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Pan-Cancer Genomic and Clinical Landscape of Human Deubiquitinases Identifies Recurrent Cancer-Associated DUBs and Candidate Substrate Networks

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Abstract

Deubiquitinases (DUBs) are key regulators of ubiquitin-mediated signaling and control essential cellular processes such as protein turnover, DNA repair, apoptosis, and immune responses. Despite their importance in cancer, a comprehensive multi-omics characterization of DUBs across malignancies remains limited. Here, we performed an integrated pan-cancer analysis using transcriptomic, genomic, and clinical data from The Cancer Genome Atlas (TCGA), combined with experimentally validated DUB–substrate interactions and public proteomic datasets from gastric cancer, esophageal cancer, and glioma. Our analysis revealed substantial heterogeneity in DUB expression across cancers, with distinctive relative expression profiles of DUBs including USP7, USP10, USP11, USP14, USP22, OTUB1, and BAP1. Comparative analysis of ESCA and STAD identified eighteen shared cancer-enriched DUBs, suggesting overlap in their relative expression patterns in upper gastrointestinal cancers. Genomic profiling showed frequent copy-number amplifications (USP13, OTUD7B, EIF3H, USP21, USP36) and deletions (OTUD7A, USP2, USP28, USP19, BAP1), while mutation analysis highlighted USP34, USP9X, USP29, USP24, and USP26 as genes with high observed mutation counts. Survival analysis identified several prognostic DUBs, including USP1, USP4, OTUD7A, DESI2, PSMD14, and UCHL1. Integration of public proteomic datasets with curated DUB–substrate interactions highlighted candidate associations with pathways related to NF-κB signaling, apoptosis, cell-cycle regulation, and DNA damage response. Several DUBs, including USP7, USP10, USP11, USP14, and USP22, showed high connectivity within the constructed networks, prioritizing them for further functional investigation. Overall, this study provides a comprehensive pan-cancer atlas of DUB alterations and highlights candidate DUBs and pathways with potential diagnostic, prognostic, and therapeutic relevance.

Keywords: deubiquitinases (DUBs); pan-cancer analysis; multi-omics; ubiquitin signaling; DUB–substrate network

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1. Introduction

Protein ubiquitination is one of the most versatile and extensively utilized post-translational modifications in eukaryotic cells, regulating virtually every aspect of cellular

physiology, including protein stability, signal transduction, DNA repair, cell-cycle progression, immune responses, chromatin remodeling, and programmed cell death [1–4]. The ubiquitin system functions through the coordinated actions of ubiquitin-activating (E1), ubiquitin-conjugating (E2), and ubiquitin ligase (E3) enzymes that covalently attach ubiquitin molecules to target proteins [5–7]. Depending on the type and topology of ubiquitin chains formed, ubiquitination can direct proteins toward proteasomal degradation or modulate their localization, activity, and interaction networks [8–10]. Given its central role in maintaining cellular homeostasis, dysregulation of ubiquitin signaling has emerged as a hallmark of numerous human diseases, particularly cancer [11,12].

The reversibility of ubiquitination is mediated by deubiquitinases (DUBs), a specialized class of proteases that remove ubiquitin modifications from substrate proteins [13–15]. By antagonizing ubiquitin ligases, DUBs provide an essential regulatory layer controlling the amplitude, duration, and specificity of ubiquitin-dependent signaling [16]. The human genome encodes more than 100 DUBs that can be classified into several catalytic families, including ubiquitin-specific proteases (USPs), ovarian tumor proteases (OTUs), ubiquitin C-terminal hydrolases (UCHs), Machado–Joseph disease proteases (MJD/Josephins), and JAB1/MPN/MOV34 metalloproteases (JAMM/MPN+) [14,15]. Although these families differ in structure and catalytic mechanisms, they collectively regulate a broad spectrum of biological processes by controlling the ubiquitination status of thousands of cellular proteins [17,18].

Accumulating evidence demonstrates that DUBs play pivotal roles in tumor initiation, progression, metastasis, and therapeutic resistance [19–21]. Numerous DUBs have been shown to regulate major oncogenic signaling pathways, including TP53, NF-κB, Wnt/β-catenin, PI3K–AKT–mTOR, TGF-β, MYC, and receptor tyrosine kinase signaling [22–24]. For example, USP7 stabilizes MDM2 and modulates TP53 signaling [25,26], USP22 contributes to transcriptional regulation and cancer stemness [27–29], USP14 controls proteasome activity and cellular proteostasis [30,31], while BAP1 functions as a critical tumor suppressor involved in chromatin regulation and DNA damage repair [32,33]. Similarly, OTUD family members regulate inflammatory and innate immune pathways through the modulation of key signaling intermediates such as TRAF proteins, RIPK1, and NF-κB components [34–36]. These observations collectively indicate that DUBs occupy central positions within cancer-associated signaling networks.

Recent advances in genomic and transcriptomic profiling have revealed widespread alterations in DUB genes across diverse cancer types. Somatic mutations, copy-number alterations, chromosomal rearrangements, and aberrant transcriptional regulation have been reported for numerous DUBs in both hematological and solid malignancies [37,38]. Loss-of-function mutations in BAP1 are recurrently observed in mesothelioma, uveal melanoma, renal cell carcinoma, and cholangiocarcinoma [32,39], whereas amplification and overexpression of DUBs such as USP13, USP21, USP36, and PSMD14 have been associated with tumor progression and poor clinical outcomes [40–42]. In addition, several DUBs have emerged as promising therapeutic targets owing to their enzymatic tractability and their involvement in oncogenic signaling pathways.

Despite these advances, our current understanding of DUB biology remains fragmented. Most studies have focused on individual DUBs within specific cancer contexts, resulting in a largely gene-centric view of deubiquitinase function. Consequently, the broader landscape of DUB dysregulation across cancers remains incompletely characterized. Important questions remain unanswered regarding which DUBs are recurrently altered across malignancies, how genomic and transcriptional alterations interact to shape DUB activity, which DUBs possess prognostic significance across multiple tumor types, and whether common substrate networks and biological pathways are consistently regulated

by cancer-associated DUBs. Addressing these questions requires a systematic pan-cancer framework capable of integrating multiple molecular layers.

The emergence of large-scale cancer consortia, including The Cancer Genome Atlas (TCGA), Pan-Cancer Atlas, and Clinical Proteomic Tumor Analysis Consortium (CPTAC), has created unprecedented opportunities to investigate cancer biology at a systems level. TCGA provides comprehensive transcriptomic, genomic, copy-number, and clinical datasets spanning thousands of patients across dozens of cancer types, while CPTAC and other large-scale proteomic studies offer direct insights into protein-level alterations and signaling network rewiring. Integrative analyses combining these complementary datasets have proven highly effective for identifying clinically relevant molecular vulnerabilities and uncovering regulatory mechanisms that cannot be resolved using a single data modality alone.

Importantly, because DUBs exert their biological functions through substrate regulation rather than merely through changes in gene expression, integration of proteomic information is essential for understanding the functional consequences of DUB dysregulation. Substrate-centered analyses can reveal how alterations in DUB abundance influence downstream signaling pathways and biological programs, thereby providing mechanistic insights that extend beyond genomic and transcriptomic observations. Such approaches are particularly valuable for identifying signaling hubs and pathway-level dependencies that may represent therapeutic opportunities.

In the present study, we performed a comprehensive pan-cancer characterization of the human deubiquitinase family by integrating transcriptomic, genomic, clinical, and proteomic datasets. Using TCGA Pan-Cancer cohorts, we systematically analyzed DUB expression patterns, somatic mutations, copy-number alterations, and prognostic associations across multiple cancer types. We further incorporated experimentally validated DUB–substrate interactions and public cancer proteomics datasets from gastric cancer, esophageal cancer, and glioma to identify recurrent substrate networks and biological pathways associated with dysregulated DUBs. Through this multi-layered approach, we generated a comprehensive atlas of DUB alterations across human cancers, identified recurrent candidate DUBs with potential biological and clinical significance, and uncovered convergent signaling pathways that may represent shared vulnerabilities across tumor types.

Our findings provide a systems-level framework for understanding DUB biology in cancer and establish a resource for prioritizing deubiquitinases for mechanistic investigation and therapeutic development. By integrating genomic alterations, transcriptional dysregulation, clinical outcomes, and substrate-centered proteomic networks, this study highlights the central role of deubiquitination in cancer pathogenesis and reveals recurrent DUB-associated signaling programs that transcend individual tumor types.

2. Results

2.1. Multi-Omics Framework for Systematic Pan-Cancer Characterization of Human Deubiquitinases

To comprehensively investigate the molecular and clinical landscape of human deubiquitinases (DUBs) across cancer types, we developed an integrated multi-omics analytical framework that combines genomic, transcriptomic, proteomic, and clinical datasets derived from The Cancer Genome Atlas (TCGA) and publicly available cancer proteomics resources (Figure 1). The study workflow was designed to systematically characterize DUB expression patterns, genomic alterations, mutational landscapes, prognostic significance, substrate interaction networks, and associated biological pathways across diverse human malignancies.

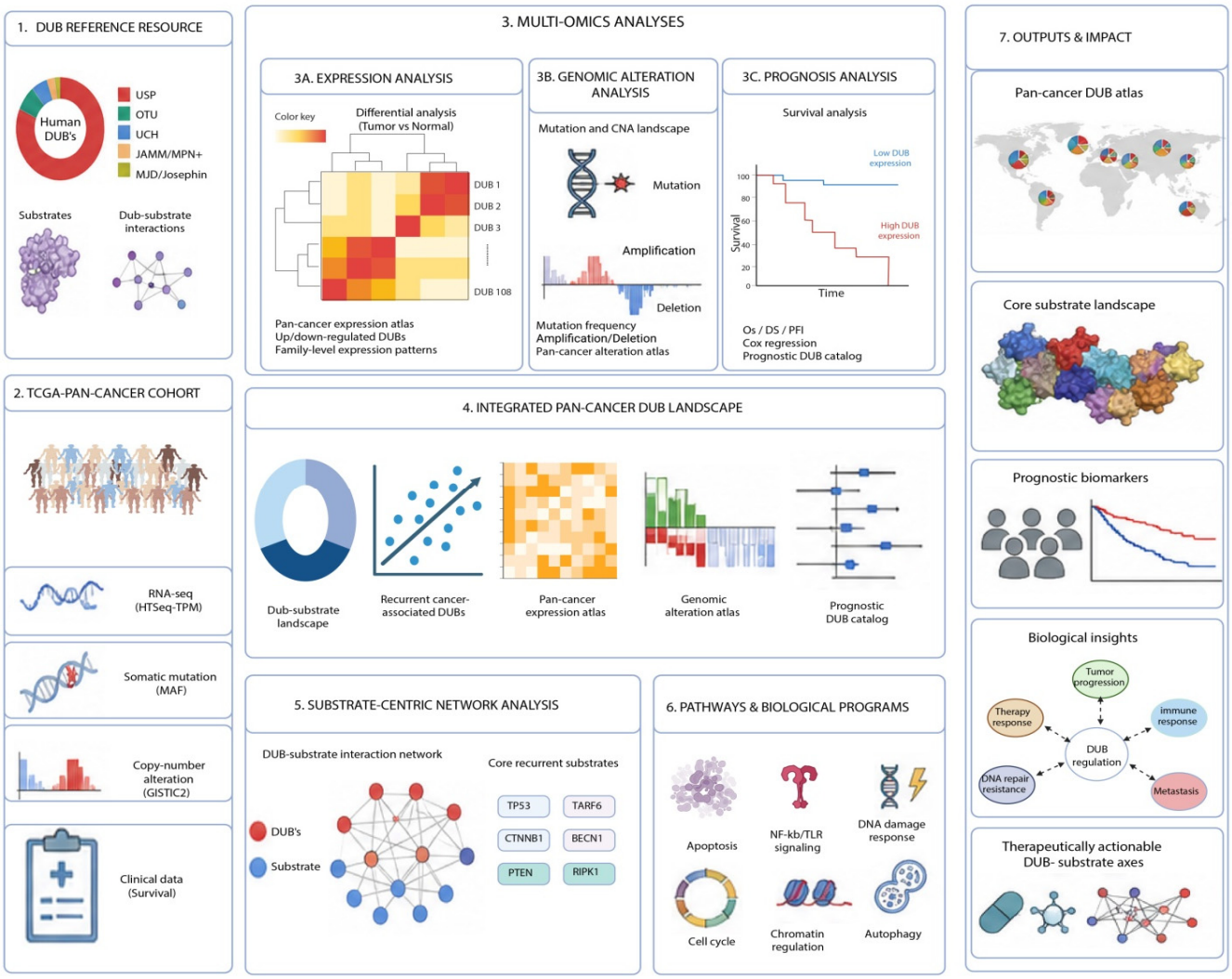


Figure 1. Overview of the multi-omics workflow for pan-cancer characterization of human deubiquitinases. A comprehensive analytical framework was developed to investigate the expression, genomic alteration, prognostic significance, substrate interactions, and biological functions of human deubiquitinases (DUBs) across TCGA cancer cohorts. The workflow integrates transcriptomic, genomic, clinical, and proteomic datasets to generate a pan-cancer DUB atlas, identify recurrent cancer-associated DUBs, construct substrate-centered interaction networks, and define biological pathways and candidate DUB–substrate relationships for further investigation. Created in BioRender. https://BioRender.com/g6z55r5 (accessed on 23 October 2026).

The analysis began with the establishment of a curated reference resource comprising all known human DUBs and their experimentally validated substrate interactions (Figure 1, Section 1). Multi-dimensional molecular data, including RNA sequencing (HTSeq-TPM), somatic mutation profiles (MAF), copy-number alteration data (GISTIC2), and clinical survival information, were subsequently collected from TCGA pan-cancer cohorts (Figure 1, Section 2).

Using these datasets, we performed three complementary layers of molecular analysis. First, transcriptomic profiling was used to define pan-cancer expression patterns, identify highly expressed DUBs, and determine tumor-specific DUB enrichment signatures. Second, genomic analyses were conducted to characterize recurrent somatic mutations and copy-number alterations affecting DUB genes across cancers. Third, survival analyses were performed to identify prognostically relevant DUBs and establish a pan-cancer prognostic catalog of deubiquitinase family members (Figure 1, Section 3).

To prioritize biologically relevant candidates, results from expression, genomic, and clinical analyses were integrated into a unified pan-cancer DUB landscape (Figure 1, Section 4). Candidate DUBs identified through this integrative strategy were subsequently investigated within substrate-centered interaction networks to identify recurrent substrates and key regulatory hubs (Figure 1, Section 5). Functional interpretation was achieved through pathway enrichment analyses, revealing biological programs and signaling pathways associated with dysregulated DUB activity across cancers (Figure 1, Section 6).

Finally, integration of molecular, clinical, and proteomic evidence generated a comprehensive pan-cancer DUB atlas that highlights recurrent cancer-associated DUBs, prognostic biomarkers, core substrate networks, and therapeutically actionable DUB–substrate axes (Figure 1, Section 7). This framework provides a systems-level resource for understanding the role of deubiquitination in cancer biology and establishes a foundation for identifying candidate DUBs for future mechanistic and therapeutic investigations.

2.2. Comprehensive Classification of the Human Deubiquitinase Family and Their Substrate Interaction Landscape

Deubiquitinases (DUBs) are a diverse group of proteases that regulate protein ubiquitination by removing ubiquitin moieties from target substrates, thereby controlling protein stability, localization, and activity [20,43,44]. Through this reversible process, DUBs play critical roles in maintaining cellular homeostasis and regulating key biological processes, including cell cycle progression, DNA damage response, signal transduction, and immune regulation. Dysregulation of DUB activity has been increasingly implicated in the development and progression of various diseases, particularly cancer, where aberrant ubiquitin signaling can influence tumor growth, metastasis, and therapeutic resistance [45,46].

To establish a comprehensive framework for subsequent pan-cancer analyses, we curated the human DUB reference repertoire and classified genes according to their reported catalytic families, including USP, OTU, UCH, MJD/Josephin, JAMM/MPN+, MINDY and ZUFSP (Figure 2A). The USP family constituted the largest class, whereas the remaining families represented progressively smaller subsets of the curated DUB repertoire. Gene-level annotations, including identifiers, family assignments, catalytic status, aliases, and notes on noncanonical or uncertain entries, in Supplementary Figure S1 and Supplementary Table S2.

To further investigate the functional organization of the DUB family, experimentally supported DUB–substrate relationships were compiled to construct a comprehensive interaction network (Figure 2B). This network encompassed several hundred curated DUB–substrate interactions, linking dozens of DUBs to a broad spectrum of protein substrates. The resulting network revealed extensive substrate sharing among multiple DUBs while simultaneously highlighting several enzymes with highly connected interaction profiles. Notably, certain DUBs exhibited pronounced hub-like behavior; with USP7 emerging as one of the most extensively connected enzymes, interacting with at least 81 experimentally validated substrates. Similarly, other prominent DUBs such as USP9X, USP10, and USP14 were associated with large substrate repertoires, each interacting with dozens of targets. In contrast, several DUBs displayed relatively restricted substrate repertoires, often interacting with fewer than five substrates, consistent with more specialized biological functions.

A subset of DUBs emerged as highly connected nodes in the curated interaction network, reflecting the larger number of reported substrate associations for these enzymes. These connectivity patterns provide a framework for prioritizing DUBs for further investigation but do not, by themselves, establish that the enzymes actively regulate all connected substrates in the cancer contexts examined. The curated network therefore serves as a reference resource for generating hypotheses about DUB–substrate relationships and guiding future functional studies.

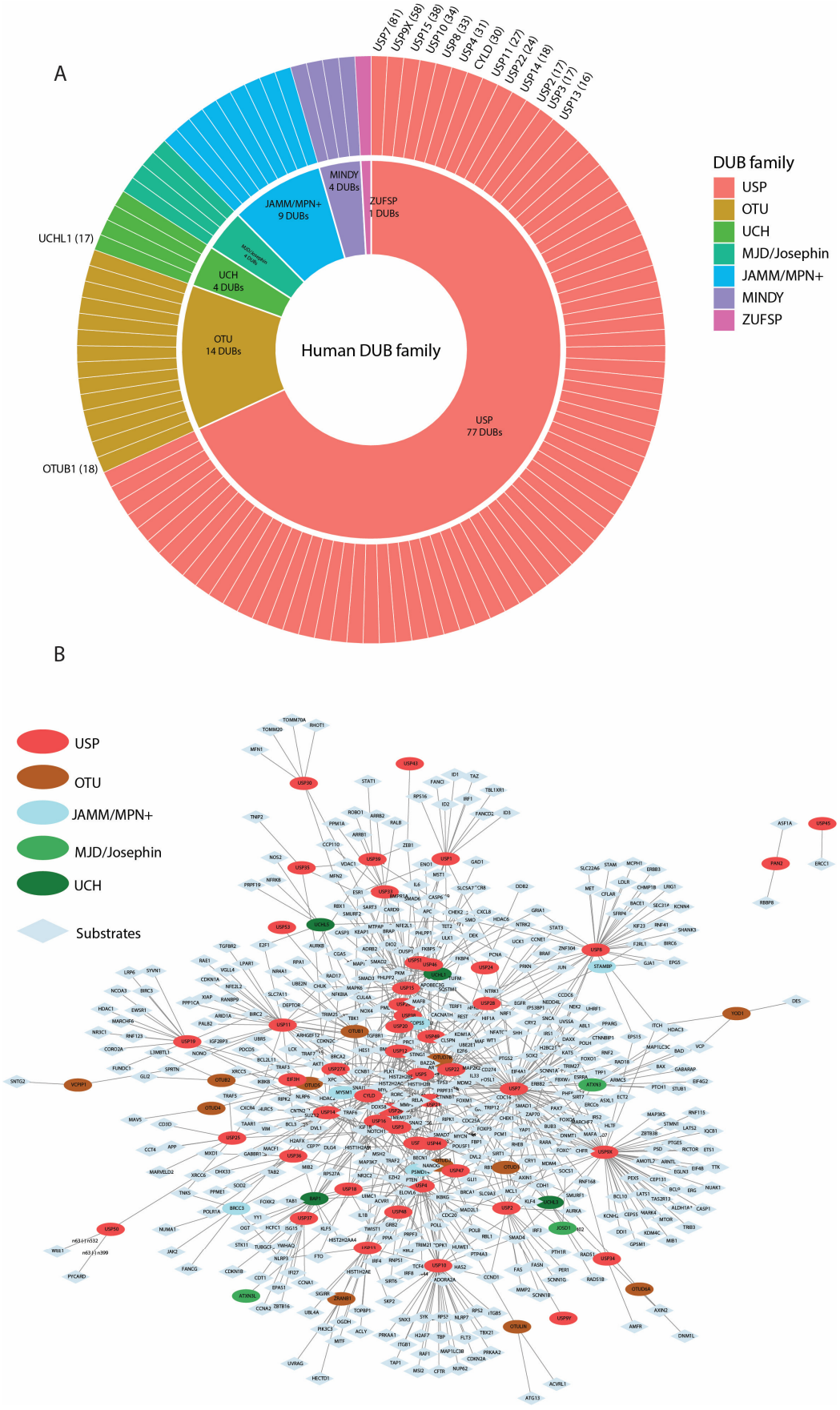


Figure 2. Classification of the human deubiquitinase (DUB) family and landscape of known DUB–substrate associations. (**A**) Sunburst plot showing the classification of human DUBs into seven

families: ubiquitin-specific proteases (USP), ovarian tumor proteases (OTU), ubiquitin C-terminal hydrolases (UCH), Machado–Joseph disease domain proteases (MJD/Josephin), JAMM/MPN+ metalloproteases, MYND-type zinc finger proteases (MINDY), and zinc finger USP proteases (ZUFSP). Inner segments represent DUB families, and outer segments represent individual DUBs. Numbers within the family segments indicate the number of DUBs in each family. (**B**) Network of known DUB–substrate associations obtained from UbiBrowser 2.0 and visualized using Cytoscape version 3.10. Colored oval nodes represent DUBs grouped by family, while light-blue diamond nodes represent associated substrates. Edges indicate known-substrate associations; their presence does not establish that the interactions are active in the cancer contexts analyzed.

2.3. Pan-Cancer Transcriptomic Profiling Reveals Recurrent and Cancer-Specific Expression Patterns of Human Deubiquitinases

To characterize the transcriptional landscape of human deubiquitinases (DUBs) across human malignancies, we examined their relative expression profiles across the TCGA cancer cohorts and generated a pan-cancer expression atlas (Figure 3A). Unsupervised hierarchical clustering revealed substantial variation in DUB expression patterns among cancer types. Some DUBs displayed comparatively high expression across multiple cohorts, whereas others showed distinct relative expression profiles in particular cancer types. These patterns provide a descriptive overview of DUB expression heterogeneity across cancers; however, they do not, by themselves, establish tumor-specific overexpression or demonstrate causal changes in deubiquitination activity.

To identify recurrently abundant DUBs, DUBs were ranked according to their normalized expression across the pan-cancer cohort. The top 20 highly expressed DUBs included USP19, COPS6, BAP1, USP5, USP22, USP11, DESI1, JOSD2, OTUD5, MPND, JOSD1, USP14, PSMD7, USP10, USP39, OTUB1, EIF3H, EIF3F, USP17L13, and USP7 (Figure 3B). Notably, members of the USP family predominated among the most abundantly expressed genes, although representatives from the OTU, JAMM/MPN+, UCH, and MJD/Josephin families were also consistently expressed, indicating that highly expressed DUBs span multiple catalytic classes. We next examined DUBs showing comparatively high relative expression in particular cancer cohorts. Based on the expression-ranking and visualization approach, 20 candidate DUBs were prioritized for further inspection (Figure 3C). We next examined DUBs showing comparatively high relative expression in particular cancer cohorts. Based on the expression-ranking and visualization approach, 20 candidate DUBs were prioritized for further inspection (Figure 3C). These candidates displayed distinct relative expression patterns across the analyzed cohorts, with several showing comparatively high expression in gastrointestinal cancer datasets, including esophageal carcinoma (ESCA) and stomach adenocarcinoma (STAD). Because this prioritization was based on relative expression profiles rather than formal tumor-versus-normal differential-expression testing, these genes are referred to as candidates with distinctive cohort-associated expression patterns rather than confirmed tumor-specific overexpressed genes. We next compared the relative expression profiles of DUBs in ESCA and STAD (Figure 3D). Several candidates displayed similar relative expression patterns across the two cohorts, while others showed differences in their cohort-level expression profiles. Comparison of the prioritized gene sets identified 18 shared DUBs, with DESI1 and BRCC3 appearing as cohort-specific candidates in ESCA and STAD, respectively (Figure 3E). These results suggest overlap in the relative expression patterns of selected DUBs between the two gastrointestinal cancer cohorts. However, because the analysis was descriptive and the cohorts contain histological and molecular heterogeneity, the observed overlap should not be interpreted as proof of a conserved tumor-specific expression program. The cohort-specific patterns also warrant cautious interpretation in the absence of formal tumor-versus-normal testing.

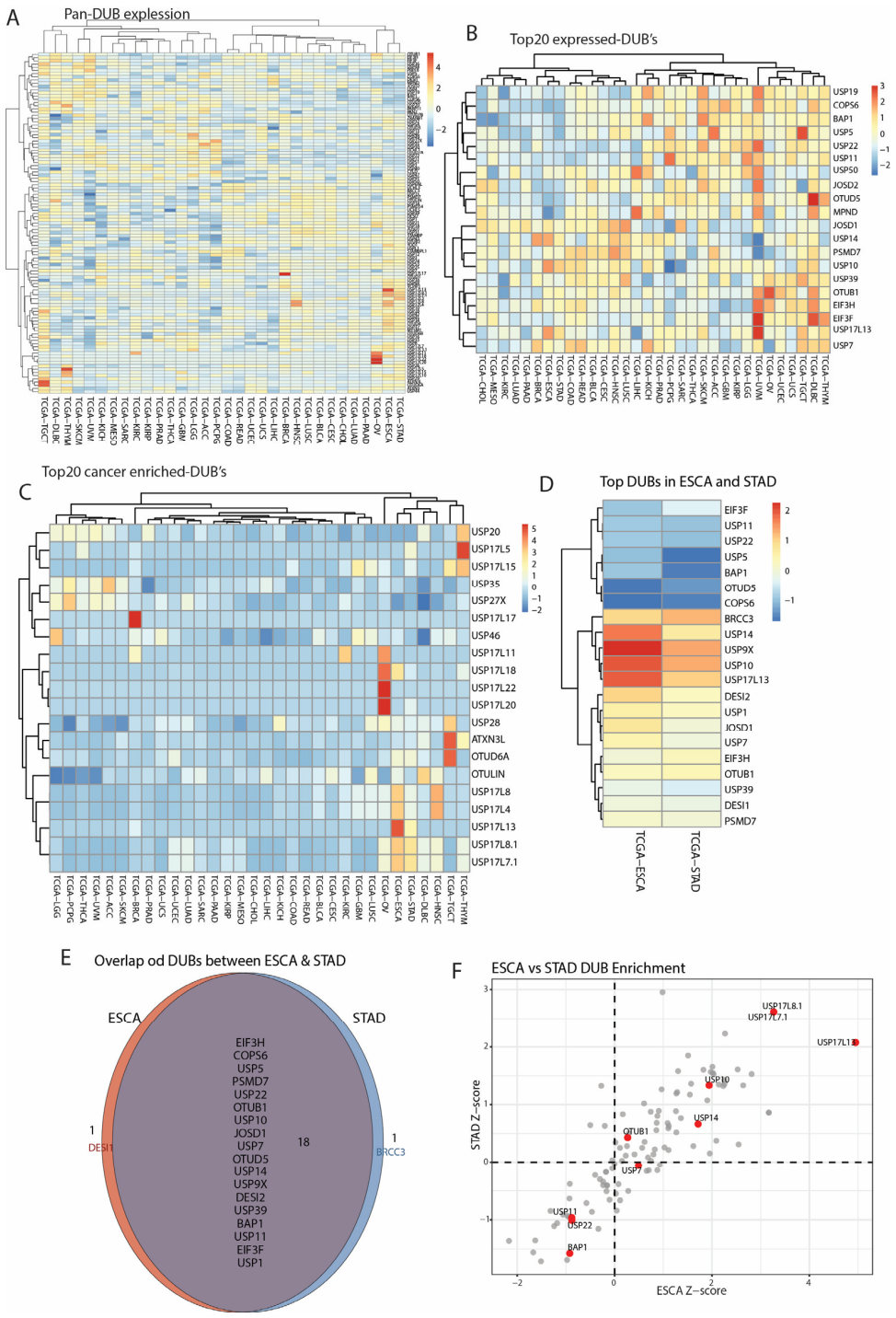


Figure 3. Pan-cancer expression landscape of human deubiquitinases characterizes relative DUB expression patterns: (**A**) Unsupervised hierarchical clustering heatmap showing the expression profiles of all human deubiquitinases (DUBs) across TCGA cancer types. Expression values are represented as Z-scores, with red indicating higher expression and blue indicating lower expression relative to the pan-cancer mean. (**B**) Heatmap of the Top 20 DUBs prioritized based on relative expression patterns across TCGA cancer cohorts. Rows represent individual DUBs and columns represent cancer types. Expression values are displayed as Z-scores following row-wise normalization. (**C**) Heatmap showing the top 20 cancer-enriched DUBs identified based on tumor-specific expression patterns across TCGA cohorts. The analysis highlights DUBs exhibiting selective enrichment within individual cancer types. (**D**) Focused comparison of relative DUB expression patterns in esophageal carcinoma (ESCA) and stomach adenocarcinoma (STAD). Heatmap visualization demonstrates the relative expression levels of candidate DUBs prioritized in these gastrointestinal malignancies. (**E**) Venn diagram illustrating

the overlap of enriched DUBs between ESCA and STAD. Eighteen DUBs were shared between the prioritized gene sets, while DESI1 and BRCC3 were identified as cohort-specific candidates. (**F**) Integrated ESCA versus STAD enrichment analysis. Each point represents an individual DUB plotted according to its expression enrichment score in ESCA (x-axis) and STAD (y-axis). Red-labeled DUBs indicate Candidates with distinctive relative expression patterns, highlighting both shared and cancer-specific expression patterns across the two gastrointestinal cancer types. Color scale: Red indicates higher relative expression, white indicates intermediate expression, and blue indicates lower relative expression.

Finally, comparison of the relative DUB expression scores in ESCA and STAD showed an overall similarity in the cohort-level expression patterns, while several DUBs displayed differences between the two cohorts (Figure 3F). These observations provide a basis for prioritizing candidates for future investigation. Nevertheless, relative expression-score comparisons do not establish statistically significant differences between tumor and normal tissues, nor do they determine whether observed differences arise from tumor biology, histological composition, or other cohort-specific factors. The results should therefore be interpreted as descriptive evidence of expression-pattern similarity and variation across ESCA and STAD.

2.4. Copy Number Alteration Profiling Identifies Recurrently Amplified and Deleted Deubiquitinases Across Human Cancers

To investigate the genomic mechanisms underlying DUB dysregulation, we systematically analyzed copy number alteration (CNA) profiles of all curated human deubiquitinases across TCGA cancers using GISTIC-derived amplification and deletion frequencies. This analysis generated a comprehensive pan-cancer CNA landscape, revealing that although most DUBs exhibited relatively stable copy number profiles, a subset of genes displayed recurrent amplification or deletion across multiple malignancies (Figure 4A). Unsupervised hierarchical clustering further demonstrated that distinct cancer types possess characteristic CNA signatures, suggesting that genomic dosage alterations contribute to cancer-specific regulation of DUB expression.

To prioritize the most recurrently altered genes, we focused on DUBs exhibiting the highest CNA frequencies across the TCGA cohort (Figure 4B). Several DUBs showed consistent amplification across multiple tumor types, whereas another subset preferentially underwent copy number loss, indicating that genomic gains and deletions represent complementary mechanisms shaping the DUB landscape in human cancers. Ranking DUBs according to their mean amplification and deletion frequencies identified distinct groups of recurrently altered genes (Figure 4C). USP13, OTUD7B, EIF3H, USP21, USP36, USP32, DESI2, USP35, USP18, and BRCC3 emerged as the most frequently amplified DUBs, whereas USP48, OTUD3, MPND, USP40, USP4, BAP1, USP19, USP28, OTUD7A, and USP2 represented the most commonly deleted genes across the pan-cancer cohort. These findings identify recurrent gene-level copy-number gains and losses across the analyzed cancer cohorts. However, because focal alterations were not distinguished from broad chromosomal events in this analysis, the observed patterns cannot establish whether individual DUBs are selectively altered independently of surrounding chromosomal regions. Because recurrent genomic alterations alone may not fully capture biological importance, we next integrated CNA profiles with transcriptomic enrichment and prognostic information to prioritize candidate cancer-associated DUBs (Figure 4D). This multi-omics framework identified several genes occupying the upper region of the composite ranking, including OTUD7A, USP4, USP27X, USP1, PSMD14, UCHL1, DESI2, OTUD5, USP17L13, USP10, USP20, COPS5, USP9X, USP42, USP39, USP2, and VCPIP1. Notably, OTUD7A, USP4, USP27X, USP1, PSMD14, DESI2, and UCHL1 consistently ranked among the highest-scoring candidates, indicating concordance between recurrent genomic alterations, transcriptional dysregulation, and clinical relevance. These findings suggest that these DUBs represent robust candidate regulators with potential functional importance across multiple cancer types.

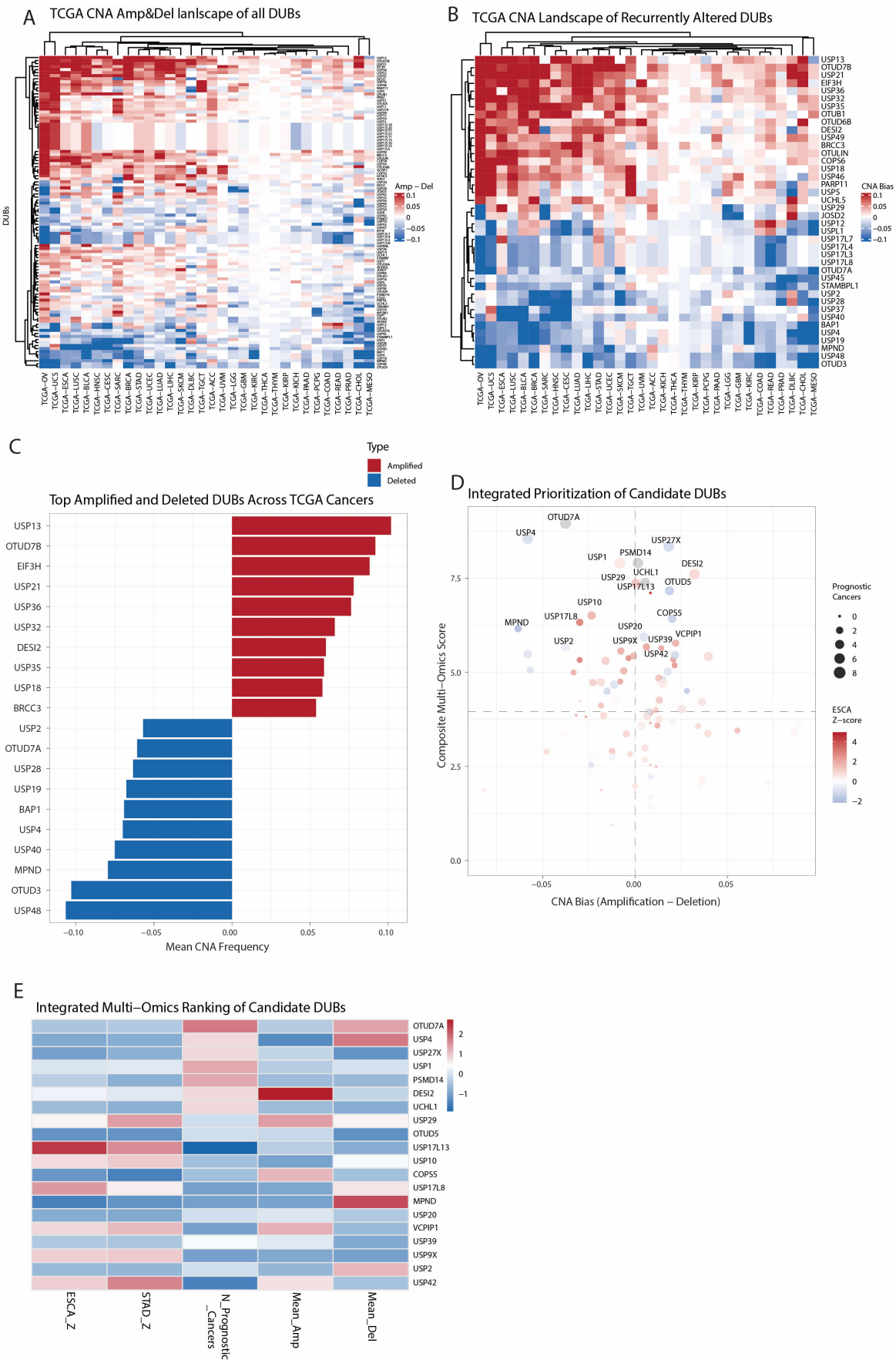


Figure 4. Copy-number alteration landscape identifies recurrently altered deubiquitinases across human cancers: (**A**) Pan-cancer copy-number alteration (CNA) landscape of human deubiquitinases

(DUBs) across TCGA cohorts. Heatmap displays mean CNA scores derived from GISTIC2 data, with red indicating amplification and blue indicating deletion events. Rows represent DUB genes and columns represent cancer types. (**B**) CNA landscape of recurrently altered DUBs across TCGA cancers. The heatmap highlights DUBs exhibiting frequent amplification or deletion events across multiple tumor types, revealing distinct patterns of genomic alteration among DUB family members. (**C**) Top amplified and deleted DUBs across human cancers. Bar plots show the mean CNA frequency of recurrently amplified (red) and deleted (blue) DUBs. Positive values indicate amplification bias, whereas negative values indicate deletion bias. (**D**) Integrated prioritization of candidate DUBs based on genomic, transcriptomic, and clinical features. Each point represents an individual DUB plotted according to CNA bias (amplification minus deletion frequency) and a composite multi-omics score. Point size reflects the number of cancer types in which the DUB exhibited prognostic significance, whereas color represents ESCA expression enrichment (Z-score). (**E**) Integrated multi-omics ranking of candidate DUBs. Heatmap summarizes the relative contribution of expression enrichment in ESCA and STAD, prognostic significance, mean amplification frequency, and mean deletion frequency. DUBs are ranked according to their overall multi-omics prioritization score. Color scales: In heatmaps, red denotes amplification or higher relative scores, whereas blue denotes deletion or lower relative scores. In panel (**D**), color intensity corresponds to ESCA enrichment scores and point size reflects prognostic recurrence across cancer types.

Finally, the integrated multi-omics ranking summarized the relative contribution of transcriptomic enrichment in esophageal (ESCA) and gastric (STAD) cancers, prognostic significance, and CNA burden for the highest-priority DUBs (Figure 4E). Rather than relying on a single molecular feature, this integrative analysis demonstrated that several candidate DUBs consistently ranked highly across multiple independent datasets, thereby identifying robust biomarkers for subsequent functional investigation. Collectively, these results establish a comprehensive pan-cancer atlas of DUB copy number alterations and provide an integrated framework for prioritizing biologically and clinically relevant deubiquitinases.

2.5. Recurrent Somatic Mutations Preferentially Target Distinct Deubiquitinases Across Human Cancers

To comprehensively characterize the mutational landscape of human deubiquitinases (DUBs), we analyzed somatic mutations across the TCGA pan-cancer cohort and quantified both mutation burden and mutation prevalence for all curated DUBs. Several DUBs exhibited recurrent genetic alterations across multiple cancer types, indicating that somatic mutations represent an additional mechanism contributing to DUB dysregulation during tumorigenesis (Figure 5A–D). Ranking DUBs by the observed number of somatic mutation events identified USP34, USP9X, PRPF8, USP29, USP24, USP26, OTUD4, USP31, BAP1, and USP4 among the genes with the highest mutation counts across the analyzed TCGA cohorts (Figure 5A). When mutation prevalence was normalized to the number of TCGA samples analyzed, USP34 (4.67%) exhibited the highest mutation frequency, followed by USP9X (4.13%), USP29 (3.37%), USP26 (2.80%), and USP24 (2.79%), identifying these enzymes as recurrently altered members of the human DUB family (Figure 5B). These counts describe the observed mutational burden and should not be interpreted as evidence that a gene is under positive selection or is a cancer driver. Because mutation counts were not adjusted for gene length or gene-specific background mutability, differences between genes may partly reflect these factors. Mutation prevalence, calculated relative to the number of analyzed samples, provides a complementary measure of how frequently a gene was altered in the cohort, but does not by itself establish functional significance.

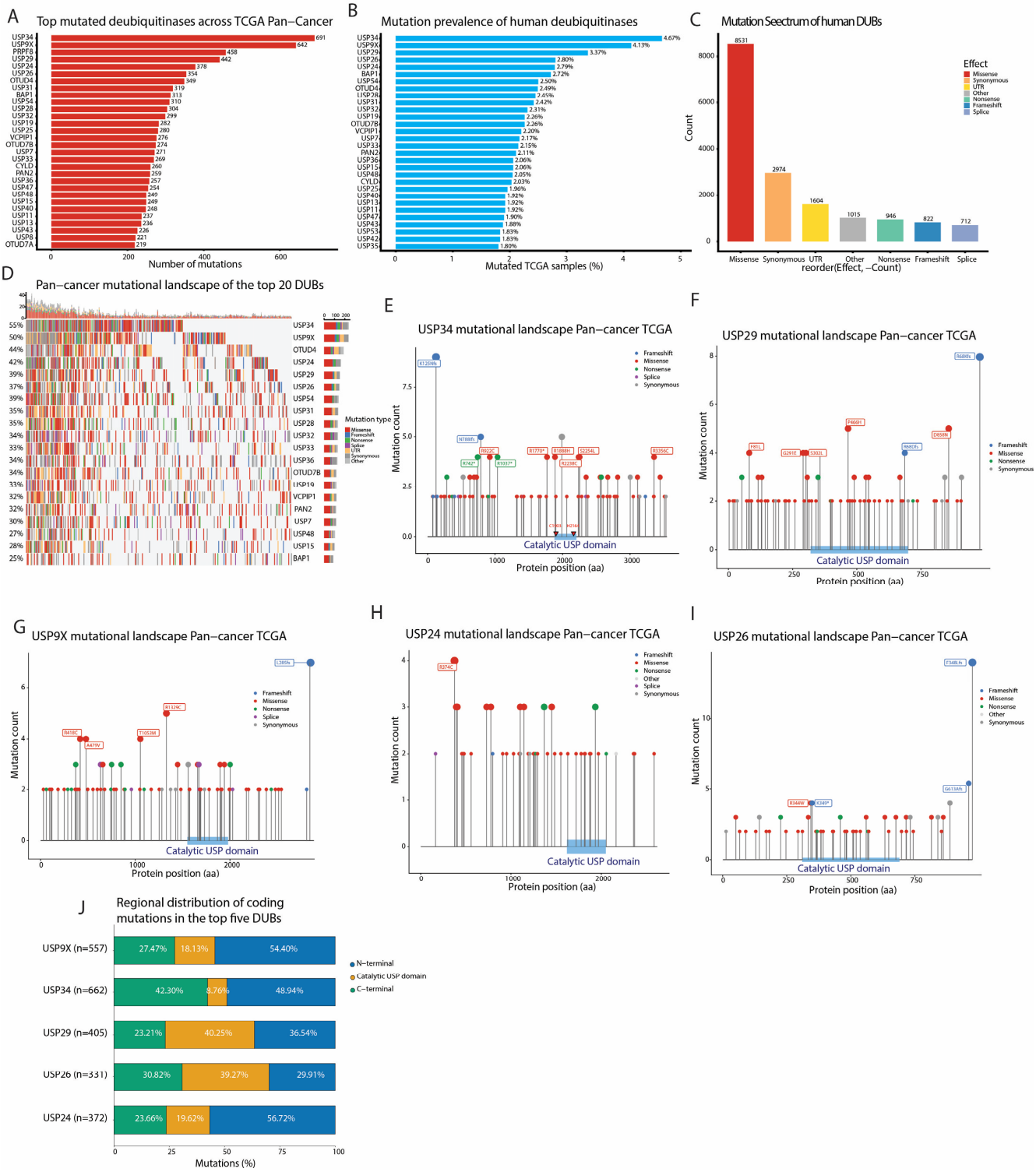


Figure 5. Pan-cancer mutational landscape of human deubiquitinases reveals recurrently altered DUBs and distinct protein domain mutation patterns: (**A**) Top mutated deubiquitinases (DUBs) across TCGA pan-cancer cohorts ranked according to the total number of observed somatic mutations. Bar lengths indicate mutation counts across all analyzed tumor samples. (**B**) Mutation prevalence of human DUBs across TCGA cancers. Bars represent the percentage of tumor samples harboring at least one somatic mutation in each DUB gene. (**C**) Global mutation spectrum of human DUBs. Bar plot summarizes the distribution of mutation classes observed across all DUB genes, including missense, synonymous, untranslated region (UTR), nonsense, frameshift, splice-site, and other mutation types. (**D**) Oncoprint representation of the top 20 most frequently mutated DUBs across TCGA cancers. Columns represent individual tumor samples and rows represent DUB genes. Colors indicate mutation

classes, illustrating the prevalence and heterogeneity of DUB alterations across cancers. (**E**–**I**) Protein-level mutational landscapes of the five most frequently mutated DUBs: USP34 (**E**), USP29 (**F**), USP9X (**G**), USP24 (**H**), and USP26 (**I**). Lollipop plots display the position and frequency of recurrent somatic mutations along each protein sequence. Mutation colors indicate mutation type, and the blue shaded region denotes the catalytic USP domain. Selected recurrent mutation hotspots are annotated. (**J**) Regional distribution of coding mutations within the top five mutated DUBs. Stacked bar plots show the proportion of mutations located within the N-terminal region, catalytic USP domain, and C-terminal region of each protein. Percentages were calculated using mutations with known amino acid positions and highlight gene-specific differences in mutation localization. Color coding: Mutation classes are indicated by distinct colors in panels (**C**–**I**). In panel (**J**), blue denotes N-terminal mutations, orange denotes mutations within the catalytic USP domain, and green denotes C-terminal mutations.

We next examined the spectrum of mutation classes across all DUBs. Missense mutations represented the predominant alteration type (8531 events), followed by synonymous mutations (2974), untranslated region (UTR) variants (1604), other mutation classes (1015), nonsense mutations (946), frameshift mutations (822), and splice-site mutations (712) (Figure 5C). These findings indicate that amino acid altering missense mutations account for the majority of somatic alterations affecting human DUBs. To visualize mutation distribution across individual tumors, we generated an oncoprint summarizing the twenty most frequently mutated DUBs (Figure 5D). Although mutation frequencies varied among cancer types, recurrent alterations were observed across a broad spectrum of malignancies, demonstrating that DUB mutations are widely distributed throughout the TCGA cohort rather than being restricted to a single tumor lineage.

Because USP34, USP9X, USP29, USP24, and USP26 represented the five most frequently mutated DUBs, we next mapped individual mutations onto their respective protein architectures to investigate whether mutations preferentially accumulated within functional domains (Figure 5E–I). Protein-level mutation mapping revealed that recurrent mutations were distributed throughout the protein sequence, with several recurrent hotspots occurring outside the catalytic USP domain. Nevertheless, each protein also harbored mutations within the catalytic region, suggesting that both catalytic and non-catalytic regions may contribute to altered DUB function in cancer.

To further examine the regional distribution of coding mutations, each protein was divided into N-terminal, catalytic USP domain, and C-terminal regions, and the proportion of mutations within each segment was quantified (Figure 5J). The catalytic USP domain accounted for 40.25% of all coding mutations in USP29 and 39.27% in USP26, whereas USP34 (8.76%), USP9X (18.13%), and USP24 (19.62%) exhibited comparatively fewer mutations within their catalytic domains, with the majority occurring in the flanking N- and C-terminal regions. These observations suggest that while catalytic-domain mutations are relatively common in certain DUBs, non-catalytic regions constitute major hotspots of somatic alteration, suggesting that cancer-associated mutations may perturb protein–protein interactions, regulatory motifs, or subcellular localization independently of catalytic activity.

Importantly, the protein architecture analysis revealed that recurrent cancer-associated mutations are not uniformly distributed across DUB proteins but instead exhibit gene-specific regional preferences, suggesting distinct mechanisms by which somatic mutations may influence deubiquitinase function. Collectively, these findings establish a comprehensive pan-cancer mutational atlas of human deubiquitinases and demonstrate that recurrent somatic mutations preferentially affect a subset of DUBs while exhibiting distinct positional distributions across functional protein domains, highlighting candidate enzymes for future mechanistic investigation.

2.6. Prognostic Landscape of Deubiquitinases Across Human Cancers

To determine the clinical significance of deubiquitinases across cancer types, we performed univariate Cox proportional hazards analyses using TCGA survival data and evaluated associations between DUB expression and patient outcomes across multiple malignancies (Figure 6A–G). Analysis of the prognostic landscape revealed substantial heterogeneity in the number of survival-associated DUBs across cancer types (Figure 6A). Among all tumors examined, lower-grade glioma (LGG) exhibited the highest number of prognostic DUBs, followed by kidney renal clear cell carcinoma (KIRC), pancreatic adenocarcinoma (PAAD), kidney renal papillary cell carcinoma (KIRP), and uveal melanoma (UVM), indicating that DUB dysregulation may have particularly strong clinical relevance in these malignancies.

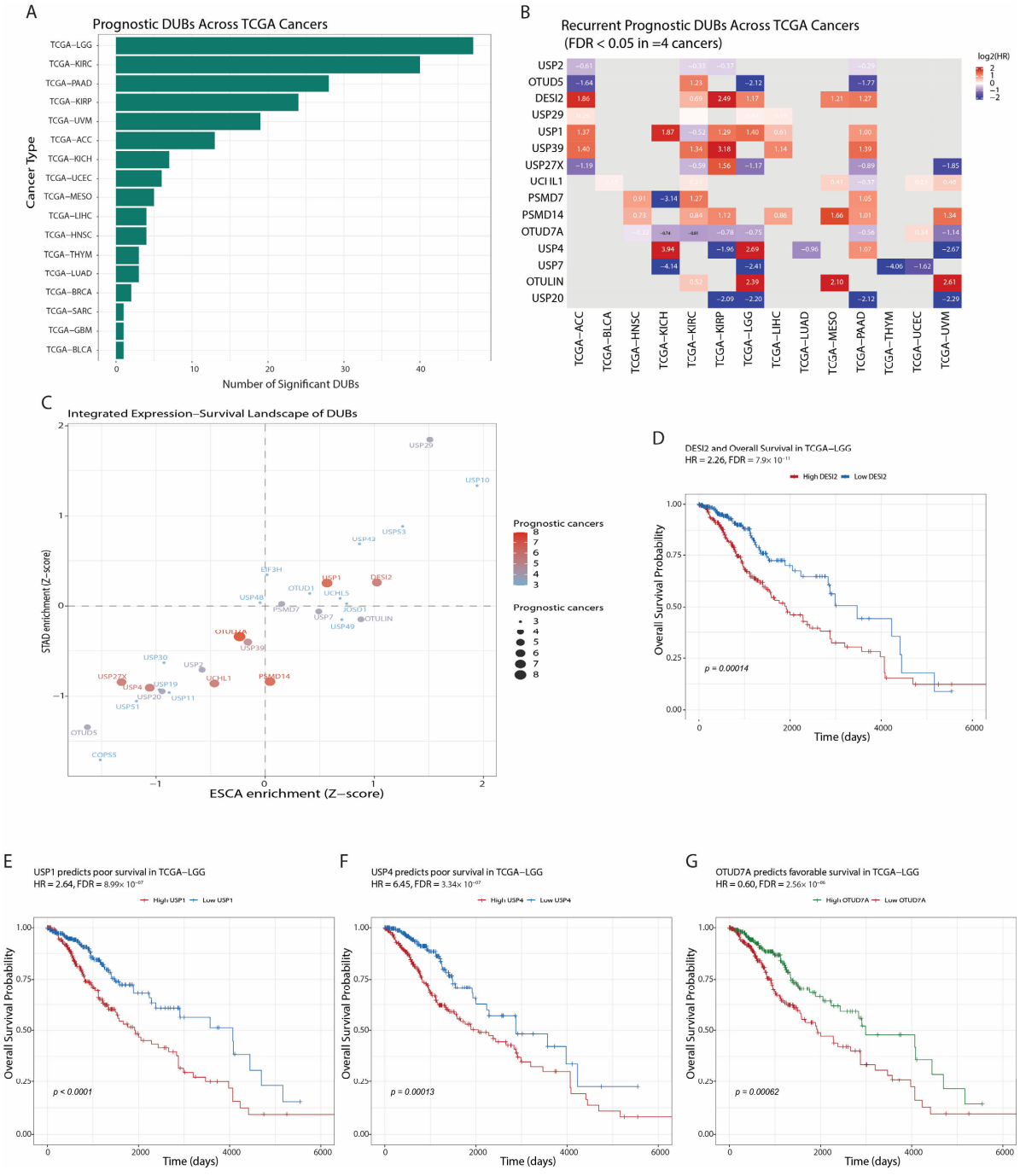


Figure 6. Pan-cancer prognostic landscape identifies recurrent survival-associated deubiquitinases across human cancers: (**A**) Distribution of prognostic deubiquitinases (DUBs) across TCGA cancer

types. Bar plot showing the number of DUBs significantly associated with overall survival in each cancer cohort based on univariate Cox proportional hazards analysis. Cancer types are ranked according to the total number of prognostic DUBs identified. (**B**) Recurrent prognostic DUBs across TCGA cancers. Heatmap displaying DUBs significantly associated with patient survival in at least four independent cancer types. Red indicates unfavorable prognostic associations (hazard ratio > 1), whereas blue indicates favorable prognostic associations (hazard ratio < 1). The color intensity reflects the magnitude of the hazard ratio. (**C**) Integrated expression–survival landscape of DUBs in esophageal carcinoma (ESCA) and stomach adenocarcinoma (STAD). Each point represents an individual DUB plotted according to its expression enrichment score and prognostic recurrence across cancers. Point size corresponds to the number of cancer types in which the DUB exhibits significant prognostic associations, while color intensity represents ESCA enrichment. Candidate DUBs occupying the upper-right quadrant combine strong expression enrichment with recurrent prognostic significance. (**D**–**G**) Kaplan–Meier survival analyses of representative prognostic DUBs in glioblastoma. Patients were stratified into high- and low-expression groups based on median gene expression levels. Kaplan–Meier curves depict overall survival differences associated with DESI2 (**D**), USP1 (**E**), USP4 (**F**), and OTUD7A (**G**) expression. Hazard ratios (HR), false discovery rate (FDR)-adjusted *p* values, and sample sizes are indicated within each panel. Color scales: In panel (**B**), red denotes poor prognostic associations and blue denotes favorable prognostic associations. In panel (**C**), color intensity represents ESCA enrichment scores, while point size reflects the number of cancer types exhibiting significant survival associations.

To identify recurrent prognostic regulators, we next focused on DUBs that were significantly associated with patient survival in at least four independent cancer types (Figure 6B). Several DUBs displayed consistent prognostic associations across multiple tumors, including USP1, USP29, USP39, DESI2, OTUD5, PSMD7, PSMD14, OTUD7A, USP4, USP7, OTULIN, and USP20. Notably, both favorable and unfavorable prognostic effects were observed depending on cancer context, highlighting the tissue-specific biological functions of DUB-mediated signaling pathways. To integrate tumor-specific expression patterns with prognostic information, we generated an expression-survival landscape using ESCA and STAD as representative gastrointestinal cancers (Figure 6C). This analysis identified several DUBs that combined strong transcriptional dysregulation with recurrent prognostic significance. In particular, USP29, USP10, USP53, USP43, DESI2, USP1, OTUD7A, UCHL5, and USP39 occupied prominent positions within the integrated landscape, suggesting potential functional importance in tumor progression.

Because LGG exhibited the greatest number of prognostic DUBs, representative Kaplan–Meier analyses were performed for selected candidates (Figure 6D–G). Elevated expression of DESI2, USP1, and USP4 was associated with significantly poorer overall survival, whereas increased OTUD7A expression predicted improved patient outcomes. These findings demonstrate that individual DUBs can function either as risk-associated or protective biomarkers depending on their biological roles within specific tumor contexts.

Collectively, these analyses establish a comprehensive pan-cancer prognostic atlas of human deubiquitinases and identify recurrent survival-associated DUBs that may serve as clinically relevant biomarkers and potential therapeutic targets across multiple malignancies.

2.7. Proteomic Interrogation of DUB-Associated Substrate Networks Reveals Conserved and Cancer-Specific Regulatory Programs

To investigate DUB-associated substrate networks at the proteome level, we integrated publicly available proteomics datasets from gastric cancer, esophageal cancer, and glioblastoma (Figure 7A–I). Gastric cancer proteomic data were obtained from Li et al. [47], and esophageal cancer data were obtained from Li et al. [48], the brain tumor proteomic dataset was obtained from Wang et al. [49], which characterized human glioblastoma. Accordingly, the brain tumor proteomic analyses described below refer to glioblastoma rather than lower-grade glioma. Differential protein abundance analysis identified extensive proteomic remodeling in both gastric

and esophageal cancers (Figure 7A,B). Numerous proteins exhibited significant upregulation or downregulation relative to matched controls, indicating widespread alterations in signaling pathways and cellular regulatory networks. We next overlaid recurrently dysregulated DUBs onto experimentally supported DUB–substrate interaction networks to investigate whether altered DUB expression was accompanied by coordinated changes in substrate abundance. In gastric cancer, network analysis revealed extensive connectivity between highly expressed DUBs and proteins involved in cell-cycle regulation, chromatin remodeling, DNA damage response, and inflammatory signaling pathways (Figure 7C). Central network hubs included USP10, USP14, USP5, USP22, USP1, and OTUB1, which were connected to multiple dysregulated substrates, suggesting broad regulatory influence on tumor-associated proteomic programs.

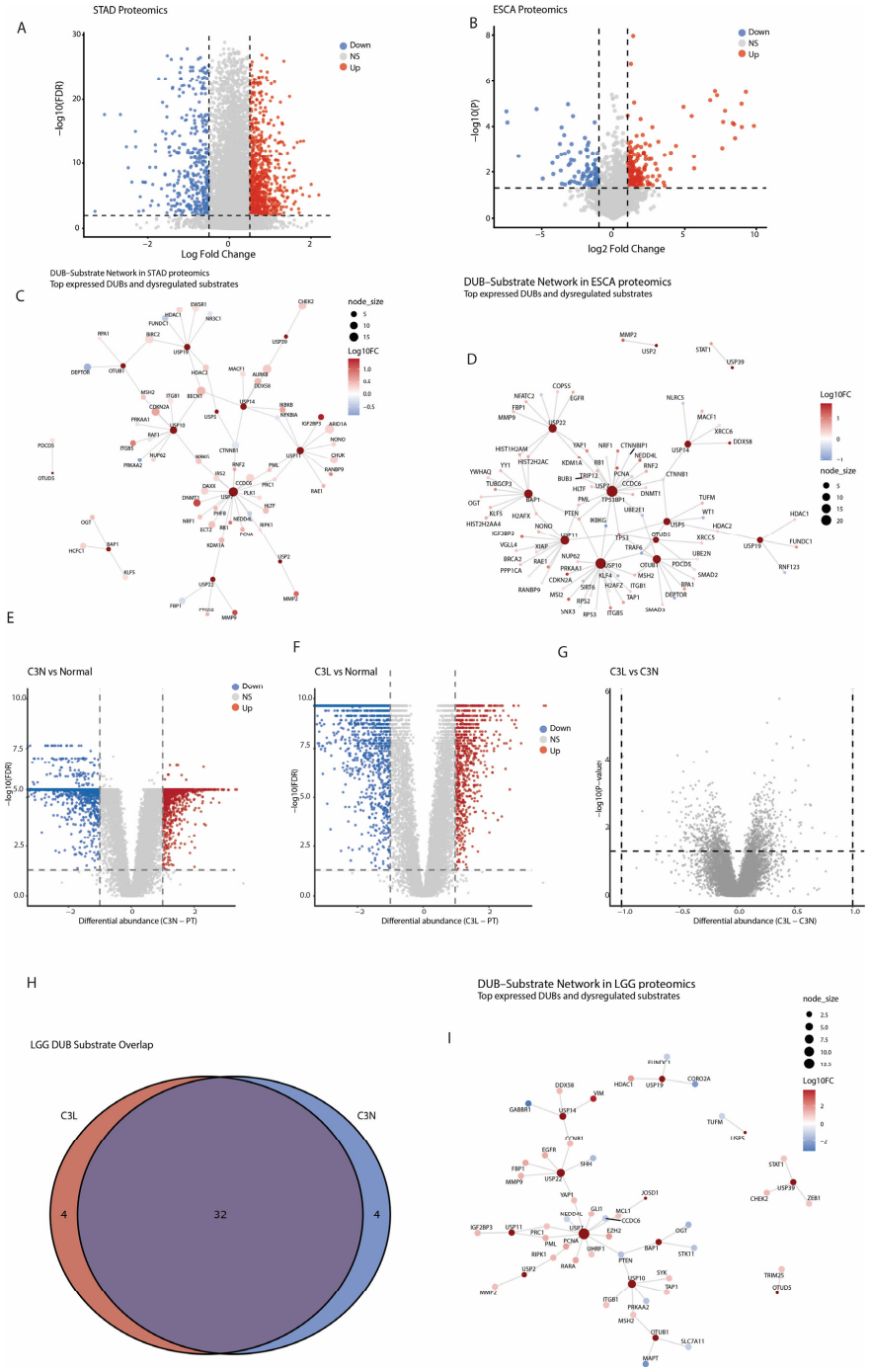


Figure 7. Integration of cancer proteomics datasets identifies DUB-associated substrate networks in gastric cancer, esophageal cancer, and glioblastoma: (**A**) Volcano plot showing differentially expressed

proteins in gastric cancer proteomic datasets. Significantly upregulated and downregulated proteins were identified based on fold-change and statistical significance thresholds. Selected dysregulated proteins are annotated. (**B**) Volcano plot showing differentially expressed proteins in esophageal cancer proteomic datasets. Differentially expressed proteins were identified using the same criteria as in panel (**A**). (**C**) DUB–substrate interaction network constructed using significantly dysregulated proteins identified in gastric cancer. Red nodes represent upregulated proteins, blue nodes represent downregulated proteins, and maroon nodes represent DUBs. Edges indicate experimentally validated DUB–substrate interactions. (**D**) DUB–substrate interaction network generated from dysregulated proteins identified in esophageal cancer. The network highlights candidate DUBs and their associated substrates potentially contributing to esophageal tumor biology. (**E**–**G**) Volcano plots illustrating differential protein abundance analyses in lower-grade glioma (LGG) proteomic datasets. Comparisons include tumor versus matched normal tissues and subtype-specific analyses. Significantly altered proteins are highlighted and annotated. (**H**) Venn diagram showing the overlap of dysregulated DUB-associated substrates identified across glioblastoma comparisons. Shared and unique substrate proteins are indicated for each dataset. (**I**) Integrated DUB–substrate interaction network generated from recurrent dysregulated proteins identified in LGG. Green nodes represent DUBs, whereas colored nodes represent dysregulated substrate proteins. Highly connected DUBs occupy central positions within the network and are linked to multiple cancer-associated substrates. Node and edge annotations: maroon nodes represent DUBs, red nodes indicate upregulated proteins, blue nodes indicate downregulated proteins, and gray edges denote experimentally validated DUB–substrate interactions. Node size is proportional to interaction connectivity. Proteomic datasets: Gastric cancer proteomic data were obtained from Li et al. [47], esophageal cancer proteomic data from Li et al. [48], and glioblastoma proteomic data from Wang et al. [49].

2.8. Integration of Proteomic Substrate Networks Identifies Conserved Biological Programs Regulated by Recurrent DUBs Across Human Cancers

To investigate the biological processes potentially regulated by recurrently dysregulated deubiquitinases, we integrated substrate interaction networks derived from gastric cancer, esophageal cancer, and lower-grade glioma proteomic datasets and performed pathway enrichment analyses on DUB-associated substrates (Figure 8A–D). Comparative analysis of DUB–substrate connectivity revealed substantial overlap in substrate-centered regulatory networks across cancer types (Figure 8A). Among the recurrent DUBs, USP7, USP10, USP11, USP22, USP14, BAP1, OTUB1, USP19, USP5, and OTUD5 exhibited the highest substrate connectivity, indicating that these enzymes occupy central positions within cancer-associated regulatory networks. Notably, several DUBs displayed consistent substrate associations across ESCA, STAD, and LGG, suggesting conserved functional roles across diverse tumor contexts.

To identify biological pathways enriched among recurrent DUB substrates, we performed Gene Ontology (GO), KEGG, and Reactome analyses using the shared substrate repertoire (Figure 8B). GO enrichment demonstrated significant overrepresentation of pathways related to intrinsic apoptotic signaling, regulation of apoptotic processes, response to oxidative stress, and signaling pathways involved in tumor necrosis factor (TNF) responses. KEGG analysis highlighted enrichment of apoptosis, NF-κB signaling, proteasome function, Salmonella infection, and hepatitis virus-related pathways, whereas Reactome analysis identified strong enrichment for TNF receptor signaling, Toll-like receptor (TLR) cascades, TRAF6-mediated signaling, and NF-κB activation pathways.

To obtain a broader systems-level view, we next analyzed the top recurrent substrates associated with dysregulated DUBs across all cancer types (Figure 8C). This expanded analysis revealed enrichment of biological programs governing cell death regulation, inflammatory signaling, stress responses, protein modification processes, and signal transduction pathways, indicating that DUB-mediated substrate regulation converges on fundamental hallmarks of cancer biology. Integration of DUBs, representative substrates, and en-

riched pathways generated a unified DUB–substrate–pathway network (Figure 8D). In this framework, recurrent DUBs including USP7, USP10, USP11, USP14, and USP22 were connected to key regulatory substrates such as TRAF6, RIPK1, NFKBIA, IKBKB, BECN1, PTEN, MCL1, TP53, and CTNNB1, which collectively mapped to three major biological programs: NF-κB/TLR signaling, apoptosis regulation, and cell-cycle control. The enrichment results identify biological processes and signaling pathways overrepresented among the selected DUB-associated substrate sets. These associations suggest candidate functional connections between DUB–substrate networks and pathways involved in apoptosis, inflammatory signaling, and cell-cycle regulation. However, pathway enrichment and network connectivity do not demonstrate that these pathways are directly regulated by the identified DUBs in the analyzed tumors. The results should therefore be interpreted as hypotheses about potentially relevant biological relationships rather than definitive evidence of active regulatory mechanisms.

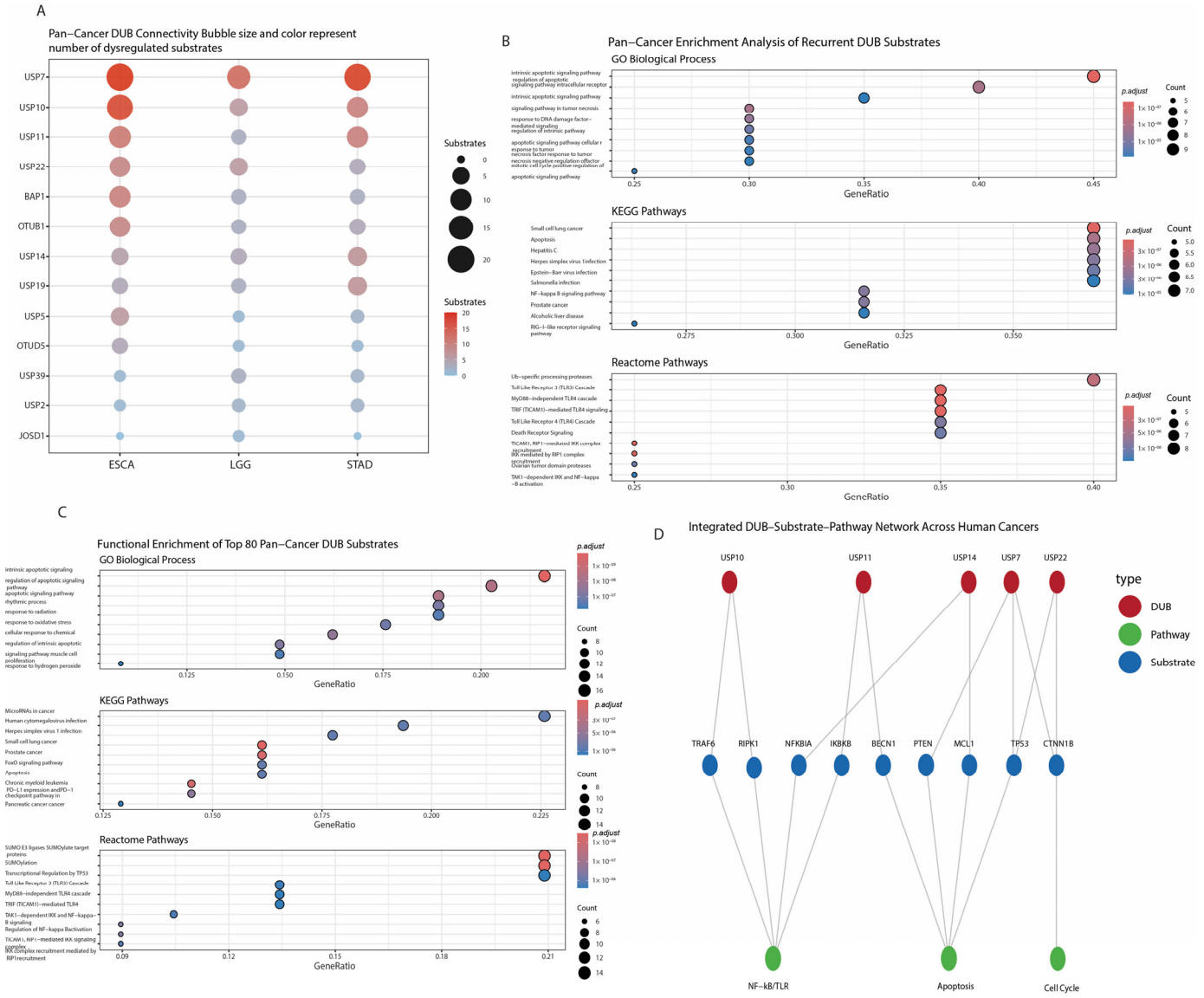


Figure 8. Functional characterization of recurrent DUB-associated substrates reveals convergent cancer signaling networks. (**A**) Pan-cancer DUB connectivity analysis across ESCA, STAD, and glioblastoma proteomic dataset. Bubble plot showing the number of dysregulated substrates associated with recurrent DUBs identified from DUB–substrate interaction analyses. Bubble size and color intensity

correspond to the number of dysregulated substrates linked to each DUB in the respective cancer type. USP7, USP10, USP11, USP22, and BAP1 exhibited the highest substrate connectivity across multiple tumor contexts. (**B**) Functional enrichment analysis of recurrent DUB-associated substrates shared across cancer types. Gene Ontology (GO) Biological Process, KEGG, and Reactome pathway analyses were performed using substrates recurrently associated with dysregulated DUBs in ESCA, STAD, and LGG. Bubble size indicates gene count, color represents adjusted *p* value, and the x-axis denotes gene ratio. Enriched pathways were predominantly associated with apoptosis, inflammatory signaling, NF-κB/TLR signaling, immune regulation, and cellular stress responses. (**C**) Functional enrichment analysis of the top 80 pan-cancer DUB substrates. GO Biological Process, KEGG, and Reactome analyses identified significant enrichment of pathways involved in apoptotic signaling, regulation of protein modification, cytokine-mediated signaling, proteasome function, NF-κB activation, and cancer-associated signaling cascades. Bubble size reflects the number of genes contributing to each pathway and color intensity corresponds to enrichment significance. (**D**) Integrated DUB–substrate–pathway network summarizing the major biological programs regulated by recurrent DUBs across cancers. Red nodes represent DUBs, blue nodes represent representative substrate proteins, and green nodes denote significantly enriched biological pathways. Edges indicate experimentally validated DUB–substrate interactions and substrate pathway associations. The network highlights convergence of multiple DUBs on key cancer-related pathways including NF-κB/TLR signaling, apoptosis, and cell-cycle regulation through substrates such as TRAF6, RIPK1, NFKBIA, IKBKB, PTEN, TP53, and CTNNB1.

Collectively, these analyses demonstrate that recurrently altered DUBs converge on a limited number of highly interconnected signaling pathways despite substantial heterogeneity across cancer types. The enrichment of NF-κB signaling, apoptosis, and cell-cycle regulatory networks suggests that dysregulated deubiquitination may promote tumor progression through coordinated modulation of inflammatory signaling, survival pathways, and cellular proliferation programs.

Taken together, our multi-layered pan-cancer analysis establishes a comprehensive atlas of human deubiquitinases across transcriptional, genomic, mutational, proteomic, and clinical dimensions. By integrating TCGA-based molecular profiling with independent cancer proteomics datasets, we identify recurrently altered DUBs that are consistently associated with tumor-specific expression patterns, genomic alterations, patient survival, and substrate centered signaling networks. These analyses highlight USP7, USP10, USP11, USP14, USP22, OTUD5, OTUB1, and BAP1 as prominent regulatory hubs and reveal convergence of DUB-mediated signaling on NF-κB/TLR signaling, apoptosis, and cell-cycle control pathways. Collectively, our findings provide a systems-level framework for understanding DUB biology in cancer and establish a resource for prioritizing candidate deubiquitinases as biomarkers and therapeutic targets.

3. Discussion

Deubiquitinases (DUBs) have emerged as critical regulators of cellular homeostasis through their ability to remove ubiquitin modifications from substrate proteins, thereby controlling protein stability, localization, signaling activity, and degradation [50–52]. Dysregulation of ubiquitin-dependent signaling is increasingly recognized as a hallmark of cancer [44,53]; however, a systematic understanding of the DUB landscape across human malignancies has remained incomplete. In the present study, we performed an integrated pan-cancer analysis of the human DUB family using transcriptomic, genomic, clinical, and proteomic datasets to generate a comprehensive atlas of DUB dysregulation across cancers. By integrating expression profiles, somatic mutations, copy-number alterations (CNAs), survival associations, and substrate-centered proteomic networks, we identified recurrent DUBs that may serve as key drivers of tumor progression and potential therapeutic targets.

Our analysis revealed heterogeneous DUB expression patterns across TCGA cancer types, with several DUBs, including USP7, USP10, USP11, USP14, USP22, OTUD5, and

BAP1, displaying distinctive relative expression profiles across multiple malignancies. These findings extend previous gene-focused studies by placing established cancer-associated DUBs within a broader pan-cancer context. For example, USP7 has been extensively investigated because of its regulation of MDM2 and the p53 tumor-suppressor pathway [54–58], whereas USP22 has been implicated in transcriptional regulation, cancer stem-cell properties, and tumor progression [59]. The recurrence of these DUBs in our pan-cancer prioritization is consistent with their established relevance to cancer biology. However, their relative expression patterns do not necessarily indicate increased enzymatic activity or a uniform oncogenic function across tumor types. DUB functions are dependent on cellular context, substrate availability, and the signaling pathways in which they participate. The overlap in DUB expression patterns observed between esophageal carcinoma (ESCA) and stomach adenocarcinoma (STAD) therefore provides a basis for investigating shared regulatory candidates in upper gastrointestinal cancers, rather than demonstrating that the same functional mechanisms operate in both malignancies.

Our genomic analysis identified recurrent copy-number alterations affecting several DUB genes, including gains involving USP13, USP21, and USP36 and losses involving BAP1, USP2, USP19, USP28, and OTUD7A. These patterns are broadly consistent with previous reports linking selected DUBs to oncogenic signaling or tumor-suppressive functions [32]. BAP1 is a particularly well-established example of a tumor-suppressive DUB, with inactivating alterations reported in several malignancies, including mesothelioma, uveal melanoma, and renal cell carcinoma [60]. Conversely, USP13, USP21, and USP36 have been implicated in the stabilization of cancer-associated proteins and the maintenance of oncogenic signaling in particular cellular contexts [42,61,62]. Our analysis extends these observations by placing these genes within a broader cross-cancer genomic landscape. Nevertheless, gene-level copy-number alterations do not establish whether a particular DUB is functionally activated or inactivated, and the presence of a genomic alteration alone does not demonstrate that it drives the associated cancer phenotype. The observed alterations should therefore be interpreted as genomic features that prioritize candidates for further investigation, rather than direct evidence of functional dependence.

One of the most striking findings of this study was the extensive mutational burden observed within specific DUB genes. USP34, USP9X, USP29, USP24, and USP26 emerged as the most frequently mutated DUBs across TCGA cohorts. Previous studies have linked USP34 to Wnt/β-catenin signaling and DNA damage responses, whereas USP9X has been reported to function as either an oncogene or tumor suppressor depending on tumor context [63]. Interestingly, our protein architecture analyses demonstrated that mutations are not uniformly distributed across DUB proteins but instead display gene-specific regional preferences. In several DUBs, mutations clustered within or adjacent to catalytic USP domains, suggesting potential effects on substrate recognition, enzymatic activity, or protein stability. Such non-random mutation patterns support the notion that selective pressure acts on specific structural regions of DUB proteins during tumor evolution.

The survival analyses revealed substantial variation in the associations between DUB expression and patient outcomes across cancer types. USP1 and USP4 were among the recurrent prognostic candidates identified in our analysis, consistent with previous investigations implicating these enzymes in DNA repair, replication-stress responses, and cancer-cell survival [64–66]. These established biological roles provide a rationale for further investigating their clinical associations, but they do not independently validate the prognostic results observed here. These established biological roles provide a rationale for further investigating their clinical associations, but they do not independently validate the prognostic results observed here. Similarly, the recurrent associations identified for OTUD7A, DESI2, PSMD14, and UCHL1 provide candidates for follow-up investigation,

although their prognostic implications may differ between tumor types. Importantly, the survival analyses were based on univariate Cox regression and were not adjusted for clinical covariates. Consequently, the observed associations cannot establish independent prognostic value or demonstrate that altered DUB expression directly influences patient outcomes. Further validation in independent cohorts and multivariable models will be needed to determine whether these candidates provide prognostic information beyond established clinical factors.

To place the genomic and transcriptomic findings in a protein-level context, we integrated public proteomic datasets with curated DUB–substrate interactions. This approach identified candidate associations between recurrently prioritized DUBs and proteins showing altered abundance in the analyzed cancer datasets. USP7, USP10, USP11, USP14, and USP22 showed high connectivity within the constructed networks, consistent with the extensive substrate associations reported for several of these enzymes in previous studies. For example, the established involvement of USP7 in p53-associated regulation and of USP22 in transcriptional control provides biological context for investigating their network-associated substrates [67,68]. However, high connectivity in a curated interaction network reflects the available interaction annotations and does not establish that a DUB actively regulates every connected substrate in a particular tumor. Likewise, altered substrate abundance cannot by itself establish a causal relationship with DUB activity, because protein abundance is influenced by transcription, translation, degradation, and other regulatory processes. The network analysis therefore extends the existing literature by prioritizing candidate DUB–substrate relationships across selected cancer proteomes, while generating hypotheses that require cancer-context-specific functional validation.

Functional enrichment analysis identified overrepresented biological processes and pathways associated with the selected DUB-linked substrate sets, particularly those related to NF-κB and inflammatory signaling, apoptosis, and cell-cycle regulation. These findings are biologically plausible given the established involvement of ubiquitin-dependent signaling in these processes. The convergence of substrate associations on these pathways suggests that the curated DUB–substrate relationships may provide a useful framework for identifying candidate connections between DUB biology and cancer-associated signaling. Nevertheless, pathway enrichment does not demonstrate direct regulation by the prioritized DUBs, nor does it establish that the same regulatory relationships are active across different tumor types. Accordingly, these findings should be interpreted as hypothesis-generating associations that can guide future mechanistic studies rather than as evidence of a common, experimentally established deubiquitination program across cancers [69–71]. The observation that multiple DUBs converge upon common substrate networks and signaling pathways suggests the existence of coordinated deubiquitination programs that shape cancer phenotypes across tissue types. This convergence may explain why DUB-targeted therapies have shown broad antitumor potential despite marked heterogeneity among cancers.

Collectively, this study provides a systematic framework for prioritizing cancer-associated DUBs through the integration of genomic, transcriptomic, clinical, and protein-level information. Candidates such as USP1, USP4, USP7, USP10, USP11, USP22, OTUD7A, DESI2, and PSMD14 warrant further investigation based on their observed molecular alterations and clinical associations. Their potential translational relevance will depend on independent validation, cancer-context-specific functional characterization, and assessment of target selectivity, therapeutic efficacy, and possible toxicity. Given the growing interest in selective DUB inhibitors, the candidates identified in this study may help guide future investigations into biomarker development and therapeutic strategies targeting the ubiquitin system. However, further experimental and clinical validation is required before their utility in precision oncology can be established.

Study Limitations and Future Directions

Despite the comprehensive nature of this pan-cancer analysis, several limitations should be acknowledged. First, our study primarily relies on publicly available datasets, including TCGA and selected proteomic cohorts, which may introduce biases related to sample composition, sequencing depth, and clinical heterogeneity. Although these datasets provide extensive coverage across cancer types, they may not fully capture rare tumor subtypes or intra-tumoral heterogeneity. Second, the analyses presented here are largely correlative, and while they identify strong associations between DUB alterations and clinical or molecular features, they do not establish direct causal relationships. Functional validation through in vitro and in vivo experimental models will be essential to confirm the biological roles of candidate DUBs identified in this study. We are currently pursuing detailed biochemical and molecular biological investigations of multiple candidate DUBs prioritized through this pan-cancer analysis to further elucidate their functional roles and underlying regulatory mechanisms in cancer. These ongoing investigations constitute a separate research effort and are beyond the scope of the present study. Accordingly, the candidates identified here should be considered priorities for further investigation rather than experimentally validated cancer drivers or therapeutic targets.

An additional limitation concerns the interpretation of the pan-cancer transcriptomic analysis. The expression patterns were primarily characterized using Z-score-based visualization and relative expression rankings, which facilitate comparisons across cancer cohorts but do not independently establish statistically significant differences between tumor and normal tissues. Accordingly, the DUBs prioritized in this analysis should be interpreted as candidates with distinctive relative expression patterns rather than confirmed tumor-associated differentially expressed genes. Formal tumor-versus-normal differential-expression analyses incorporating effect-size estimates and multiple-testing correction would provide additional evidence to validate these candidates. This distinction is particularly important for ESCA and STAD, in which histological and molecular heterogeneity may contribute to the observed expression patterns.

The genomic and survival analyses also have limitations. Mutation rankings were based on observed mutation counts and sample-level prevalence without adjustment for gene length or gene-specific background mutability; consequently, genes with high observed mutation counts should not automatically be interpreted as cancer drivers. Similarly, the gene-level copy-number analysis did not independently distinguish focal alterations from broad chromosomal gains or losses, limiting conclusions about gene-specific copy-number events. In addition, survival associations were evaluated using univariate Cox regression and were not adjusted for clinical covariates. The identified prognostic associations therefore require further evaluation in multivariable models and independent patient cohorts before their independent prognostic value can be established.

Third, while we incorporated proteomic datasets to infer DUB–substrate interactions, these datasets are limited to a subset of cancer types and may not fully represent the dynamic and context-dependent nature of ubiquitination signaling. Additionally, post-translational modifications such as ubiquitination are highly transient and regulated by multiple factors, including E3 ligases and cellular stress conditions, which were not comprehensively addressed in this analysis. Future studies integrating ubiquitinomics, phosphoproteomics, and single-cell multi-omics approaches will provide a more detailed understanding of DUB-mediated regulatory networks. Furthermore, differences in cohort size, histological and molecular subtypes, tumor purity, clinical characteristics, and data-generation and processing workflows may influence cross-cancer and cross-cohort comparisons. Consequently, shared DUB expression patterns should be interpreted as associations observed across the analyzed datasets rather than evidence of universally conserved regulatory programs.

In summary, while our study provides a comprehensive framework for understanding DUB dysregulation across cancers, further experimental validation and integration of emerging multi-omics technologies will be essential to translate these findings into clinical applications.

4. Materials and Methods

4.1. Compilation of the Human Deubiquitinase (DUB) Reference Dataset

A reference list of human deubiquitinases (DUBs) was assembled using published literature and curated DUB resources, including UbiBrowser 2.0 [72]. Candidate genes were organized according to their reported catalytic families, including ubiquitin-specific proteases (USP), ovarian tumor proteases (OTU), ubiquitin C-terminal hydrolases (UCH), Machado–Joseph disease proteases (MJD/Josephin), JAB1/MPN/MOV34 metalloproteases (JAMM/MPN+), MINDY and ZUFSP family. Gene symbols, UniProt identifiers, family assignments, catalytic status, and recognized aliases were compiled in Supplementary Table S1. Proteins with noncanonical, uncertain, or potentially modifier-specific activities were annotated separately to distinguish established ubiquitin deubiquitinases from related proteins whose inclusion requires qualification. Experimentally supported DUB–substrate interactions were compiled from available resources and literature for construction of the DUB–substrate interaction network.

4.2. TCGA Pan-Cancer Transcriptomic Dataset Acquisition

Normalized RNA-sequencing (RNA-seq) gene expression data for The Cancer Genome Atlas (TCGA) Pan-Cancer cohorts were downloaded using the UCSC Xena Browser. The expression matrices representing 33 tumor types were obtained and processed in R (version 4.5.0). The expression matrix used in the analysis was the GDC-PANCAN.htseq_fpkm-uq.tsv file. Sample-level clinical and survival information and gene-level copy-number data were obtained from the corresponding phenotype, survival, and GISTIC files. Expression values corresponding to the curated DUB gene list were extracted for downstream analyses. To identify cancer-enriched DUBs, expression values were Z-score normalized across cancer types. Hierarchical clustering was performed using Euclidean distance and complete linkage methods. Pan-cancer expression heatmaps were generated using the pheatmap package (version 1.0.13). Highly expressed DUBs and cancer-enriched DUBs were identified based on relative expression rankings and enrichment scores across tumor types. For descriptive expression profiling, mean expression values were summarized for each DUB across the 32 analyzed TCGA cancer cohorts. DUBs were ranked by mean expression and by their maximum observed expression Z-score to identify candidates with high expression across cohorts or elevated expression in at least one cohort. These rankings were used for exploratory prioritization and were not, by themselves, interpreted as evidence of statistically significant tumor-specific enrichment. The expression summaries and rankings are provided in the accompanying analysis data. Sample identifiers were mapped to TCGA project identifiers using the matched sample metadata. The resulting matched dataset covered 32 TCGA cancer cohorts, with cohort sizes ranging from 36 samples for CHOL to 1097 for BRCA. Cohort-specific sample counts are provided in Supplementary Table S1. Sample counts were determined separately for each analytical modality because the number of evaluable samples differed between the matched dataset and the survival-analysis results.

4.3. Somatic Mutation Analysis

Somatic mutation data for TCGA samples were obtained in Mutation Annotation Format (MAF) from the Genomic Data Commons (GDC) resource. Mutation records were mapped to the curated DUB gene list, and mutation prevalence was calculated for each gene

and cancer cohort as the percentage of analyzed samples containing at least one qualifying mutation. Mutation classes, including missense, nonsense, splice-site, frameshift, synonymous, and untranslated-region variants, were summarized to characterize the mutational landscape of the DUB family. For selected genes, mutations were mapped to protein coordinates using protein-domain annotations to examine their distribution across functional regions. Mutation counts and sample-level mutation prevalence were used as descriptive measures of the mutational landscape. Mutation counts were not normalized for gene length or adjusted for gene-specific background mutability; therefore, the ranking of genes by observed mutation counts was interpreted descriptively rather than as a measure of positive selection or driver-gene probability.

4.4. Copy Number Alteration Analysis

Gene-level copy-number alteration (CNA) data generated using GISTIC2 were obtained from the TCGA Pan-Cancer resource. CNA values for the curated DUB genes were summarized across cancer cohorts to characterize amplification and deletion patterns. Mean amplification and deletion frequencies were used to compare recurrent CNA patterns across cancer types, and the results were visualized using heatmaps and ranking plots. The CNA summaries were subsequently considered alongside expression and survival features during candidate prioritization. The CNA analysis summarized gene-level amplification and deletion patterns using the available GISTIC-derived data. The analysis was not designed to independently distinguish focal copy-number alterations from broad chromosomal gains or losses. Consequently, the observed CNA frequencies were interpreted as gene-level copy-number patterns and were not taken as evidence that individual DUBs were affected by focal, gene-specific alterations.

4.5. Survival and Prognostic Analysis

Univariate Cox proportional hazards regression was performed separately for each DUB gene within each cancer cohort using the R survival package. Hazard ratios (HRs), p-values, patient counts, and event counts were recorded, and p-values were adjusted for multiple testing using the Benjamini–Hochberg false discovery rate (FDR) procedure. DUBs with FDR-adjusted p-values < 0.05 were considered significantly associated with overall survival. Kaplan–Meier analyses were performed for selected DUBs by stratifying patients into high- and low-expression groups using median expression as the cutoff. The prognostic recurrence score for each DUB was defined as the number of cancer types in which it showed a significant survival association (FDR < 0.05). DUBs with significant associations in at least four cancer types were considered recurrently prognostic for descriptive prioritization. The number of patients and observed survival events varied by cancer cohort and is summarized for all 32 analyzed cohorts in the accompanying Supplementary Data. For each cancer cohort, 110 DUB genes were evaluated in the saved survival-analysis results. The Cox regression analyses were univariate and were not adjusted for clinical covariates, including age, sex, or tumor stage. The recurrence threshold and median expression cutoff were used for exploratory prioritization; permutation testing and systematic comparisons with alternative expression cutoffs, including quartiles, were not performed.

4.6. Multi-Omics Prioritization of Candidate DUBs

Candidate DUBs were prioritized by integrating transcriptomic, copy-number, and clinical-survival features. The expression component included relative enrichment scores for ESCA and STAD, while the clinical component captured the number of cancer cohorts in which each DUB was significantly associated with survival. CNA features included mean amplification and deletion frequencies across cancer cohorts. These features were visualized

together to compare candidate DUBs across molecular and clinical dimensions. The precise normalization, weighting, and aggregation procedure used to calculate the final prioritization score will be specified alongside its mathematical definition and implementation in the public analysis repository.

4.7. Acquisition and Processing of Public Proteomics Datasets

Publicly available mass spectrometry-based processed proteomics datasets were used to investigate DUB-associated protein networks in human cancers. For gastric cancer (STAD), proteomic data were obtained from the study by Li et al. [47], who profiled tumor tissues from patients receiving chemotherapy or anti-HER2-based therapy. The original study included treatment-response comparisons, and its differentially abundant protein lists were not equivalent to tumor-versus-adjacent-normal comparisons. Accordingly, these proteins were interpreted in the context of the original study design. For esophageal cancer (ESCA), proteomic data were obtained from Li et al. [48], who performed proteomic profiling of early-stage gastrointestinal cancer FFPE samples, including tumor and paired normal tissues from EESCC cases. The original study also compared EESCC with early-stage duodenal adenocarcinoma (EDAC). For glioblastoma,, proteomic data were obtained from Wang et al. [49], who reported a proteogenomic and metabolomic characterization of human glioblastoma. This dataset was used for the glioblastoma proteomic analysis and was not treated as a lower-grade glioma (LGG) dataset. For each proteomics dataset, the reported protein-abundance comparisons and differential-expression criteria were used to define the proteins included in downstream analyses. Differentially abundant proteins were then intersected with the curated DUB–substrate interaction resource to identify candidate substrate associations. The glioblastoma dataset was analyzed separately from the gastric and esophageal cancer datasets.

4.8. DUB–Substrate Network Construction

DUB–substrate interactions were obtained from the known-substrate dataset in UbiBrowser 2.0. This resource distinguishes literature-curated known interactions from computationally predicted interactions. The present analysis used the known-substrate dataset rather than the predicted interaction dataset. These curated associations were integrated with the differentially abundant proteins identified in the selected cancer proteomic datasets to construct candidate DUB–substrate networks. Although the known interactions are supported by published literature or datasets, their inclusion does not establish that each interaction is active in the specific cancer context examined in this study. Network visualizations were generated using Cytoscape (version 3.10). Interaction counts and network topology metrics, including degree centrality and connectivity, were calculated to identify highly connected DUBs and recurrent substrate hubs. The resulting networks were used to prioritize highly connected DUBs and associated substrates for further investigation. Network hub status was interpreted as a measure of connectivity within the curated interaction network rather than direct evidence of regulatory activity, functional importance, or therapeutic relevance. Because the network was assembled from previously reported interactions, it may be influenced by differences in research attention and the availability of published interaction evidence. The network analysis was therefore considered hypothesis-generating, and predicted functional relationships were not regarded as experimentally validated findings of the present study.

4.9. Functional Enrichment Analysis

Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG), and Reactome pathway enrichment analyses were performed using the clusterProfiler package in R.

Enrichment analyses were conducted for recurrent DUB-associated substrates identified across proteomic datasets. Pathways with adjusted p-values < 0.05 were considered significantly enriched. Enrichment results were visualized using bubble plots in which bubble size represented gene count and color intensity represented statistical significance.

4.10. Statistical Analysis and Data Visualization

Analyses and visualizations were performed in R (version 4.5.0), with network visualizations generated using Cytoscape (version 3.10). For survival analysis, DUBs with Benjamini–Hochberg FDR-adjusted p-values below 0.05 were considered significantly associated with survival. For functional enrichment analyses, pathways with adjusted p-values below 0.05 were considered significantly enriched. The statistical tests, effect-size measures, multiple-testing correction procedures, and decision thresholds used for each analytical modality are specified in the corresponding Methods subsections.

4.11. Data Availability Statement

The publicly available TCGA/GDC datasets analyzed in this study are identified in the Materials and Methods section. The analysis scripts, processed result tables, and DUB–substrate network files will be will be available on author request.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijms272210366/s1.

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Abbreviations

DUB	Deubiquitinase
TCGA	The Cancer Genome Atlas
CPTAC	Clinical Proteomic Tumor Analysis Consortium
CNA	Copy Number Alteration
USP	Ubiquitin-Specific Protease
OTU	Ovarian Tumor Protease
UCH	Ubiquitin C-terminal Hydrolase
JAMM	JAB1/MPN/MOV34 Metalloprotease
MJD	Machado–Joseph Disease Protease
ESCA	Esophageal Carcinoma
STAD	Stomach Adenocarcinoma
LGG	Lower-Grade Glioma
RNA-seq	RNA Sequencing

MAF	Mutation Annotation Format
GISTIC	Genomic Identification of Significant Targets in Cancer
GO	Gene Ontology
KEGG	Kyoto Encyclopedia of Genes and Genomes
FDR	False Discovery Rate
HR	Hazard Ratio
NF-κB	Nuclear Factor Kappa B
TLR	Toll-Like Receptor
PTM	Post-Translational Modification
TPM	Transcripts Per Million
DUB–Substrate	Deubiquitinase–Substrate
PPI	Protein–Protein Interaction

References

1. Barbour, H.; Nkwe, N.S.; Estavoyer, B.; Messmer, C.; Gushul-Leclaire, M.; Villot, R.; Uriarte, M.; Boulay, K.; Hlayhel, S.; Farhat, B.; et al. An inventory of crosstalk between ubiquitination and other post-translational modifications in orchestrating cellular processes. *iScience* **2023**, *26*, 106276. [CrossRef] [PubMed]
2. Damgaard, R.B. The ubiquitin system: From cell signalling to disease biology and new therapeutic opportunities. *Cell Death Differ.* **2021**, *28*, 423–426. [CrossRef] [PubMed]
3. Hu, H.; Sun, S.-C. Ubiquitin signaling in immune responses. *Cell Res.* **2016**, *26*, 457–483. [CrossRef] [PubMed]
4. Wang, Y.; Wang, F. Post-Translational Modifications of Deubiquitinating Enzymes: Expanding the Ubiquitin Code. *Front. Pharmacol.* **2021**, *12*, 685011. [CrossRef] [PubMed]
5. Ebner, P.; Versteeg, G.A.; Ikeda, F. Ubiquitin enzymes in the regulation of immune responses. *Crit. Rev. Biochem. Mol. Biol.* **2017**, *52*, 425–460. [CrossRef] [PubMed]
6. Kim, Y.; Kim, E.-K.; Jang, H.H. Fine-Tuning Protein Fate: Mechanisms of E1, E2, and E3 Enzymes and Deubiquitinases in Cell Signaling. *Int. J. Mol. Sci.* **2026**, *27*, 3404. [CrossRef] [PubMed]
7. Randles, L.; Walters, K.J. Ubiquitin and its binding domains. *Front. Biosci.* **2012**, *17*, 2140–2157. [CrossRef] [PubMed]
8. Bialek, W.; Collawn, J.F.; Bartoszewski, R. Ubiquitin-Dependent and Independent Proteasomal Degradation in Host-Pathogen Interactions. *Molecules* **2023**, *28*, 6740. [CrossRef] [PubMed]
9. Chen, X.; Htet, Z.M.; López-Alfonzo, E.; Martin, A.; Walters, K.J. Proteasome interaction with ubiquitinated substrates: From mechanisms to therapies. *FEBS J.* **2020**, *288*, 5231–5251. [CrossRef] [PubMed]
10. Ikeda, F.; Dikic, I. Atypical ubiquitin chains: New molecular signals. 'Protein Modifications: Beyond the Usual Suspects' review series. *EMBO Rep.* **2008**, *9*, 536–542. [CrossRef] [PubMed]
11. Duan, S.; Pagano, M. Ubiquitin ligases in cancer: Functions and clinical potentials. *Cell Chem. Biol.* **2021**, *28*, 918–933. [CrossRef] [PubMed]
12. Yang, X.; Lan, T.; Zhang, B.; Xue, T.; Qi, W.; Xie, K.; Cai, Y.; Liu, C.; Wu, H.; Yang, X.; et al. Targeting ubiquitination in disease and therapy. *Signal Transduct. Target. Ther.* **2025**, *10*, 424. [CrossRef] [PubMed]
13. Basar, M.A.; Beck, D.B.; Werner, A. Deubiquitylases in developmental ubiquitin signaling and congenital diseases. *Cell Death Differ.* **2020**, *28*, 538–556. [CrossRef] [PubMed]
14. Lange, S.M.; Armstrong, L.A.; Kulathu, Y. Deubiquitinases: From mechanisms to their inhibition by small molecules. *Mol. Cell* **2022**, *82*, 15–29. [CrossRef] [PubMed]
15. Snyder, N.A.; Silva, G.M. Deubiquitinating enzymes (DUBs): Regulation, homeostasis, and oxidative stress response. *J. Biol. Chem.* **2021**, *297*, 101077. Correction in *J. Biol. Chem.* **2026**, *302*, 113504. https://doi.org/10.1016/j.jbc.2026.113504. [CrossRef] [PubMed]
16. Wilkinson, K.D. DUBs at a glance. *J. Cell Sci.* **2009**, *122*, 2325–2329. [CrossRef] [PubMed]
17. Sowa, M.E.; Bennett, E.J.; Gygi, S.P.; Harper, J.W. Defining the Human Deubiquitinating Enzyme Interaction Landscape. *Cell* **2009**, *138*, 389–403. [CrossRef] [PubMed]
18. Trulsson, F.; Akimov, V.; Robu, M.; van Overbeek, N.; Berrocal, D.A.P.; Shah, R.G.; Cox, J.; Shah, G.M.; Blagoev, B.; Vertegaal, A.C.O. Deubiquitinating enzymes and the proteasome regulate preferential sets of ubiquitin substrates. *Nat. Commun.* **2022**, *13*, 2736. [CrossRef] [PubMed]
19. He, M.; Zhou, Z.; Wu, G.; Chen, Q.; Wan, Y. Emerging role of DUBs in tumor metastasis and apoptosis: Therapeutic implication. *Pharmacol. Ther.* **2017**, *177*, 96–107. [CrossRef] [PubMed]
20. Xian, Y.; Ye, J.; Tang, Y.; Zhang, N.; Peng, C.; Huang, W.; He, G. Deubiquitinases as novel therapeutic targets for diseases. *Medcomm* **2024**, *5*, e70036. [CrossRef] [PubMed]
21. Xiao, S.; Tian, L.; Gan, X.; Xu, X.; Liao, M.; Song, D.; Yu, Y.; Qin, W.; Zhang, R.; Lyu, H.; et al. Role of Ubiquitin-regulated EMT in Cancer Metastasis and Chemoresistance. *Int. J. Biol. Sci.* **2025**, *21*, 6081–6112. [CrossRef] [PubMed]

22. Bhattacharya, S.; Chakraborty, D.; Basu, M.; Ghosh, M.K. Emerging insights into HAUSP (USP7) in physiology, cancer and other diseases. *Signal Transduct. Target. Ther.* **2018**, *3*, 17. [CrossRef] [PubMed]
23. Serratore, V.; Lucibello, M.; Malanga, D.; Viglietto, G.; De Marco, C. AKT and DUBs: A bidirectional relationship. *Cell. Mol. Biol. Lett.* **2025**, *30*, 77. [CrossRef] [PubMed]
24. Wang, Y.; Mao, A.; Liu, J.; Li, P.; Zheng, S.; Tong, T.; Li, Z.; Zhang, H.; Ma, L.; Lin, J.; et al. USP10 strikes down β-catenin by dual-wielding deubiquitinase activity and phase separation potential. *Cell Chem. Biol.* **2023**, *30*, 1436–1452.e10. [CrossRef] [PubMed]
25. Hu, M.; Gu, L.; Li, M.; Jeffrey, P.D.; Gu, W.; Shi, Y. Structural Basis of Competitive Recognition of p53 and MDM2 by HAUSP/USP7: Implications for the Regulation of the p53–MDM2 Pathway. *PLoS Biol.* **2006**, *4*, e27. [CrossRef] [PubMed]
26. Qi, S.-M.; Cheng, G.; Cheng, X.-D.; Xu, Z.; Xu, B.; Zhang, W.-D.; Qin, J.-J. Targeting USP7-Mediated Deubiquitination of MDM2/MDMX-p53 Pathway for Cancer Therapy: Are We There Yet? *Front. Cell Dev. Biol.* **2020**, *8*, 233. [CrossRef] [PubMed]
27. Liu, K.; Gao, Q.; Jia, Y.; Wei, J.; Chaudhuri, S.M.; Wang, S.; Tang, A.; Mani, N.L.; Iyer, R.; Cheng, Y.; et al. Ubiquitin-specific peptidase 22 controls integrin-dependent cancer cell stemness and metastasis. *iScience* **2024**, *27*, 110592. [CrossRef] [PubMed]
28. Ma, Y.; Fu, H.-L.; Wang, Z.; Huang, H.; Ni, J.; Song, J.; Xia, Y.; Jin, W.-L.; Cui, D.-X. USP22 maintains gastric cancer stem cell stemness and promotes gastric cancer progression by stabilizing BMI1 protein. *Oncotarget* **2017**, *8*, 33329–33342. [CrossRef] [PubMed]
29. Zhang, X.-Y.; Varthi, M.; Sykes, S.M.; Phillips, C.; Warzecha, C.; Zhu, W.; Wyce, A.; Thorne, A.W.; Berger, S.L.; McMahon, S.B. The Putative Cancer Stem Cell Marker USP22 Is a Subunit of the Human SAGA Complex Required for Activated Transcription and Cell-Cycle Progression. *Mol. Cell* **2008**, *29*, 102–111. [CrossRef] [PubMed]
30. Kim, E.; Park, S.; Lee, J.H.; Mun, J.Y.; Choi, W.H.; Yun, Y.; Lee, J.; Kim, J.H.; Kang, M.-J.; Lee, M.J. Dual Function of USP14 Deubiquitinase in Cellular Proteasomal Activity and Autophagic Flux. *Cell Rep.* **2018**, *24*, 732–743. [CrossRef] [PubMed]
31. Srinivasan, V.; Soliymani, R.; Ivanova, L.; Eriksson, O.; Peitsaro, N.; Lalowski, M.; Karelson, M.; Lindholm, D. USP14 is crucial for proteostasis regulation and α-synuclein degradation in human SH-SY5Y dopaminergic cells. *Heliyon* **2025**, *11*, e42031. [CrossRef] [PubMed]
32. Masclef, L.; Ahmed, O.; Estavoyer, B.; Larrivée, B.; Labrecque, N.; Nijnik, A.; Affar, E.B. Roles and mechanisms of BAP1 deubiquitinase in tumor suppression. *Cell Death Differ.* **2021**, *28*, 606–625. [CrossRef] [PubMed]
33. Yu, H.; Pak, H.; Hammond-Martel, I.; Ghram, M.; Rodrigue, A.; Daou, S.; Barbour, H.; Corbeil, L.; Hébert, J.; Drobetsky, E.; et al. Tumor suppressor and deubiquitinase BAP1 promotes DNA double-strand break repair. *Proc. Natl. Acad. Sci. USA* **2013**, *111*, 285–290. [CrossRef] [PubMed]
34. Oikawa, D.; Gi, M.; Kosako, H.; Shimizu, K.; Takahashi, H.; Shiota, M.; Hosomi, S.; Komakura, K.; Wanibuchi, H.; Tsuruta, D.; et al. OTUD1 deubiquitinase regulates NF-κB- and KEAP1-mediated inflammatory responses and reactive oxygen species-associated cell death pathways. *Cell Death Dis.* **2022**, *13*, 694. [CrossRef] [PubMed]
35. Tang, X.; Li, Y.; Liu, Y. Dissecting the dual role of OTU family proteins in tumor progression and immune escape. *Front. Immunol.* **2025**, *16*, 1544341. [CrossRef] [PubMed]
36. Wu, B.; Qiang, L.; Zhang, Y.; Fu, Y.; Zhao, M.; Lei, Z.; Lu, Z.; Wei, Y.-G.; Dai, H.; Ge, Y.; et al. The deubiquitinase OTUD1 inhibits colonic inflammation by suppressing RIPK1-mediated NF-κB signaling. *Cell. Mol. Immunol.* **2022**, *19*, 276–289. [CrossRef] [PubMed]
37. Ge, Z.; Leighton, J.S.; Wang, Y.; Peng, X.; Chen, Z.; Chen, H.; Sun, Y.; Yao, F.; Li, J.; Zhang, H.; et al. Integrated Genomic Analysis of the Ubiquitin Pathway across Cancer Types. *Cell Rep.* **2018**, *23*, 213–226.e3. [CrossRef] [PubMed]
38. Grade, M.; Difilippantonio, M.J.; Camps, J. Patterns of Chromosomal Aberrations in Solid Tumors. In *Recent Results in Cancer Research*; Springer International Publishing: New York, NY, USA, 2015; pp. 115–142. [CrossRef] [PubMed]
39. Andrici, J.; Goeppert, B.; Sioson, L.; Clarkson, A.; Renner, M.; Stenzinger, A.; Tayao, M.; Watson, N.; Farzin, M.; Toon, C.W.; et al. Loss of BAP1 Expression Occurs Frequently in Intrahepatic Cholangiocarcinoma. *Medicine* **2016**, *95*, e2491. [CrossRef] [PubMed]
40. Guo, J.; Zhao, Y.; Sui, H.; Liu, L.; Liu, F.; Yang, L.; Gao, F.; Wang, J.; Zhu, Y.; Li, L.; et al. USP21-mediated G3BP1 stabilization accelerates proliferation and metastasis of esophageal squamous cell carcinoma via activating Wnt/beta-Catenin signaling. *Oncogenesis* **2024**, *13*, 23. [CrossRef] [PubMed]
41. Ma, C.; Wang, D.; Tian, Z.; Gao, W.; Zang, Y.; Qian, L.; Xu, X.; Jia, J.; Liu, Z. USP13 deubiquitinates and stabilizes cyclin D1 to promote gastric cancer cell cycle progression and cell proliferation. *Oncogene* **2023**, *42*, 2249–2262. [CrossRef] [PubMed]
42. Zhuang, T.; Zhang, S.; Liu, D.; Li, Z.; Li, X.; Li, J.; Yang, P.; Zhang, C.; Cui, J.; Fu, M.; et al. USP36 promotes tumorigenesis and tamoxifen resistance in breast cancer by deubiquitinating and stabilizing ERalpha. *J. Exp. Clin. Cancer Res.* **2024**, *43*, 249. [CrossRef] [PubMed]
43. D'Arcy, P.; Wang, X.; Linder, S. Deubiquitinase inhibition as a cancer therapeutic strategy. *Pharmacol. Ther.* **2015**, *147*, 32–54. [CrossRef] [PubMed]
44. Li, J.; Liu, S.; Li, S. Mechanisms underlying linear ubiquitination and implications in tumorigenesis and drug discovery. *Cell Commun. Signal.* **2023**, *21*, 340. [CrossRef] [PubMed]

45. Antao, A.M.; Tyagi, A.; Kim, K.-S.; Ramakrishna, S. Advances in Deubiquitinating Enzyme Inhibition and Applications in Cancer Therapeutics. *Cancers* **2020**, *12*, 1579. [CrossRef] [PubMed]
46. Bolhuis, D.L.; Emanuele, M.J.; Brown, N.G. Friend or foe? Reciprocal regulation between E3 ubiquitin ligases and deubiquitinases. *Biochem. Soc. Trans.* **2024**, *52*, 241–267. [CrossRef] [PubMed]
47. Li, Y.; Xu, C.; Wang, B.; Xu, F.; Ma, F.; Qu, Y.; Jiang, D.; Li, K.; Feng, J.; Tian, S.; et al. Proteomic characterization of gastric cancer response to chemotherapy and targeted therapy reveals potential therapeutic strategies. *Nat. Commun.* **2022**, *13*, 5723. Correction in *Nat. Commun.* **2022**, *13*, 6749. https://doi.org/10.1038/s41467-022-34238-0. [CrossRef] [PubMed]
48. Li, L.; Liu, H.; Li, Y.; Guo, C.; Wang, B.; Shen, D.; Zhang, Q.; Ding, C. Integrative proteomic characterization of trace FFPE samples in early-stage gastrointestinal cancer. *Proteome Sci.* **2022**, *20*, 5. [CrossRef] [PubMed]
49. Wang, L.-B.; Karpova, A.; Gritsenko, M.A.; Kyle, J.E.; Cao, S.; Li, Y.; Rykunov, D.; Colaprico, A.; Rothstein, J.H.; Hong, R.; et al. Proteogenomic and metabolomic characterization of human glioblastoma. *Cancer Cell* **2021**, *39*, 509–528.e20. [CrossRef] [PubMed]
50. Akizuki, Y.; Kaypee, S.; Ohtake, F.; Ikeda, F. The emerging roles of non-canonical ubiquitination in proteostasis and beyond. *J. Cell Biol.* **2024**, *223*, e202311171. [CrossRef] [PubMed]
51. Liu, P.; Chen, Z.; Guo, Y.; He, Q.; Pan, C. Recent advances in small molecule inhibitors of deubiquitinating enzymes. *Eur. J. Med. Chem.* **2025**, *287*, 117324. [CrossRef] [PubMed]
52. Ventii, K.H.; Wilkinson, K.D. Protein partners of deubiquitinating enzymes. *Biochem. J.* **2008**, *414*, 161–4175. [CrossRef] [PubMed]
53. Liao, Y.; Zhang, W.; Liu, Y.; Zhu, C.; Zou, Z. The role of ubiquitination in health and disease. *MedComm* **2024**, *5*, e736. [CrossRef] [PubMed]
54. Uma Devi, A.; Shukla, P.K. Decoding the Oncogenic Role of USP22 Through Pan-Cancer Genomic and Epigenetic Analysis. *Cancer Rep.* **2026**, *9*, e70572. [CrossRef] [PubMed]
55. Gao, D.; Zhang, Z.; Xu, R.; He, Z.; Li, F.; Hu, Y.; Chen, H.; Lu, J.; Cao, X.; Liu, Y.; et al. The Prognostic Value and Immune Infiltration of USP10 in Pan-Cancer: A Potential Therapeutic Target. *Front. Oncol.* **2022**, *12*, 829705. [CrossRef] [PubMed]
56. Hang, C.; Gong, C.; Fang, Y.; Chen, L.; Zhu, J. Ubiquitin-specific protease 14 (USP14) promotes proliferation and metastasis in pancreatic ductal adenocarcinoma. *J. Mol. Histol.* **2021**, *52*, 187–196. [CrossRef] [PubMed]
57. Lu, J.; Zhao, H.; Yu, C.; Kang, Y.; Yang, X. Targeting Ubiquitin-Specific Protease 7 (USP7) in Cancer: A New Insight to Overcome Drug Resistance. *Front. Pharmacol.* **2021**, *12*, 648491. [CrossRef] [PubMed]
58. Sturgill, I.R.; Raab, J.R.; Hoadley, K.A. Expanded detection and impact of *BAP1* alterations in cancer. *NAR Cancer* **2024**, *6*, zcae045. [CrossRef] [PubMed]
59. Lin, Z.; Tan, C.; Qiu, Q.; Kong, S.; Yang, H.; Zhao, F.; Liu, Z.; Li, J.; Kong, Q.; Gao, B.; et al. Ubiquitin-specific protease 22 is a deubiquitinase of CCNB1. *Cell Discov.* **2015**, *1*, 15028. [CrossRef] [PubMed]
60. Kuznetsoff, J.N.; Owens, D.A.; Lopez, A.; Rodriguez, D.A.; Chee, N.T.; Kurtenbach, S.; Bilbao, D.; Roberts, E.R.; Volmar, C.H.; Wahlestedt, C.; et al. Dual Screen for Efficacy and Toxicity Identifies HDAC Inhibitor with Distinctive Activity Spectrum for BAP1-Mutant Uveal Melanoma. *Mol. Cancer Res.* **2021**, *19*, 215–222. [CrossRef] [PubMed]
61. Gu, B.; Chang, S.Y.; Li, K.K.; Wang, B.Q.; Ke, S.B.; Zhang, X.D.; Du, R.L. USP13 promotes colorectal cancer progression by deubiquitinating and stabilizing HIF-1alpha. *Cell Commun. Signal.* **2025**, *23*, 502. [CrossRef] [PubMed]
62. Hou, P.; Ma, X.; Zhang, Q.; Wu, C.J.; Liao, W.; Li, J.; Wang, H.; Zhao, J.; Zhou, X.; Guan, C.; et al. USP21 deubiquitinase promotes pancreas cancer cell stemness via Wnt pathway activation. *Genes Dev.* **2019**, *33*, 1361–1366. [CrossRef] [PubMed]
63. Yang, B.; Zhang, S.; Wang, Z.; Yang, C.; Ouyang, W.; Zhou, F.; Zhou, Y.; Xie, C. Deubiquitinase USP9X deubiquitinates beta-catenin and promotes high grade glioma cell growth. *Oncotarget* **2016**, *7*, 79515–79525. [CrossRef] [PubMed]
64. Tan, L.; Shan, H.; Han, C.; Zhang, Z.; Shen, J.; Zhang, X.; Xiang, H.; Lu, K.; Qi, C.; Li, Y.; et al. Discovery of Potent OTUB1/USP8 Dual Inhibitors Targeting Proteostasis in Non-Small-Cell Lung Cancer. *J. Med. Chem.* **2022**, *65*, 13645–13659. [CrossRef] [PubMed]
65. Unda, B.K.; Chalil, L.; Yoon, S.; Kilpatrick, S.; Irwin, C.; Xing, S.; Murtaza, N.; Cheng, A.; Brown, C.; Afonso, A.; et al. Impaired OTUD7A-dependent Ankyrin regulation mediates neuronal dysfunction in mouse and human models of the 15q13.3 microdeletion syndrome. *Mol. Psychiatry* **2023**, *28*, 1747–1769. [CrossRef] [PubMed]
66. Xu, Z.; Lei, Z.; Peng, S.; Fu, X.; Xu, Y. Dysregulation of deubiquitinases in gastric cancer progression. *Front. Oncol.* **2024**, *14*, 1456710. [CrossRef] [PubMed]
67. Brooks, C.L.; Gu, W. p53 regulation by ubiquitin. *FEBS Lett.* **2011**, *585*, 2803–2809. [CrossRef] [PubMed]
68. Rawat, R.; Starczynowski, D.T.; Ntziachristos, P. Nuclear deubiquitination in the spotlight: The multifaceted nature of USP7 biology in disease. *Curr. Opin. Cell. Biol.* **2019**, *58*, 85–94. [CrossRef] [PubMed]
69. Javaid, N.; Choi, S. Toll-like Receptors from the Perspective of Cancer Treatment. *Cancers* **2020**, *12*, 297. [CrossRef] [PubMed]
70. Mao, H.; Zhao, X.; Sun, S.C. NF-kappaB in inflammation and cancer. *Cell. Mol. Immunol.* **2025**, *22*, 811–839. [CrossRef] [PubMed]

71. Qian, C.; Cao, X.; Hoffmann, J.A. Toll-like receptors in innate immunity and inflammation: From fundamental biology to clinic insights. *Immun. Inflamm.* **2026**, *2*, 22. [CrossRef] [PubMed]
72. Wang, X.; Li, Y.; He, M.; Kong, X.; Jiang, P.; Liu, X.; Diao, L.; Zhang, X.; Li, H.; Ling, X.; et al. UbiBrowser 2.0: A comprehensive resource for proteome-wide known and predicted ubiquitin ligase/deubiquitinase–substrate interactions in eukaryotic species. *Nucleic Acids Res.* **2021**, *50*, D719–D728. [CrossRef] [PubMed]

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