissue samples from (D). (G) Relative mRNA expression of Il1a, Ilb, Ccl2, Ccl3, Tnf, Ccl4, Ccr1, Tgfb in mice liver tissue samples from (D). (B-C) n = 4 mice in siCtrl group, n = 6 mice in siKyat2 group. (D-G) n = 6 mice per group. Paired student’s t test was used. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. Data are presented as the mean ± SEM.

Supplementary Figure 6. Nicotinate phosphoribosyltransferase (Naprt) inhibitor 2-hydroxynicotinic acid (2-HNA) has a neglectable effect on hepatic IR-induced liver injury. (A) Representative images of gross morphology of livers from mice with 15 mg/kg 2-HNA or vehicle injection by i.p. for 24 h. (B-C) Survival probability and serum ALT/AST levels of mice from (A). (A-C) n = 3 mice per group. Paired student’s t test was used. Data are presented as the mean ± SEM.

Supplementary Figure 7. FK866 enhances immune infiltration and inflammatory response in the livers subjected to hepatic IR insult. (A-B) IHC staining of Ly6B in sham (A) and hepatic IR-injured livers (B) treated with 15 mg/kg FK866 or vehicle. (C) Relative mRNA expression of Tnf, Ccl2 and Ccl4 in sham or IR-injured livers treated with FK866 or vehicle. *P < 0.05 by Student’s t test. (A-C) n = 4 mice per group. Data are presented as the mean ± SEM.

Supplementary Figure 8. Lip-1 mitigates FK866-induced immune infiltration and inflammatory response in the lives subjected to hepatic IR insult. (A-B) IHC staining of Ly6B (A) and mRNA levels of Il1a, Ccl3, Ccl4, Ccr1, Ccl2 and Cxcl13 (B) in livers from IR-injured mice treated as indicated. n = 4 mice per group. Unpaired student’s t test was used. *P < 0.05, **P < 0.01, ***P < 0.001. Data are presented as the mean ± SEM.

Supplementary Figure 9. NMN effectively alleviates immune infiltration and inflammatory response by IR injury. (A-B) IHC staining of CD45 (A) and relative mRNA expression of Il33 and Cxcl13 (B) in livers from IR-injured mice treated with NMN or vehicle. n = 4 mice per group. Unpaired student’s t test was used. **P < 0.01, ***P < 0.001. Data are presented as the mean ± SEM.
Supplementary Figure 1.
Supplementary Figure 4.
Supplementary Figure 5.

A

B

C

D

E

F

G

Supplementary Figure 5.
Supplementary Figure 6.
Supplementary Figure 8.
Supplementary Figure 9.
Supplementary Figure S.
Supplementary Materials and Methods

Key Resources Table

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| **Antibodies**      |        |            |
| Aadat (Kyat2)       | Proteintech | 13031-1-AP |
| Kmo                 | Proteintech | 10698-1-AP |
| Kyat3               | Santa   | sc-166922  |
| TDO2                | Proteintech | 15880-1-AP |
| AFMID               | Proteintech | 19522-1-AP |
| MDA                 | ENZO    | 01072110   |
| Ly6B                | abcam   | ab53457    |
| E06                 | Avanti  | 330001S    |
| Kynu                | Proteintech | 11796-1-AP |
| CD45                | Servicebio | GB11066  |
| Cox2                | Abclonal | A1253      |
| GAPDH               | Abways  | AB0037     |
| 4-Hydroxynonenal    | Abcam   | ab46545    |
| Kyat1               | Abcam   | Ab194296   |
| p-eIF2α             | CST     | #3398      |

**Experimental Models:**

- **Organisms/Strains**
  - Mouse:C57BL/6J
    - Charles River Laboratories, Beijing, China

**Chemicals, and Recombinant Proteins**

| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| Liproxstatin-1      | Selleck | S7699      |
| FK866               | Topscience | T2644    |
| NMN                 | Topscience | T4721    |
| 2-HNA               | Energy-Chemical | A010247 |
| ISRIB               | MedChemExpress | HY-12495 |
| In vivo-jetPEI™     | PolyPlus | 201-10G   |
| Oligonucleotides | Sequence                      | Supplier      |
|-----------------|-------------------------------|---------------|
| m_Il1α_F        | CGAAGACTA CAGTTCTGC CATT     | Sangon Biotech|
| m_Il1α_R        | GACGTTTCA GAGGTTCTC AGAG     | Sangon Biotech|
| m_Il1b_F        | GCAACTGTT CCTGAACTC AACT     | Sangon Biotech|
| m_Il1b_R        | ATCTTTTGG GGTCCGTC AACT      | Sangon Biotech|
| m_Tnf_F         | CCCTCACAC TCAGATCAT CTTCT    | Sangon Biotech|
| m_Tnf_R         | GCTACGACG TGGGCTACA G         | Sangon Biotech|
| m_Ccl2_F        | TTTAAAAACC TGGATCGGA ACCAA   | Sangon Biotech|
| m_Ccl2_R        | GCATTAGCT TCAGATTAC GGGT     | Sangon Biotech|
| m_Ccl3_F        | GCAACCAAG TCTTCCTCG ACG       | Sangon Biotech|
| m_Ccl3_R        | TTGGACCCA GGTCTCTTT GGG       | Sangon Biotech|
| m_Ccl4_F        | TTCCTGCTG TTCTTCCTAC ACCT    | Sangon Biotech|
| m_Ccl4_R        | CTGTCTGCC TCTTTGGTC          | Sangon Biotech|
| Gene | Forward Primer Sequence | Reverse Primer Sequence | Supplier |
|------|-------------------------|-------------------------|----------|
| m_Tgfb_F | AG | CTCCCCGTGG CTTCTAGTG C | Sangon Biotech |
| m_Tgfb_R | AG | GCCTTAGTT TGGACAGGA TCTG | Sangon Biotech |
| m_Ccr1_F | AG | CTGAGGGCC CGAACTGTT AC | Sangon Biotech |
| m_Ccr1_R | AG | GGCTAGGGCC CCAGGTGAT | Sangon Biotech |
| m_IL33_F | AG | TCCAACTCC AAGATTTCC CCG | Sangon Biotech |
| m_IL33_R | AG | CATGCAGTA GACATGGCA GAA | Sangon Biotech |
| m_Cxcl13_F | AG | ATATGTGTG AATCCTCGT GCCA | Sangon Biotech |
| m_Cxcl13_R | AG | GGGAGTTGA AGACAGACT TTTGC | Sangon Biotech |
| siRNA | Sequence | Supplier |
| m_siKyat2-1 | cctaagaccttgatacagaat | GenePharma |
| m_siKyat2-3 | ggtgaccgcaagaaagaaatc | GenePharma |