

Supplemental Figure Legends

Fig. S1 Bioinformatic analysis of *Hsp* mRNA from the publicly available data for mouse KI/R (GSE172042).

- A. Heatmap of 29 *Hsps* mRNA levels in kidneys of sham and KI/R mice.
- B. mRNA levels of 29 *Hsps* in sham kidneys. Note that *Hspa12a* mRNA showed the highest level among these 29 *Hsps* in normal kidneys.
- C. Effects of KI/R on mRNA levels of 29 *Hsps* in kidneys. Data are mean \pm SD, * $P < 0.05$ and ** $P < 0.01$ by Student's two-tailed unpaired *t*- test or Mann–Whitney U test. $n = 2-3$.

Fig. S2 Effects of KI/R on expression of HSPs.

Expression of HSP32, HSP47, HSP60 and HSP70 were examined by immunoblotting in mouse kidneys following KI/R. Blots for GAPDH served as loading controls. Data are mean \pm SD, ** $P < 0.01$ by Student's two-tailed unpaired *t*- test. $n = 3$.

Fig. S3 Identification of the isolated primary TEC.

The primary TEC that isolated from mouse kidneys were immunostained with the epithelial marker CK-18 (red). DAPI was used to counter stain nuclei. Scale bar = 50 μm .

Fig. S4 Time-effect on HSPA12A expression in primary TEC after reperfusion.

The time-effect on HSPA12A expression in primary TEC at 0 h, 3 h, 6 h and 12 h after reperfusion were examined by immunoblotting. Blots for GAPDH served as loading controls. Data are mean \pm SD, $**P < 0.01$ by one-way ANOVA followed by Tukey's test. $n = 4$.

Fig. S5 HSPA12A increases nuclear abundance of c-Myc in TEC after H/R.

Nuclear fractions were prepared from H/R-treated TEC. c-Myc protein expression was examined by immunoblotting. Blots for H3 served as loading controls. Data are mean \pm SD, $*P < 0.05$ by Student's two-tailed unpaired t - test. $n = 4$.

Fig. S6 Effects of HSPA12A on c-Myc mRNA and protein levels in primary TEC upon H/R.

The following experiments were performed in primary TEC after H/R.

A. The mRNA levels of c-Myc were analyzed by RT-PCR. $*P < 0.05$ and $*P < 0.05$ by two-way ANOVA followed by Tukey's test. $n = 4$.

B. The protein levels of c-Myc were examined by immunoblotting. Blots for GAPDH served as loading controls. Data are mean \pm SD, $*P < 0.05$ and $**P < 0.01$ by two-way ANOVA followed by Tukey's test. $n = 4$.

C. Immunostaining for c-Myc and ubiquitin was performed. DAPI staining was used to indicate nuclei. Data are mean \pm SD, $**P < 0.01$ by Student's two-tailed unpaired t - test. $n = 3$.

Fig. S7 Knockdown of c-Myc in TEC after H/R.

c-Myc knockdown in TEC was confirmed by immunoblotting. $**P < 0.01$ by Mann–Whitney U test. $n = 5$ /group.

Fig. S8 C646 reverses the HSPA12A-increased c-Myc nuclear abundance in TEC.

Nuclear fractions were prepared from H/R-treated TEC in the presence or absence of C646. c-Myc protein in nuclear fraction was examined by immunoblotting. Blots for H3 served as loading controls. Data are mean \pm SD, $**P < 0.01$ by one-way ANOVA followed by Tukey's test. $n = 4$.

Fig. S9 Effects of HSPA12A on expression of glycolysis-related genes in primary TEC.

- A.** ATP production rate was analyzed by the seahorse assay in primary TEC following H/R. Data are mean \pm SD, $*P < 0.05$ by Student's two-tailed unpaired *t*-test $n = 4$.
- B.** Expression of the indicated genes were examined by immunoblotting in primary TEC following H/R. Blots for GAPDH served as loading controls. Data are mean \pm SD, $**P < 0.01$ by two-way ANOVA followed by Tukey's test. $n = 3$.

Fig. S10 Effects of HSPA12A on expression of glycolysis-related genes in mouse kidneys following KI/R.

- A.** Lactate contents were analyzed in serum of mice following KI/R. Data are mean \pm SD, $**P < 0.01$ by Mann–Whitney U test. $n = 5$.

B. Protein expressions of glycolysis-related genes were determined by immunoblotting in KI/R kidneys of mice. Data are mean \pm SD, $**P < 0.01$ by Student's two-tailed unpaired *t*-test. $n = 5$ for GLUT1 groups and $n = 6$ for the other groups.

Fig. S11 2-DG abolishes the HSPA12A-increased lactate generation and proliferation of TEC after H/R.

- A.** The brief diagram of glycolysis pathway. 2-DG was used to block lactate generation.
- B.** Lactate contents in culture medium of H/R-treated TEC were measured in the presence or absence of 2-DG. Data are mean \pm SD, $**P < 0.01$ by one-way ANOVA followed by Brown-Forsythe and Welch test. $n = 5$.
- C.** Cell proliferation in H/R-treated TEC was indicated by EdU incorporation in the presence or absence of 2-DG. Data are mean \pm SD, $**P < 0.01$ by one-way ANOVA followed by Tukey's test. $n = 3$. Scale bar = 50 μm .

Fig. S12 Effect of HSPA12A on nuclear abundance of Hif1 α in KI/R kidney.

- A. Experimental setting of primary TEC.**
- B.** Anti-Flag-HSPA12A immunoprecipitates prepared from H/R-treated TEC were immunoblotted with Hif1 α . Input or IgG-immunoprecipitates served as positive or negative controls. Note that Hif1 α was not recovered in Flag-tagged HSPA12A immunoprecipitates.
- C. Experimental setting of mouse kidneys.**
- D.** Following KI/R, nuclear fraction was prepared from kidneys for immunoblotting

against Hif1 α . Blots for LaminA/C served as loading controls. Data are mean \pm SD,

** $P < 0.01$ by Student's two-tailed unpaired t -test. $n = 5$.

Fig. S13 Hif1 α inhibition diminishes the HSPA12A-induced increase of proliferation of TEC after H/R.

Cell proliferation was examined by EdU incorporation in H/R-treated *Hspa12a*^{O/E} TEC in the presence or absence of YC-1. Data are mean \pm SD, ** $P < 0.01$ by one-way ANOVA followed by Tukey's test. $n = 3$. Scale bar = 50 μ m.

Supplemental Tables

Table S1. SiRNA sequence used in the experiments

| Species | Gene name | Sequence (5'-3') | |
|---------|------------|------------------|-----------------------|
| Mus | <i>Myc</i> | Sence | CCGUACAGCCCUAUUUCAUTT |
| | | Antisence | AUGAAAUAGGGCUGUACGGTT |

Table S2. Antibodies used in the experiments

| Antibody | Source | Company | Catalog No. |
|-----------------------------|--------|---------------------|-------------|
| Anti-HSPA12A | Rabbit | Abcam | ab200838 |
| Anti- α -Tubulin | Mouse | ProteinTech | 66031-1-Ig |
| Anti-GAPDH | Rabbit | Bioworld Technology | AP0063 |
| Anti-c-Myc | Rabbit | ProteinTech | 10828-1-AP |
| Anti-L-lactyl lysine (Klac) | Rabbit | PTM BIO | PTM-1401RM |
| Anti-H3 | Rabbit | ProteinTech | 17168-1-AP |
| Anti-Flag | Mouse | Sigma-Aldrich | F1804 |
| Anti-PKM2 | Rabbit | ProteinTech | 60268-1-Ig |
| Anti-LDHA | Rabbit | Abcam | ab101562 |
| Anti-GLUT1 | Rabbit | Cell Signaling | #12939S |
| Anti-GLUT4 | Rabbit | Abcam | ab33780 |
| Anti-MCT4 | Rabbit | ProteinTech | 22787-1-AP |
| Anti-Hif1 α | Rabbit | Abcam | ab2185 |
| Anti-Lamin A/C | Rabbit | ProteinTech | 10298-1-AP |
| Anti-IgG | Mouse | SantaCruz | sc-2025 |
| Anti-Cytokeratin 18 | Rabbit | Abcam | ab181597 |
| Anti-Ki-67 | Rabbit | Cell Signaling | #9449 |

Table S3. Primers used in the experiments

| Gene name | Primers | |
|-------------------------|----------------|-------------------------|
| <i>Cyclinb1 for mus</i> | Forward | AGAGCTATCCTCATTGACTGGC |
| | Reverse | AACATGGCCGTTACACCGAC |
| <i>Cyclind2 for mus</i> | Forward | TGAATTACCTGGACCGTTTCTTG |
| | Reverse | AGAGTTGTTCGGTGTAATGCAC |
| <i>Rcc1 for mus</i> | Forward | ACACAGGTCCCACAACACAG |
| | Reverse | GCCTTCGACTGAAGTGTCCC |
| <i>Cdk4 for mus</i> | Forward | ATGGCTGCCACTCGATATGAA |
| | Reverse | TGCTCCTCCATTAGGAACTCTC |
| <i>Cdc25a for mus</i> | Forward | TCCCTGACGAGAATAAATTCCT |
| | Reverse | TCGATGAGGTGAAAGGTGTCG |
| <i>Actin for mus</i> | Forward | ATGACCCAAGCCGAGAAGG |
| | Reverse | CGGCCAAGTCTTAGAGTTGTTG |