Supplementary Materials
Kaseda, S., et al.

Contents

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

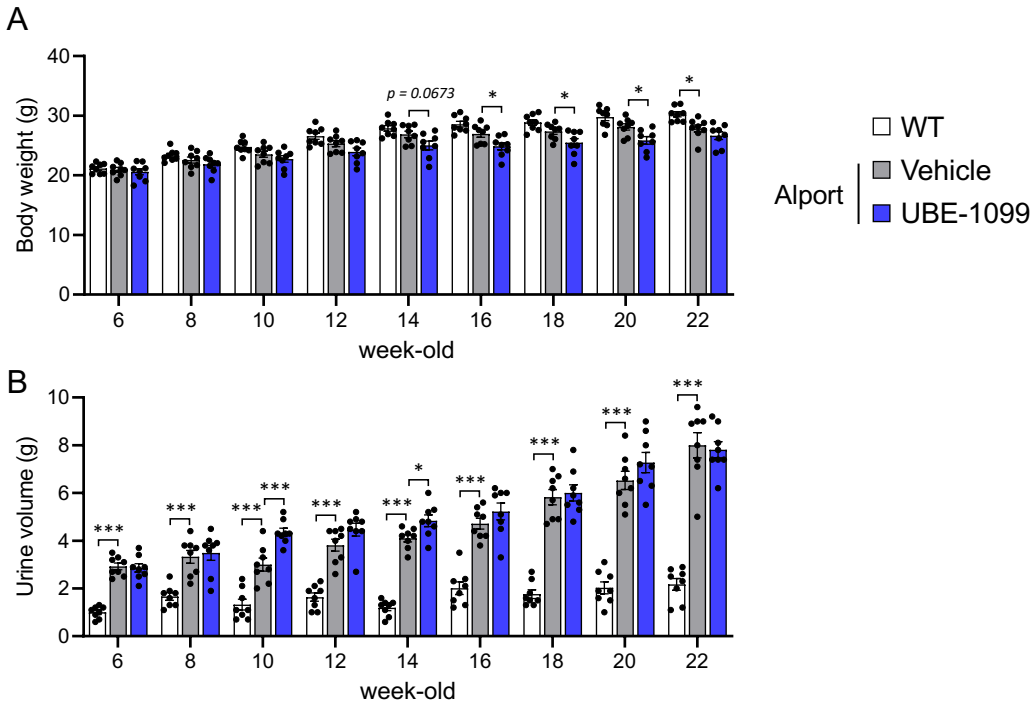

Figure S1. UBE-1099 slightly reduced the body weight and increased the urine volume in Alport mice

(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 ± SE (n = 8 per group). P values were assessed by Dunnett`s test. (*p<0.05, ***p<0.001).

Figure S2

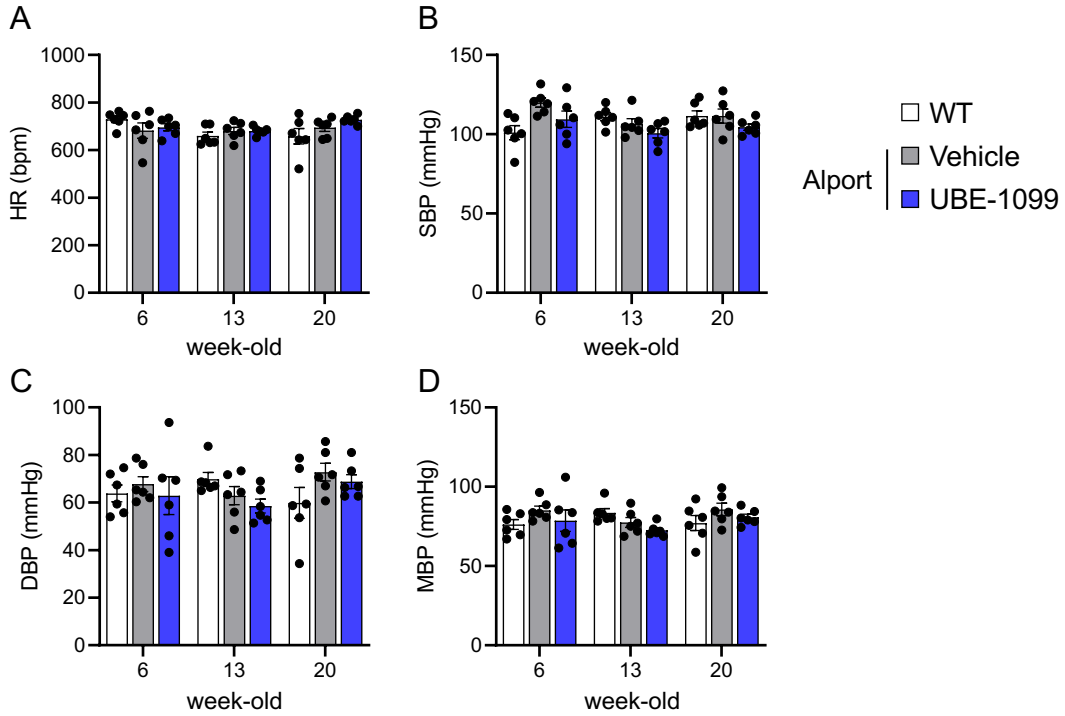

Figure S2. UBE-1099 did not affect the heart rate and blood pressure in Alport mice

(A-D) Heart rate (HR), Systolic blood pressure (SBR), Diastolic blood pressure (DBP) and Mean blood pressure MBP) were measured by BP-98A-l (Softron). Data are presented as mean ± SE (n = 6 per group).

Figure S3

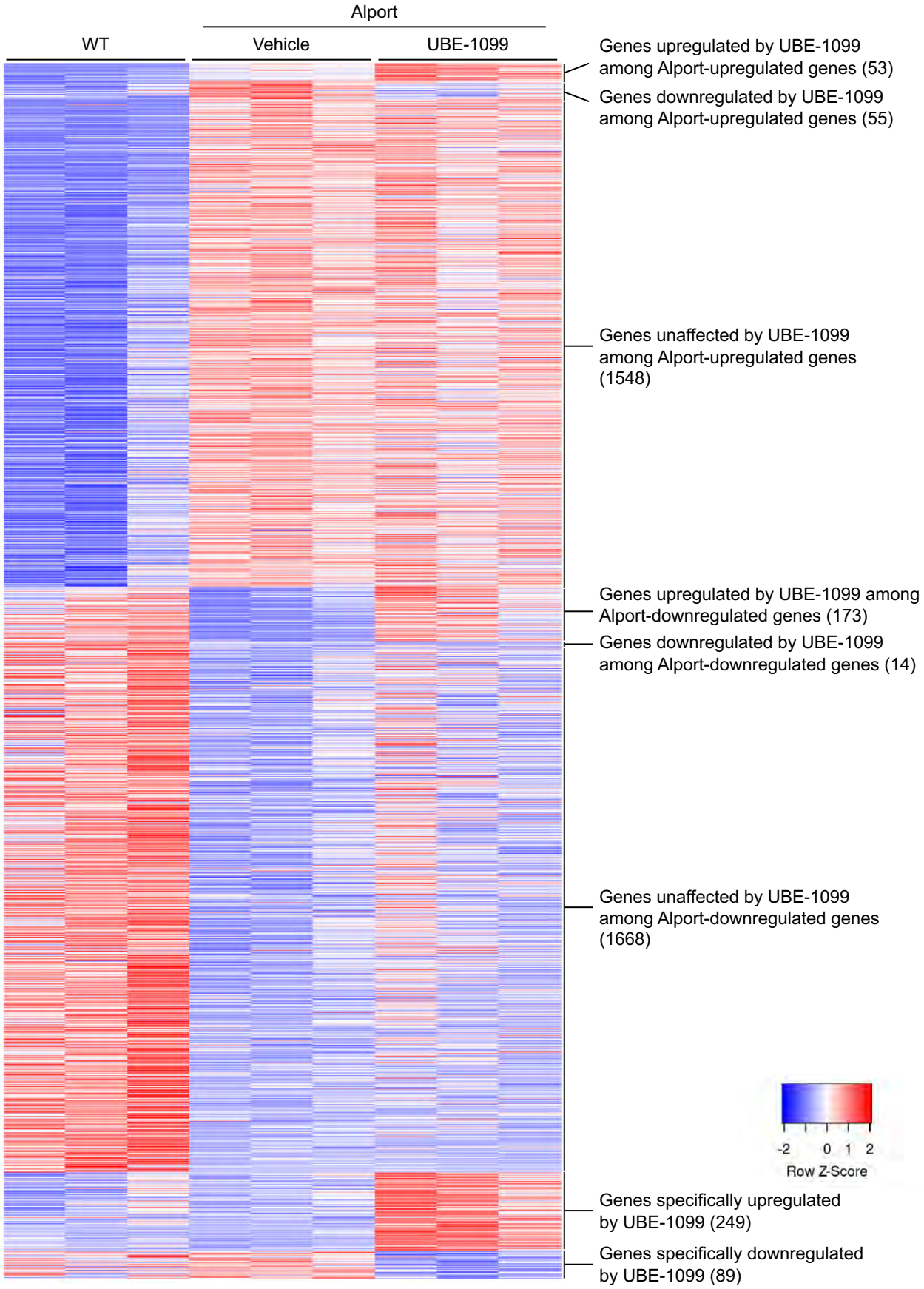

Figure S3. Transcriptome analysis reveals the comprehensive effects of UBE-1099 in the glomeruli of Alport mice

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

Figure S4

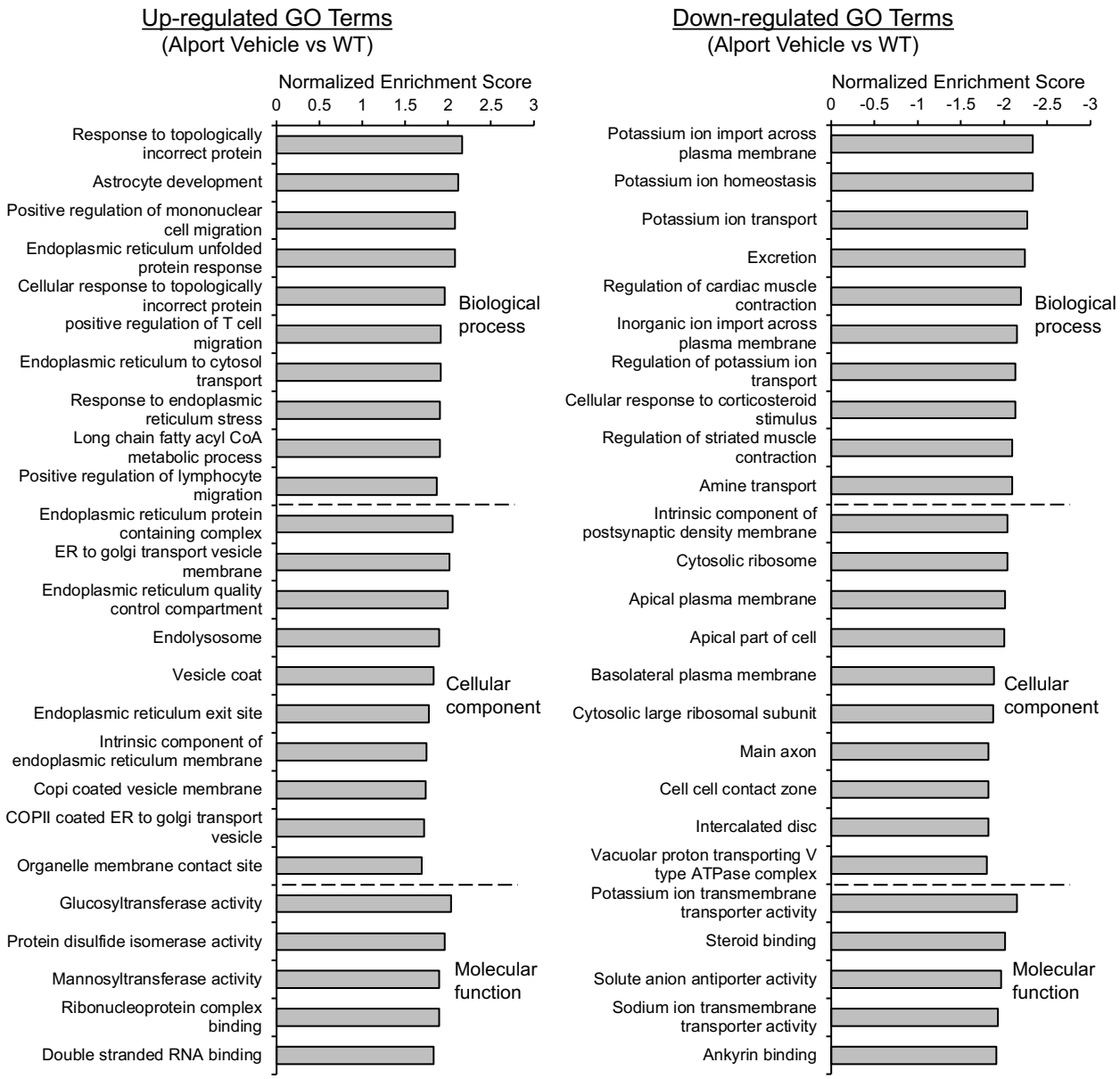

Figure S4. Dysregulated GO terms in the glomeruli of Alport mice

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

Figure S5

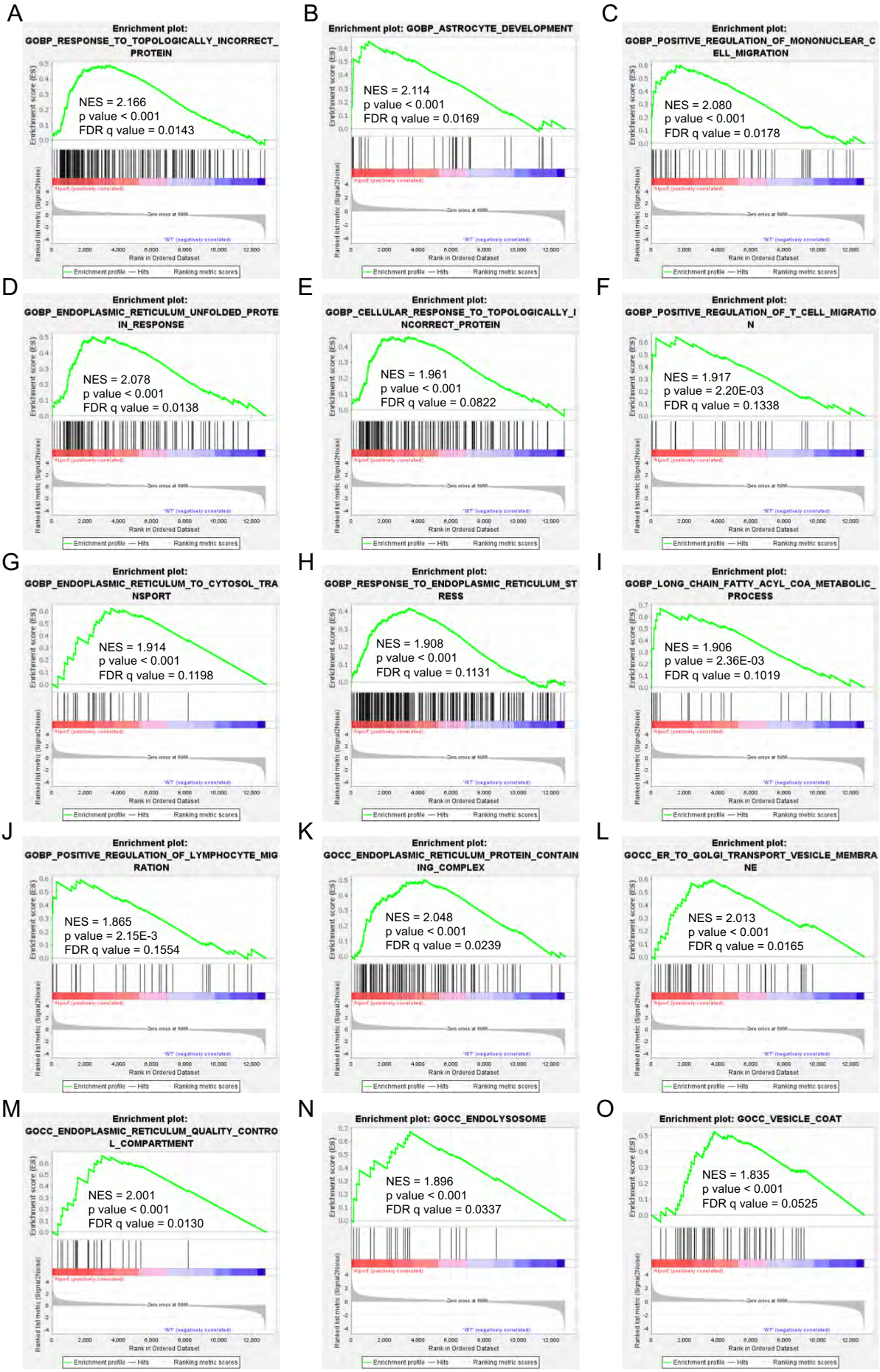

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Figure S5

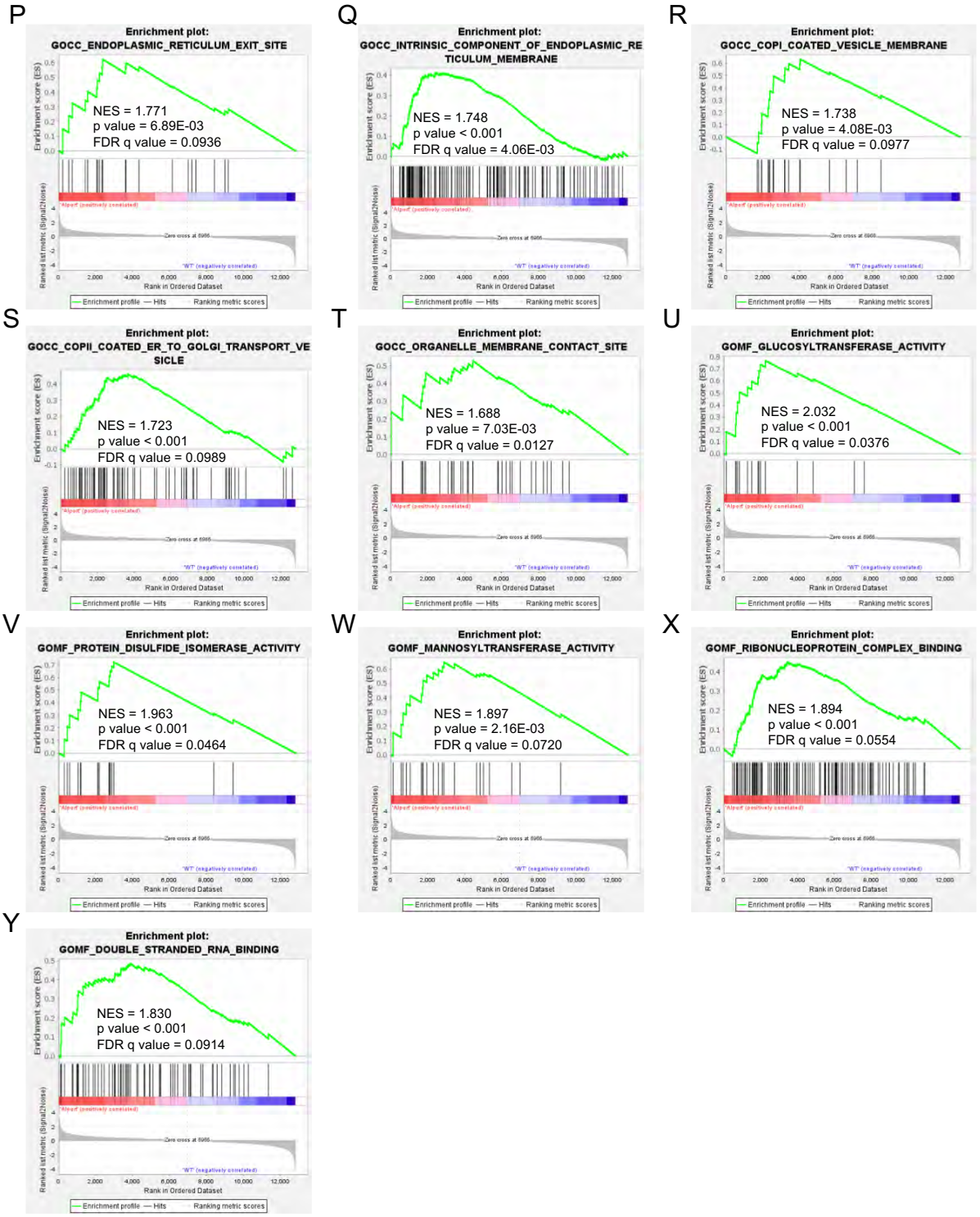

Figure S5. Up-regulated GO terms for Alport vehicle vs WT

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

Figure S6

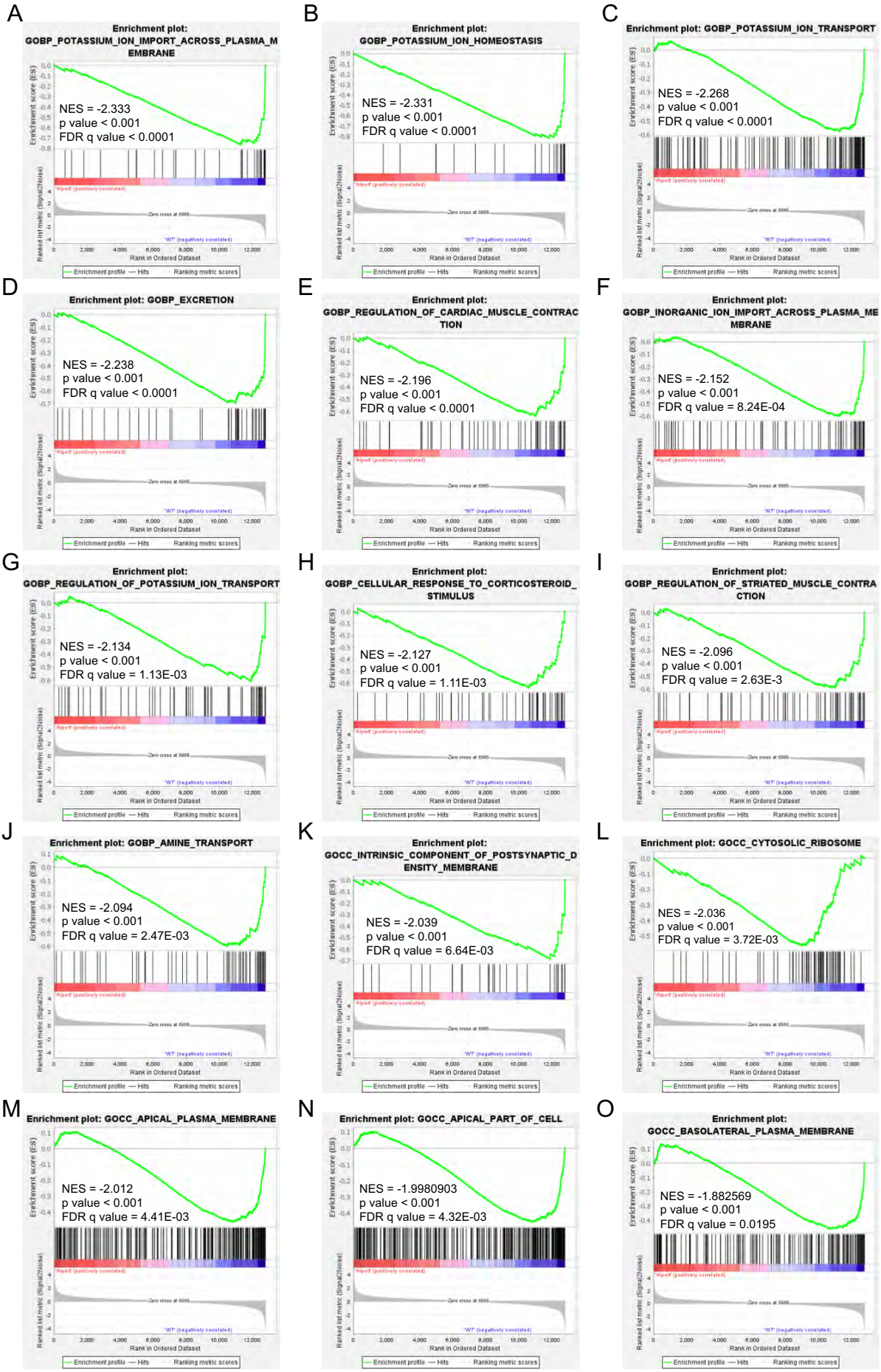

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Figure S6

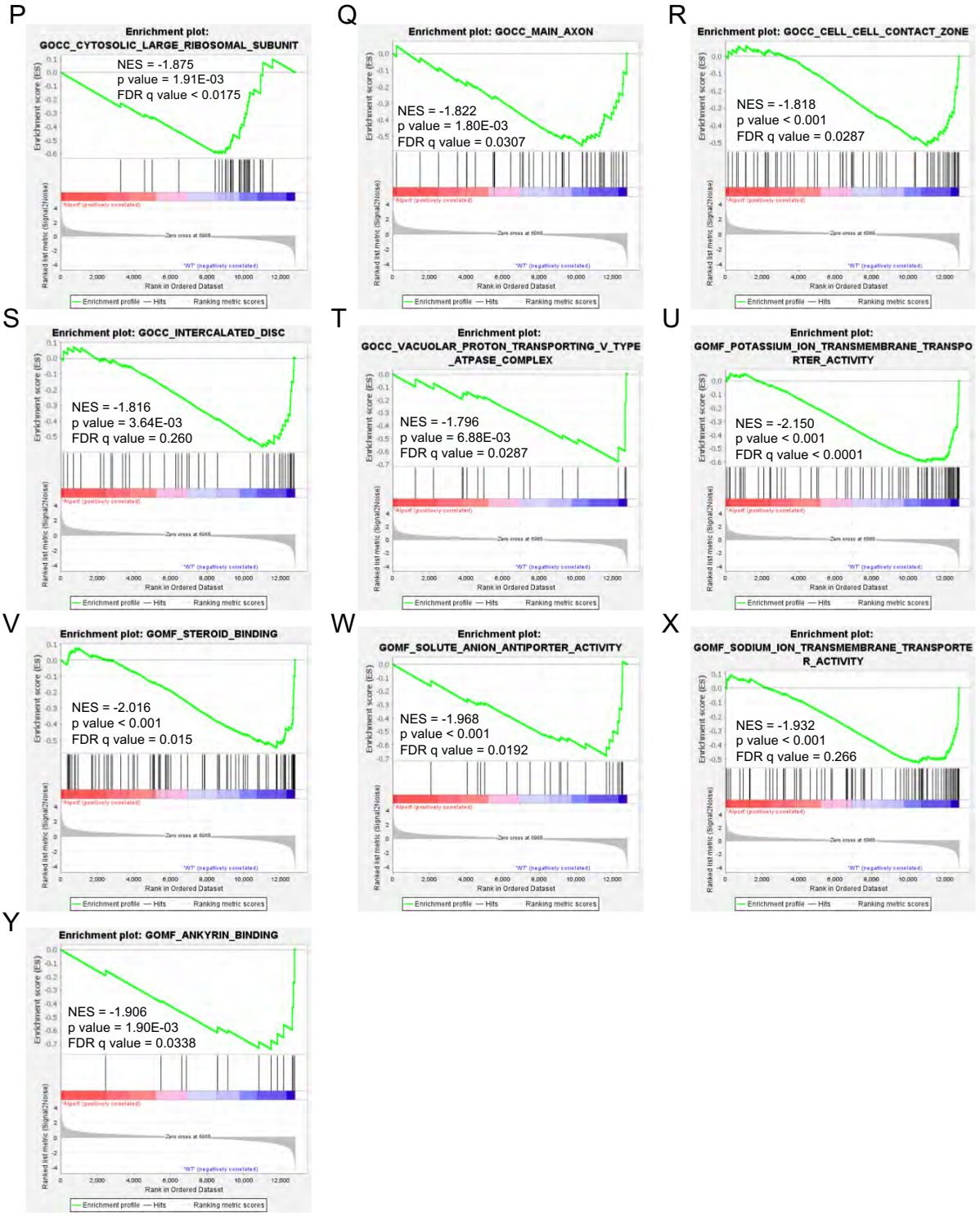

Figure S6. Down-regulated GO terms for Alport Vehicle vs WT

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

Figure S7

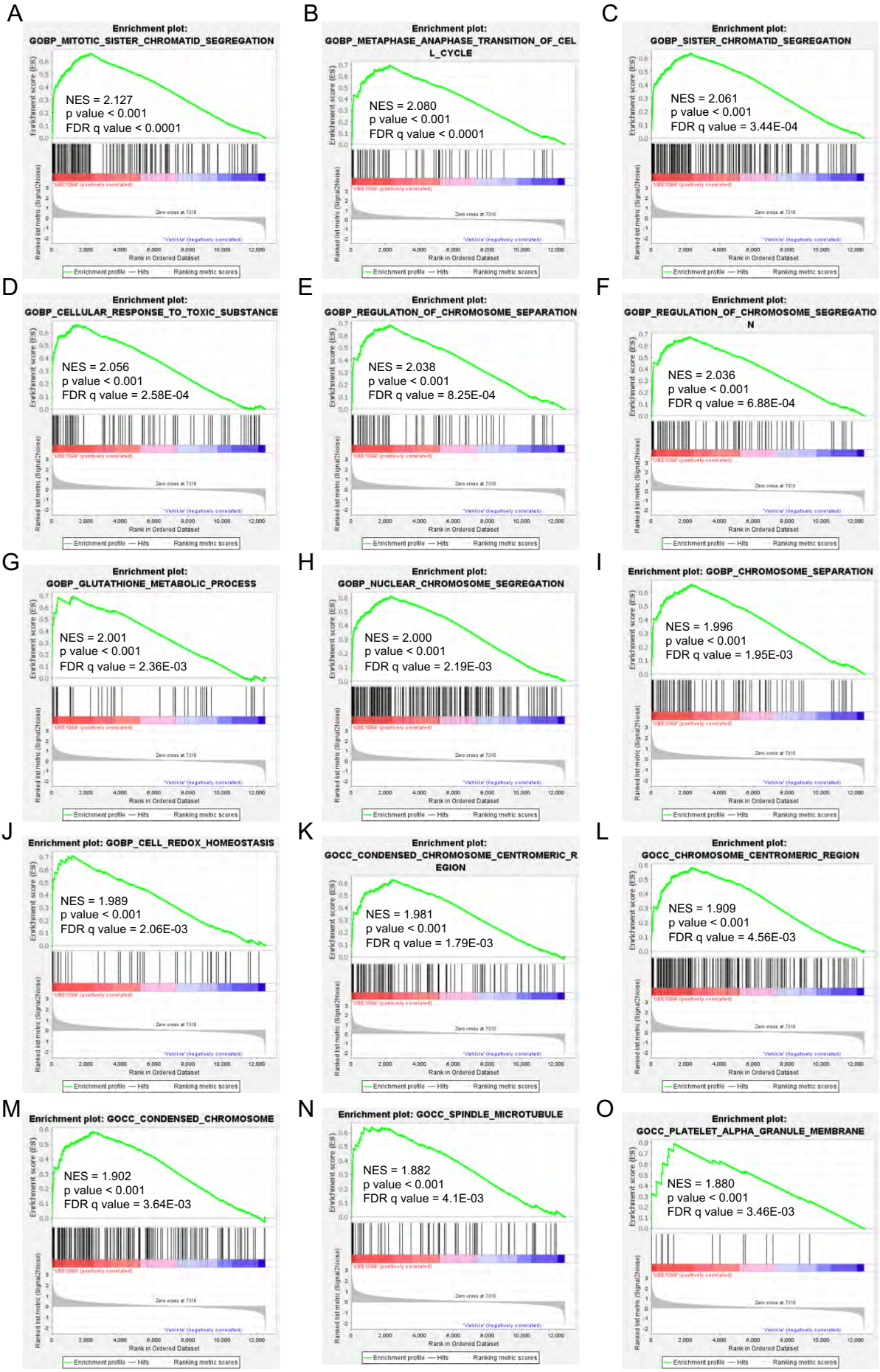

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Figure S7

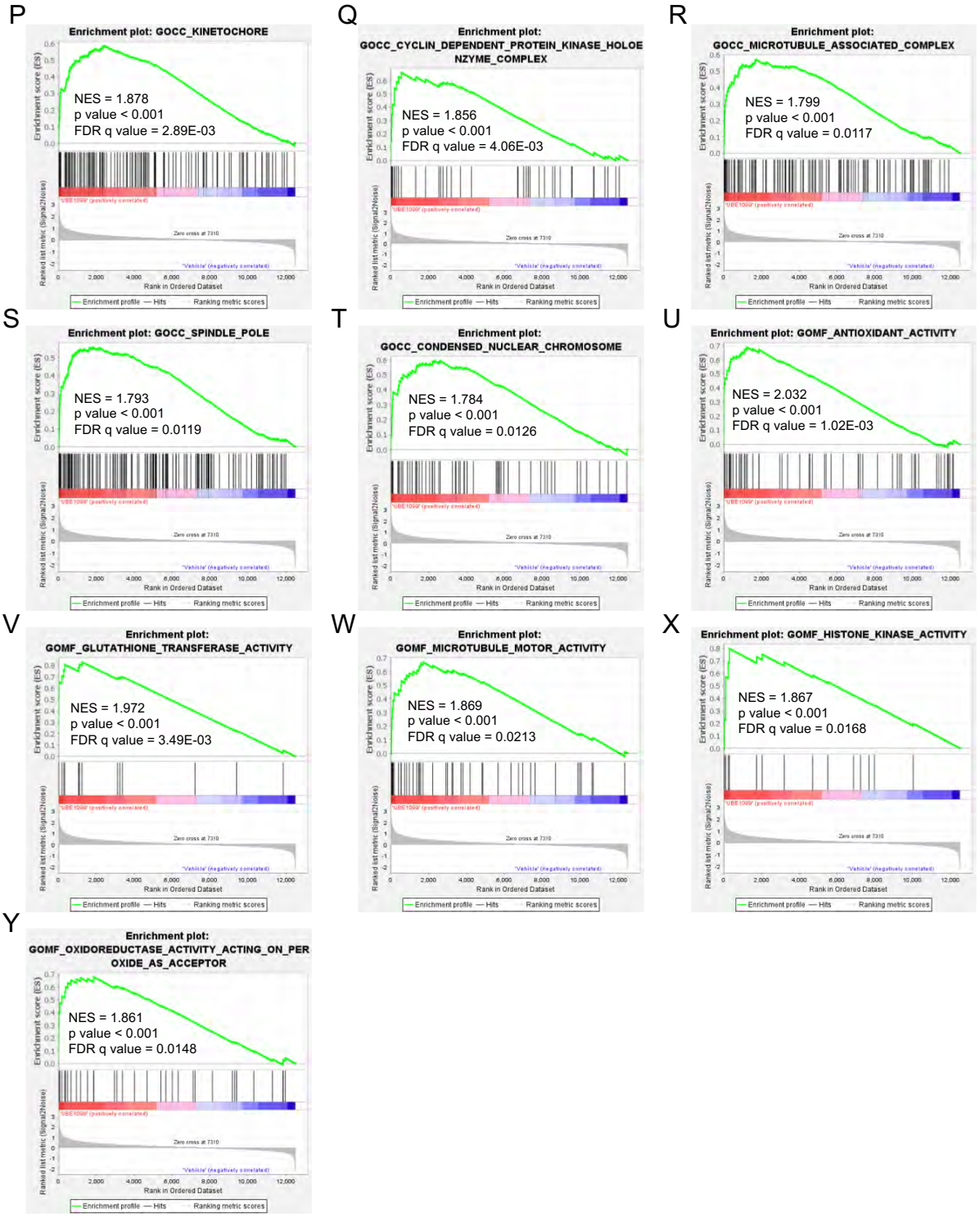

Figure S7. Up-regulated GO terms for Alport UBE-1099 vs Alport Vehicle

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

Figure S8

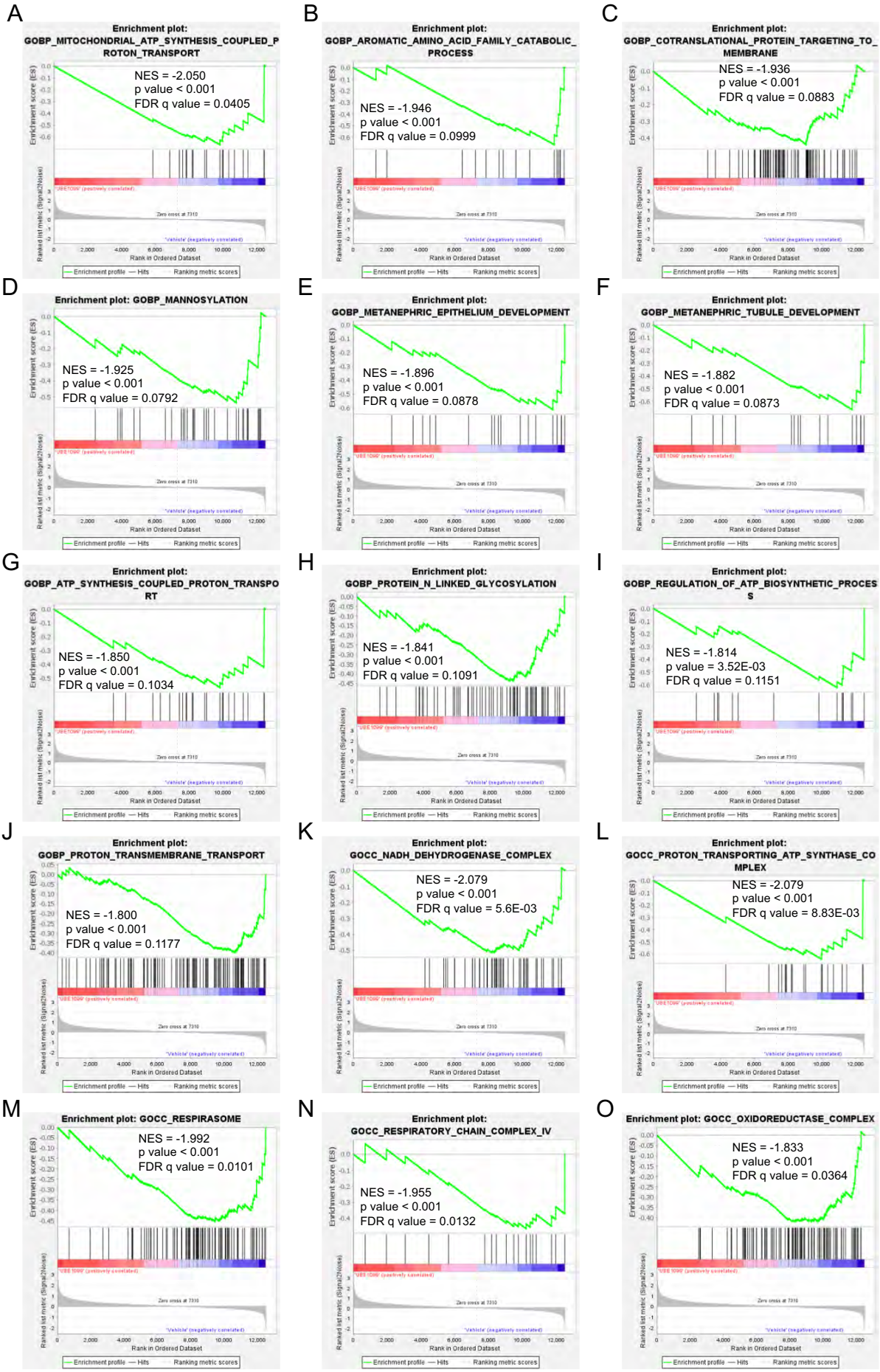

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Figure S8

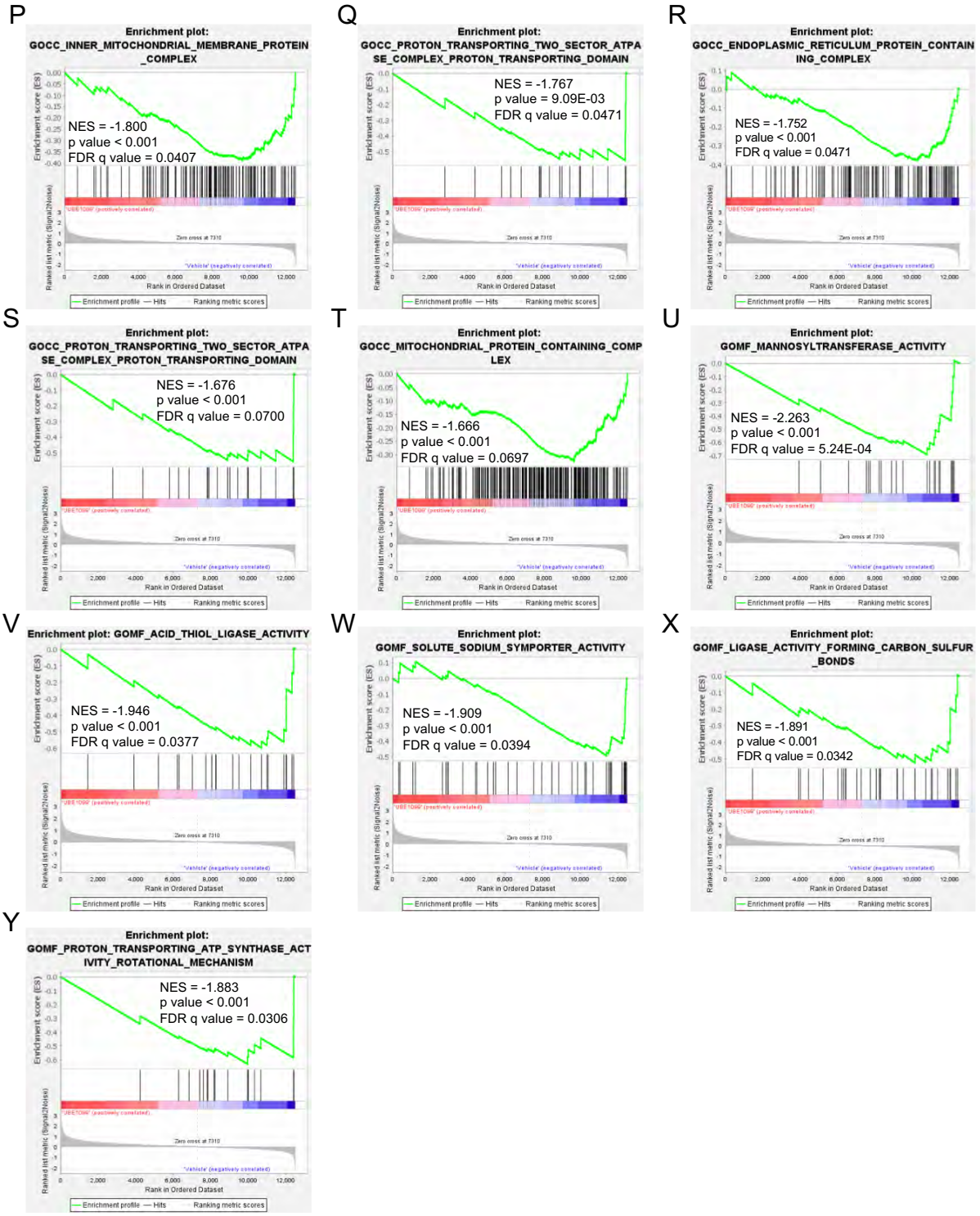

Figure S8. Down-regulated GO terms for Alport UBE-1099 vs Alport Vehicle

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

Figure S9

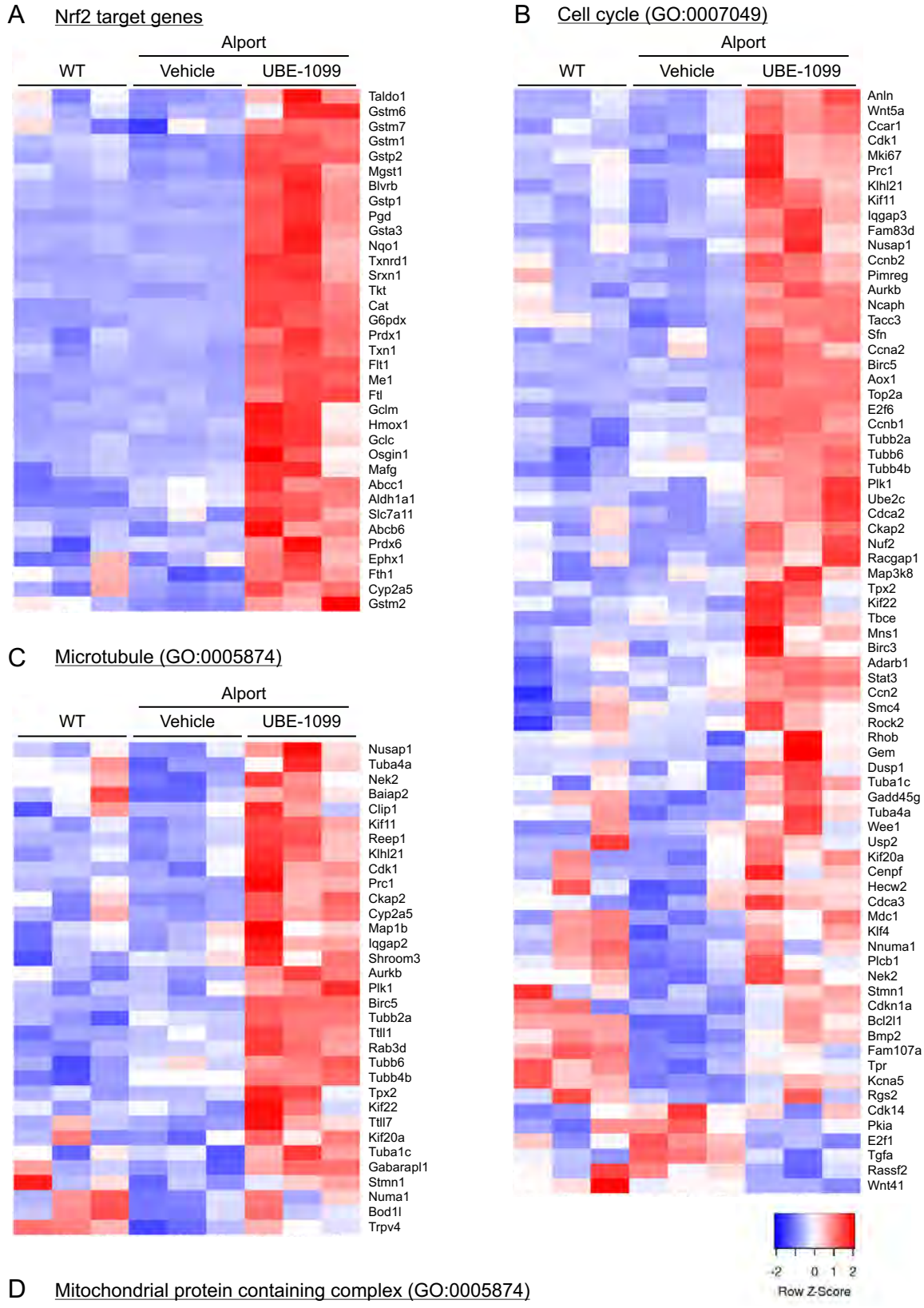

Figure S9. UBE1099 altered genes in each condition

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

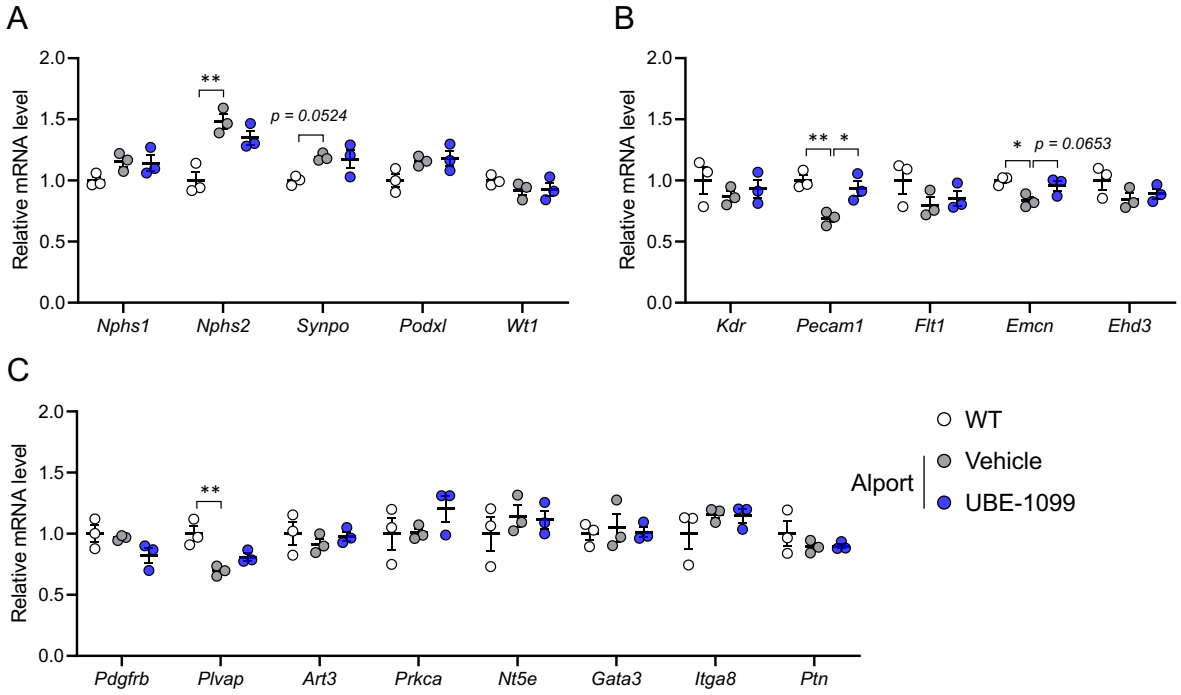

Figure S10. Expression level of cell specific markers in the glomerular cell

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 ± SE (n = 3 per group). P values were assessed by Dunnett's test. (*p<0.05, **p<0. 01).

Figure S11

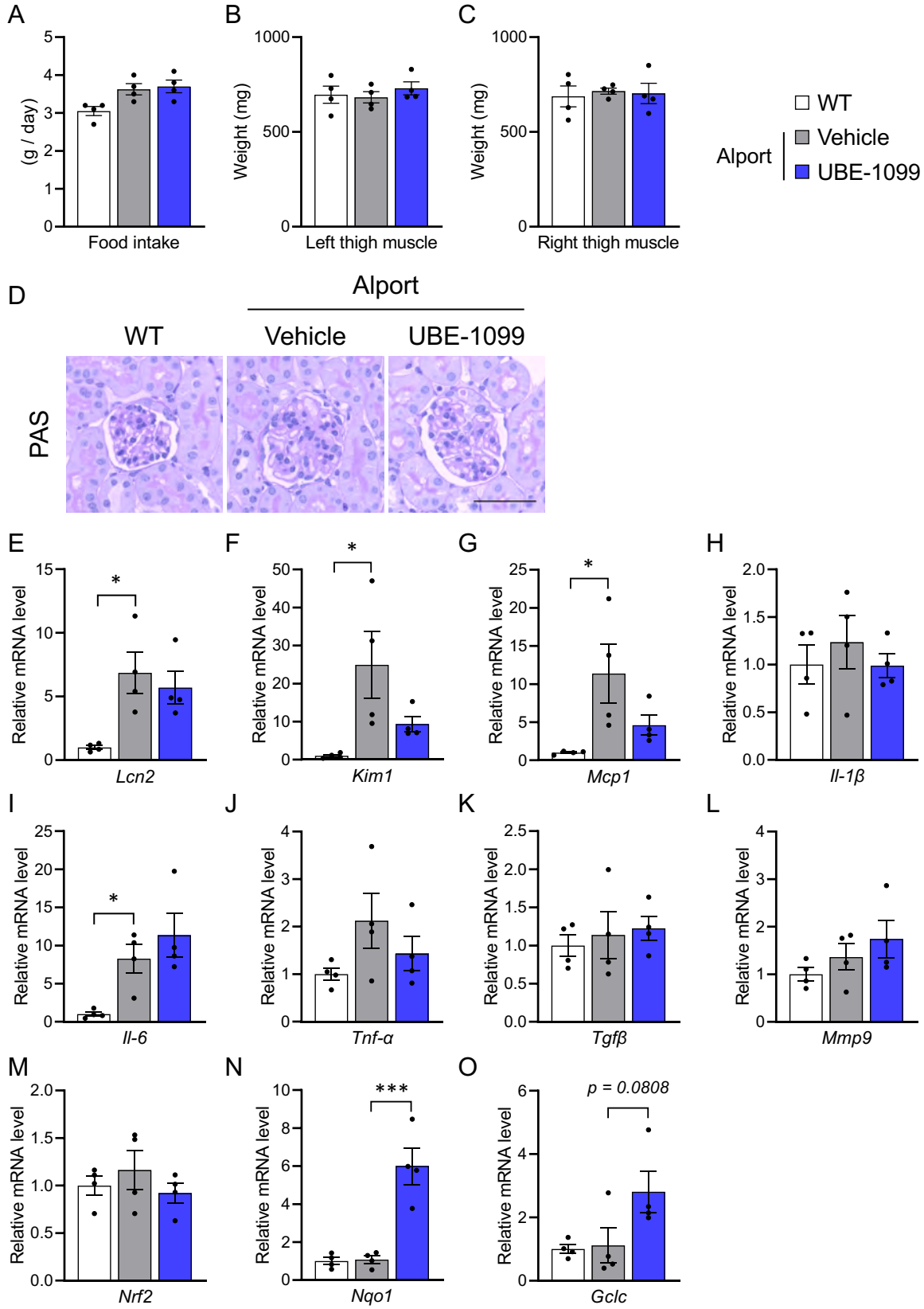

Figure S11. UBE-1099 did not affect the food intake and muscle weight in Alport mice and did not worsen the early renal pathology (A-C) Food intake and thigh muscle weight were measured at 10 weeks old. (D) Renal sections of 10-week-old wild-type and Alport mice were analyzed by PAS staining. Representative images are shown. Scale bars = 50 μm. (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 ± SE (n = 4 per group). P values were assessed by Dunnett's test. (*p<0.05, ***p<0.001).

Figure S12

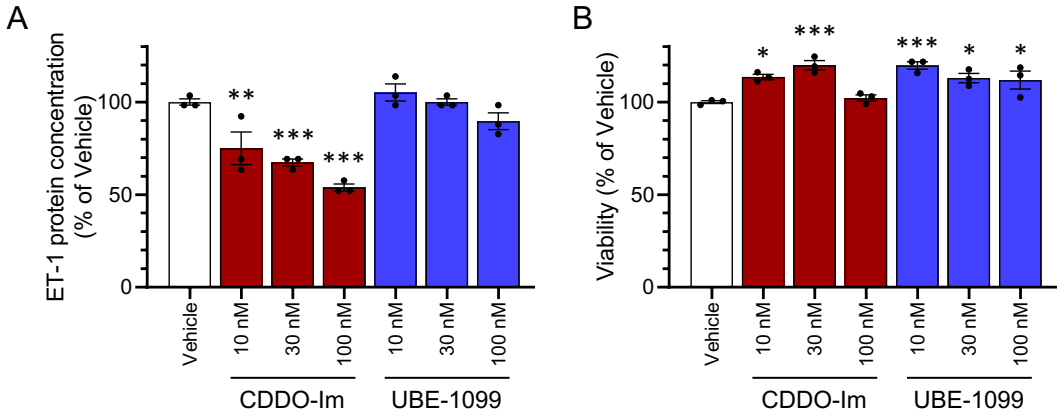

Figure S12. UBE-1099 did not affect the endothelin expression

(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 ± 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

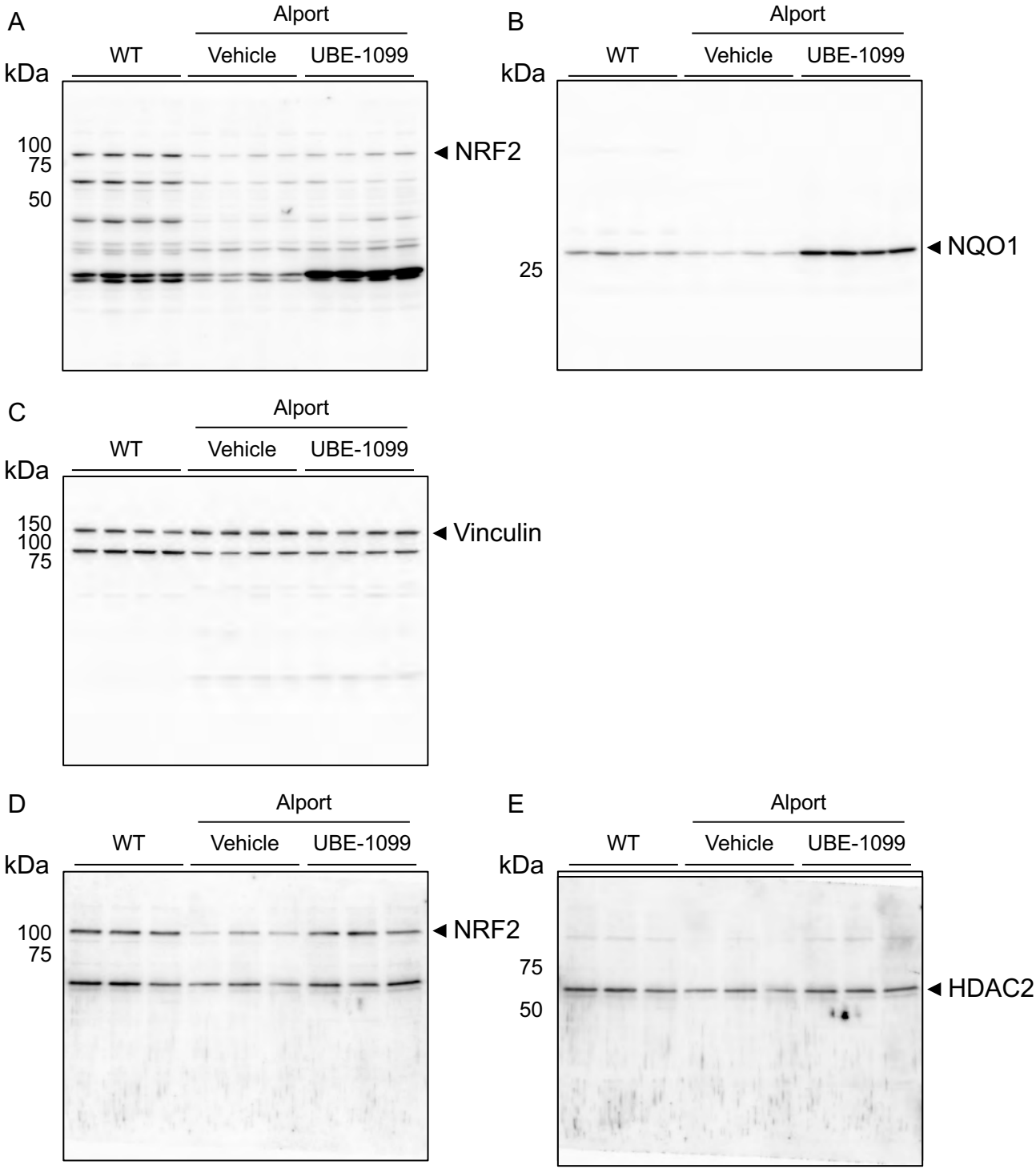

Figure S13. Full length blots for Figure 5A, B

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.