SUPPLEMENTAL MATERIAL

Large-scale proteomic assessment of urinary extracellular vesicles highlights their reliability in reflecting protein changes in the kidney

The following Supplemental Tables are in Excel spreadsheet format:

Supplemental Table 1. All identified and quantified proteins from uEV and kidney under control diet

Supplemental Table 2. All identified and quantified proteins from uEV and kidney under high K^+ diet

Supplemental Table 3. All identified and quantified proteins from uEV and kidney under high K^+ diet (short-term – 2 day)

Supplemental Table 4. Protein list that passed the criteria of abundance RSD<10% (in both uEV and kidney) plus fold change smaller than 5 (between uEV and kidney) under both control and high K^+ diets

Supplemental Table 5. All LFQ quantifiable proteins in uEVs and kidney

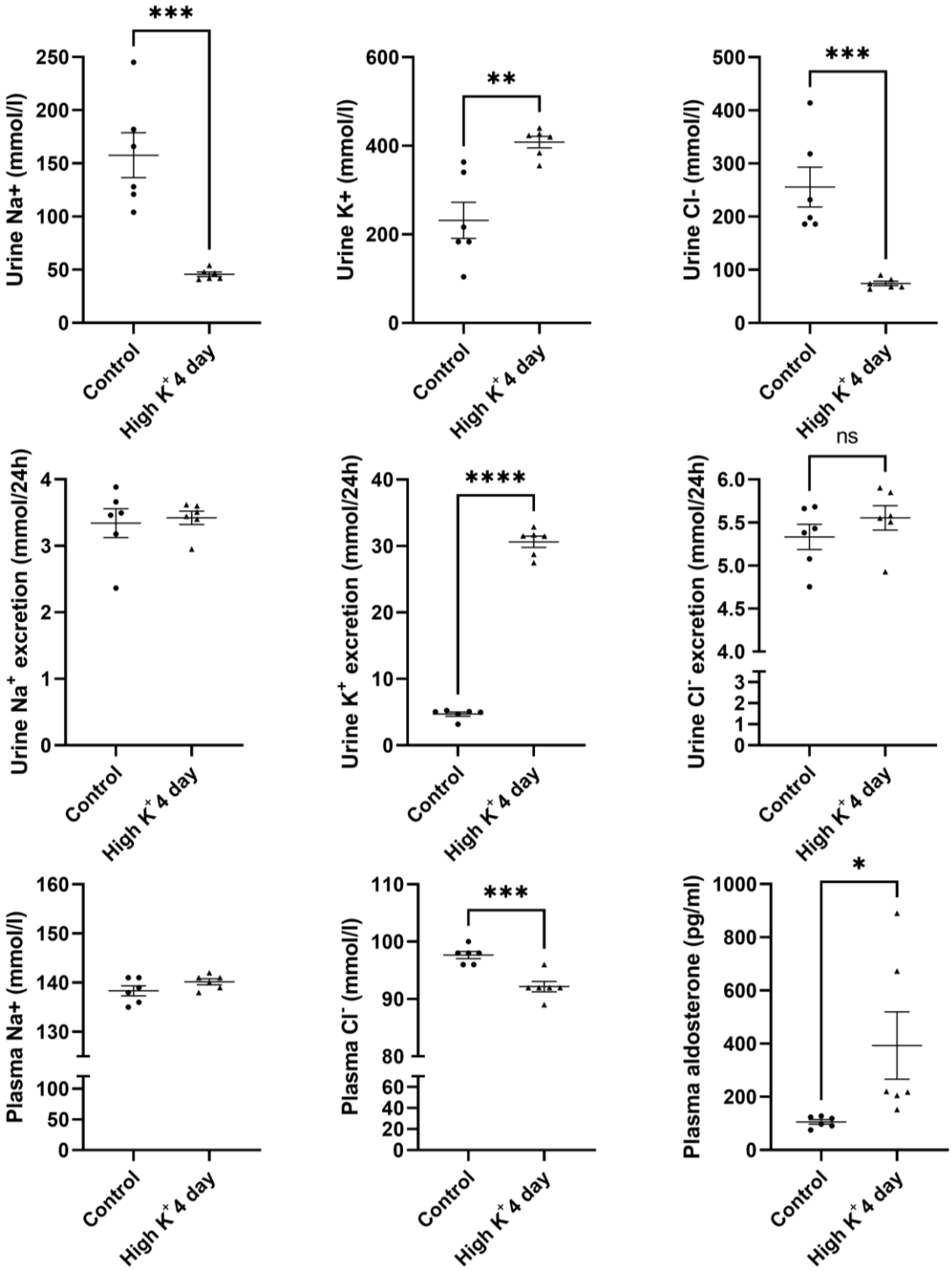

Figure S1. Measured physiological parameters of rats fed either a control diet (0.4% Na^+, 0.8% K^+) or a high K^+ diet (0.4% Na^+, 5% K^+ as potassium citrate) for four days. Urine data are from day 3 – 4 and plasma values are from termination at day 4. Pairwise comparisons of data meeting the statistical assumptions of normality and variance homogeneity were performed using Students two-sided t-test. * p<0.05, ** p<0.01, *** p<0.001, ****p<0.0001.

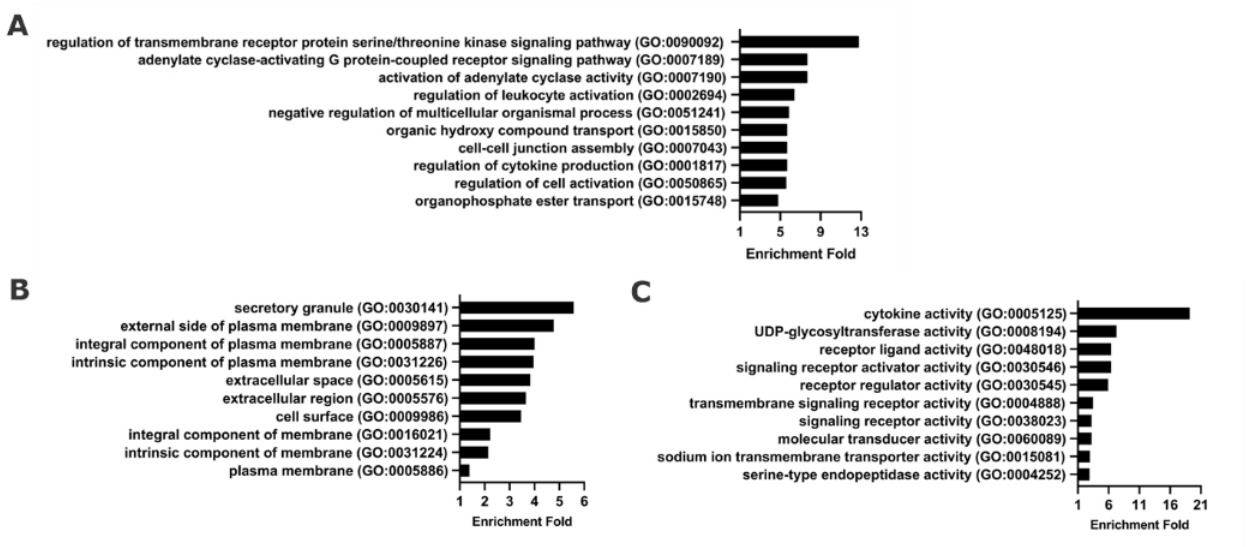

Figure S2. Gene Ontology overrepresentation analysis on uEV abundant proteins under control diet. (A) biological processes; (B) cellular components; (C) molecular functions.

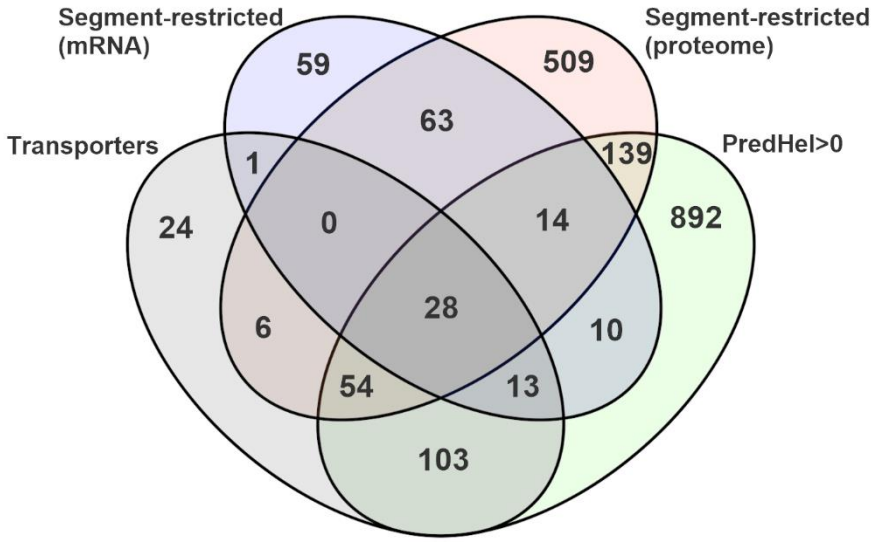

Figure S3. Venn diagram of four categories under control diet: transporter proteins, segment-restricted proteins screened by transcriptomics (mRNA) and proteomics (proteome) databases, and predicted transmembrane proteins (PredHel>0). The algorithm used to predict transmembrane helices may in some cases miss "weak" transmembrane helices, hence explaining why some transporters do not fall into the PredHel>0.

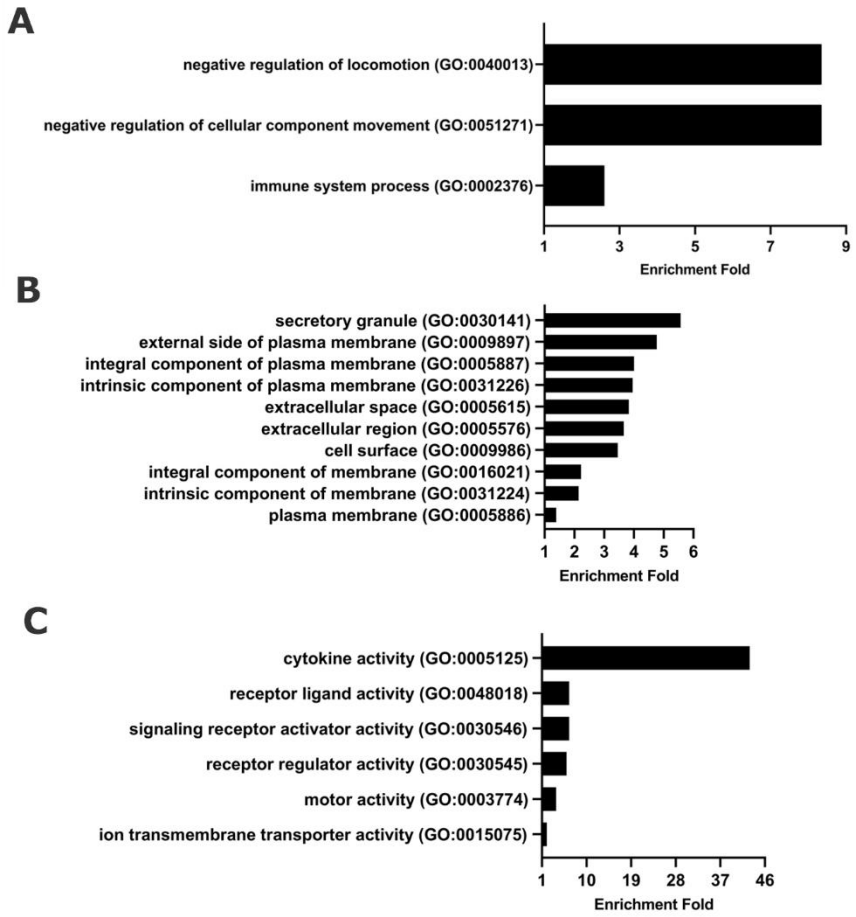

Figure S4. Gene Ontology overrepresentation analysis on uEV abundant proteins under high K^+ diet. (A) biological processes; (B) cellular components; (C) molecular functions.

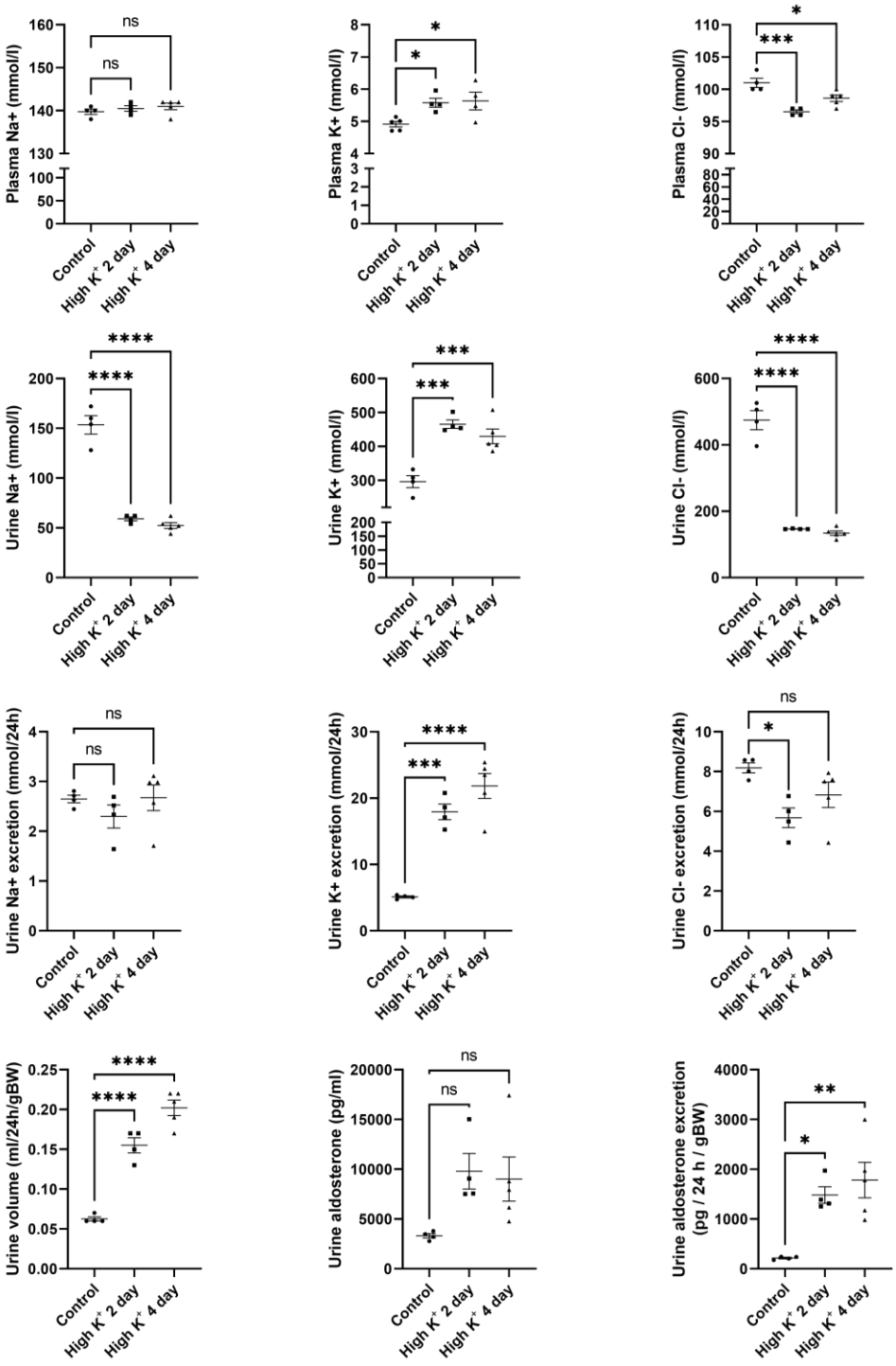

Figure S5. Measured physiological parameters of rats fed either a control (0.3% Na^+, 1.05% K^+) or a high potassium citrate (0.3% Na^+, 5.25% K^+) diet for 2 or 4 days. Urine data are from period 24 - 48 h or 72 - 96 h after start of diet. Plasma values are from termination at day 2 or 4. Comparisons were made using one-way ANOVAs followed by a Tukey multiple comparison test. Each dot represents a sample from an individual rat. * p<0.05, ** p<0.01, *** p<0.001, **** p <0.0001.

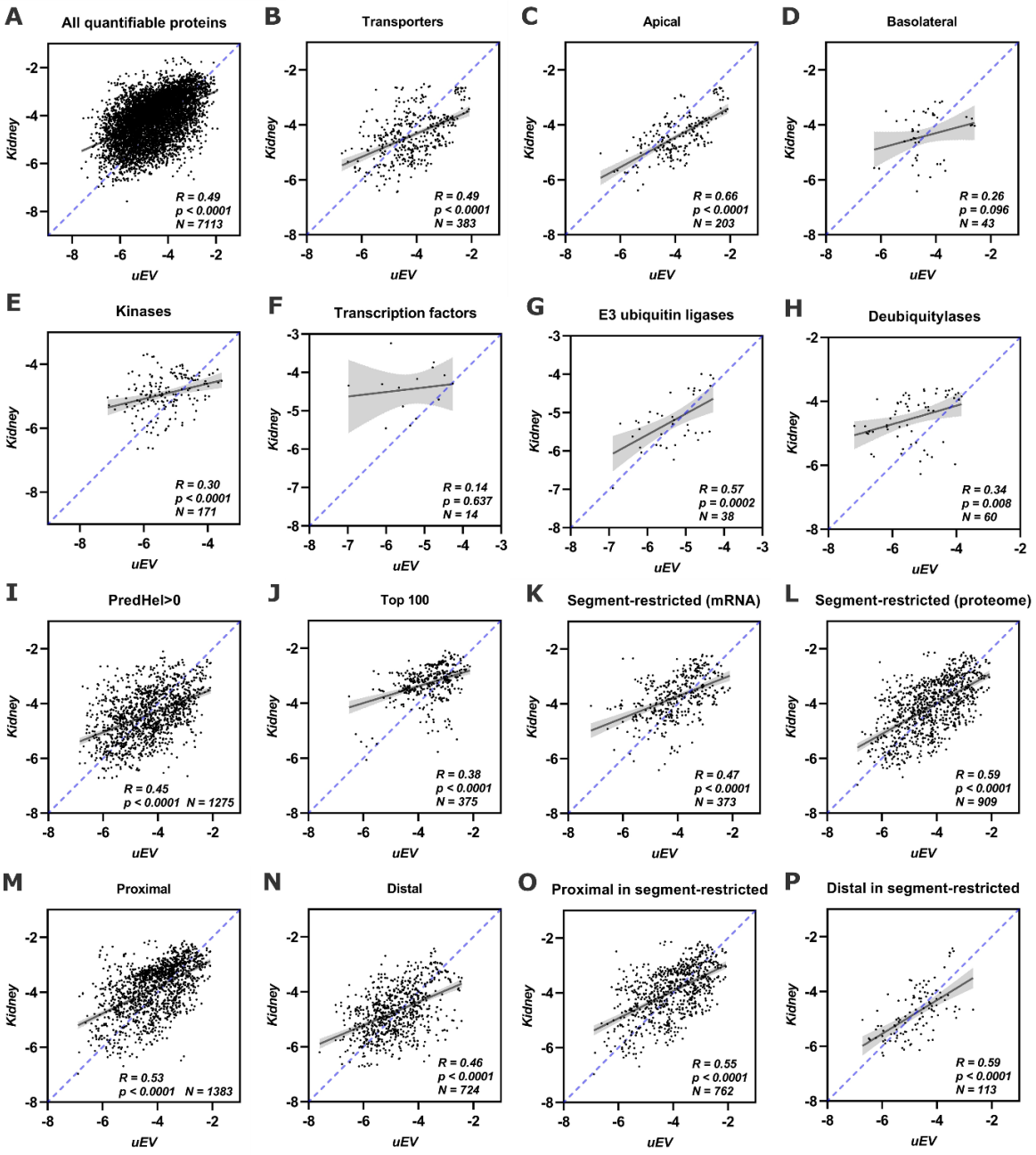

Figure S6. Correlations of absolute protein abundances between uEV and kidney on a short-term high K^+ diet (2 days) showed similar trend to control diet and high K^+ diet (4 days). X axis denotes normalized log10 iBAQ percentages for uEV, while Y axis denotes the same for kidney. R denotes Pearson's correlation coefficient, p denotes the significance of Pearson's correlation, N denotes the number of data points in the figure. The blue dashed diagonal denotes perfect positive correlation, the black line inside the cluster of dots denotes the actual linear regression line, and the grey area denotes the 95% confidence interval.

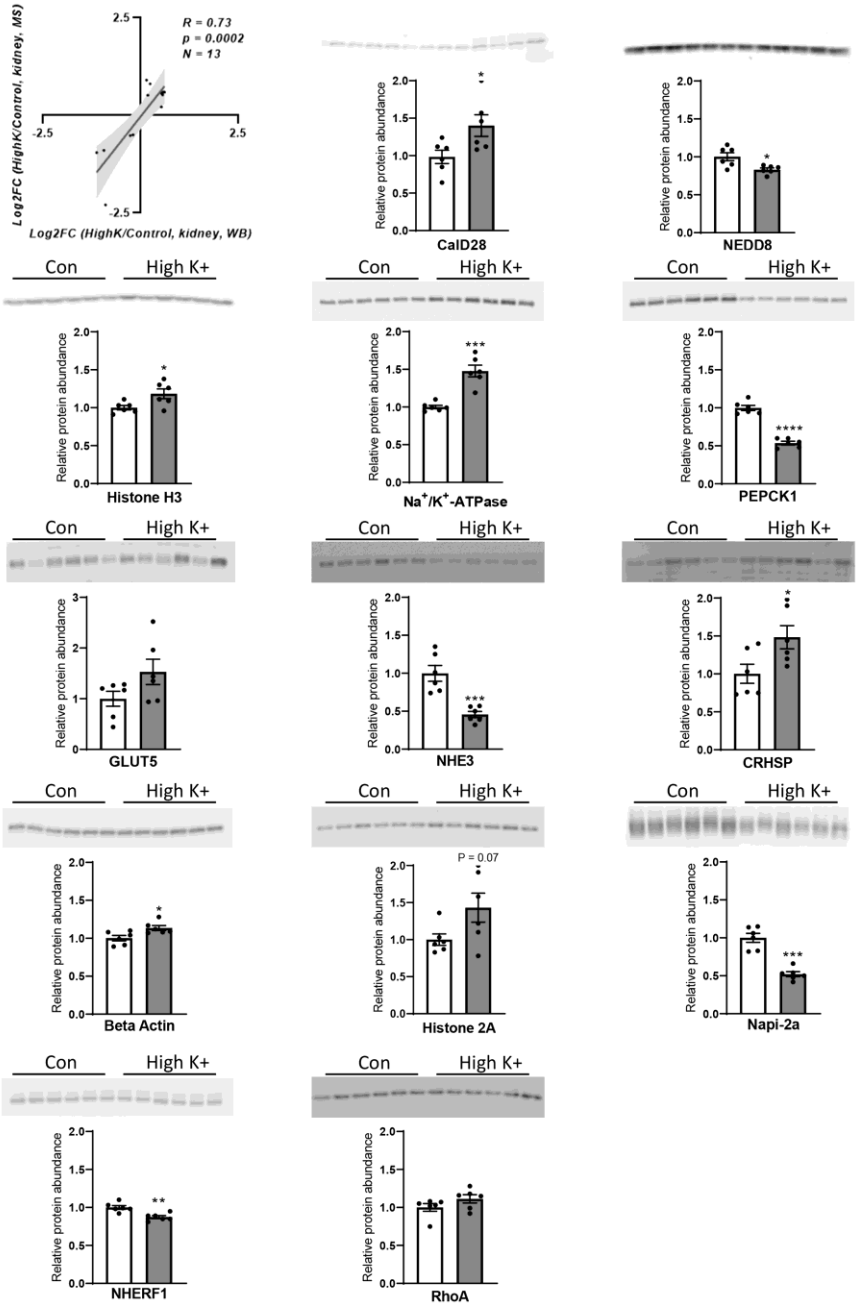

Figure S7. Immunoblotting of 13 randomly selected proteins in kidney samples from rats fed a control or high K^+ diet for 4 days. The top left panel shows the average fold change for each protein observed using mass spectrometry relative to the average fold change detected using immunoblotting. R denotes Pearson's correlation coefficient, p denotes the significance of Pearson's correlation, N denotes the number of data points in the figure. The black line inside the cluster of dots denotes the actual linear regression line, and the grey area denotes the 95% confidence interval. The other individual panels show the representative immunoblots for each protein and the quantification of the results. Pairwise comparisons of data meeting the statistical assumptions of normality and variance homogeneity were performed using Students two-sided t-test. * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

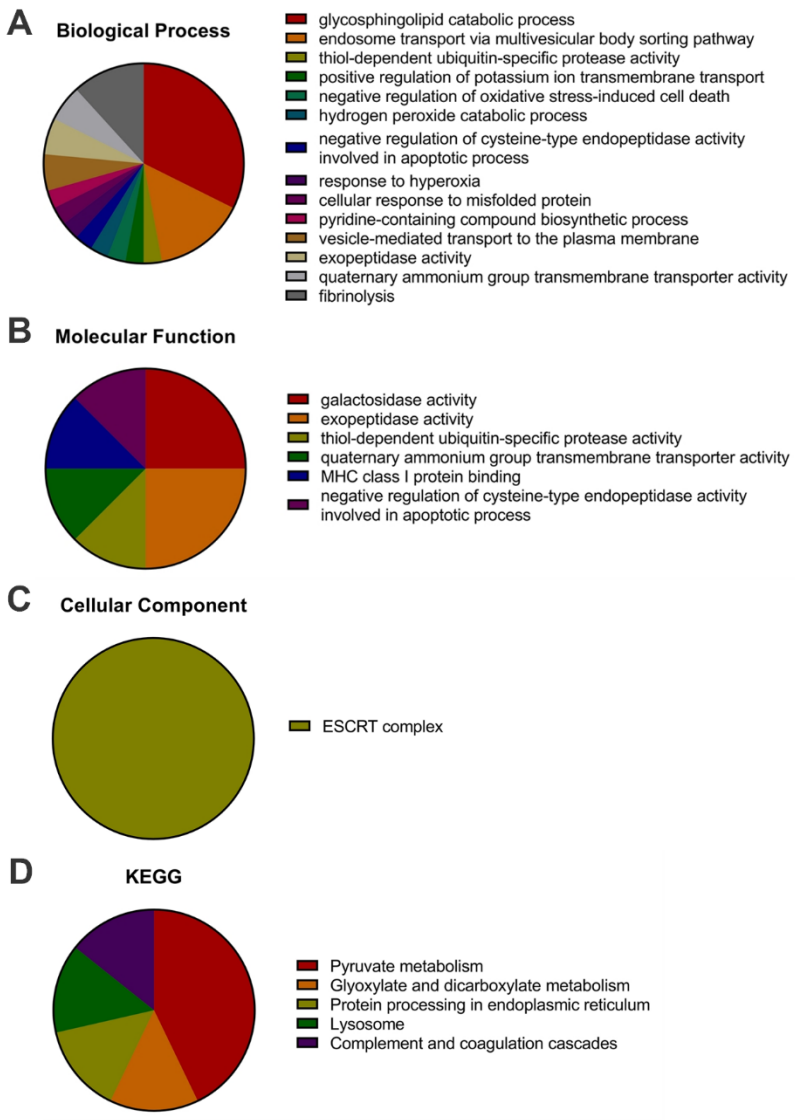

Figure S8. Gene Ontology functional analysis on proteins that have large changes in uEVs but small changes in kidney. (A) biological processes; (B) cellular components; (C) molecular functions; (D) KEGG pathways.

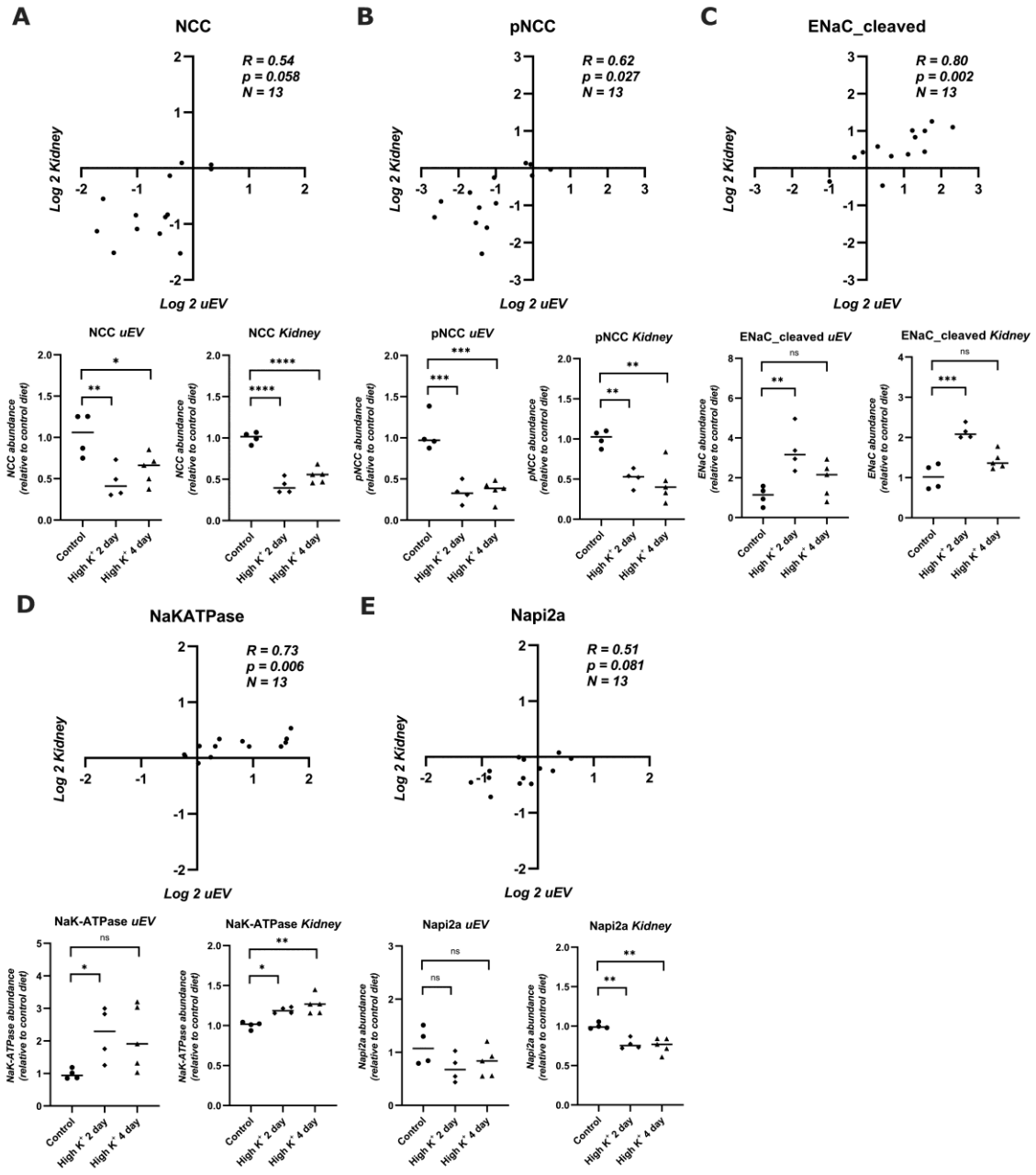

Figure S9. Correlation of protein abundances in uEV and kidney following dietary K^+ manipulation. Abundances of the sodium chloride cotransporter NCC (A), the active phosphorylated form of NCC (pT58-NCC) (B), the cleaved (active) form of the epithelial sodium channel αENaC (C), the α subunit of the Na-K-ATPase (D) and the sodium/phosphate cotransporter NaPi-2a (E) were assessed in uEVs and kidney samples isolated from control or high K^+ diet fed rats using immunoblotting. For correlation analysis, axes denote normalized log2 densitometry values for proteins in uEV or kidney, R denotes Spearman's correlation coefficient, p denotes the significance of Spearman's correlation and N denotes the sample size. Values <1 or >1, indicate a reduction or an increase in protein abundance with the high dietary K^+ manipulation, respectively. For dot plots, the relative change in protein abundance in uEV or kidney is shown. Comparisons were made using one-way ANOVAs followed by a Tukey multiple comparison test. Each dot represents a sample from an individual rat. * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.

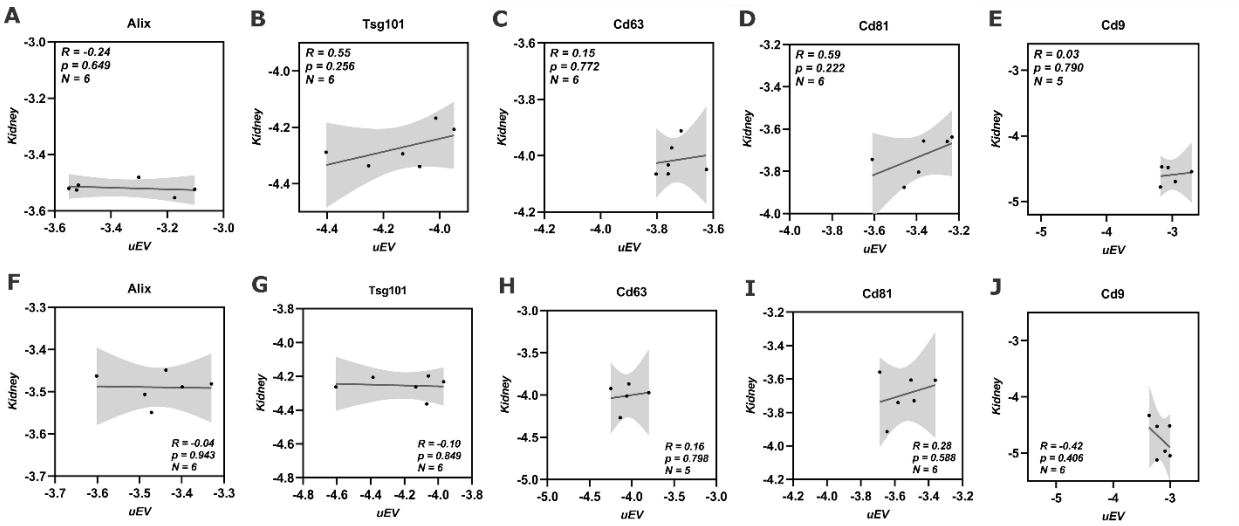

Figure S10. Correlations of the individual absolute protein levels for five well establish uEV marker proteins in six rats. A-E: normal diet; F-J: high K^+ diet. R denotes Pearson's correlation coefficient, the black line inside the cluster of dots denotes actual linear regression line, and the grey area denotes 95% confidence interval. X axis denotes normalized log10 iBAQ percentages for uEV, while Y axis denotes the same for kidney.