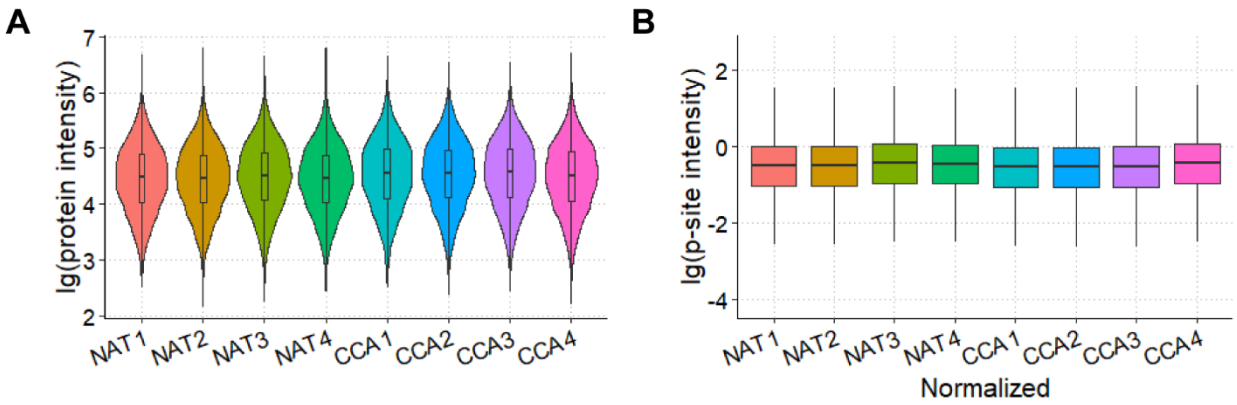



Figure S1. Additional analysis of proteomic and phosphoproteomic profiling for CCAs.
A, B. The intensity distribution of quantified proteins (A) and normalized p-sites (B) in all CCA patients.

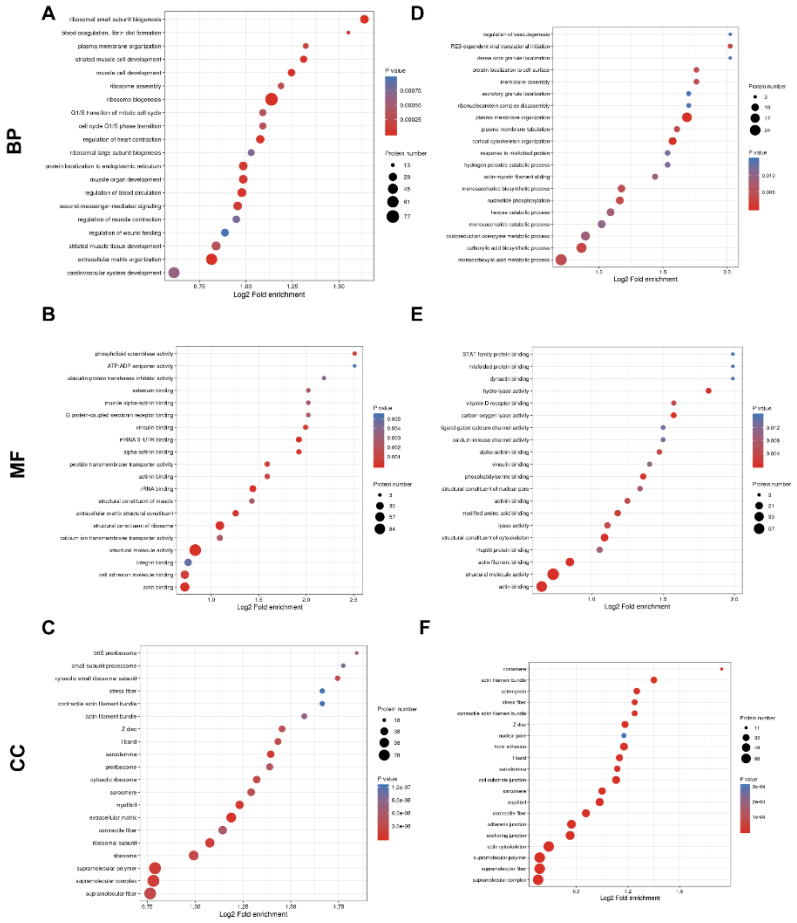



Figure S2. Gene Ontology (GO) enrichment analysis of the DEPs **(A-C)** and DPPs **(D-F)** based on the biological process (BP), molecular function (MF), and cellular component (CC).

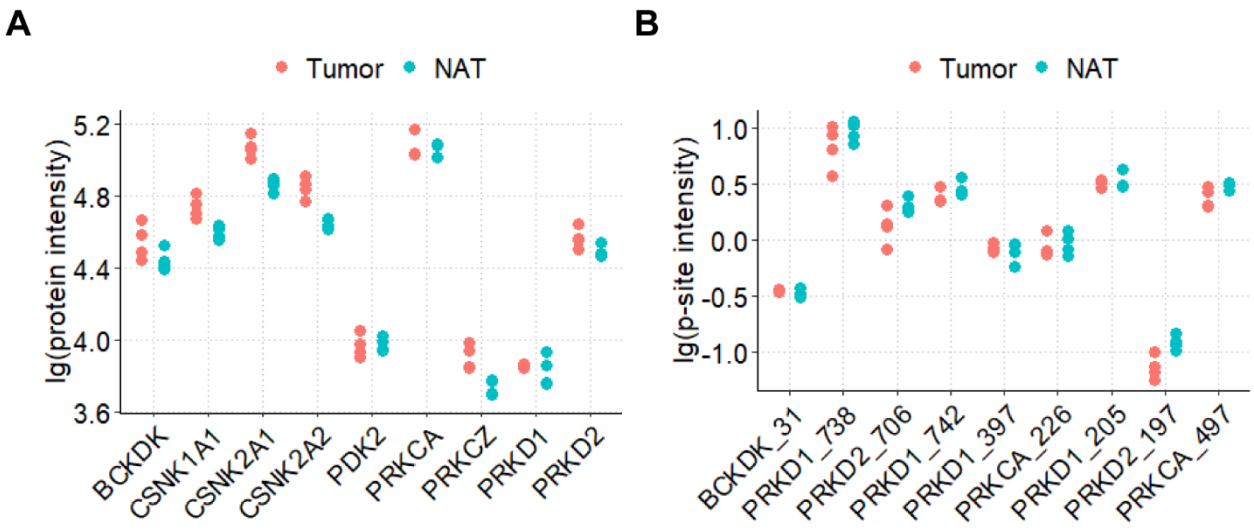



Figure S3. The protein abundance and phosphorylation modification of potentially CCA-associated PKs from proteomic and phosphoproteomic data. A. The protein abundance of 11 potentially CCA-associated PKs from proteomic data, if available. **B.** The phosphorylation modification of potentially CCA-associated PKs from phosphoproteomic data, if available.

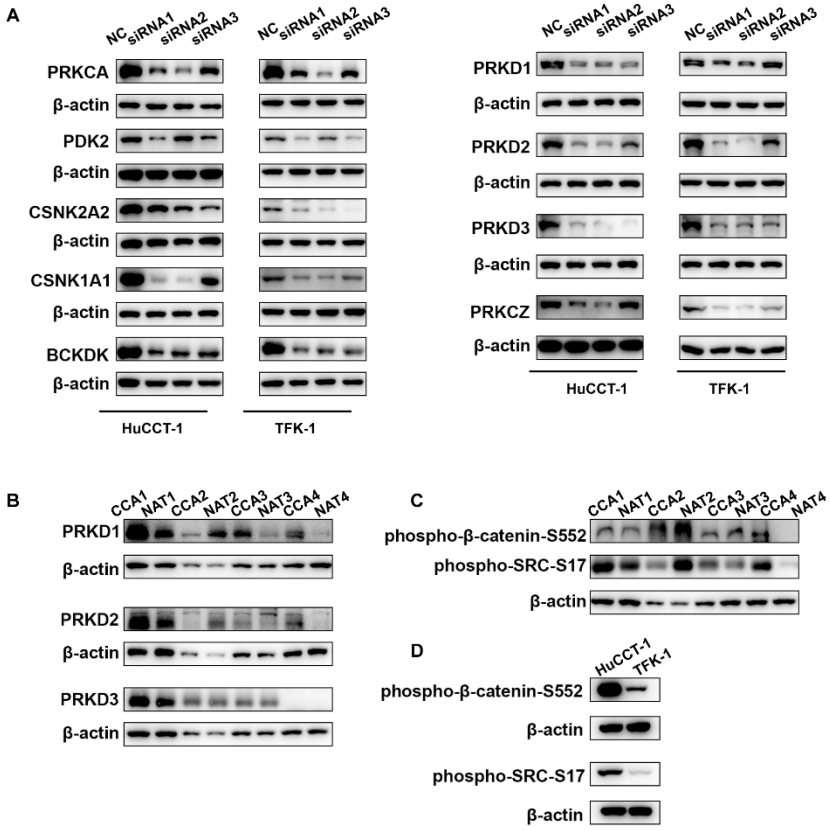
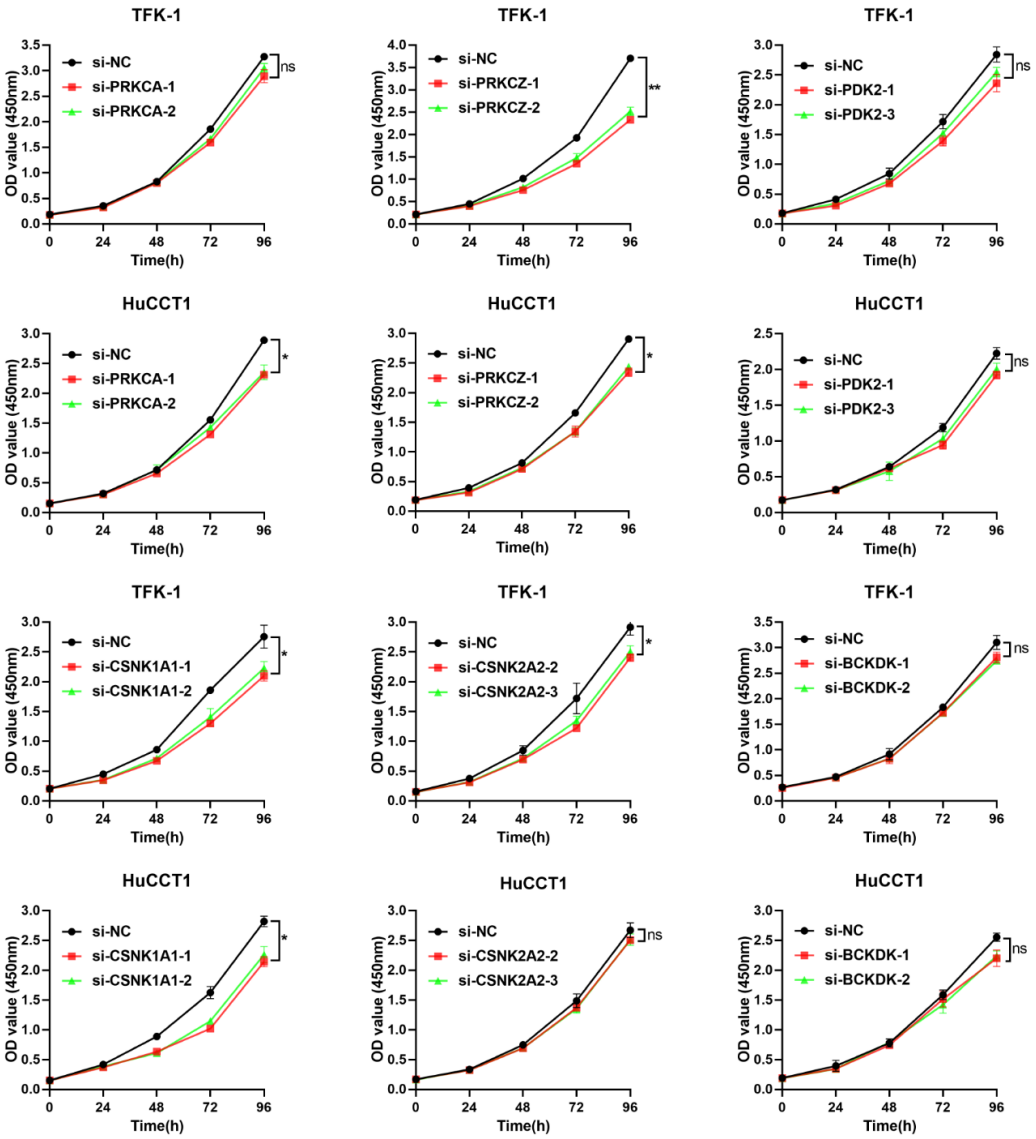



Figure S4. Western blot assay validated the expression of the candidate PKs and substrates in CCA tissues and cell lines. **A.** Western blot for measuring the expression of PK treated with siRNA for 48 h, including PRKCA, PDK2, CSNK2A2, CSNK1A1,BCKDK, PRKD1, PRKD2, PRKD3, and PRKCZ. **B.** The expression of PRKD1, PRKD2, and PRKD3 in CCA tissues and pared NATs was measured by western blot. **C.** The expression of PRKD-relative substrates in CCA tissues and pared NATs was validated by western blot, including phospho-β-catenin-S552 and phospho-Src-S17. **D**. The expression of phospho-β-catenin-S552 and phospho-Src-S17 in TFK-1 and HuCCT1 were measured by western blot assay.




.

Figure S5. The CCK-8 assay of PK candidates. **A-F**. The proliferation of TFK-1 and HuCCT1 transfected with specific siRNAs for PK candidates, including PRKCA (**A**), PRKCZ (**B**), PDK2 (**C**), CSNK1A1 (**D**), CSNK2A2 (**E**), and BCKDK (**F**). *p < 0.05, **p < 0.01, ***p < 0.001, determined by two-way ANOVA. Data are presented with means ± SDs and from three independent experiments.

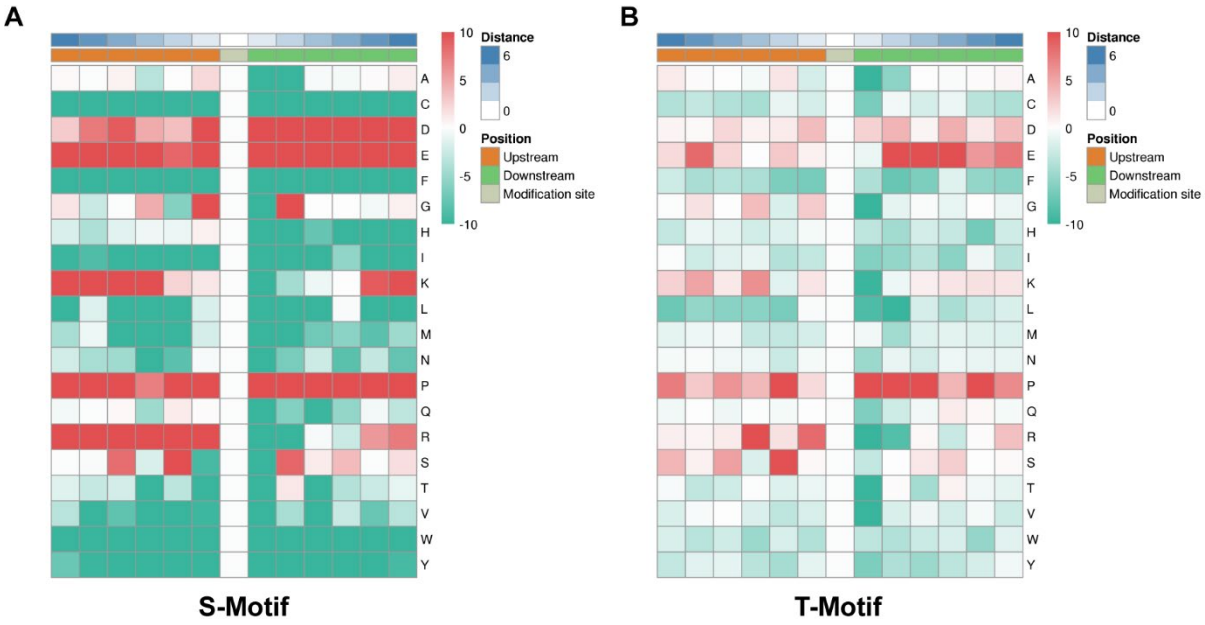



Figure S6. The heatmap of the amino acid profile of the serine-phosphosites **(A)** and threonine-phosphosites **(B)**, featuring the enrichment (red) and depletion (green) of the amino acids at every position (from -10 to +10) on both sides of the phospho-site.