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Supplemental information

Hypoxic preconditioning protects against ischemic kidney injury through the IDO1/kynurenine pathway

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Suppl. Figure 1

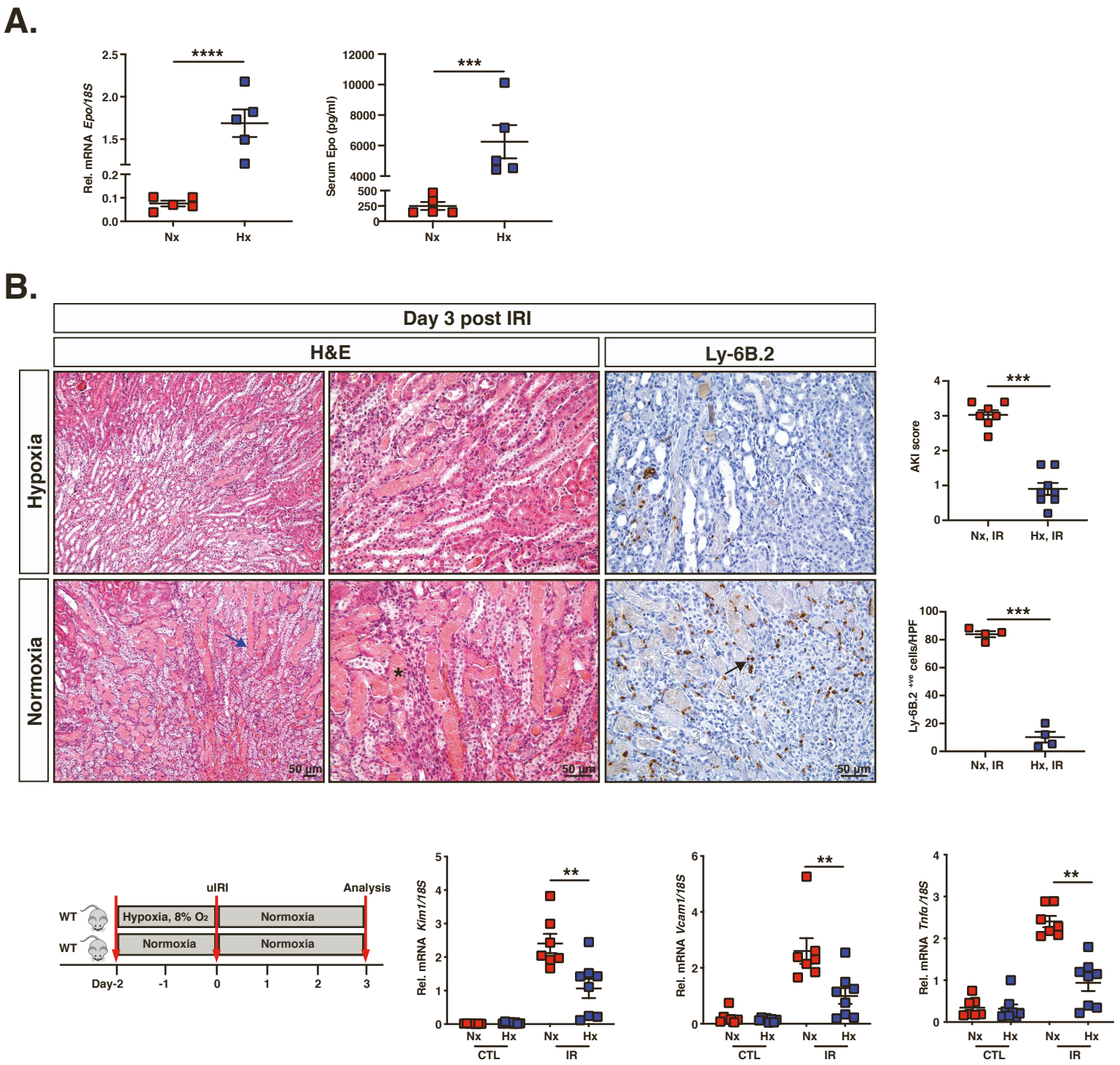
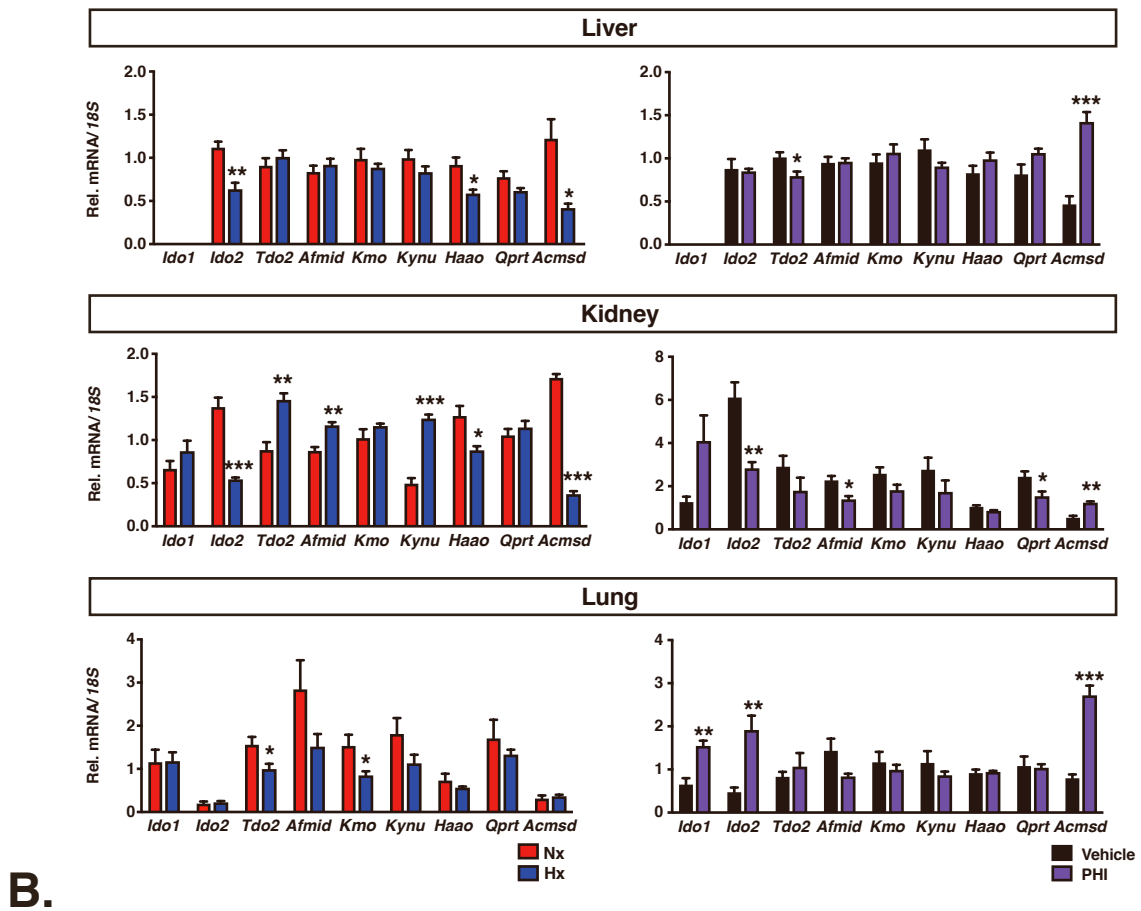
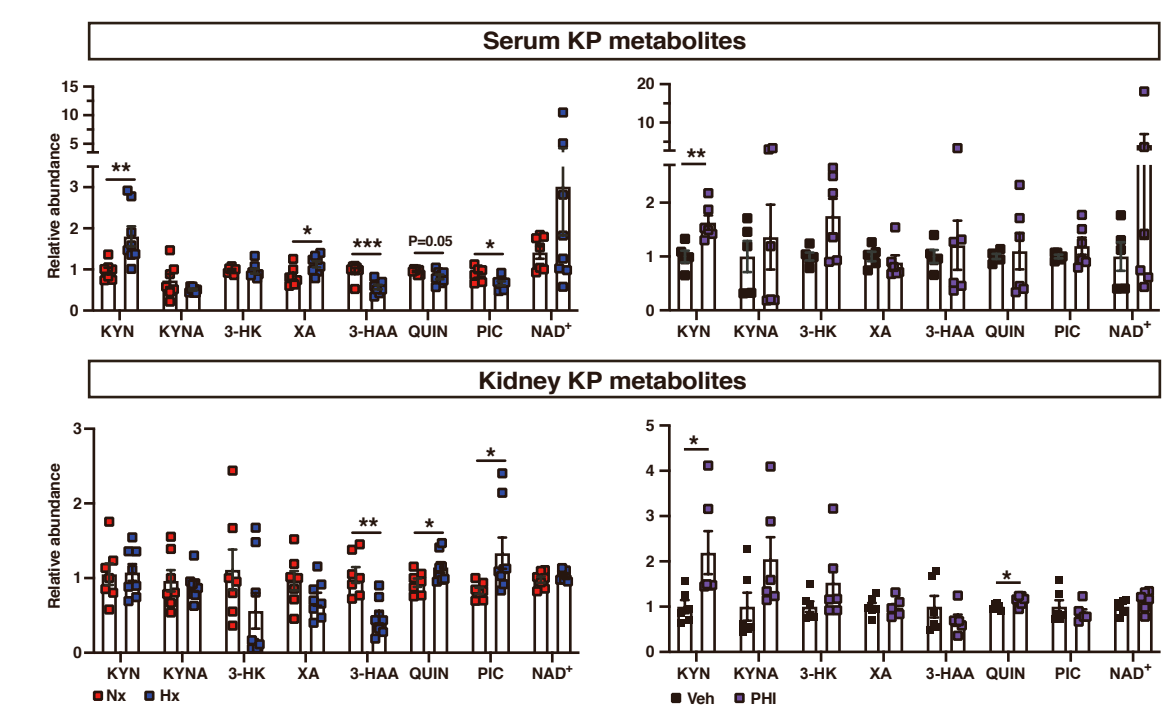


Figure S1, Related to Figure 1. Hypoxic preconditioning attenuates postischemic kidney injury and inflammation. (A) Shown are renal *Epo* mRNA levels (left graph) and serum Epo concentrations (right graph) in mice exposed to hypoxia **(**8% O_2, 2 days). (n=5). **(B)** Overview of the experimental protocol and representative images of H&E and Ly-6B.2-stained sections of injured kidneys from wild-type mice subjected to HP compared to normoxia 3 days after unilateral renal IRI. Arrow points to a dilated tubule; asterisk indicates a tubule with cast formation. Right panels demonstrate scoring of tubular injury (n=8-7) and quantification of Ly-6B.2^{+ve} cells/HPF in post-ischemic kidneys of the indicated experimental conditions (n=4). Bottom graphs show *Kim1, Vcam1, and Tnfa* mRNA levels in day 3 post-IRI and CTL kidneys from wild type mice subjected to HP compared to normoxia. (n=8-7). Error bars represent S.E.M; **, P<0.01; ***, P<0.001; ****, P<0.0001. Statistics were determined by two-tailed t-test for (**A**) and by one-way ANOVA with Sidak correction for (**B**). Scale bar indicates 50 μm. Hx, hypoxia; Nx, normoxia; uIRI, unilateral ischemia reperfusion injury; WT, wild-type; IR, kidney subjected to uIRI; CTL, contralateral kidney.

Suppl. Figure 2





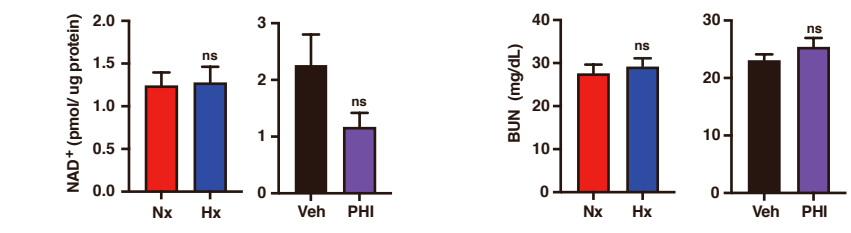


Figure S2, Related to Figure 2. Hypoxic preconditioning and pharmacologic PHD inhibition generate distinct transcriptional responses in kynurenine pathway genes in the liver, kidney and lung without altering kidney NAD^+ content. (A) Transcript levels of genes encoding KP enzymes following hypoxia exposure compared to normoxia and PHD inhibition compared to vehicle. RT-PCR analysis was performed in RNA extracted from livers (upper panels), kidneys (middle panels), and lungs (lower panels) (n=5). **(B)** Levels of serum and kidney KP metabolites in conditions indicated in (A) (n=7-8 for Hx vs Nx, n=6 for PHI vs vehicle). **(C)** Kidney NAD^+ levels (n=7 for Hx vs Nx, n=4 for PHI vs vehicle) and **(D)** serum BUN levels (n=8-7 for Hx vs Nx, n=10-7 for PHI vs vehicle). Error bars represent S.E.M; *, P<0.05; **, P<0.01; ***, P<0.001; ns, not statistically significant. Statistics were determined by two-tailed t-test. PHI, prolyl hydroxylase inhibitor. *Afmid,* arylformamidase; *Kmo,* kynurenine 3-monooxygenase*; Kynu,* kynurerinase; *Haao,* 3-hydroxyanthranilate 3,4-dioxygenase; *Qprt,* quinolinate phosphoribosyltransferase*; Acmsd,* alpha-amino-beta-carboxy-muconate-semialdehyde decarboxylase. KYN, kynurenine; KYNA, kynurenic acid; 3-HK, 3-hydroxykynurenine; XA, xanthurenic acid; 3-HAA, 3-hydroxyanthranilic acid; QUIN; quinolinic acid, PIC; picolinic acid. BUN, blood urea nitrogen.

Suppl. Figure 3

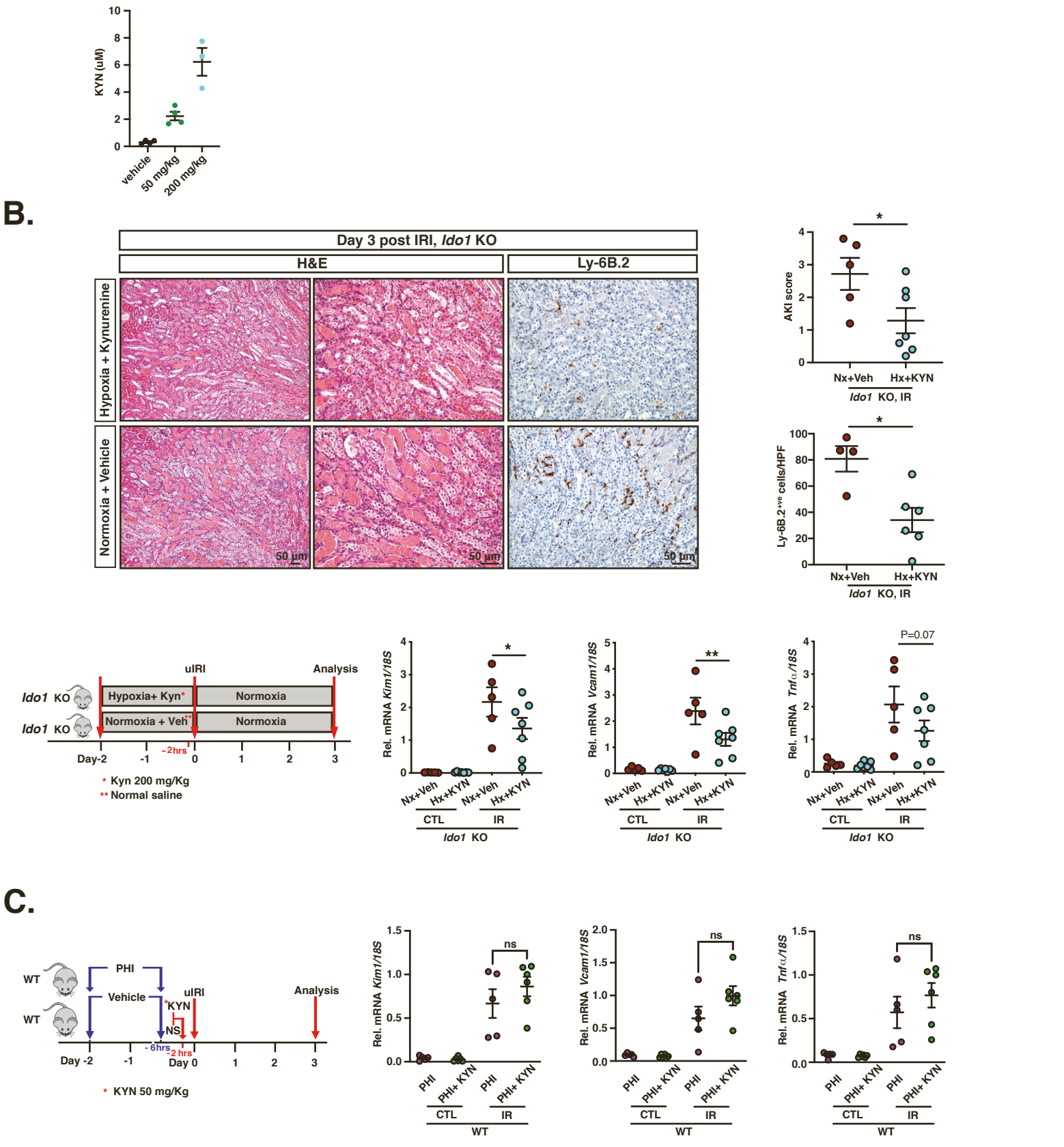
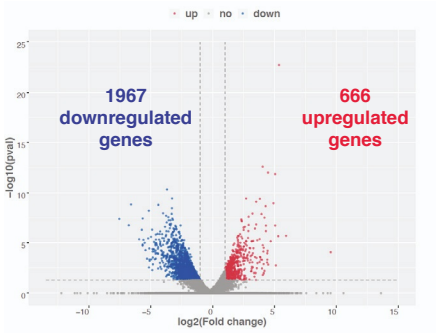
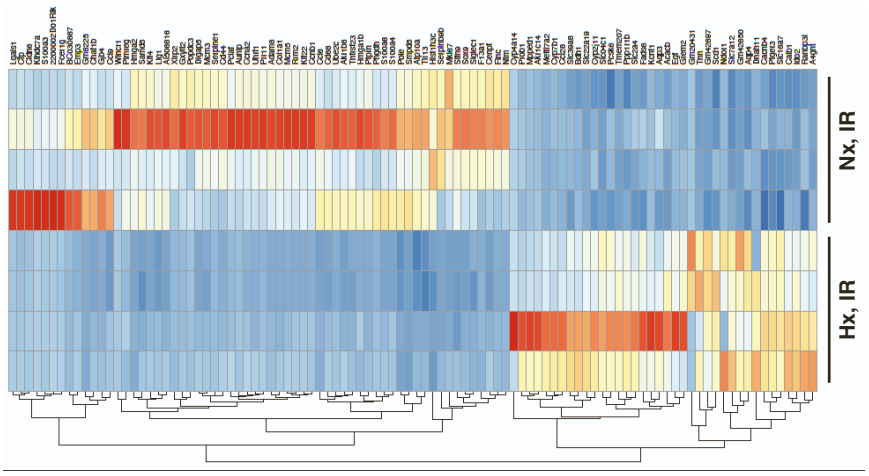


Figure S3, Related to Figure 3. Exogenous kynurenine administration restores the hypoxic preconditioning in mice lacking *Ido1*. **(A)** Serum kynurenine levels achieved at 2 hrs post i.p. administration of kynurenine at doses 50 mg/kg and 200 mg/kg compared to vehicle (normal saline). **(B)** Schematic of the experimental protocol, representative images of H&E and Ly-6B.2-stained sections of day 3 post-IRI kidneys from *Ido1* KO mice subjected to HP and i.p. kynurenine administration (200 mg/kg) compared to normoxic *Ido1* KO mice treated with vehicle. Right panels demonstrate scoring of tubular injury (n=6-8) and quantification of Ly-6B.2^{+ve} cells/HPF (n=4-6) in post-ischemic kidneys of the indicated experimental conditions. Bottom graphs show *Kim1, Vcam1*, *and Tnfa* mRNA levels in IR and CTL kidneys (n=5-7). **(C)** Schematic of the experimental protocol, in which animals were pretreated with a PHD inhibitor (at 2 days and 6 hrs prior to renal IRI) and received either one single injection of kynurenine (50 mg/kg) or normal saline 2 hrs prior to IRI. Right graphs show *Kim1, Vcam1*, *and Tnfa* transcript levels in IR and CTL kidneys (n=5-6) at the indicated experimental conditions. Error bars represent S.E.M. For AKI scores and Ly-6B.2^{+ve} cell counts, statistics were determined by two-tailed t-test. All other comparisons were performed by one-way ANOVA with Sidak correction. *, $P<0.05$; **, $P<0.01$; ns, not statistically significant. KYN, kynurenine. Scale bar indicates 50 μm.

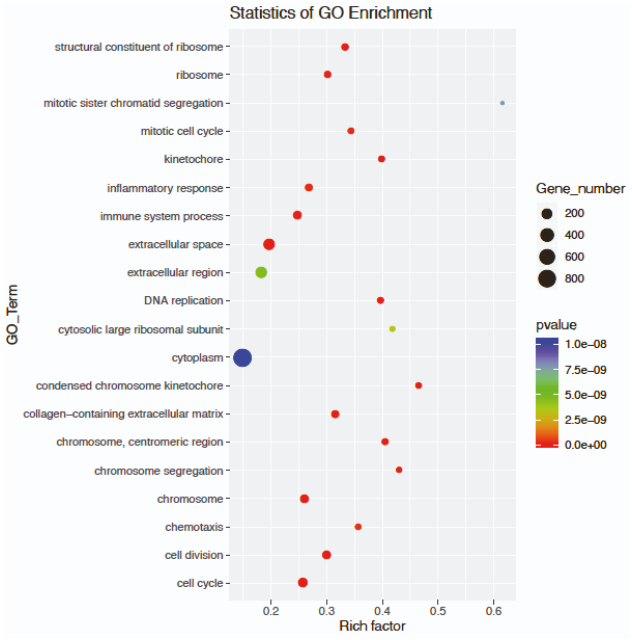
Suppl. Figure 4

A.

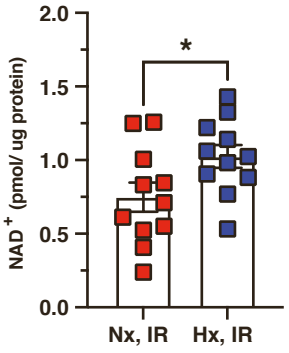




B.



C.



D.

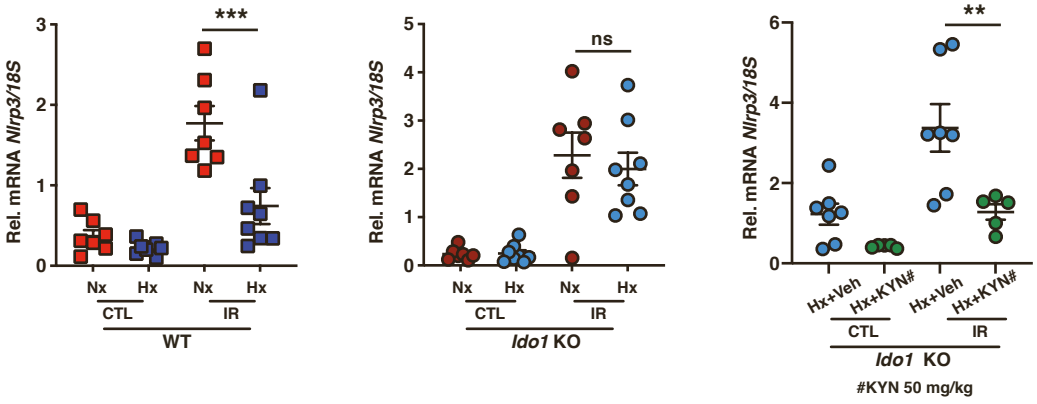


Figure S4, Related to Figure 4. RNA-sequencing performed in day 3 post-ischemic kidneys from wild type subjected to hypoxic preconditioning compared to normoxia. (A) (Left) Volcano plot of the differentially expressed genes (DEGs) in postischemic kidneys between the HP and the normoxia groups. Red indicates up-regulated genes, and blue indicates down-regulated genes. The grey area shows the gene expression below the threshold criteria (log2FC > 1 or <-1 and P value<0.05). (Right) Heat map of log10(FPKM) across all the samples using the top 100 most DEGs in post-ischemic kidneys subjected to HP compared to the normoxia group. Each column corresponds to a specific gene and each row corresponds to a sample. **(B)** The GO enrichment graph displays the top 20 pathways. The number of DEGs enriched in GO terms, p value, and rich factor are shown in scatterplot. Rich factor = (number of DEGs in GO term)/(total number of genes in GO term). **(C)** NAD^+ levels in postischemic kidney tissue from mice subjected to HP compared to normoxia (n=11/group, measured by colorimetric assay). **(D)** Shown are *Nlrp3* mRNA levels in day 3 post-ischemic and contralateral kidneys. Left graph: wild-type mice subjected to HP compared to normoxia (n=7-8); middle graph: *Ido1* KO mice subjected to HP compared to normoxia (n=7-8); right graph: *Ido1* KO mice subjected to HP and kynurenine administration compared to HP/vehicle treated *Ido1* KO mice (n=7-5). Error bars represent S.E.M; **, P<0.01; ***, P<0.001; ns, not statistically significant. Veh, vehicle; WT, wild-type; KYN, kynurenine.