

# Supplementary Materials

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### 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**

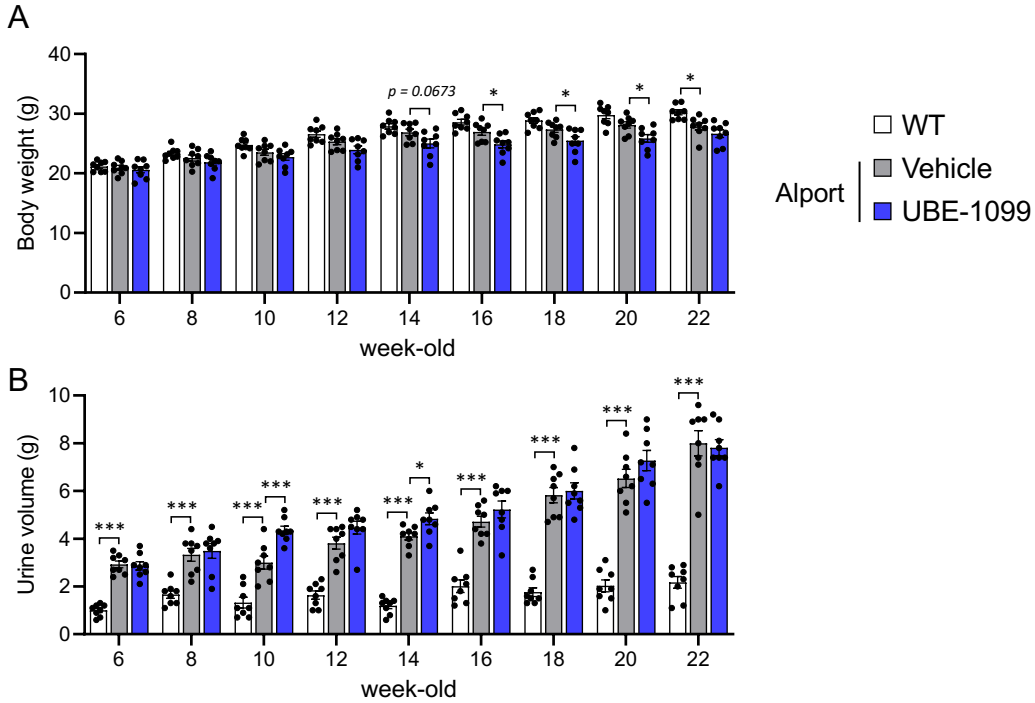
### **Endothelin-1 concentration measurement**

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

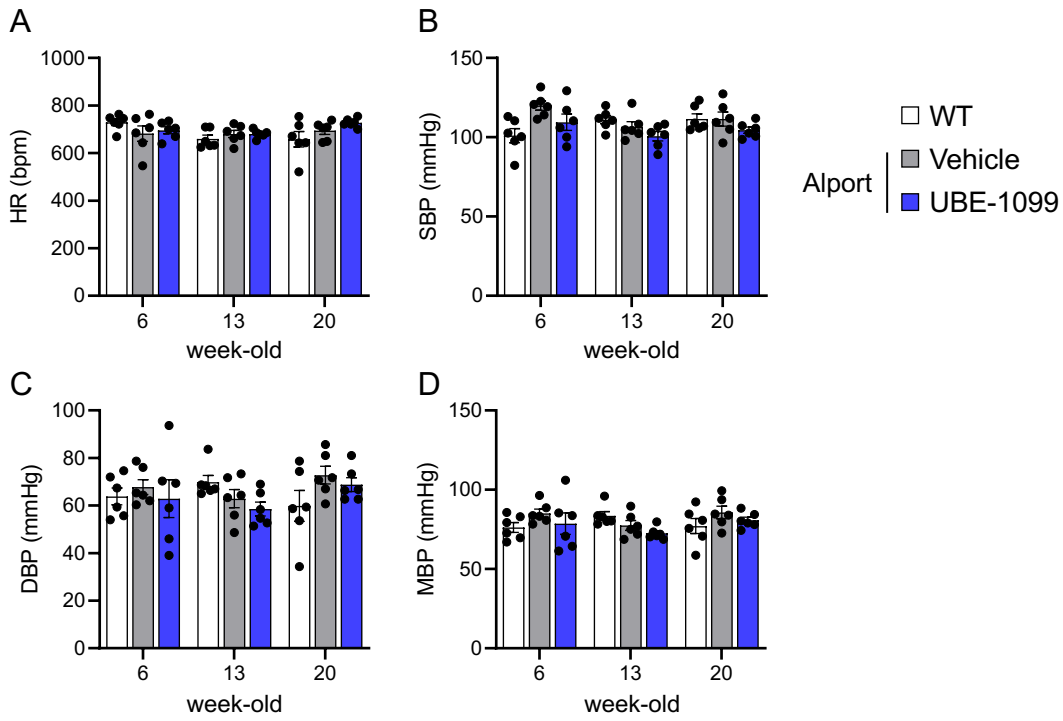
## Figure S1



**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. (\* $p < 0.05$ , \*\*\* $p < 0.001$ ).

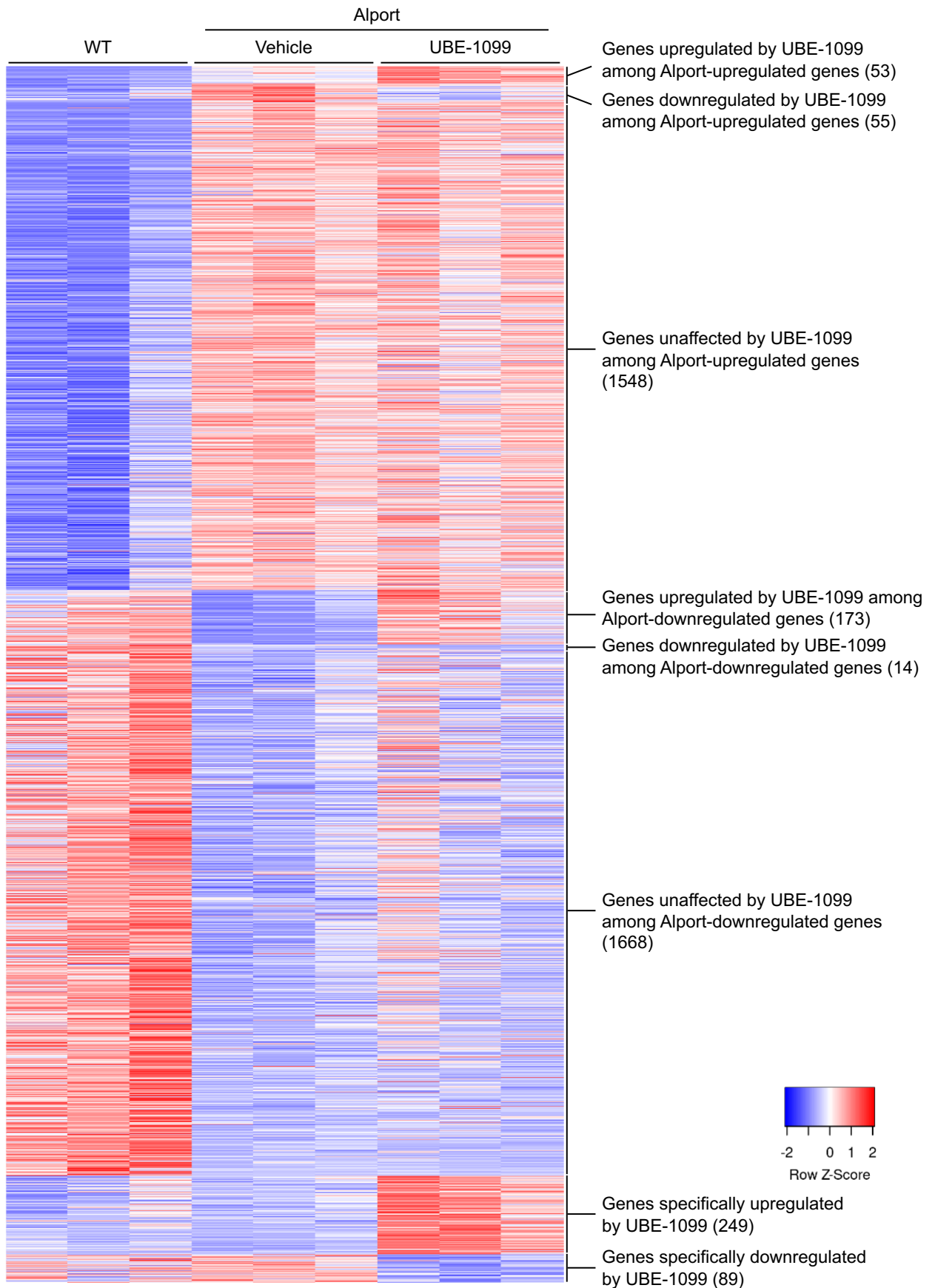
**Figure S2**



**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).

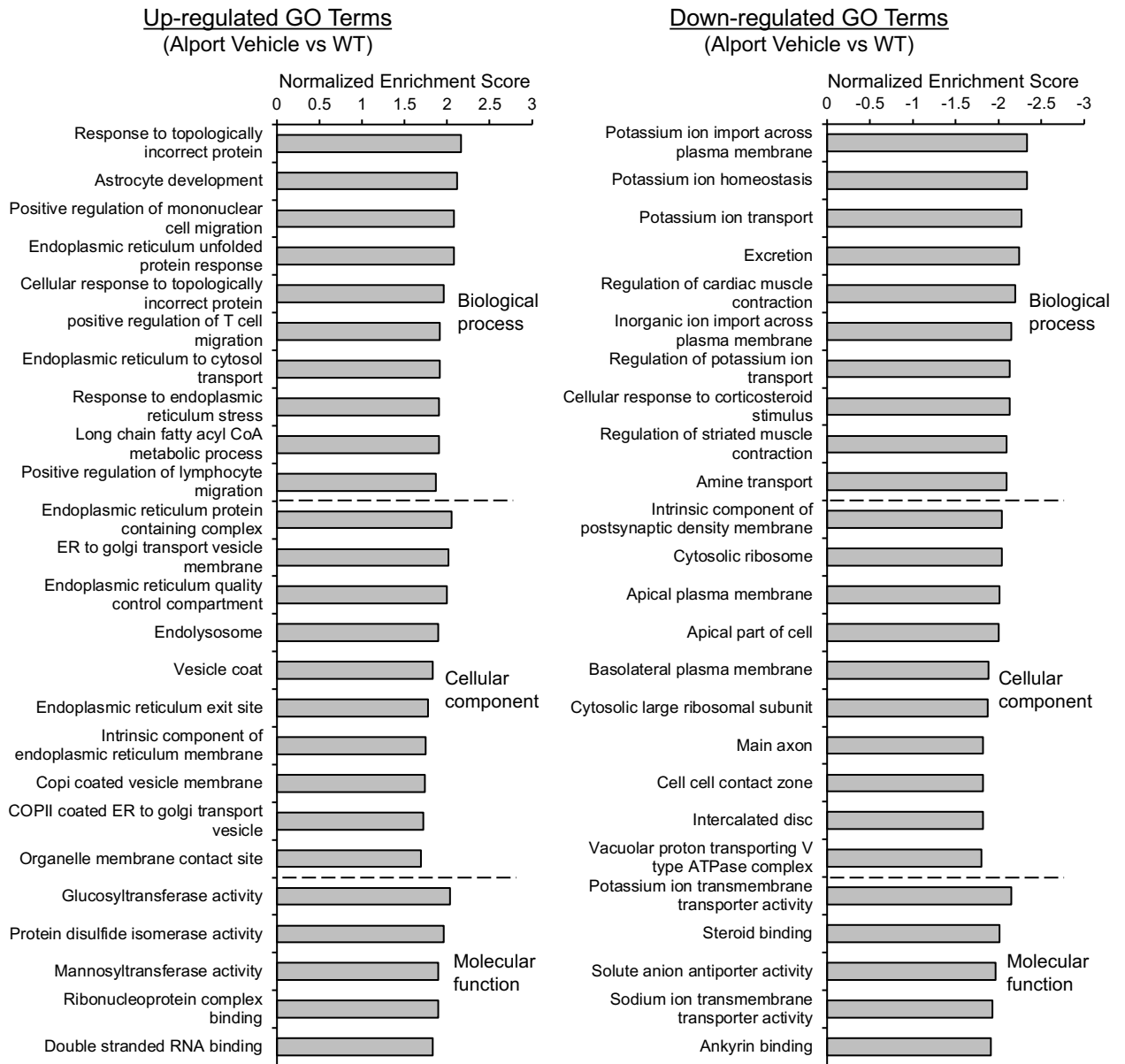
**Figure S3**



**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  $< -1.2$ ,  $p < 0.05$ ).

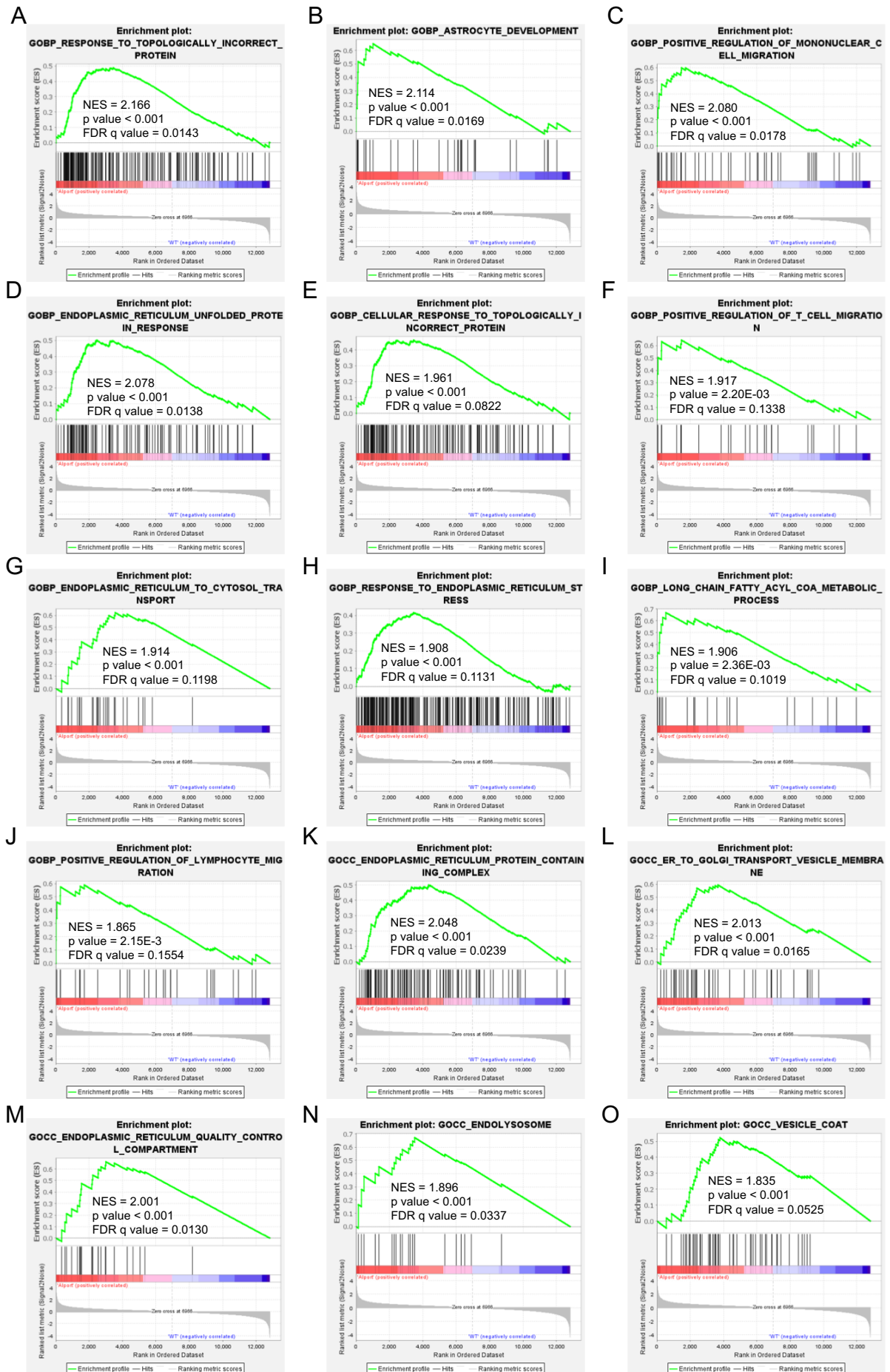
**Figure S4**



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

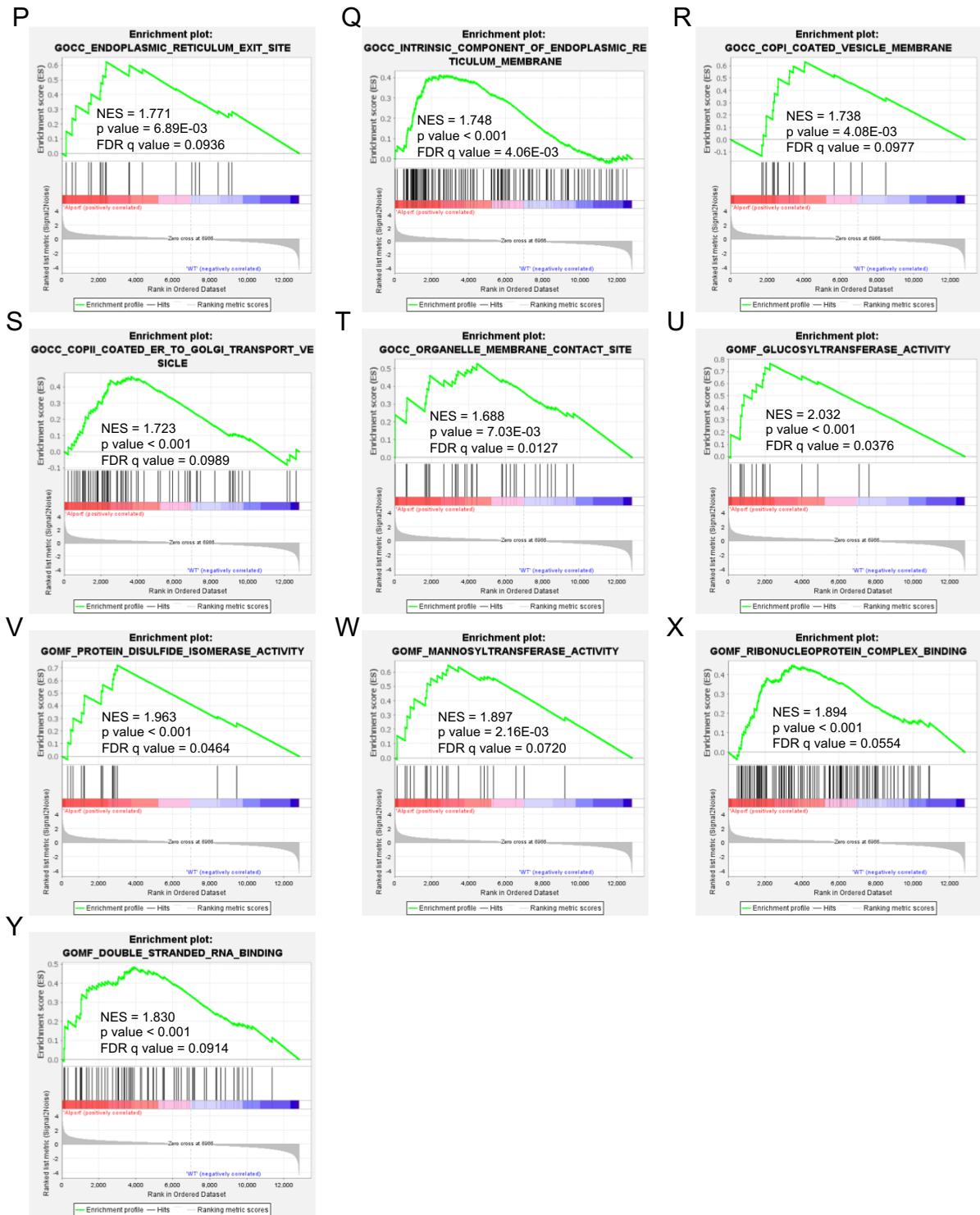
Gene Set Enrichment Analysis (GSEA) for Alport Vehicle vs WT

**Figure S5**



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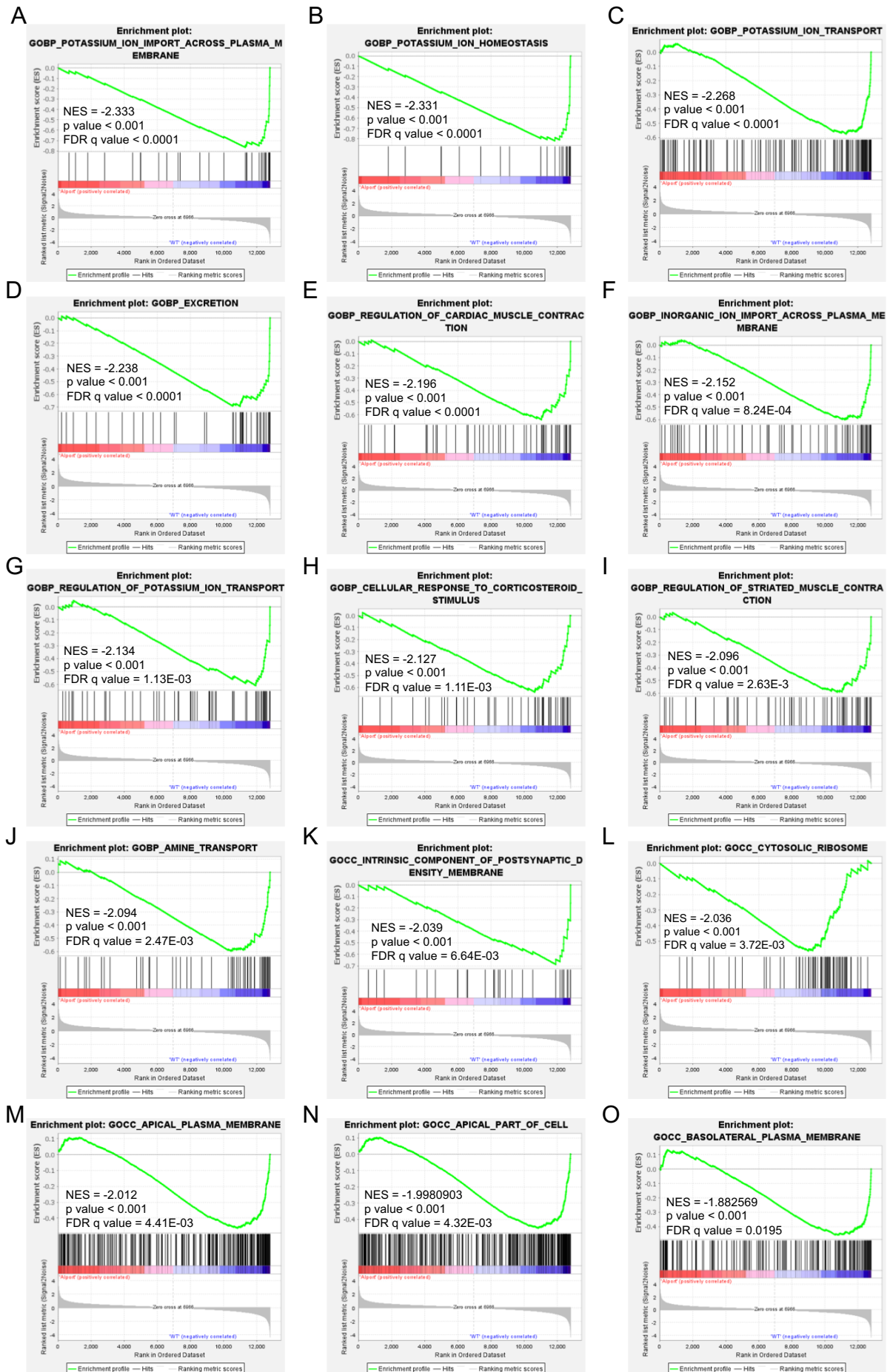
**Figure S5**



**Figure S5. Up-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.

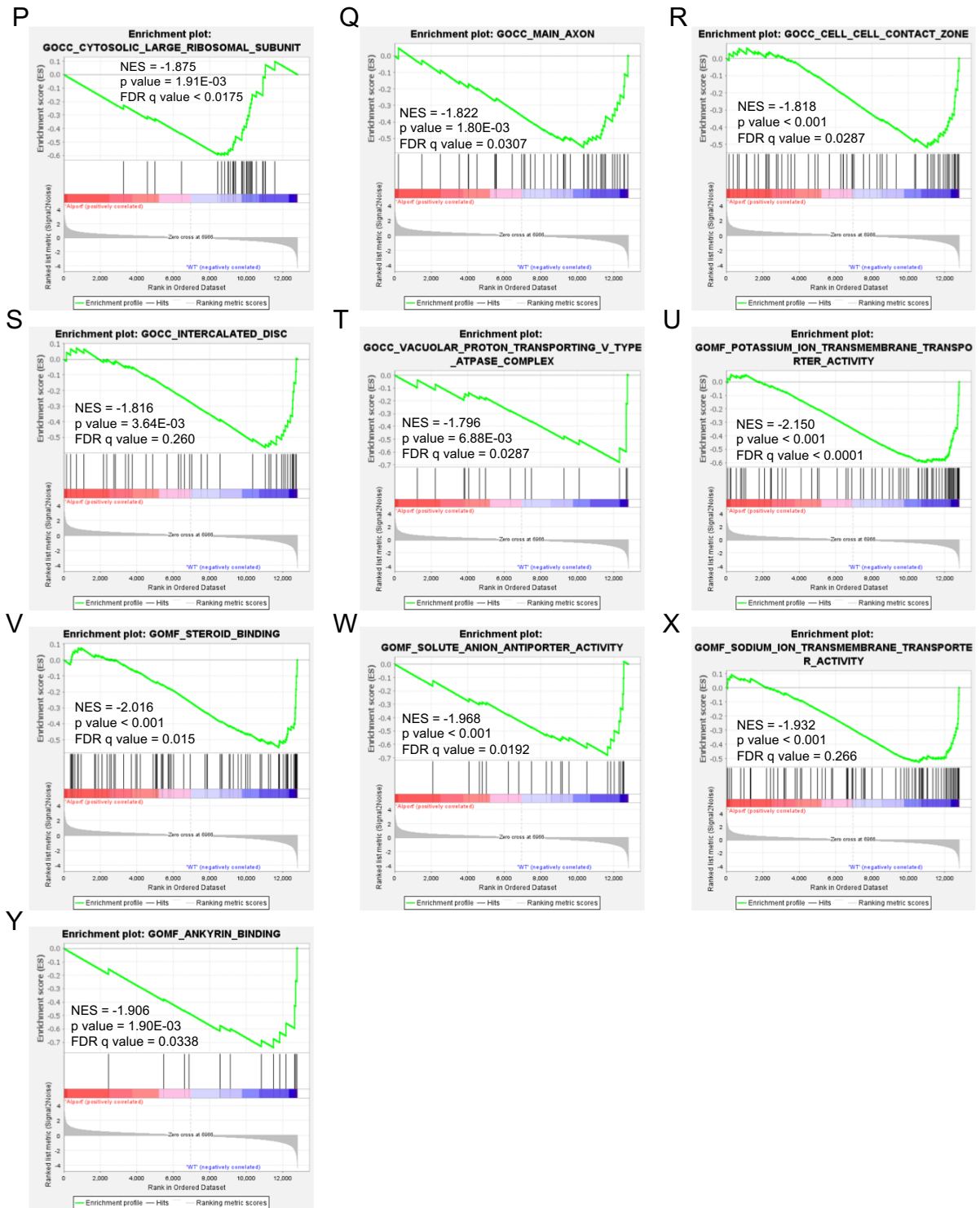
**Figure S6**



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**Figure S6**

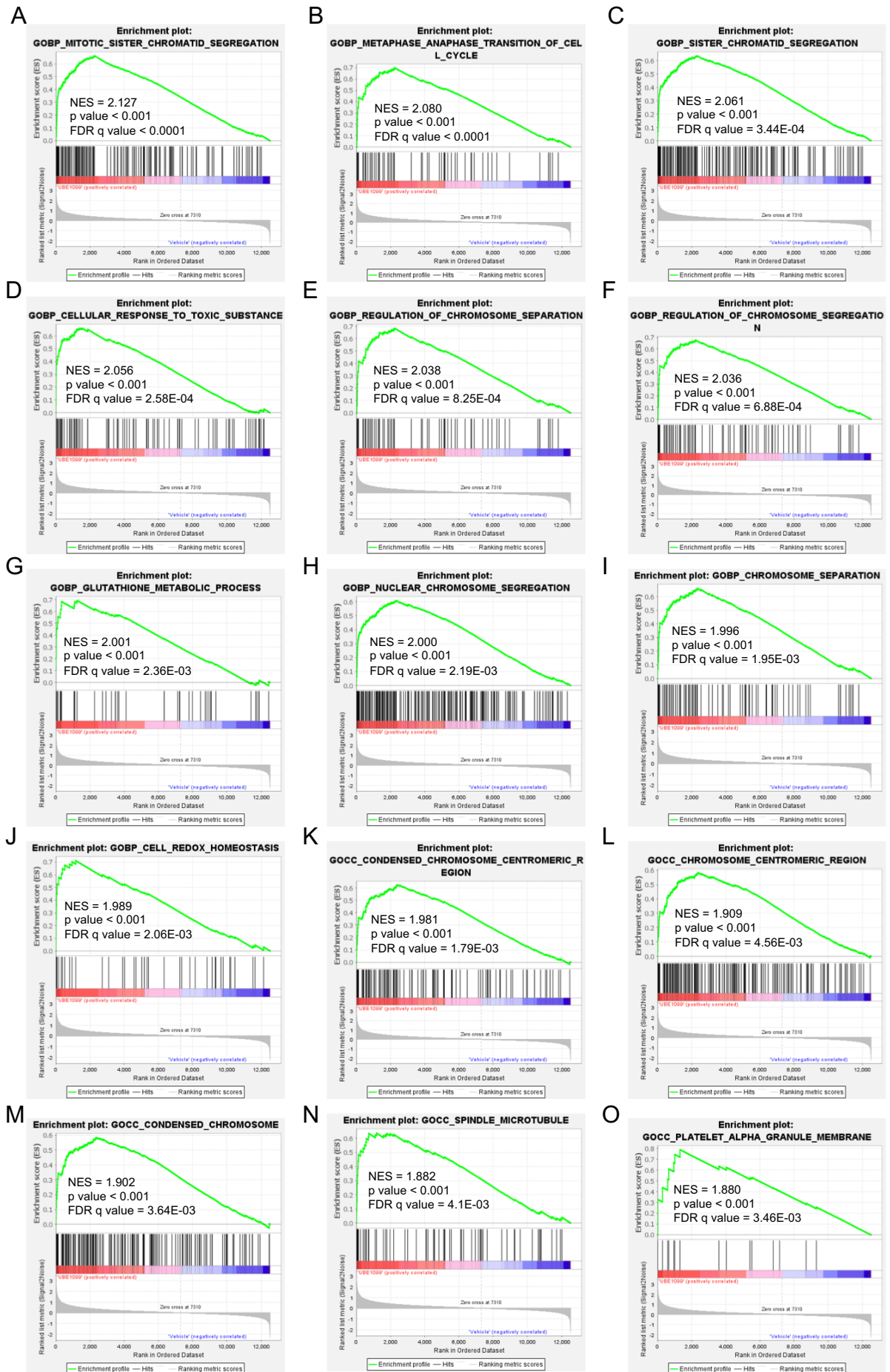


**Figure S6. Down-regulated GO terms for Alport Vehicle vs WT**

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

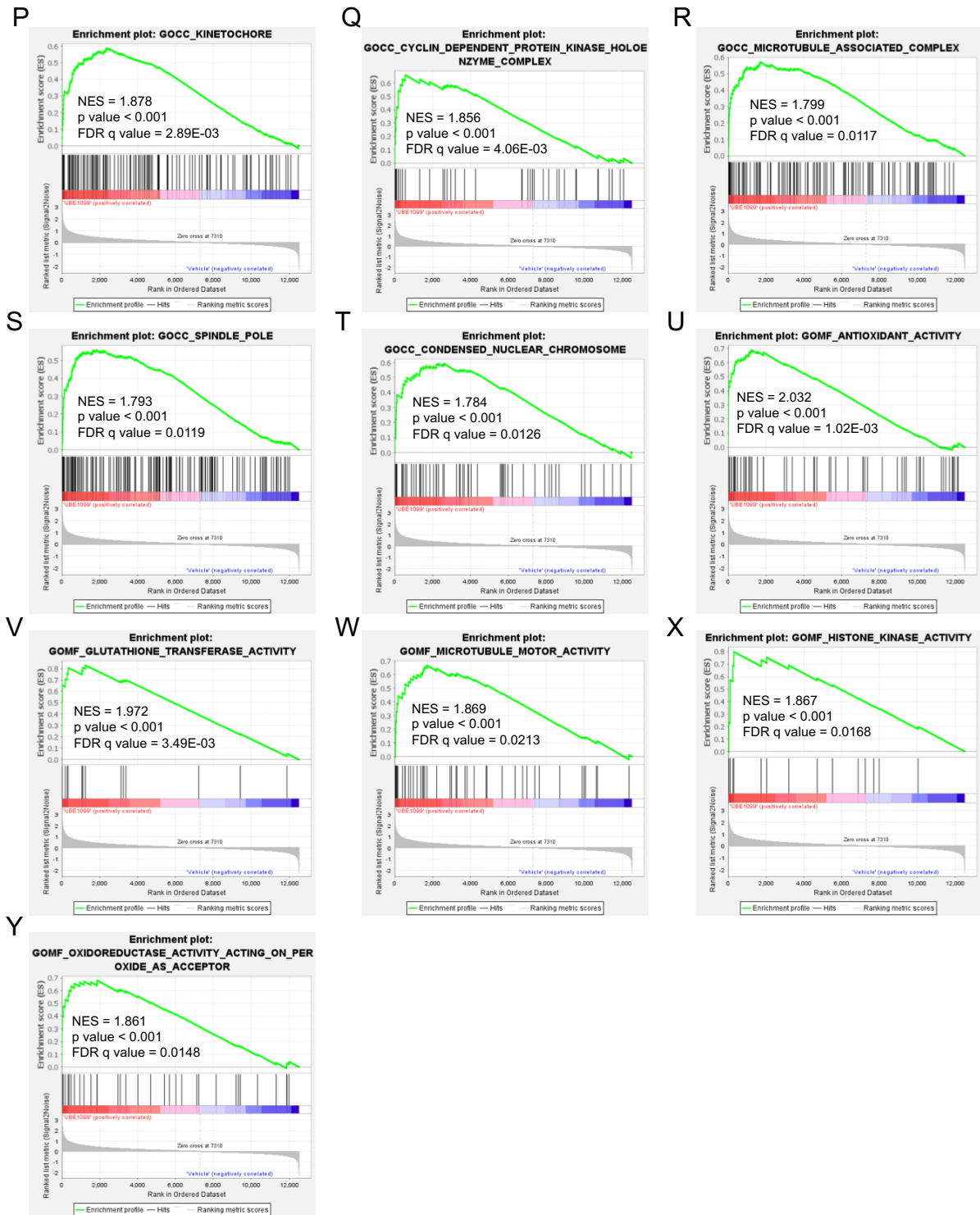


Figure S7



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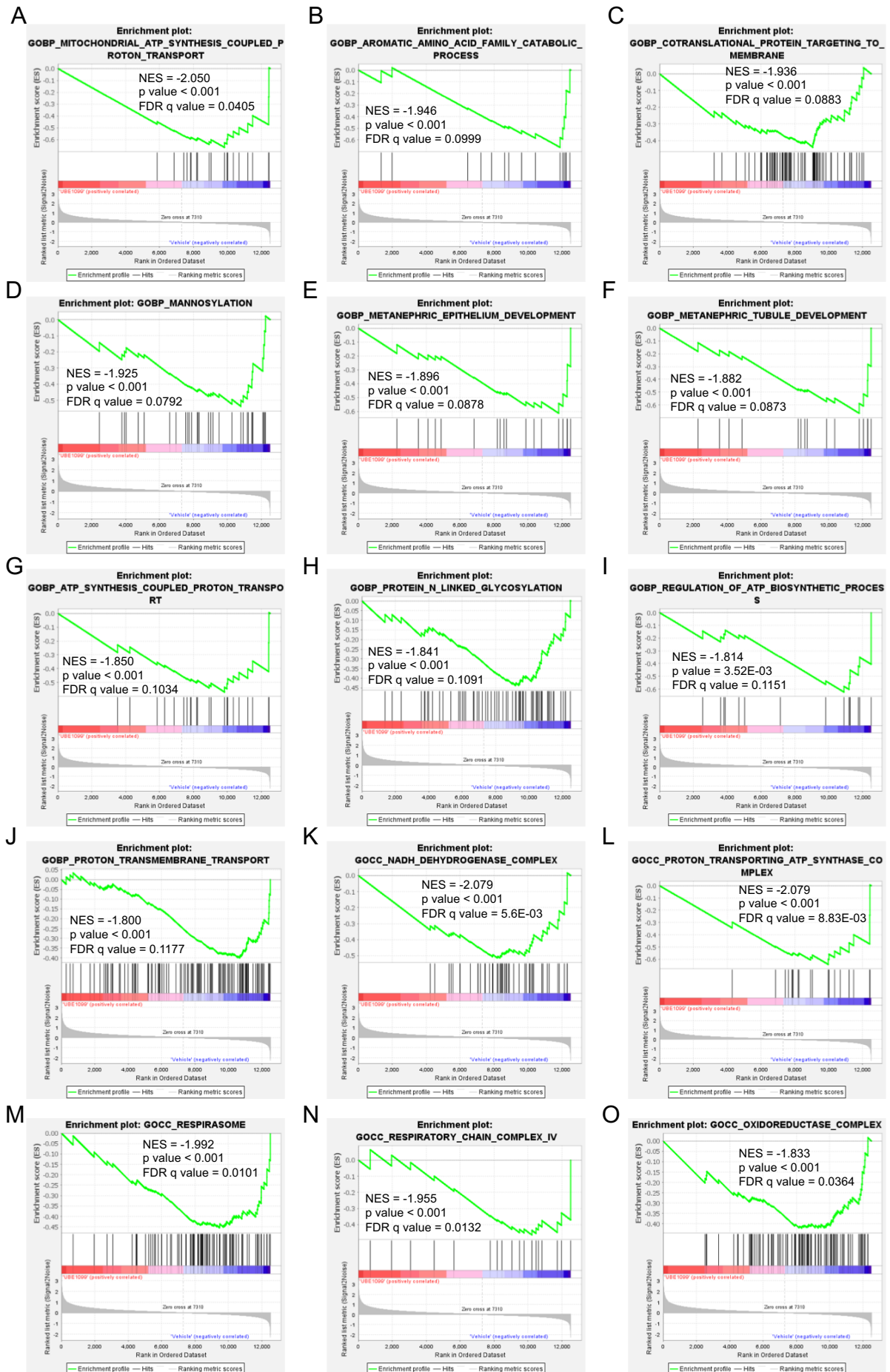
**Figure S7**



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

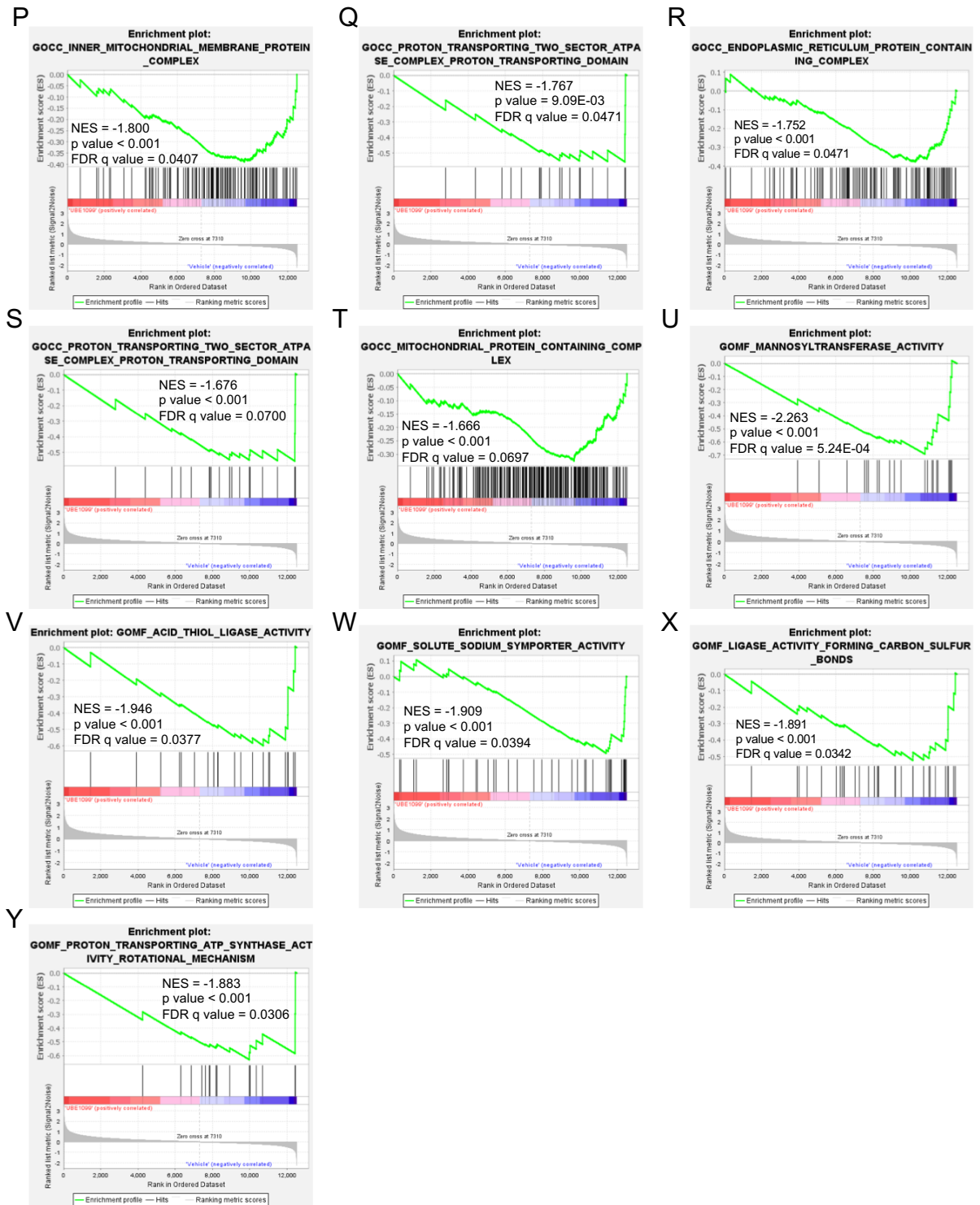
Gene Set Enrichment Analysis (GSEA) data for up-regulated GO terms in Figure 6B. NES = Normalized Enrichment Score.

**Figure S8**



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**Figure S8**

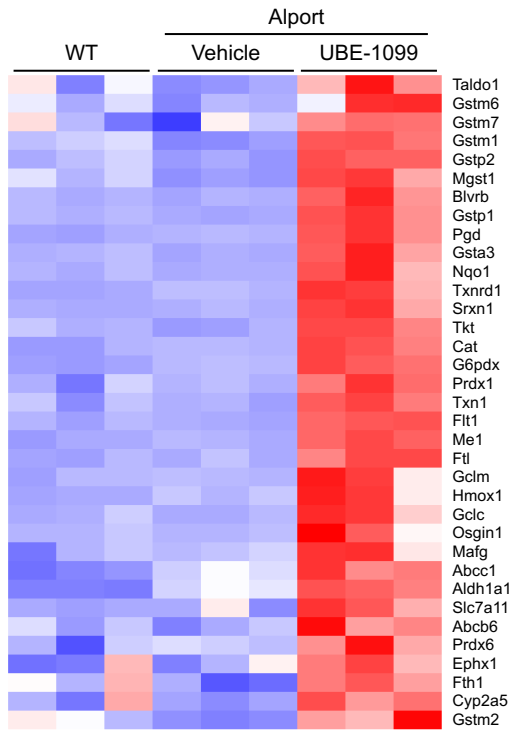


**Figure S8. Down-regulated GO terms for Alport UBE-1099 vs Alport Vehicle**

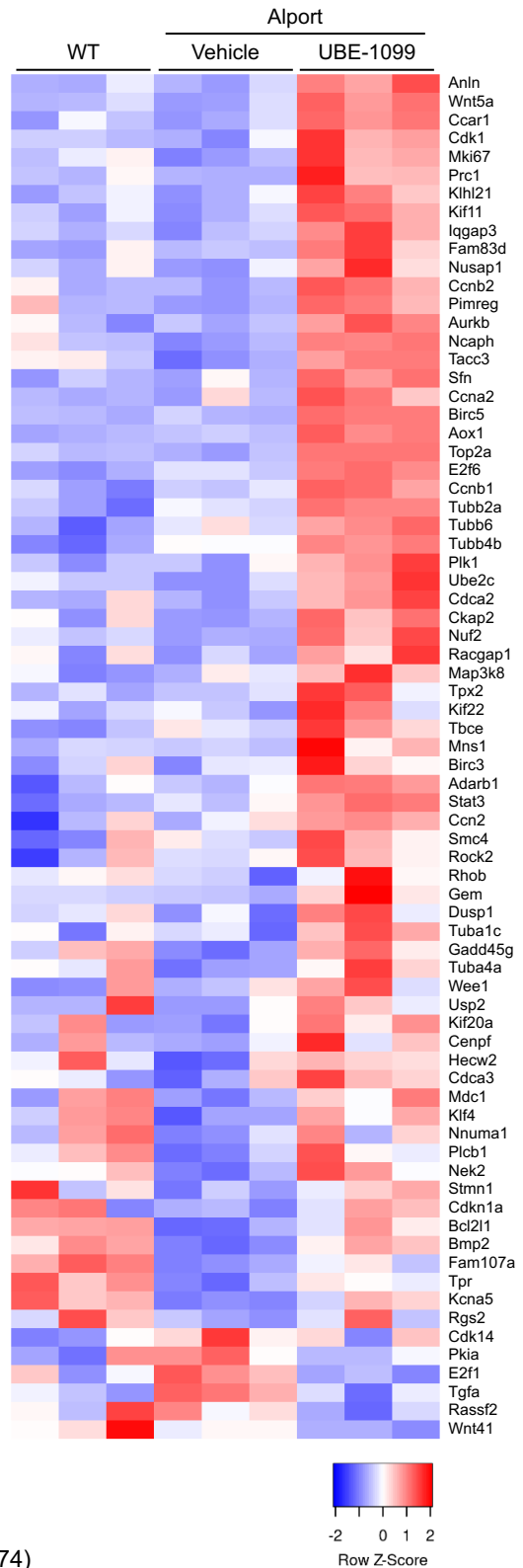
Gene Set Enrichment Analysis (GSEA) data for down-regulated GO terms in Figure 6B. NES = Normalized Enrichment Score.

**Figure S9**

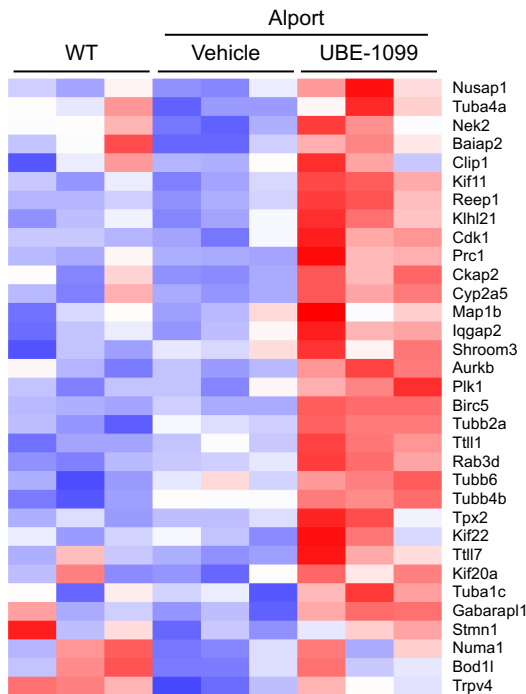
**A** Nrf2 target genes



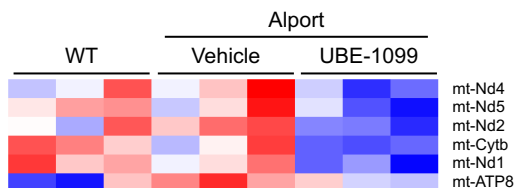
**B** Cell cycle (GO:0007049)



**C** Microtubule (GO:0005874)



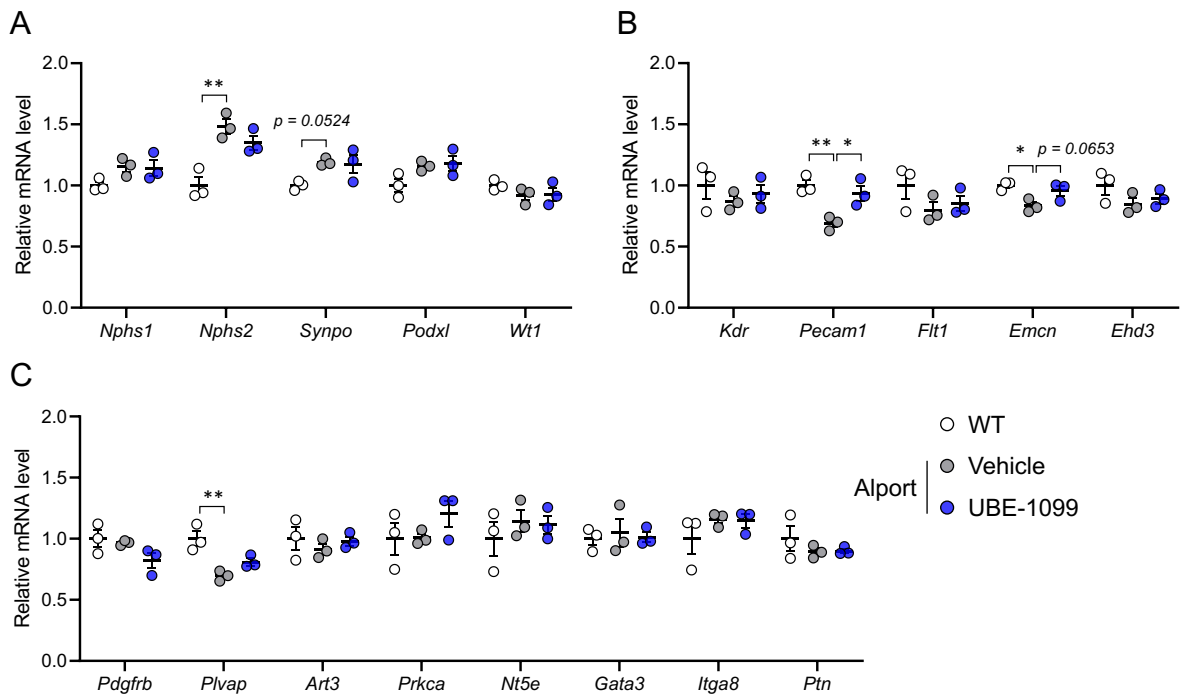
**D** Mitochondrial protein containing complex (GO:0005874)



**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).

**Figure S10**

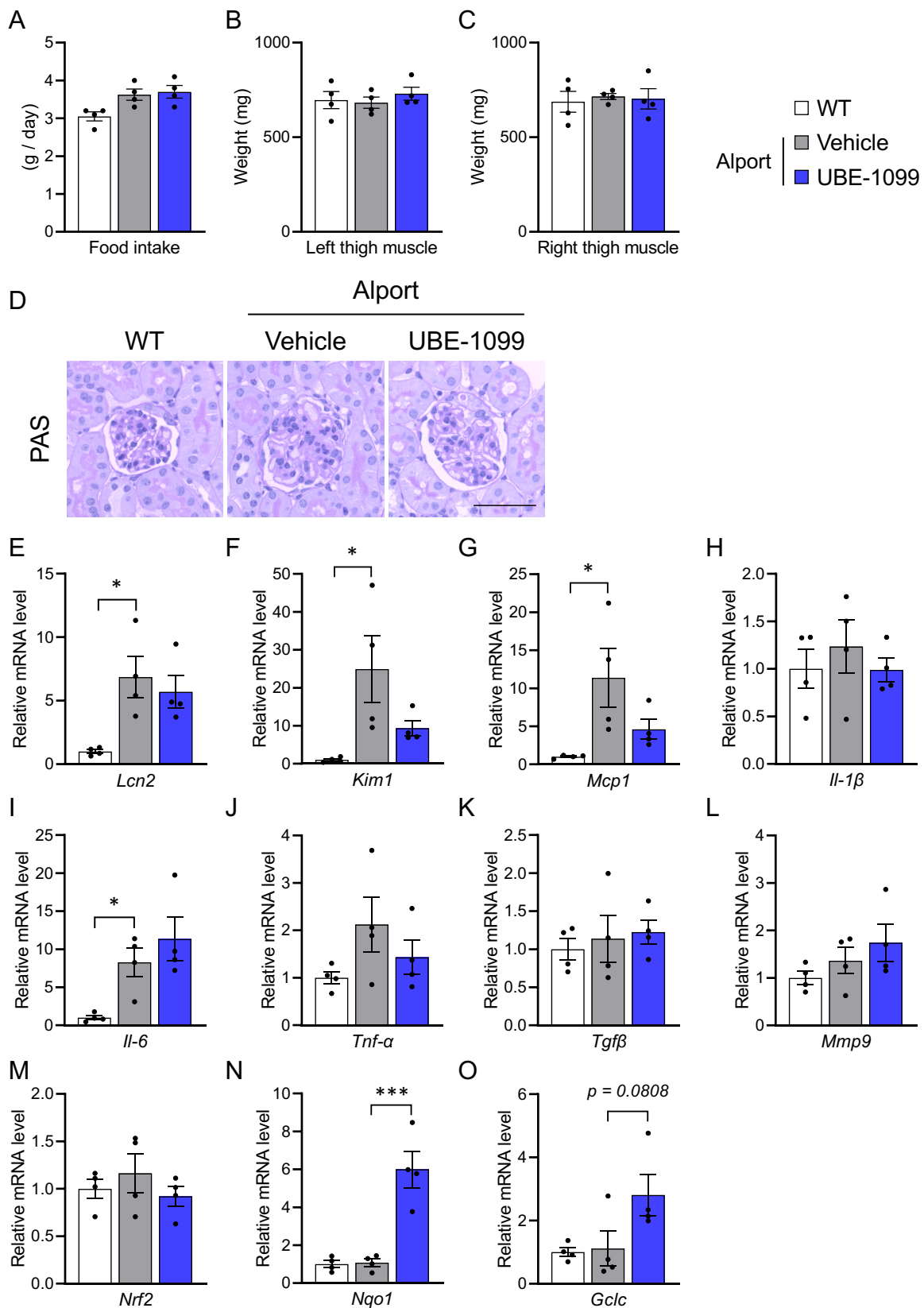


**Figure S10. Expression level of cell specific markers in the glomerular cell**

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. (\* $p < 0.05$ , \*\* $p < 0.01$ ).

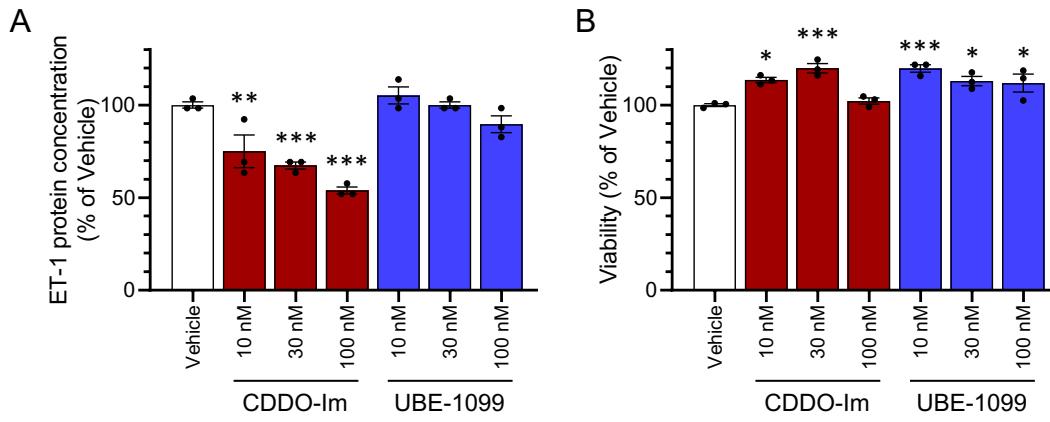


**Figure S11**



**Figure S11. UBE-1099 did not affect the food intake and muscle weight in Alport mice and did not worsen the early renal pathology (A-C)** Food intake and thigh muscle weight were measured at 10 weeks old. (D) Renal sections of 10-week-old wild-type and Alport mice were analyzed by PAS staining. Representative images are shown. Scale bars = 50  $\mu$ m. (E-O) Total RNA was isolated from renal tissues of 10-week-old wild-type and Alport mice. The level of the indicated mRNA was measured and normalized to the level 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**

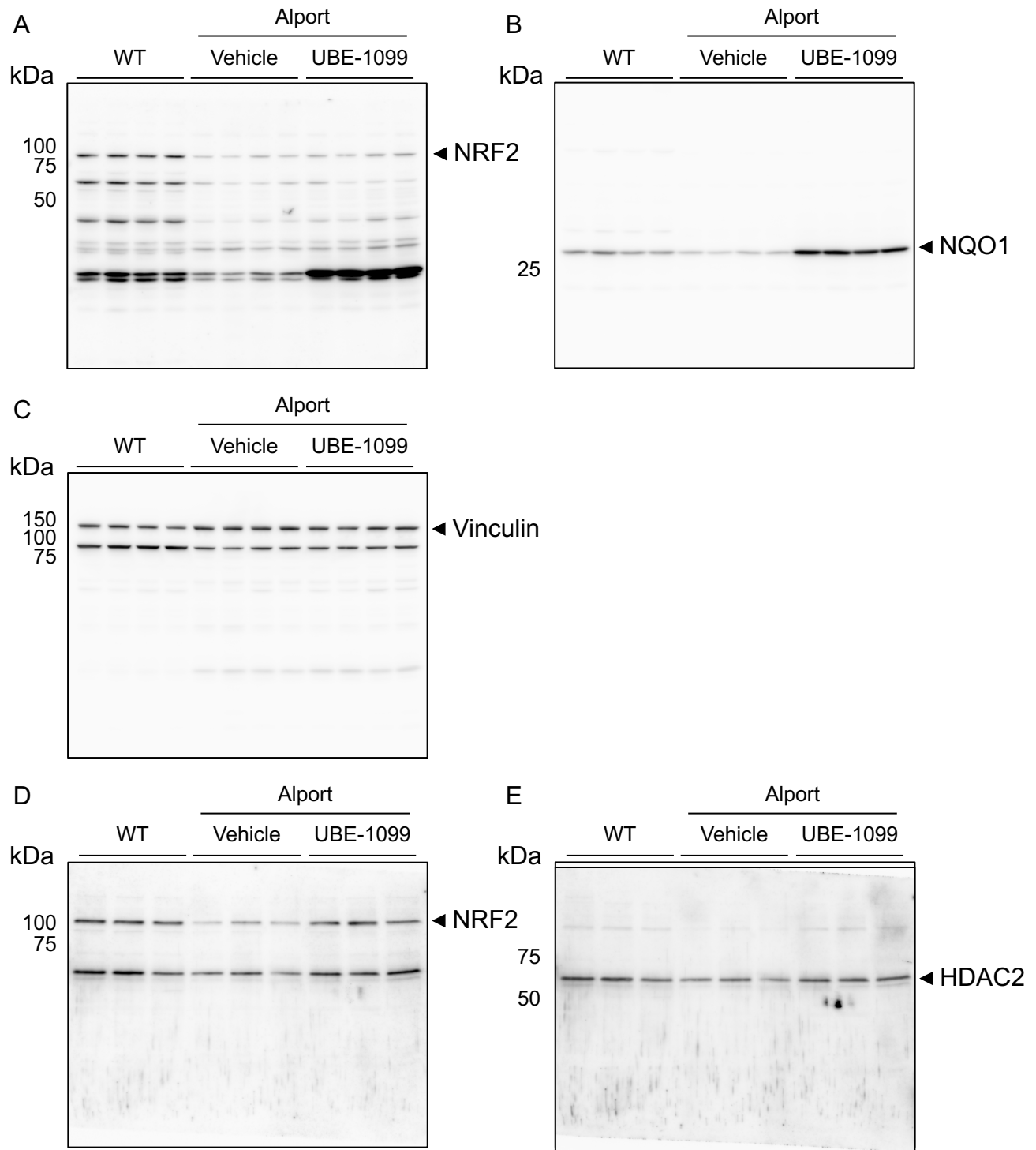


**Figure S12. UBE-1099 did not affect the endothelin expression**

(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**



**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.