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Supplementary Materials

Asymptomatic Hyperuricemia Promotes Recovery from Ischemic Organ Injury by Modulating the Phenotype of Macrophages

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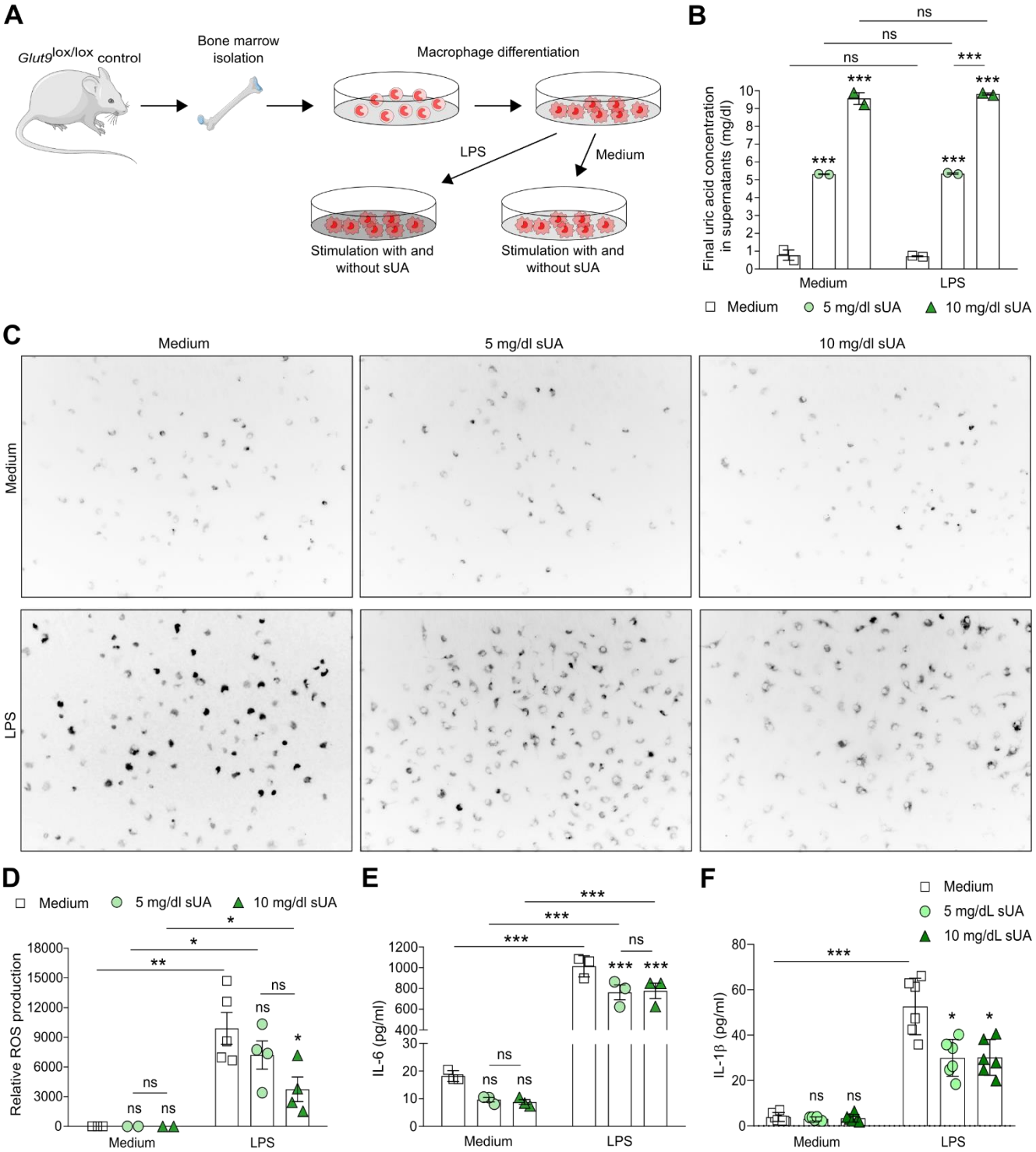
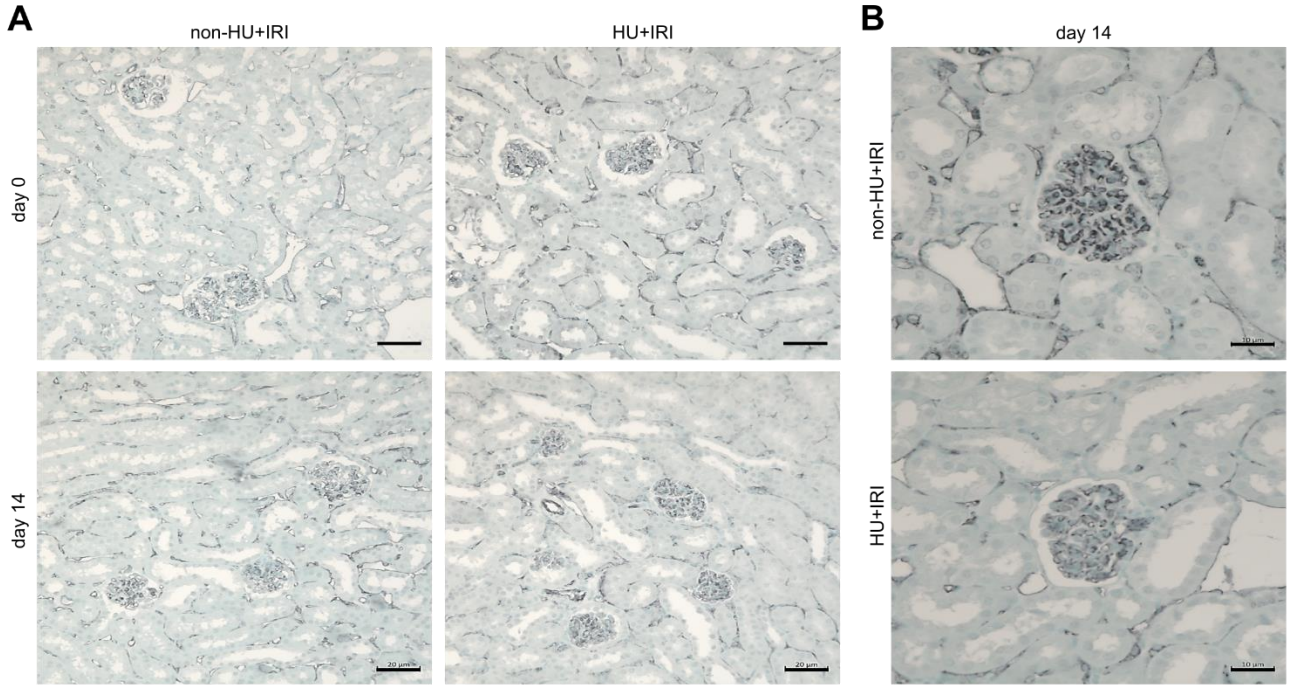
Supplementary Table S1. Mouse primer sequences.

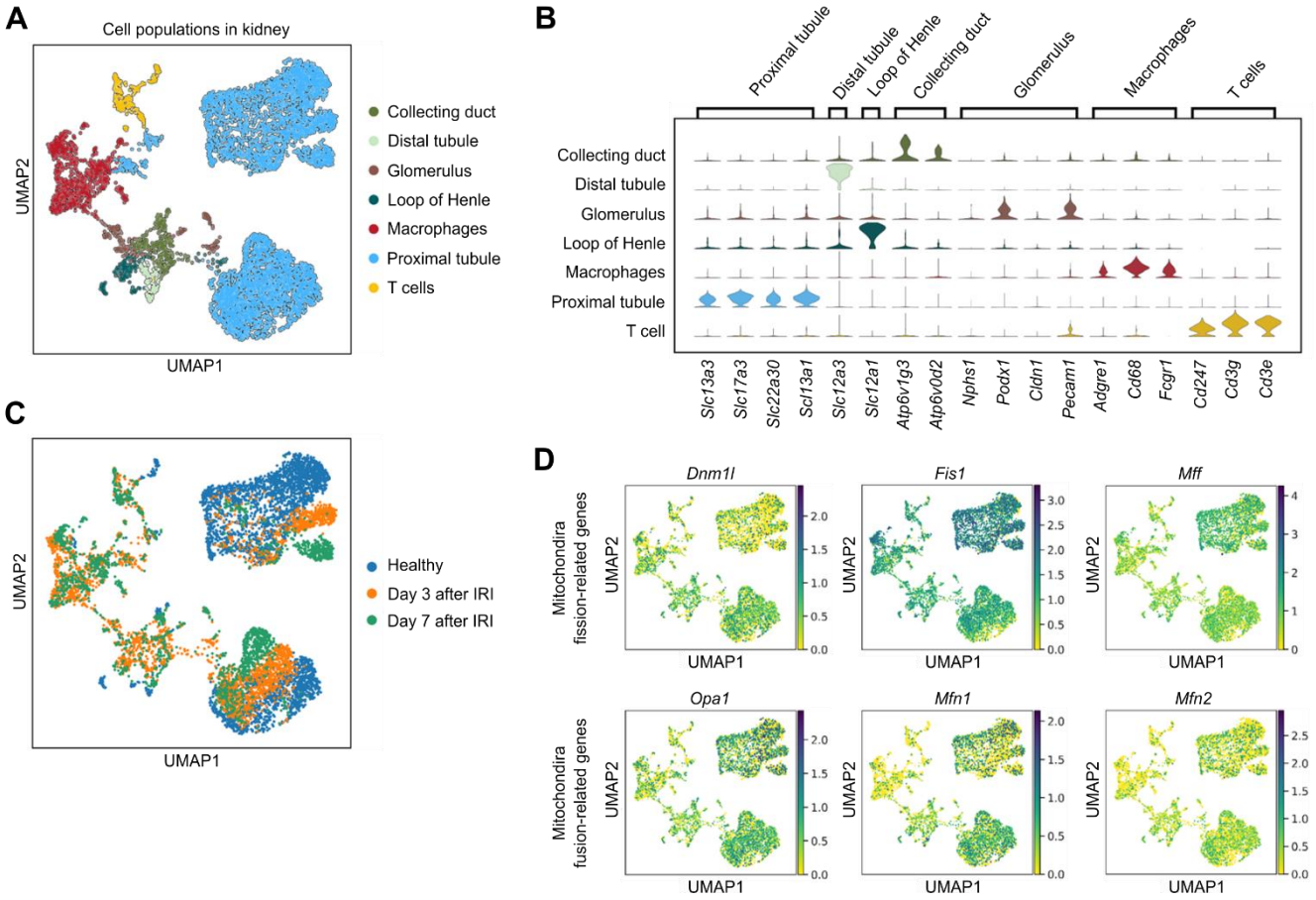
Gene name	Primer Sequences
KIM-1	Forward: TCAGCTCGGGAATGCACAA Reverse: TGGTTGCCTTCCGTGTCTCT
Col1α1	Forward: ACATGTTCAGCTTTGTGGACC Reverse: TAGGCCATTGTGTATGCAGC
Fibronectin 1	Forward: GGAGTGGCACTGTCAACCTC Reverse: ACTGGATGGGGTGGGAAT
iNos	Forward: GAGACAGGGAAGTCTGAAGCAC Reverse: CCAGCAGTAGTTGCTCCTCTTC
Il6	Forward: TGATGCACTTGCAGAAAACA Reverse: ACCAGAGGAAATTTTCAATAGGC
Tnfα	Forward: AGGGTCTGGGCCATAGAACT Reverse: CCACCACGCTCTTCTGTCTAC
Arginase 1/Arg1	Forward: AGAGATTATCGGAGCGCCTT Reverse: TTTTTCCAGCAGACCAGCTT
Fizz-1	Forward: CCCTTCTCATCTGCATCTCC Reverse: CTGGATTGGCAAGAAGTTCC
Ho-1	Forward: GAAATCATCCCTTGCACGCC Reverse : CCTGAGAGGTCACCCAGGTA
Nrf-1	Forward: GCACCTTTGGAGAATGTGGT Reverse: GATAAATGCCCGAAGCTGAG
Sod	Forward: CCAGTGCAGGACCTCATTTT Reverse: GTTTACTGCGCAATCCCAAT
Cpt1	Forward: CTCCGCCTGAGCCATGAAG Reverse: CACCAGTGATGATGCCATTCT
Pparg	Forward: GGAAGACCACTCGCATTCCTT Reverse: GTAATCAGCAACCATTGGGTCA
Pgc1b	Forward: TCCTGTAAAAGCCCGGAGTAT Reverse: GCTCTGGTAGGGGCAGTGA
Glut1	Forward: GCCTGACCTTCGGATATGAGC Reverse: TGCCATAGCAGTCAATGAGGA
Slc2a9/Glut9	Forward: CTGTCCAGATGTTGTCTAGG Reverse: GTTATGATGCAGGAGCTTAGC
Drp1	Forward: GGGCACTTAAATTGGGCTCC Reverse: TGTATTCTGTTGGCGTGGAAC
Fis1	Forward: GGCTGTCTCCAAGTCCAAATC Reverse: GGAGAAAAGGGAAGGCGATG
Dnm1	Forward: GTGGACATGGTTATCTCGGAGC Reverse: GGTGGTCACAATTCGCTCCATC
Opa1	Forward: TCACCTCTGCGTTTATTTGAAGA Reverse: GGGTAGAACGGGAGGAAAGG
Mfn1	Forward: TATCGATGCCTTGCGGAGAT Reverse: GGCGAATCACAACACTTCCA
Mfn2	Forward: GGAGACCAACAAGGACTGGA Reverse: TGCACAGTGACTTTCAACCG
18s	Forward: GCAATTATTCCCCATGAACG Reverse: AGGGCCTCACTAAACCATCC



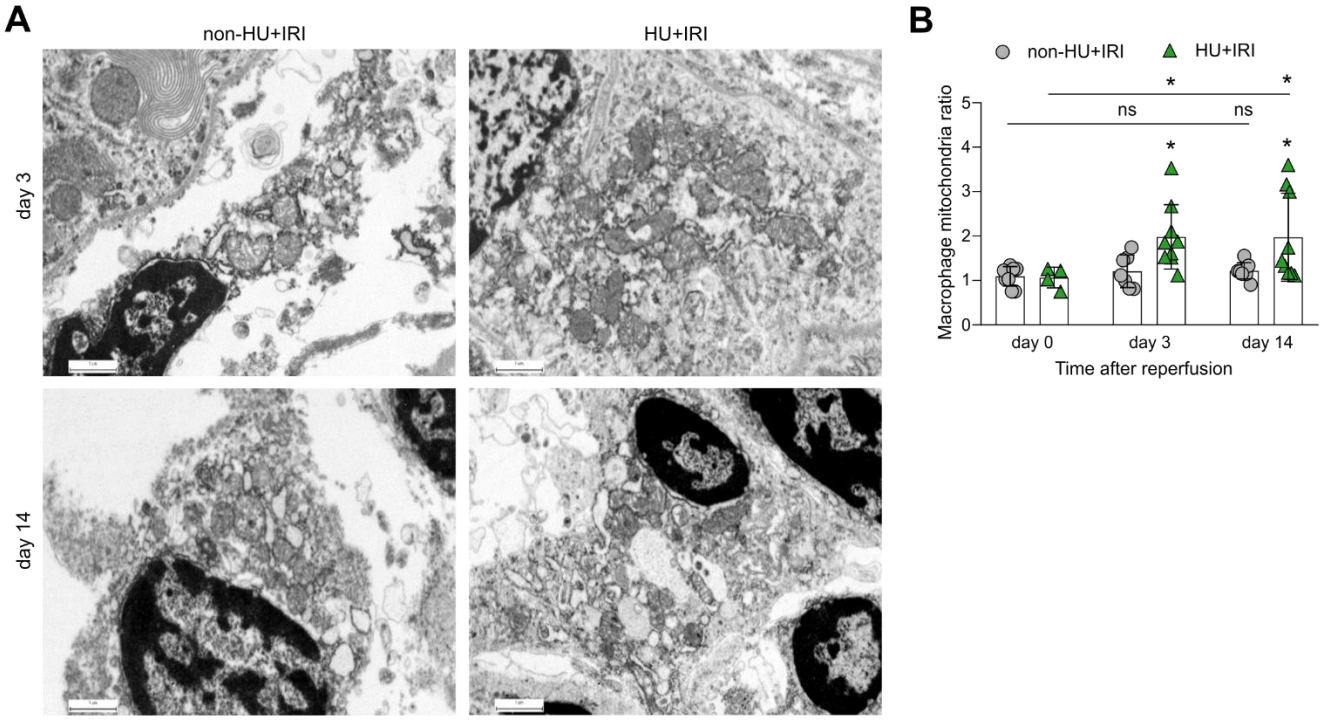
Supplementary Figure S1. Soluble uric acid inhibits the pro-inflammatory function of LPS-activated macrophages. (**A**) Schematic of experimental setup. Bone marrow–derived macrophages from *Glut9*$^{lox/lox}$ mice were primed with LPS for 24 h prior to sUA stimulation (medium; 5 mg/dL sUA; 10 mg/dL sUA). Afterwards, cells were stimulated with and without LPS (100 ng/mL) for 24 h. (**B**) Final UA concentrations after adding 5 and 10 mg/dL sUA into the macrophage culture were measured by colorimetric assay (n = 2). (**C**,**D**) Representative images (C) and quantification of ROS production (D) was detected with dihydrorhodamine 123 dye using a fluorescent microscope (n = 2-5). (**E**) Concentrations of interleukin (IL)-6 in culture supernatants were determined by ELISA (n = 3). (**F**) Concentration of interleukin (IL)-1β in culture supernatants were determined by ELISA (n = 6). Data are expressed as mean ± SEM. * p<0.05, ** p<0.01, *** p<0.001, ns, not significant; two-way ANOVA with Tukey post-test.



Supplementary Figure S2. Asymptomatic hyperuricemia does not induce endothelial dysfunction after acute organ injury. Hyperuricemic Alb-creERT2;*Glut9*$^{lox/lox}$ (HU) and *Glut9*$^{lox/lox}$ control (non-HU) mice were injected with tamoxifen and placed on a chow diet enriched with inosine to induce hyperuricemia (HU). Nephrectomy was performed (-day 7) prior to clamping of the left renal pedicle for 15 min to induce ischemia/reperfusion injury (IRI). Mice were sacrificed on day 14. (**A**,**B**) Representative images of CD31 staining of glomerular and peritubular endothelial cells on kidney sections in non-HU+IRI and HU+IRI mice on day 0 and 14. Magnification x200 (**A**) and x400 (**B**).



Supplementary Figure S3. Mitochondria gene expression profile of the kidney from mice after acute organ injury. Ischemia/reperfusion injury (IRI) was induced in wild-type C57Bl/6 mice by clamping the left renal pedicle for 30 min. Mice were sacrifice at 3 days (n = 3) and 7 days (n = 3) after IRI-induced AKI. Healthy mouse kidney (n = 1) was used as control. Single cell RNA sequencing of kidney samples was performed. (**A**) UMAP projection showing cluster distribution of mouse kidney cells. (**B**) Violin cluster characterization of cell population. (**C**) UMAP projection showing cluster distribution of mouse kidney cells in healthy mice and mice on day 3 and 7 after IRI-induced AKI. (**D**) UMAP projection showing cluster distribution of mitochondria fission- and fusion-related genes of mouse kidney cells.



Supplementary Figure S4. Asymptomatic hyperuricemia promotes mitochondria elongation in macrophages after acute organ injury. Hyperuricemic Alb-creERT2;*Glut9*$^{lox/lox}$ (HU) and *Glut9*$^{lox/lox}$ control (non-HU) mice were injected with tamoxifen and placed on a chow diet enriched with inosine to induce hyperuricemia (HU). Nephrectomy was performed (day 7) prior to clamping of the left renal pedicle for 15 min to induce ischemia/reperfusion injury (IRI). Mice were sacrificed on day 3 or 14. (**A**,**B**) Electron microscopy of mitochondria morphology in macrophages (**A**) and quantification of the mitochondrial long axis/short axis ratio (n = 9-11 mitochondria, (**B**). Data are mean ± SEM from 2-3 mice per group. * $p<0.05$, ns, not significant; two-way ANOVA with Tukey's post-test.