Supplementary Materials

Mitochondrial ROS promote mitochondrial dysfunction and inflammation in ischemic acute kidney injury by disrupting TFAM-mediated mtDNA maintenance

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Figure S1. The vehicle (DMSO/PBS) alone had no influence on renal lesions in IRI-AKI mice. (A) Serum CREA and UREAL concentrations of mice on day 5 after IRI-AKI (n = 6; *p < 0.05 vs. CON group). (B) Representative micrographs of renal H&E staining in mice with different treatments. (C) Real-time PCR analysis of Bax, ICAM-1, MCP-1, TFAM, ATP5a-1, and PGC-1α mRNA levels in kidneys of mice on day 5 after IRI-AKI (n = 3; *p < 0.05 vs. CON group). (D) mtDNA copy number in kidneys of mice on day 5 after IRI-AKI (n = 3; *p < 0.05 vs. CON group).
Figure S2. Measurement of ROS production in HK2 cells under different stress conditions.

(A) Determination of intracellular ROS in the HK2 cells under H/R conditions using the DCFH-DA staining (scale bar = 50 μm). (B) Determination of intracellular ROS and mtROS in HK2 cells under t-BHP conditions with the DCFH-DA and MitoSOX staining (Scale bar = 50 μm). (C) Determination of mtROS level in HK2 cells under t-BHP conditions using flow cytometry with MitoSOX staining.
Figure S3. TFAM degradation was enhanced in HK2 cells under oxidative stress. (A) Cell viability was determined by CCK8 assay (n = 3; *p < 0.05 vs. Control group; # p < 0.05 vs. t-BHP group). (B) Western blotting of TFAM, Lon, and p53 proteins in HK2 cells under t-BHP conditions with or without bortezomib treatment. (C) Quantitative analysis of protein expression detected by western blotting (n = 3; *p < 0.05 vs. Control group; # p < 0.05 vs. t-BHP group).
**Figure S4.** (A) Observation of mitochondria and quantification of mitochondrial length in HK2 cells under H/R conditions. HK2 cells were treated with or without MT and then stained with anti-TOM20. (B) Real-time PCR analysis of PGC-1α, ATP5a-1, and NDUFS8 mRNA levels in HK2 cells under H/R conditions with or without MT treatment (n = 3; *p < 0.05 vs. Control group; #p < 0.05 vs. H/R group).
Figure S5. (A) Double-IF staining of TOM20 (red) and TFAM (green) in the HK2 cells under H/R conditions with or without TFAM siRNA treatment (scale = 10 µm). (B) Quantitative analysis of TFAM protein expression in the HK2 cells (n = 6; *p < 0.05 vs. Control group; #p < 0.05 vs. H/R group; &p < 0.05 vs. MT group).
Figure S6. TFAM deficiency abolished the protective role of mtROS scavenger in the HK2 cells. (A) Real-time PCR analysis of TFAM mRNA levels in HK2 cells. (B) Western blotting analysis of TFAM protein levels in the HK2 cells at 48 h after transfection. (C) Real-time PCR analysis of TFAM and PGC-1α mRNA levels in HK2 cells under H/R conditions (n = 3; *p < 0.05 vs. Control group; #p < 0.05 vs. H/R group; &P < 0.05 vs. MT group).
**Figure S7.** Loss of TFAM under oxidative stress induced cytokine production in HK2 cells.

(A) Western blotting analysis of ICAM-1, Bax, and TFAM protein levels in HK2 cells under H/R conditions with various treatments. (B) Quantitative analysis of ICAM-1 and TFAM protein expression detected by western blotting (n = 3; *p < 0.05 vs. Control group; # p < 0.05 vs. H/R group; & p < 0.05 vs. MT group).
**Figure S8.** (A) Double-IF staining of TFAM (red) and dsDNA (green) in HK2 cells under H/R conditions with various treatments (scale bar = 10 μm). (B) Average size of mtDNA nucleoid in HK2 cells of different groups (n = 20 cells; *p < 0.05 vs. Control group; #p < 0.05 vs. H/R group). (C) Quantification of cytoplasmic dsDNA (yellow arrows) intensity in HK2 cells (n = 16 cells; *p < 0.05 vs. Control group; #p < 0.05 vs. H/R group; &p < 0.05 vs. MT group).
**Figure S9.** Representative images of the TFAM (red), dsDNA (green), and DAPI costaining in kidney of normal control mice (scale bar = 10 μm).
| Gene   | Sequence 5'-3'                                                                 | Species  |
|--------|-------------------------------------------------------------------------------|----------|
| TFAM   | AGCTCAGAACCAGATGCAA                                                          | Human    |
|        | CCGCCCTATAAGCATTCTTGA                                                         |          |
| PGC1-α | TGCTGAAGAGGCAAGAGACA                                                           |          |
|        | CACACACGCACACTCCATC                                                           |          |
| NDUFS8 | CATCTACTGCGGTCTTGTG                                                         |          |
|        | GGGCGTCACCAGATACAGT                                                          |          |
| ATP5a-1| AGAGGACAGGAGCCATTGTG                                                          |          |
|        | TCAGACCAACTCGCTACG                                                            |          |
| UQRC1  | CAGTCCTCTCAGGCCCACCGT                                                         |          |
|        | CCGATCTTTGCTCCCTTTGA                                                        |          |
| IL-1β  | TGGCACAAAGGGAGCAAGAAA                                                          |          |
|        | CTGGCTGATGAGACAGGAGAT                                                          |          |
| TNF-α  | TGCTGACATTGGAGTGTG                                                          |          |
|        | TGTCATCGGAGGCTGAGAAG                                                       |          |
| GAPDH  | ACCACAGTCCATGCCCCACAC          |          |
|        | TCCACCACCTTGTTGCTGTGA                                                      |          |
| TFAM   | CACCCAGATGCAAAACTTTCAAG                                                       |          |
|        | CTGCTTTTATACCTGGTCACAG                                                      |          |
| PGC1-α | CACCAAAACACAGAAACAG                                                          |          |
|        | GGGTCAGAGGAAGAGATAAAGTTG                                                      |          |
| ATP5a-1| CATTTGATGATGTTTGCG                                                          |          |
|        | TCCCCAACACGACAACTCC                                                        | Mouse    |
| Bax    | TGGAGATGAACTGGACAGCA                                                        |          |
|        | TGAAGTTGCCCATCAGAAAC                                                      |          |
| MCP-1  | AGTTGACCGTAAATCTGAGC                                                        |          |
|        | GTGGTTTGGGAAAGGTTAGTG                                                       |          |
| ICAM-1 | ACCCAAACGTGAAGCTGTGTTG                                                      |          |
|        | CACACTCCCGGAAAAACGAAT                                                      |          |
| TNF-α  | CCAGGAGAAAGTCAGCCTCCT                                                       |          |
|        | TCATCAGGGCTTGGAGCTCA                                                      |          |
| GAPDH  | CAGATCCACCCGGATATTTGGG                                                      |          |
|        | CATGACAACTTTGGCCATTGG                                                       |          |
| Gene  | Sequence 5'-3'                  | Species   |
|-------|--------------------------------|-----------|
| hB2M  | TGTTCCCTGCTGGGTAGCTCT          | Human     |
|       | CCTCCATGATGCTGCTTACA           |           |
| mtND1 | CACTTTCACACAGACATCA            |           |
|       | TGGTTAGGCTGGTGTAGGG            |           |
| COX2  | ATAACCGAGTCGTTCTGCAAT          | Mouse     |
|       | TTCAGAGCATTGGCAGTAGAA          |           |
| Rsp18 | TGTGTAGGGGACTGGTGGACA          |           |
|       | CATCACCCACTTTACCCCAAAA         |           |