

Figure S1. Differential expression of CHCHD family genes across pan-cancers. (A-G) Expression in tumor vs. normal tissues across multiple types of cancer of (A) CHCHD1, (B) CHCHD3, (C) CHCHD4, (D) CHCHD5, (E) CHCHD6, (F) CHCHD7 and (G) CHCHD10. Boxplots compare gene expression levels between normal tissues (blue) and tumor tissues (red) across indicated cancer types. Statistical significance is indicated above each cancer type. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. CHCHD, coiled-coil-helix-coiled-coil-helix domain.

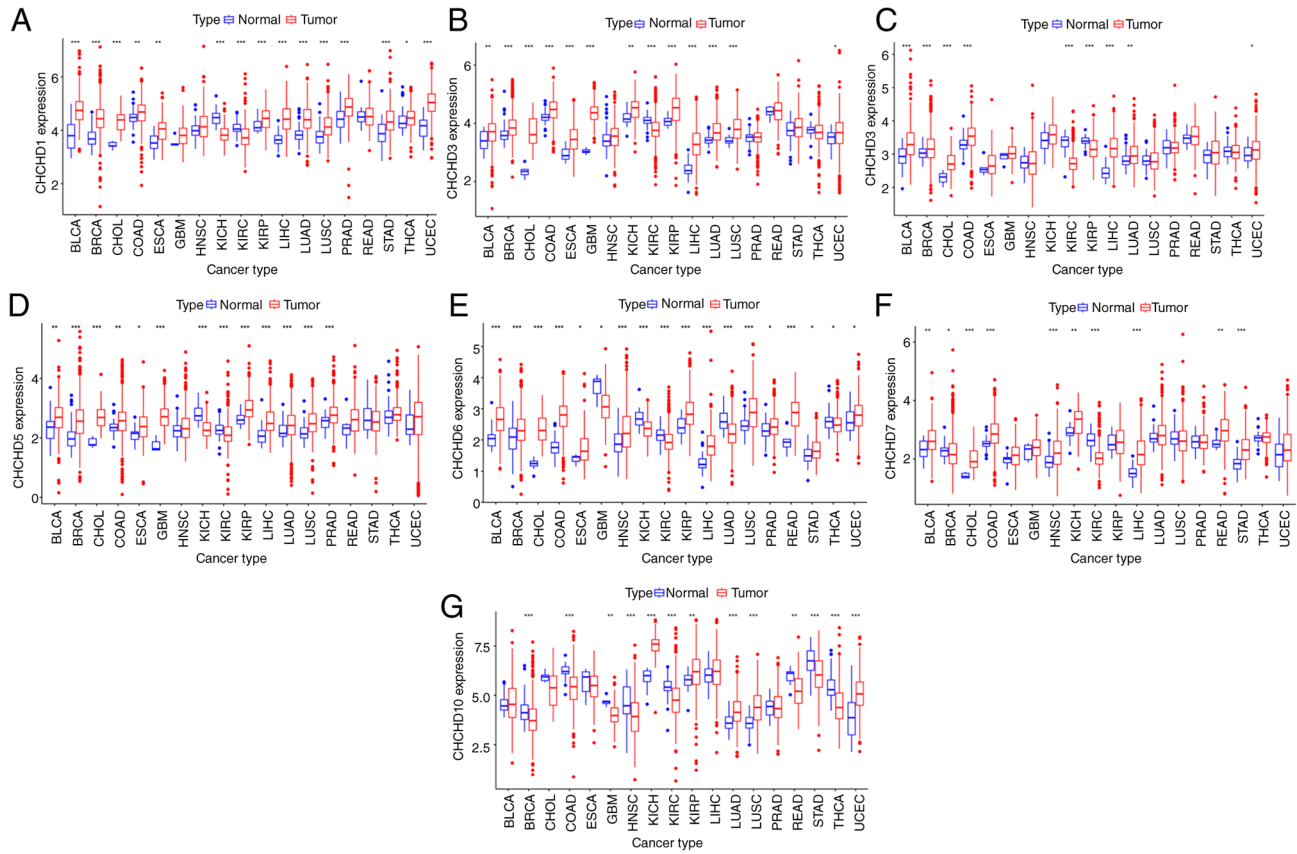


Figure S2. Genomic alteration landscape of CHCHD family genes across cancers. (A-D) Bar plots show the alteration frequency of CHCHD1-CHCHD4 across cancer types, with alterations stratified by amplification, mutation, deep deletion and structural variants (and multiple alterations where applicable). Lollipop diagrams below each panel depict the distribution of mutations along the protein sequence and annotated domains. Cancer type labels are shown using TCGA abbreviations: ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma; CHOL, cholangiocarcinoma; COAD, colorectal adenocarcinoma; DLBC, diffuse large B-cell lymphoma; ESCA, esophageal adenocarcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, brain lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma. CHCHD, coiled-coil-helix-coiled-coil-helix domain.

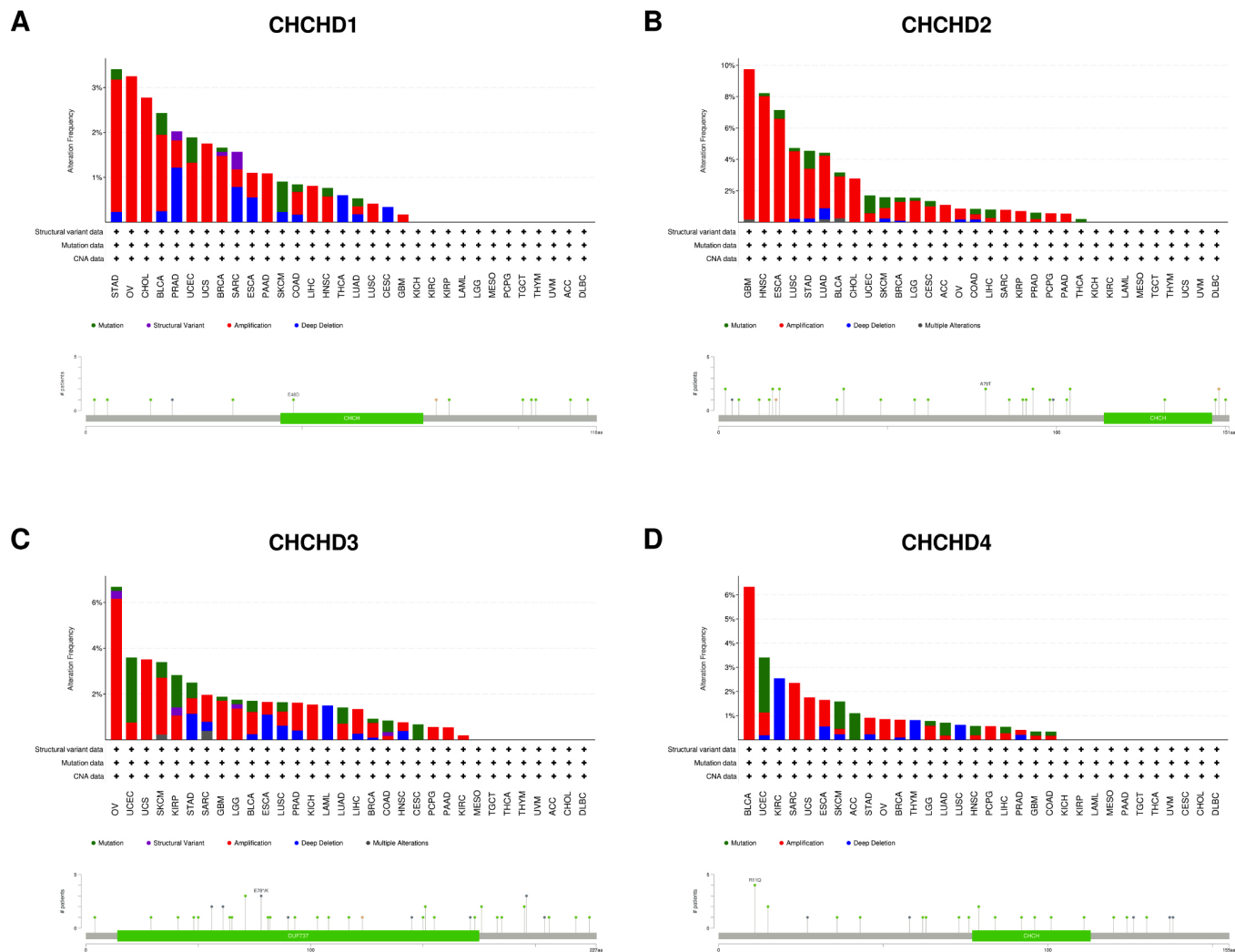


Figure S3. Genomic alteration landscape of CHCHD family genes across cancers. (A-D) Bar plots show the alteration frequency of CHCHD5, CHCHD6, CHCHD7 and CHCHD10 across cancer types, with alterations categorized as amplification, mutation, deep deletion, structural variants, and multiple alterations. Lollipop plots beneath each panel illustrate the distribution of mutations along the protein sequence and annotated domains. Cancer type labels are shown using TCGA abbreviations: ACC, adrenocortical carcinoma; BLCA, bladder urothelial carcinoma; BRCA, breast invasive carcinoma; CESC, cervical squamous cell carcinoma; CHOL, cholangiocarcinoma; COAD, colorectal adenocarcinoma; DLBC, diffuse large B-cell lymphoma; ESCA, esophageal adenocarcinoma; GBM, glioblastoma multiforme; HNSC, head and neck squamous cell carcinoma; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, brain lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma. CHCHD, coiled-coil-helix-coiled-coil-helix domain.

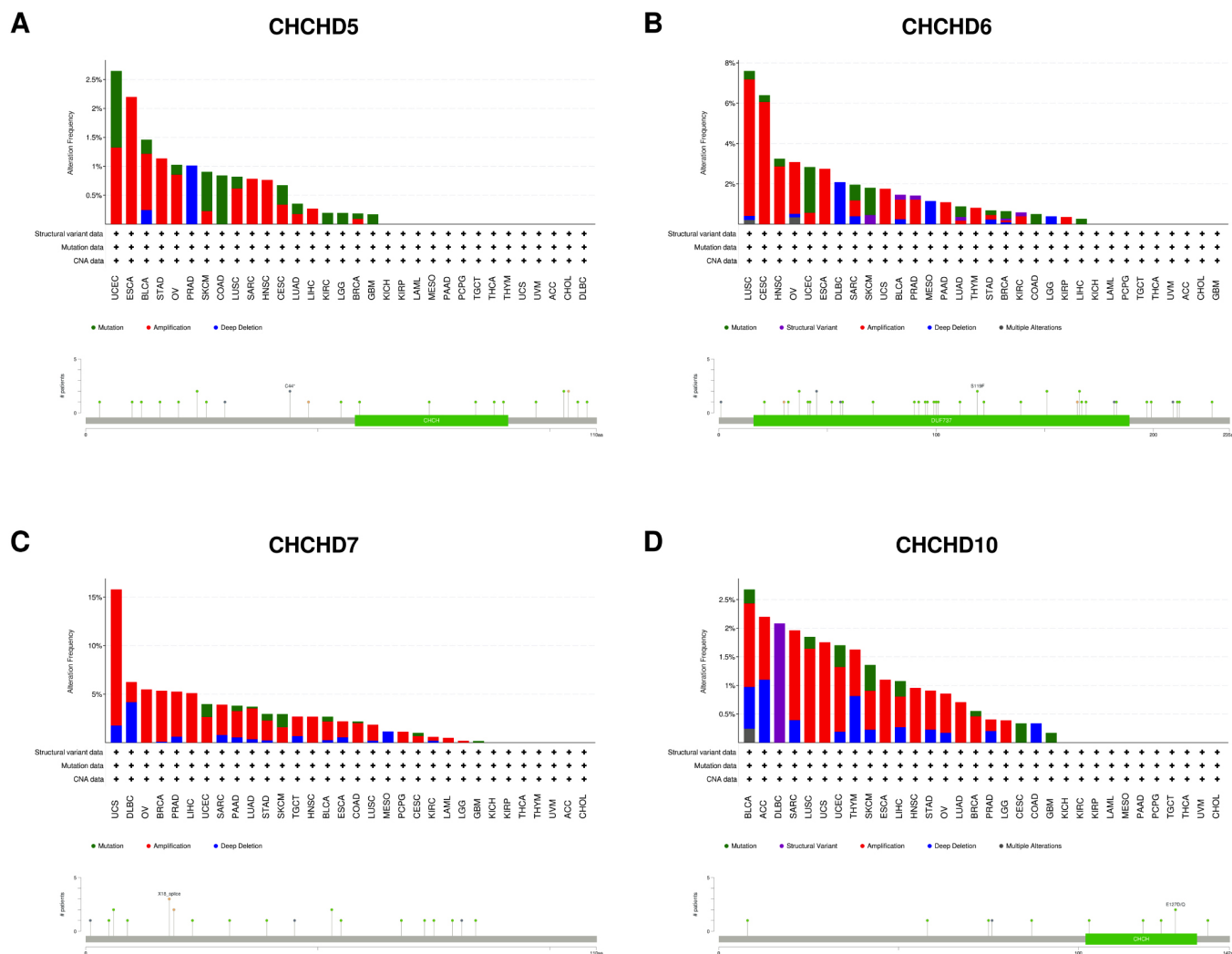


Figure S4. Upregulated CHCHD2 protein expression across multiple cancers and validation by IHC. (A) CHCHD2 protein abundance in tumor vs. normal tissues based on CPTAC proteomic data queried via UALCAN, showing significantly elevated CHCHD2 levels in LUAD, LUSC, COAD, OV, UCEC, BRCA and LIHC. (B) Representative IHC images from the Human Protein Atlas (HPA) demonstrating stronger CHCHD2 staining in tumor tissues (LUAD, LUSC, COAD, OV, UCEC, BRCA and LIHC) compared with the corresponding normal tissues. CHCHD, coiled-coil-helix-coiled-coil-helix domain; IHC, immunohistochemistry; LUAD, lung adenocarcinoma; LUSC, lung squamous cell carcinoma; COAD, colon adenocarcinoma; OV, ovarian serous cystadenocarcinoma; UCEC, uterine corpus endometrial carcinoma; BRCA, breast invasive carcinoma; LIHC, liver hepatocellular carcinoma.

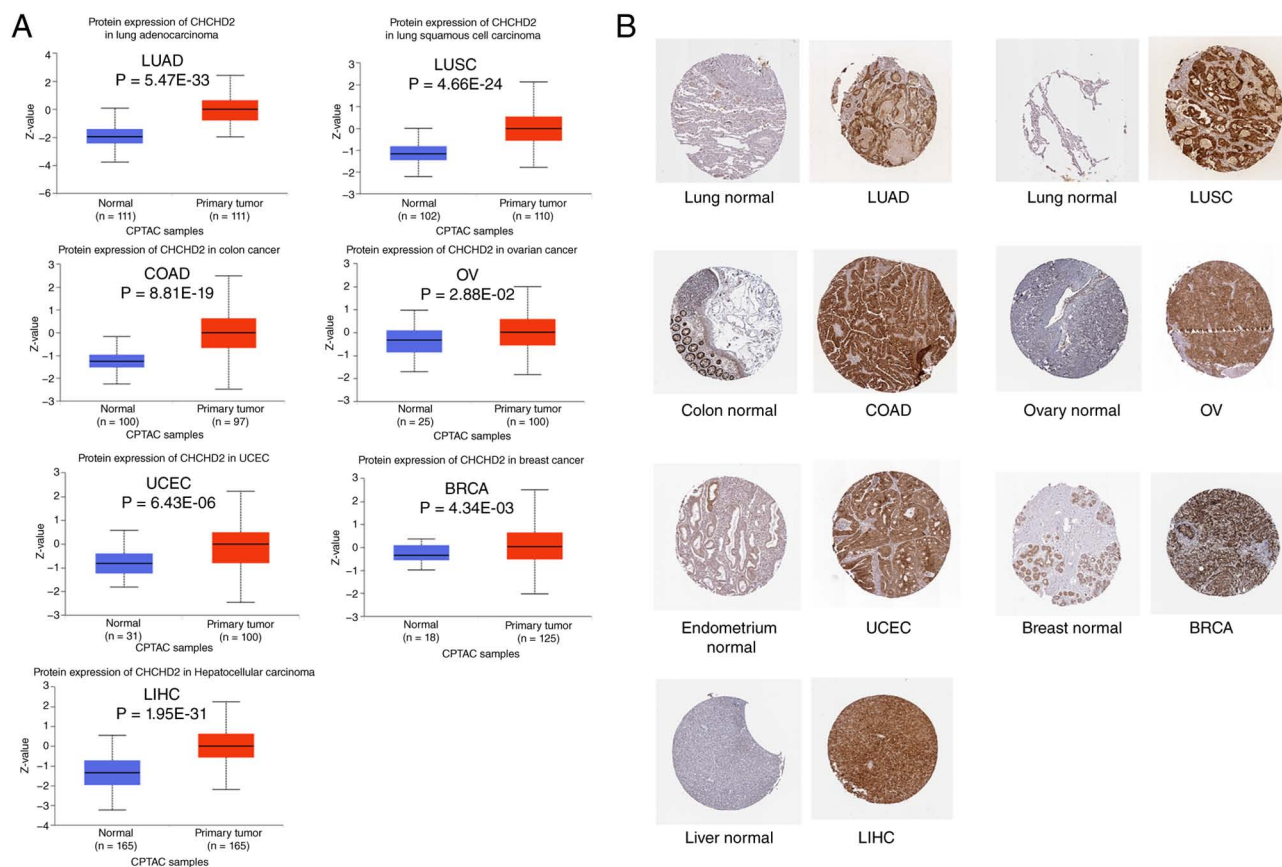


Figure S5. CHCHD2-associated co-expression network and functional enrichment of DEGs in LUAD. (A) Co-expression analysis based on TCGA LUAD RNA-seq identified the 11 genes most strongly correlated with CHCHD2, and a co-expression network was constructed. (B) Heatmap of the top 100 DEGs between CHCHD2-high and CHCHD2-low groups (total DEGs=1,150; upregulated=156, downregulated=994). (C) Gene Ontology enrichment analysis of DEGs, highlighting the most significantly enriched terms across Biological Process, Cellular Component and Molecular Function categories. (D) Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis of DEGs, showing significant enrichment in PI3K-Akt signaling, neuroactive ligand-receptor interaction, cAMP signaling, and MAPK signaling pathways. CHCHD, coiled-coil-helix-coiled-coil-helix domain; DEGs, differentially expressed genes.

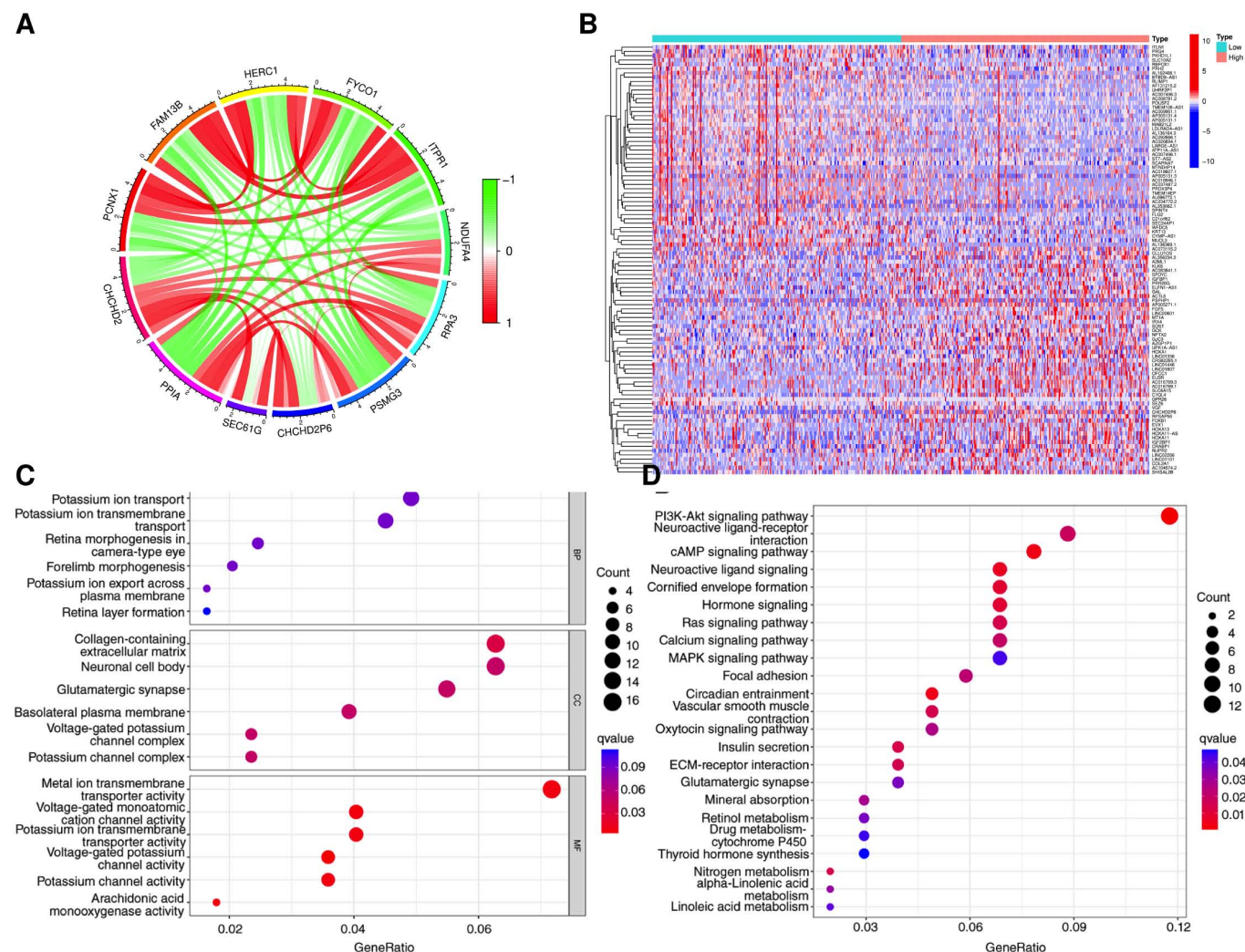


Figure S6. Association analysis of CHCHD2 with the lung adenocarcinoma tumor microenvironment, stemness features, immune subtypes and immune infiltration. (A) Spearman correlation analysis showed that CHCHD2 expression was significantly positively correlated with RNAss and DNAss, but significantly negatively correlated with stromal score, immune score, and the overall ESTIMATE score. (B) The Kruskal-Wallis test was used to compare differences in CHCHD2 expression across immune subtypes (C1-C6): CHCHD2 expression was relatively higher in C1 (wound healing) and lowest in C3 (inflammatory). (C) Correlation heatmap between CHCHD2 and 10 immune checkpoint-related genes: CHCHD2 was negatively correlated with CD40LG, TNFSF15, TNFSF14, CD28, BTLA, ADORA2A, CD160, TIGIT and NRP1, but positively correlated with TNFSF18 (all $P < 0.001$). (D and E) CIBERSORT deconvolution results comparing immune cell compositions and correlations between the CHCHD2 high- and low-expression groups: CHCHD2 was positively correlated with follicular helper T cells, activated CD4 memory T cells, and gamma delta T cells, and negatively correlated with resting CD4 memory T cells and resting mast cells. Statistical significance is indicated as * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$. CHCHD, coiled-coil-helix-coiled-coil-helix domain.

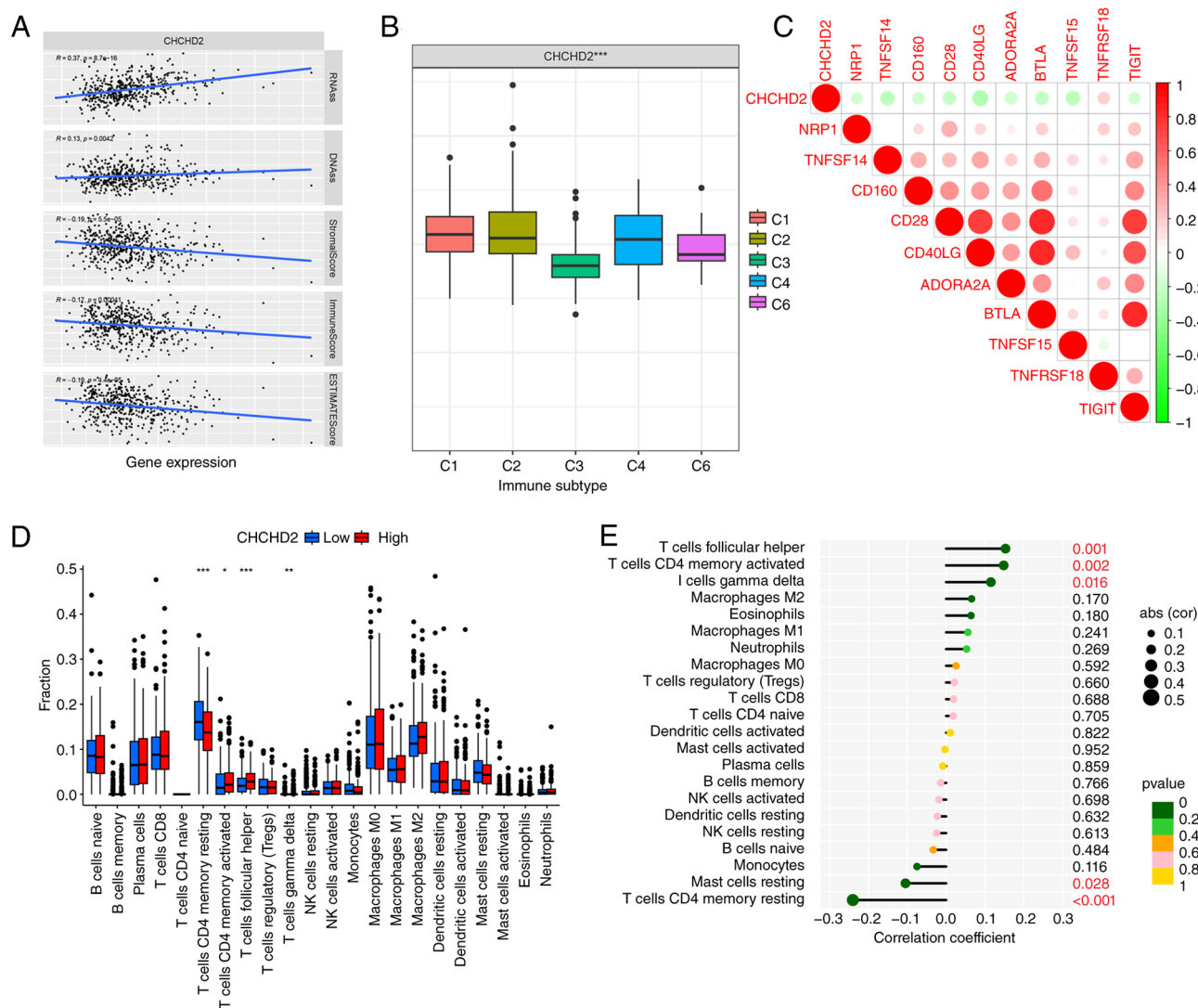


Figure S7. CHCHD2 expression across cell populations in NSCLC single-cell datasets, with validation in a LUAD-derived cohort. (A) Heatmap of the average CHCHD2 expression across major cell types in multiple NSCLC scRNA-seq datasets from TISCH2 (NSCLC_GSE127465, NSCLC_GSE131907, NSCLC_GSE99254 and NSCLC_GSE117570). (B) In NSCLC_GSE127465: UMAP plots annotated by cell type (left), CHCHD2 feature plots (middle), and violin plots showing CHCHD2 expression across cell populations (right). (C) In NSCLC_GSE131907, a LUAD-derived single-cell dataset used as the primary LUAD-relevant validation cohort: UMAP plots annotated by cell type (left), CHCHD2 feature plots (middle), and violin plots showing CHCHD2 expression across cell populations (right). (D) In NSCLC_GSE117570, an early-stage NSCLC dataset containing LUAD cases: UMAP plots annotated by cell type (left), CHCHD2 feature plots (middle), and violin plots showing CHCHD2 expression across cell populations (right). Among these datasets, NSCLC_GSE131907 was used as the primary LUAD-relevant single-cell validation cohort, whereas NSCLC_GSE127465, NSCLC_GSE99254 and NSCLC_GSE117570 were included as supportive NSCLC-level references and were not interpreted as LUAD-specific evidence. CHCHD, coiled-coil-helix-coiled-coil-helix domain; NSCLC, non-small cell lung cancer; LUAD, lung adenocarcinoma.

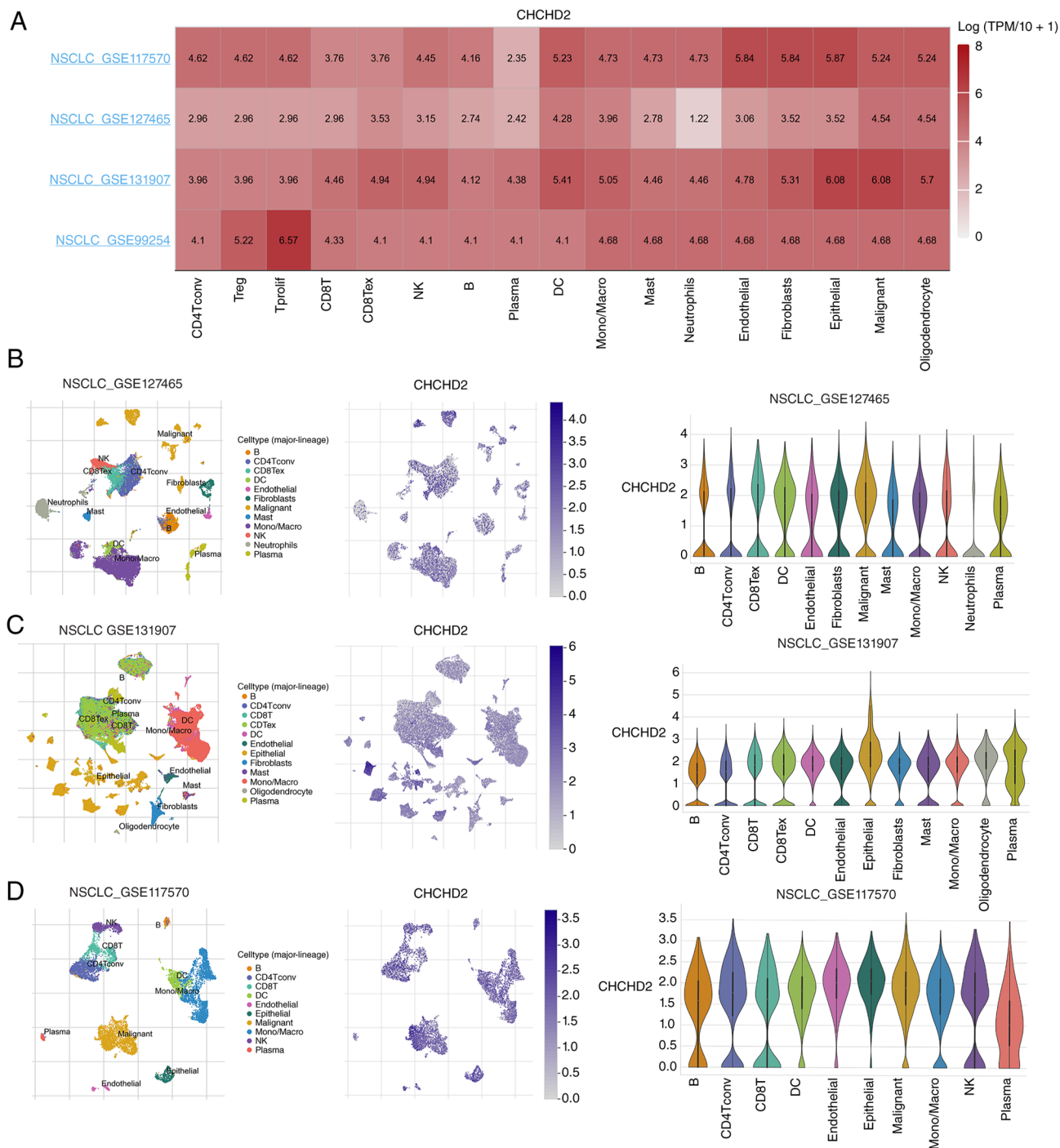


Figure S8. Association between CHCHD2 expression and drug sensitivity in human cancer cell lines. Correlation analysis of CHCHD2 expression with the response to multiple therapeutic agents. (A) Ifosfamide. (B) Lomustine. (C) Carmustine. (D) Hydroxyurea. (E) Nelarabine. (F) Chelerythrine. (G) Fenretinide. (H) Imexon. (I) Uracil mustard. (J) Acrichine. (K) Irofulven. (L) Dexamethasone. (M) PX-316. (N) Vorinostat. (O) Fluphenazine. (P) Asparaginase. Pearson's correlation coefficients and corresponding P-values are shown in each panel. CHCHD, coiled-coil-helix-coiled-coil-helix domain.

