# Supplementary Materials Kaseda, S., et al.

#### Contents

#### **Supplementary Method**

Endothelin-1 concentration measurement

#### **Supplementary Figures**

Figure S1. UBE-1099 slightly reduced the body weight and increased the urine volume in Alport mice

Figure S2. UBE-1099 did not affect the heart rate and blood pressure in Alport mice

Figure S3. Transcriptome analysis reveals the comprehensive effects of UBE-1099 in the glomeruli of Alport mice

Figure S4. Dysregulated GO terms in the glomeruli of Alport mice

Figure S5. Up-regulated GO terms for Alport vehicle vs WT

Figure S6. Down-regulated GO terms for Alport Vehicle vs WT

Figure S7. Up-regulated GO terms for Alport UBE-1099 vs Alport Vehicle

Figure S8. Down-regulated GO terms for Alport UBE-1099 vs Alport Vehicle

Figure S9. UBE1099 altered genes in each condition

Figure S10. Expression level of cell specific markers in the glomerular cell

Figure S11. UBE-1099 did not affect the food intake and muscle weight in Alport

mice and did not worsen the early renal pathology

Figure S12. UBE-1099 did not affect the endothelin expression

Figure S13. Full length blots for Figure 5A, B

## **Supplementary Method**

#### **Endothelin-1 concentration measurement**

Rat proximal tubular cells (NRK-52E cells) were treated with vehicle (dimethyl sulfoxide, 0.1% final concentration) or 10-100 nM of CDDO-Im or UBE-1099. After 24 h, cell culture media were collected and assessed for concentrations of secreted endothelin-1 (ET-1) using ELISA kit (R&D Systems, USA) according to the manufacturer's protocol. Cell viability was measured by Cell Titer-Glo Luminescent cell viability assay (Promega) according to the manufacturer's protocol.

## **Supplementary Figures**



# Figure S1. UBE-1099 slightly reduced the body weight and increased the urine volume in Alport mice

(A, B) Body weight and urine volume were measured every two weeks. Urine volume was measured using metabolic cages for 24h. Data are presented as mean  $\pm$  SE (n = 8 per group). P values were assessed by Dunnett's test. (\*p<0.05, \*\*\*p<0.001).



Figure S2. UBE-1099 did not affect the heart rate and blood pressure in Alport mice (A-D) Heart rate (HR), Systolic blood pressure (SBR), Diastolic blood pressure (DBP) and Mean blood

pressure MBP) were measured by BP-98A-1 (Softron). Data are presented as mean  $\pm$  SE (n = 6 per group).



# Figure S3. Transcriptome analysis reveals the comprehensive effects of UBE-1099 in the glomeruli of Alport mice

Heatmap shows the number of fluctuated genes in each condition (fold change > 1.2 or < -1.2, p <0.05).

# Figure S3



#### Figure S4. Dysregulated GO terms in the glomeruli of Alport mice

Gene Set Enrichment Analysis (GSEA) for Alport Vehicle vs WT

- Enric

chment profile — Hits

Ranking metric scores





Ranking metric scores (Continue to the next page)

10,000 12,000

FDR q value = 0.0525

2,000 4,000 5,000 8,000 Rank in Ordered Dataset

- Enrichment profile - Hits

Enrichm

Ranke

Ranking m

tric scores

Enrichment profile — Hits



### Figure S5. Up-regulated GO terms for Alport vehicle vs WT

6,000 8,000 Rank in Ordered Dataset 10,000 12,00

Ranking metric scores

4,000

- Hits

Ranket

Gene Set Enrichment Analysis (GSEA) data for up-regulated GO terms in Figure S4. NES = Normalized Enrichment Score.



Ranke

2,000 4,000 6,000 8,000 Rank in Ordered Dataset

- Enrichment profile - Hits

12.000

10,000

Ranking metric scores





Ranking metric scores (Continue to the next page)

- Enrichment profile - Hits

Rank

4,000

- Hits Ranking metric scores

6,000 8,000 Rank in Ordered Dataset 10,000 12,000



### Figure S6. Down-regulated GO terms for Alport Vehicle vs WT

Gene Set Enrichment Analysis (GSEA) data for up-regulated GO terms in Figure S4. NES = Normalized Enrichment Score.



(Continue to the next page)

10,000

12,000

10,000

10,000 12.000

10,000

Ranked

4,000

— Hits

6,000 8,000 Rank in Ordered Dataset 10,000

Ranking metric scores



### Figure S7. Up-regulated GO terms for Alport UBE-1099 vs Alport Vehicle

Gene Set Enrichment Analysis (GSEA) data for up-regulated GO terms in Figure 6B. NES = Normalized Enrichment Score.



- Enri

nent profile — Hits

Ranking m



Ran

- Hits

- En



(Continue to the next page)

Ranking m

Enrichment profile

Ranked list



### Figure S8. Down-regulated GO terms for Alport UBE-1099 vs Alport Vehicle

10,000

tric scores

6,000 8,000 Rank in Ordered Dataset — Hits Ranking

Gene Set Enrichment Analysis (GSEA) data for down-regulated GO terms in Figure 6B. NES = Normalized Enrichment Score.

#### А Nrf2 target genes



#### С Microtubule (GO:0005874)



#### Alport WT UBE-1099 Vehicle AnIn Wnt5a Ccar1 Cdk1 Mki67 Prc1 Klhl21 Kif11 lqgap3 Fam83d Nusap1 Ccnb2 Pimreg Aurkb Ncaph Tacc3 Sfn Ccna2 Birc5 Aox1 Top2a E2f6 Ccnb1 Tubb2a Tubb6 Tubb4b Plk1 Ube2c Cdca2 Ckap2 Nuf2 Racgap1 Map3k8 Tpx2 Kif22 Tbce Mns1 Birc3 Adarb1 Stat3 Ccn2 Smc4 Rock2 Rhob Gem Dusp1 Tuba1c Gadd45g Tuba4a Wee1 Usp2 Kif20a Cenpf Hecw2 Cdca3 Mdc1 Klf4 Nnuma1 Plcb1 Nek2 Stmn1 Cdkn1a Bcl2l1 Bmp2 Fam107a Tpr Kcna5 Rgs2 Cdk14 Pkia E2f1 Tgfa Rassf2 Wnt41 .2 0 1 2

Row Z-Score

#### D Mitochondrial protein containing complex (GO:0005874)



### Figure S9. UBE1099 altered genes in each condition

Heat map shows the altered genes of Nrf2 target (A) and indicated GO terms (B-D) in UBE1099 group compared with Alport vehicle (fold change > 1.2 or < -1.2, p < 0.05).

#### В Cell cycle (GO:0007049)



Figure S10. Expression level of cell specific markers in the glomerular cell

Relative expression levels for cell specific markers for podocyte (A), endothelial cell (B) and mesangial cell (C) were measured by RNA-seq data (TPM). Data are presented as mean  $\pm$  SE (n = 3 per group). P values were assessed by Dunnett's test. (\*p<0.05, \*\*p<0.01).

Figure S11





of Gapdh mRNA (internal control). Data are presented as mean  $\pm$  SE (n = 4 per group). P values were assessed by Dunnett's test. (\*p<0.05, \*\*\*p<0.001).



#### Figure S12. UBE-1099 did not affect the endothelin expression

(A) Endothelin-1 (ET-1) protein concentration (as % vehicle control) secreted into media from rat proximal tubule cells treated with vehicle, CDDO-Im or UBE-1099 (10-100 n M). (B) Cell viability of vehicle-, CDDO-Im- or UBE-1099-treated cells was measured by Cell Titer-Glo Luminescent cell viability assay. Data are presented as mean  $\pm$  SE (n = 3 per group). P values were assessed by Dunnett's test. (\*p<0.05, \*\*\*p<0.01, \*\*\*p<0.001 vs Vehicle).





### Figure S13. Full length blots for Figure 5A, B

The full-length blots for Figure 5A, B with the indicated antibodies. Vinculin and HDAC2 were used as loading control. Samples were derived from the same experiment, and gels/blots were processed in parallel.