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Supplemental information

**Integrated glycoproteomic characterization
of clear cell renal cell carcinoma**

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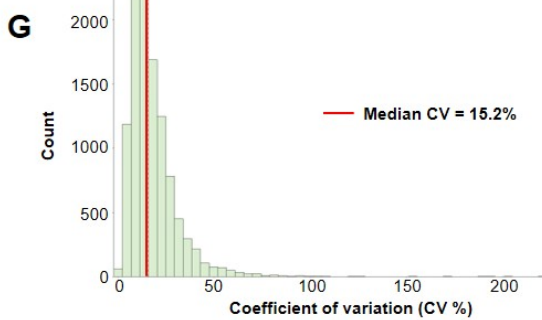
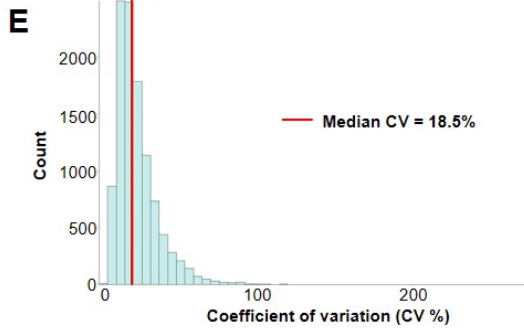
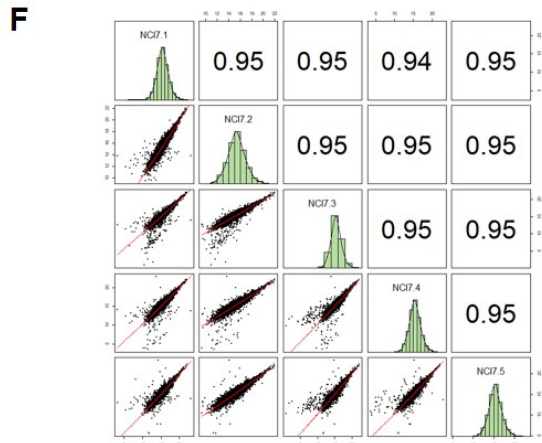
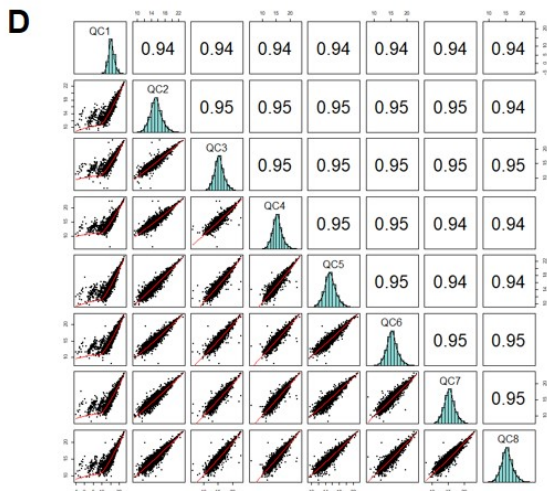
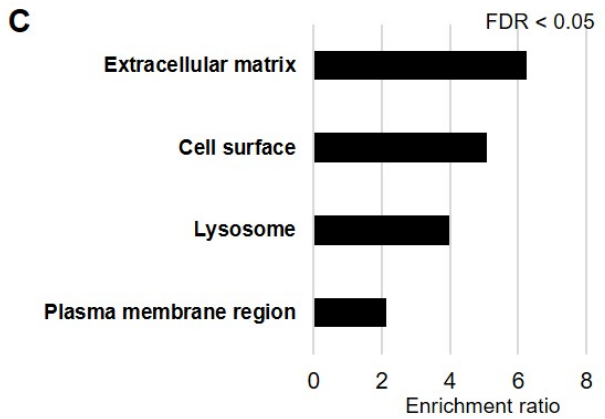
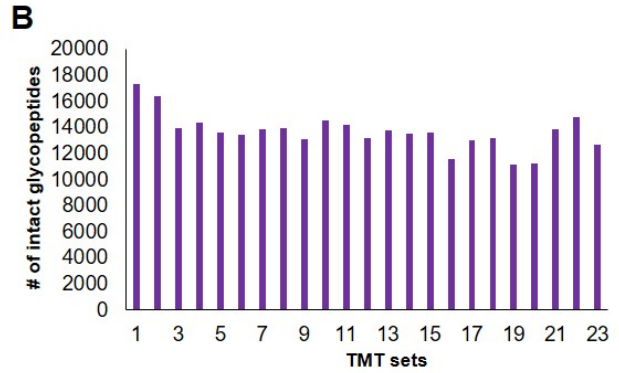
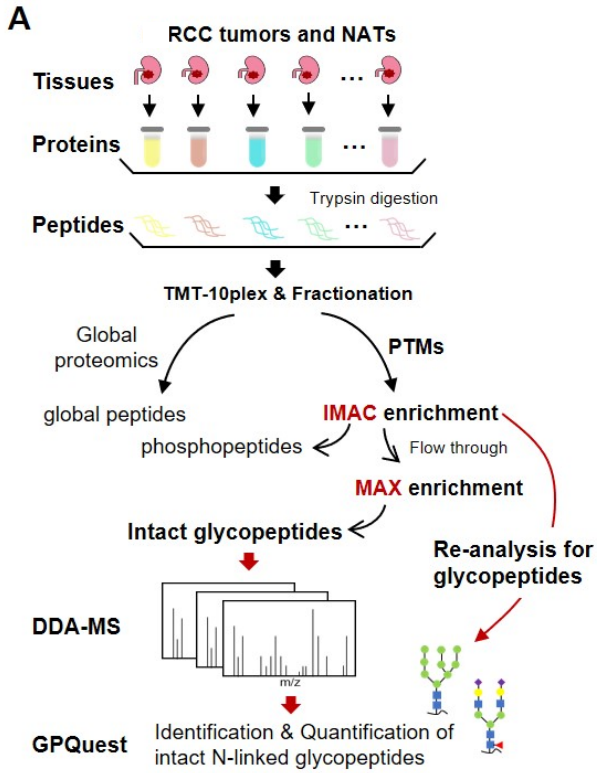


Figure S1. Experimental workflow and quality control of glycoproteomic data of ccRCC.

(A) Schematic of the experimental workflow used to analyze proteomic, phosphoproteomic, and glycoproteomic of ccRCC. **(B)** Total identified intact glycopeptides in each of the TMT-10plex set. **(C)** Enriched GO cellular components (FDR<0.05) of glycoproteins with intact glycopeptides detected in ccRCC. The p values were calculated using hypergeometric test and adjusted (FDR) using Benjamini-Hochberg method. **(D)** Reproducibility of the glycoproteomic data of ccRCC based on QC samples. **(E)** Coefficient of variation of intact glycopeptides in QC samples. **(F)** Reproducibility of the glycoproteomic data of ccRCC based on NCI-7 samples. **(G)** Coefficient of variation of intact glycopeptides in NCI-7 samples. Related to Table S1.

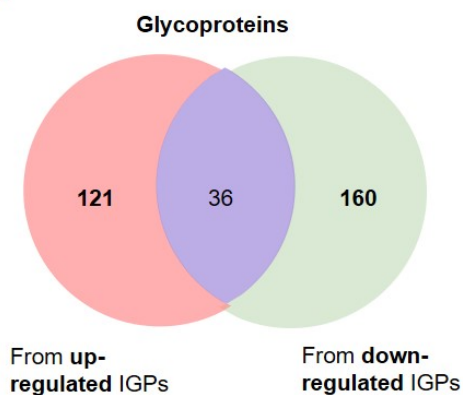
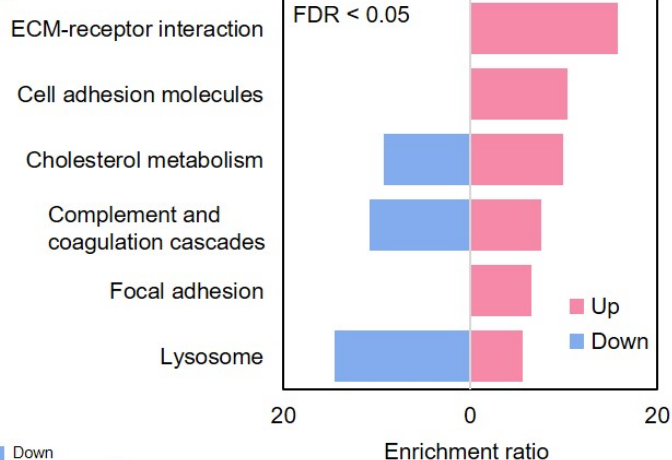
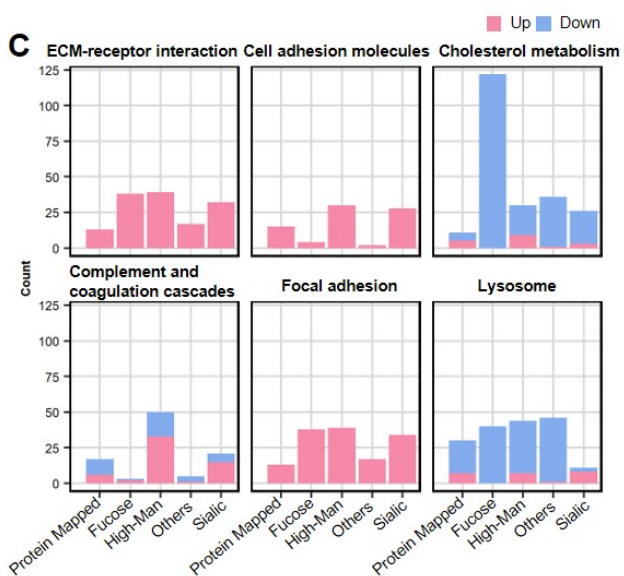
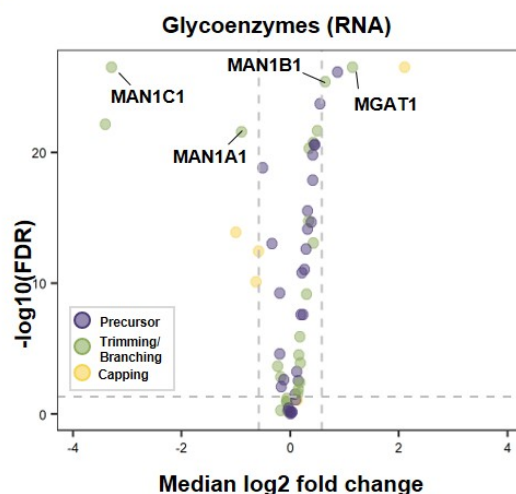
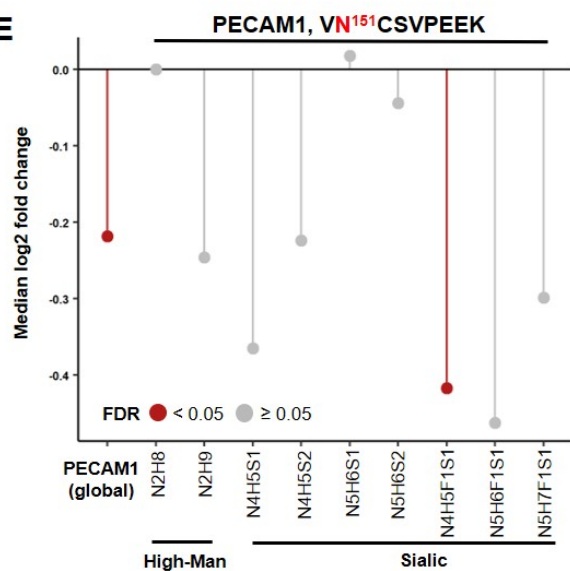
A**B****C****D****E**

Figure S2. Examination of altered glycosylation in ccRCC. **(A)** Glycoproteins corresponding to the upregulated and downregulated intact glycopeptides. **(B)** KEGG pathways enriched (FDR<0.05) using uniquely observed glycoproteins of differentially expressed intact glycopeptides. The p values were calculated using hypergeometric test and adjusted (FDR) using Benjamini-Hochberg method. **(C)** Number of glycoproteins mapped and associated glycan types for each enriched KEGG pathway. **(D)** Differential analysis of glycosylation enzymes on transcriptomic level. Significantly altered glycosylation enzymes were defined as >1.5-fold changes and FDR<0.05. **(E)** Glycosylation changes in VN¹⁵¹CSVPEEK of PECAM1 in high-grade tumors relative to low-grade tumors (FDR<0.05 as significant changes). (D and E) The p values were computed using two-sided Wilcoxon rank-sum test and adjusted (FDR) using Benjamini-Hochberg method. Related to Figure 1 and Table S2.

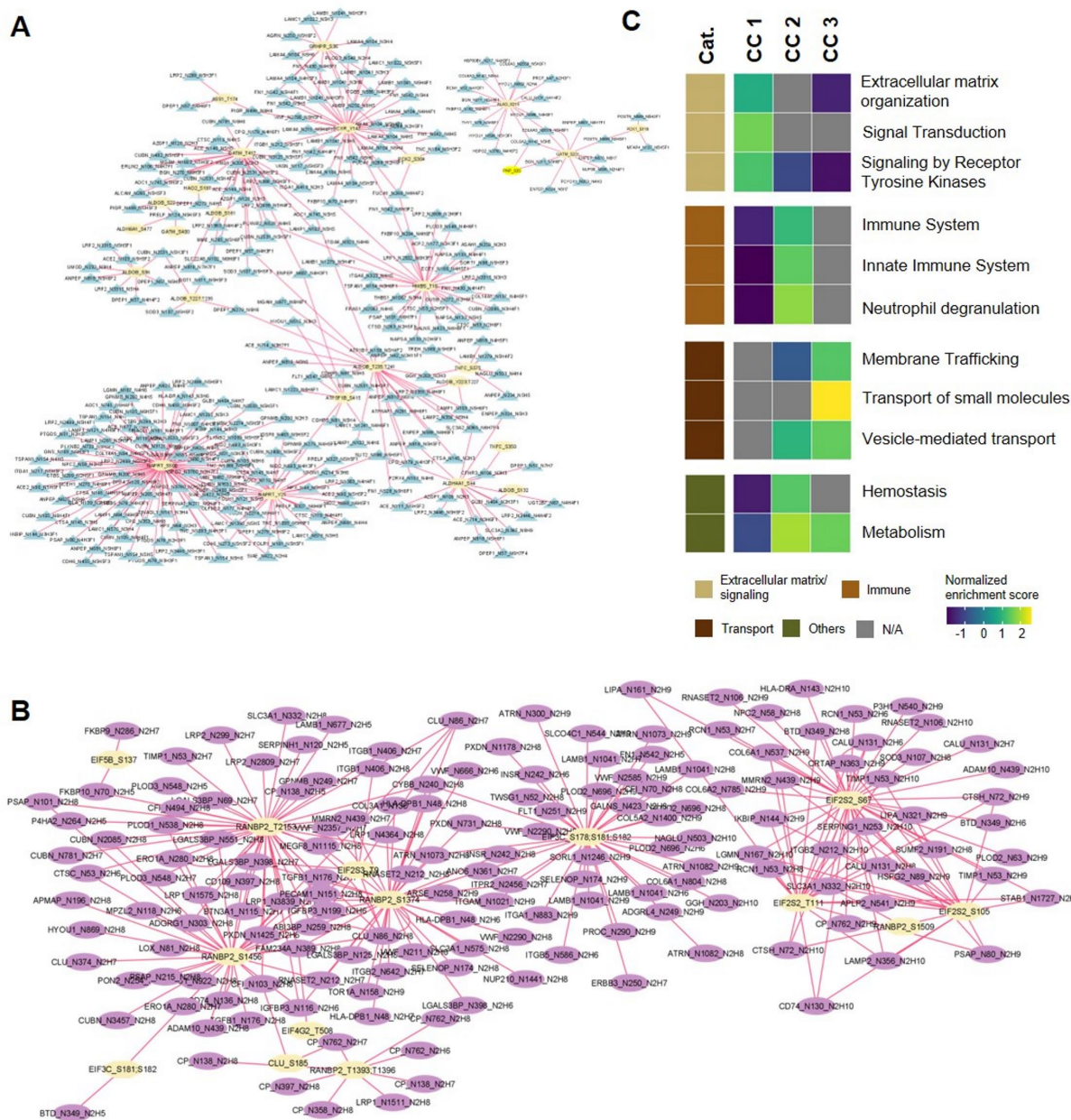


Figure S3. Cross-correlation between glycosylation and phosphorylation in ccRCC. **(A)** Networks associated with metabolic pathways. **(B)** Networks associated with RNA transport. **(C)** Pathways associated with each of the CC clusters. Related to Figure 4 and Table S5.