

Allosteric network of dynamic coupling within BAP1-UCH revealed by methyl NMR

Received: 11 September 2025

Accepted: 15 May 2026

Published online: 26 May 2026



Chih-Hsuan Lai¹, Yuan-Chao Lou², Chi-Fon Chang³, Wei-Lin Lu¹,
Manoj Kumar Sriramoju¹, Yong-Sheng Wang^{1,4}, Kuen-Phon Wu^{1,4},
Carlo Camilloni⁵ & Shang-Te Danny Hsu^{1,4,6,7} ✉

BRCA1-associated protein 1 (BAP1) is a tumor suppressor whose deubiquitinase (DUB) activity is essential for transcriptional regulation and is frequently compromised in cancer by mutations clustered in its ubiquitin carboxyl-terminal hydrolase (UCH) domain. The structural and dynamic bases of these oncogenic mutations remain elusive. Here, we introduce 22 cancer mutations and additional methyl mutations on the other 22 cancer mutation sites of BAP1-UCH to map their effects on the methyl chemical shifts. This analysis reveals an allosteric coupling network centered on a conserved leucine (L49) shared across human UCH paralogs. Strikingly, a single-carbon side-chain truncation in the L49V variant abolishes DUB activity, coinciding with disruption of correlated μ s–ms timescale motions within a phenylalanine cluster that undergoes concerted motions within the L49 hub. These findings uncover how BAP1-UCH sustains its catalytic competence through a delicately tuned dynamic network and how minute alterations can collapse this allosteric balance, providing a mechanistic link between subtle structural perturbations and oncogenesis.

BRCA1-associated protein 1 (BAP1) is a tumor suppressor deubiquitinase (DUB) that plays pivotal roles in a myriad of biological functions^{1,2}. BAP1 actively contributes to chromatin remodeling through deubiquitination of the histone H2A^{3–5}, DNA damage response and repair^{6–8}, DNA replication^{9,10}, cell proliferation^{11,12}, and cell death^{13–15}. Hundreds of cancer-associated mutations have been documented in BAP1, particularly in the N-terminal ubiquitin C-terminal hydrolase (UCH) domain^{16,17}. Individuals carrying germline mutations have a near 85% chance of developing at least one type of BAP1-associated cancer, including uveal melanoma, malignant mesothelioma, and renal cell carcinoma^{18,19}, a condition referred to as the BAP1 tumor predisposition syndrome (BAP1-TPDS) (OMIM 614327). Three guidelines have been published for clinical diagnosis and surveillance of BAP1-TPDS from the groups in Europe, Australia, and America^{18,20,21}. The family

histories of germline variant carriers are tracked worldwide^{19,22–24}. Additionally, somatic mutations are frequently observed in cancer studies, which share cancer types similar to the BAP1-TPDS^{17,25–27}. These encompass multiple mutations leading to loss of function, such as the catalytic site mutations and the nuclear localization signal (NLS) mutations^{26,27}, as well as gain-of-function mutations that result in the formation of fibrillar aggregates¹⁷.

BAP1 is a multidomain protein encompassing an N-terminal UCH domain, followed by two coiled-coil domains (CC1 and CC2), an intrinsic disorder region, a UCH37-like domain (ULD), and a C-terminal extension (CTE) (Fig. 1)^{3,4}. The CC1, CC2, and ULD are involved in many protein-protein interactions in coordination with the N-terminal UCH domain. For example, the ULD domain of BAP1-UCH binds to the deubiquitinase adaptor (DEUBAD) domain of ASXL1-3 for histone

¹Institute of Biological Chemistry, Academia Sinica, Taipei, Taiwan. ²Biomedical Translation Research Center, Academia Sinica, Taipei, Taiwan. ³Genomics Research Center, Academia Sinica, Taipei, Taiwan. ⁴Institute of Biochemical Sciences, National Taiwan University, Taipei, Taiwan. ⁵Department of Bioscience, University of Milan, Milan, Italy. ⁶International Institute for Sustainability with Knotted Chiral Meta Matter (SKCM²), Hiroshima University, Higashihiroshima, Japan. ⁷Department of Biochemistry, Microbiology, and Immunology, Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada.

✉ e-mail: sthsu@as.edu.tw

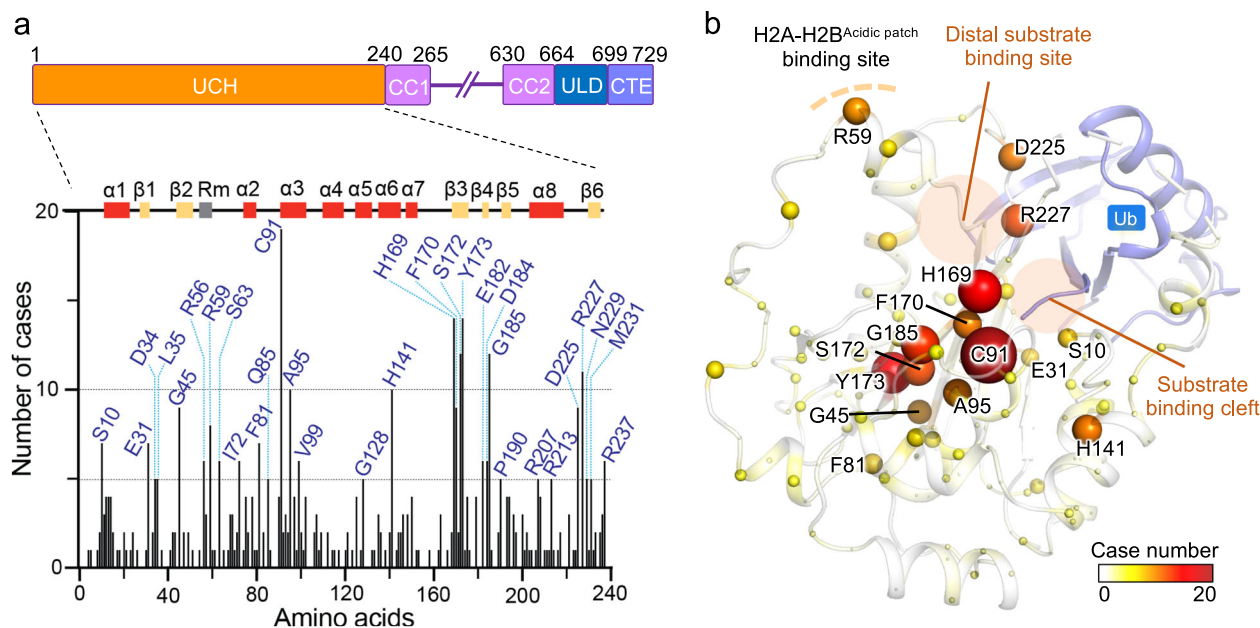


Fig. 1 | The cancer mutations of the BAP1-UCH domain. **a** BAP1 consists of a UCH domain, coiled-coil domains (CC1 and CC2), an intrinsic disorder region, a UCH37-like domain (ULD), and a C-terminal extension (CTE). BAP1-UCH harbors many cancer mutations, mostly missense mutations according to the COSMIC database (v102). The high-frequency mutations are indicated with the residue identities. The secondary structural elements are shown above; the α -helices and β -sheets are shown in red and yellow rectangles, respectively. **b** Cartoon representation of the

homology model of BAP1-UCH (white) in complex with ubiquitin (Ub; blue) based on the cryo-EM structure (PDB ID: 8HIT <https://doi.org/10.2210/pdb8HIT/pdb>). The C α atoms of the residues that are mutated in the COSMIC database are shown in spheres with varying radii proportional to the reported case numbers; they are also color-ramped from white to yellow to red to firebrick red according to the scale bar shown below.

remodeling³⁻⁵. The CTE is responsible for DNA and histone interactions^{3,28}. It also contains an atypical PY-NLS motif for nuclear import by transportin-1 (TNPO1) in competition with the ubiquitination ligase activity of UBE2O²⁹. The PY-NLS is auto-deubiquitinated by the N-terminal UCH domain, a vital regulatory process for the appropriate nuclear localization of BAP1³⁰ to deubiquitinate H2A at the K119 position³¹.

Numerous BAP1 cancer mutations have been documented in the databases. While high-frequency catalytic mutations, i.e., C91G, C91W and H169Q, directly contribute to loss of DUB activity, some other mutations distant to the catalytic site result in destabilization of BAP1-UCH and increased aggregation propensity, which could lead to cytosolic retention and, therefore, the inability for BAP1 to execute its transcription regulation activity^{16,17}. Long-range destabilization by some of these mutations has been revealed by hydrogen-deuterium exchange mass spectrometry (HDX-MS)¹⁶, however, detailed structure-activity relationship of BAP1-UCH remains elusive not least due to the lack of high-resolution structure. Recently, two cryo-EM structures of the BAP1-H2A nucleosome complex have been reported with limited resolution around the catalytic center^{3,4}. In addition to the cryo-EM structures of BAP1, the crystal structure of the *Drosophila* homolog of BAP1, Calypso, has been reported⁵, and the crystal structures of the three human paralogs of BAP1, namely UCHL1, UCHL3, and UCHL5, in the apo and ubiquitin-bound forms³²⁻³⁷. While mechanistic insights could be inferred from these crystal structures, human UCH paralogs exhibit a broad spectrum of DUB activity with very different k_{cat} and K_M values due to subtle changes in the catalytic side chain orientations and internal dynamics³⁸.

In this study, we use methyl nuclear magnetic resonance (NMR) spectroscopy to characterize the structure and dynamics of BAP1-UCH. Systematic mutagenesis was used to assign methyl chemical shift assignments and further revealed long-range chemical shift perturbations, based on which an allosteric network was established. Near the catalytic triad, L49 serves as the signaling hub of this network.

Combining a fluorogenic DUB activity assay and NMR chemical exchange analysis on the μ s-ms time scale, we revealed that a minimal perturbation by the L49V mutation led to loss of function and the decoupling of internal dynamics revealed by methyl transverse relaxation dispersion measurements, demonstrating how minute changes in protein structures can have profound impacts on the functional dynamics through allostery.

Results

Systematic mutagenesis revealed a hidden network inside BAP1-UCH

Site-specific methyl chemical shift assignments of BAP1-UCH were accomplished by (1) a combination of selective $^{13}\text{C}/^1\text{H}$ labeling of the methyl groups of isoleucine, leucine, valine and alanine with and without stereospecific methyl group labeling of the pro-S groups of leucine ($\delta 2$) and valine ($\gamma 2$) under a perdeuterated background, $[\text{U-}^2\text{H}, ^{13}\text{C}_m/\text{H}_m\text{-Ile-}\delta 1/\text{Leu-}\delta 1/2/\text{Val-}\gamma 1/2/\text{Ala-}\beta]$, for transverse relaxation optimized (TROSY) NMR spectroscopy (**Methods** and Supplementary Fig. 1), (2) systematic mutagenesis to introduce cancer-associated mutations and conservative methyl mutations, e.g., isoleucine-to-leucine or vice versa, to uniformly ^{13}C -labeled $[\text{U-}^{13}\text{C}]$ BAP1-UCH variants to identify loss of resonances and chemical shift perturbations (CSPs) due to spatial proximity ($[\text{U-}^{13}\text{C}]$ also helped the assignments of the Met- ϵ and Thr- $\gamma 2$ methyl groups; Supplementary Fig. 2), and (3) three-dimensional (3D) ^{13}C -edited nuclear Overhauser effect spectroscopy (NOESY) aided by homology modeling of BAP1-UCH to establish the spatial connectivities (Supplementary Fig. 3).

Collectively, we have generated 55 point mutations (11 of them were insoluble or too destabilized for NMR analyses; Supplementary Fig. 4 and Supplementary Table 1) for the systematic CSP analysis. 70% of the methyl-containing residues have at least one methyl group assigned (Supplementary Fig. 1). Fifty methyl probes (60% of the methyl-containing residues) have been used in CSP analysis. The point mutations R59Q, V62I, S63C, L152I, and A161G, located in the loop

regions, including the $\beta 2$ - $\alpha 2$ loop (containing a RRSRR motif, which interacted with the acidic patch of H2A and H2B^{3,4}) and the cross-over loop, did not show significant CSPs (Supplementary Fig. 2), indicating that these flexible loops do not make critical contacts with the core structure. In contrast, we have observed numerous long-range CSPs in BAP1-UCH variants that harbor cancer mutations or conserved methyl mutations. Several residues seemed to respond to distant mutations. After compiling all the observed CSPs of 44 mutants (Supplementary Fig. 2), we conducted network analysis to identify hotspots that frequently sense mutations manifested in large CSPs (**Methods**). In the network analysis, individual residues are nodes, and the connection between one particular mutation and the other residue is defined as an edge. Combining all the edges and nodes in our CSP dataset, we generated a 3D network using the homology model of BAP1-UCH as the template. The network illustrates that L49 is the node that forms the highest number of edges with various residues throughout the UCH domain, except the loop region (Fig. 2 and Supplementary Movie 1). Importantly, the catalytic mutations, C91G, H169Q, and D184N also showed reciprocal CSPs in L49, indicating that L49 is structurally coupled to the catalytic site (Supplementary Fig. 2). Following L49, L14, V171, I210, and M231 were the second most connected nodes in the network, and they were clustered close to L49 to form a continuous pathway. In contrast, the I72, M80, and I226 also showed many edge connections to different parts of BAP1-UCH, but they are located distant from the catalytic site.

L49 is a strictly conserved residue among the four human UCH paralogs (Fig. 3a). L49V is reported to be linked to breast invasive carcinoma and kidney renal clear cell carcinoma³⁹. Using ubiquitin 7-amido-4-methylcoumarin (Ub-AMC) as a model substrate, we showed that L49V completely obliterated the DUB activity of BAP1-UCH (Fig. 3b). Furthermore, introduction of a homolog mutation, L55V, to UCHL5, which is the close paralog to BAP1 relative to UCHL1 and UCHL3, the mutation also led to the functional loss for UCHL5, indicating that the pivotal role of L49 in the allosteric network of BAP1-UCH is of functional and biomedical importance (Fig. 3b). To examine how the L49V mutation may impact the structure of BAP1-UCH, we carried out small-angle X-ray scattering (SAXS), which is very sensitive to the global conformational and dynamic changes in proteins⁴⁰ (Supplementary Table 2). The raw SAXS profiles of WT and L49V were very similar; the radii of gyration (R_g) of WT (21.18 ± 0.02 Å) and L49V (21.53 ± 0.02 Å) were essentially the same. Likewise, the Kratky plots of both variants did not indicate a difference in global dynamics. The only discernible difference was the maximum global dimension, D_{max} , which was slightly larger for L49V (87 Å) compared to that of WT (80 Å), but the peak distributions of both variants were very similar (Supplementary Fig. 5). In summary, the SAXS analysis indicated that the functional loss inflicted by the L49V mutation was not linked to global conformational or dynamics changes, but more subtle dynamics changes may be at play to account for the functional impact.

Changes in functional dynamics on the μ s-ms timescale by the L49V mutation

To probe the changes in functional dynamics inflicted by the L49V mutation, we generated [U - ^2H , $^{13}C_m/^{13}H_m$ -Ile- $\delta 1$ /Leu- $\delta 1$ /2/Val- $\gamma 1$ /2] WT and L49V (Supplementary Fig. 3a) to carry out Carr-Purcell-Meiboom-Gill (CPMG) relaxation dispersion NMR experiments to compare their μ s-ms dynamics that may be linked to the DUB activity. The methyl CPMG experiments were carried out at two different magnetic fields, corresponding to the proton Larmor frequency of 600 and 850 MHz (Supplementary Fig. 6). By fitting the observed relaxation dispersion profiles to a two-site exchange model (the observable state A in exchange with a lowly populated and invisible state B), followed by global fitting to different clusters of residues that showed similar exchange behaviors, we showed that the majority of the methyl groups in

WT can be global fit to yield an exchange rate $k_{ex} = 1824 \pm 97$ s⁻¹ with the population of the invisible state B, p_B , of $3.2 \pm 0.1\%$ (Fig. 4, Table 1 and Supplementary Table 3). In this case, the relaxation dispersion profile of L49 exhibited a distinct behavior that cannot be included in the global fit (Supplementary Table 4 and Supplementary Fig. 7). In contrast, the global fitting analysis of L49V showed that the a similar group of the methyl groups in L49V as observed in WT can be global fit to yield an exchange rate $k_{ex} = 1610 \pm 76$ s⁻¹ with *ca.* fourfold increase in the population of the invisible state B, p_B , of $12.1 \pm 1.1\%$; meanwhile, a smaller cluster of only two residues, namely L97 and I101, needed to be global fit to yield a different set of kinetic parameters: an exchange rate $k_{ex} = 1212 \pm 271$ s⁻¹ with the population of the invisible state B, p_B , of $3.9 \pm 1.0\%$ (Table 1 and Supplementary Tables 3 and 4). The comparative relaxation dispersion analysis suggested a decoupling of the internal dynamics in BAP1-UCH that may be responsible for the functional loss.

When examining the chemical shift difference, $\Delta\omega$, derived from the relaxation dispersion analyses of WT and L49V, a good correlation can be found for the ^{13}C $\Delta\omega$ values of the major clusters of WT and L49V, but not for the minor cluster in L49V with its counterpart in WT, implying that the L49V mutation increased the population of the invisible state with a comparable chemical exchange rate while the minor cluster in L49V became decoupled from the rest of the core structure and drifted towards a different conformational state (Supplementary Fig. 8a). The correlation in the ^1H $\Delta\omega$ values was less obvious, which may be attributed to the higher sensitivity to local ring current effects that are exceptionally abundant in the hydrophobic core of BAP1-UCH (see the next section, Supplementary Fig. 8b).

Interdigitated side chain conformational changes of the aromatic clusters within the hydrophobic core of BAP1-UCH

BAP1-UCH has 13 phenylalanines, six tyrosines, and four tryptophans. L49 is surrounded by six phenylalanines (F15, F48, F50, F168, F170, and F228), one histidine (the catalytic H169) and one tryptophan (W52) forming an unusually dense aromatic ring cluster that we designated as aromatic cluster 1 (AC1; Fig. 5a). Adjacent to it lies another cluster, AC2, comprising two phenylalanine residues (F82 and F181), two tyrosine residues (Y173 and Y189), and one histidine residue (H193) (Fig. 5a). The AC1 and AC2 are integral parts of the hydrophobic core of BAP1-UCH. The six phenylalanines within the AC1—F15, F48, F50, F168, F170 and F228—are fully conserved between BAP1 and UCHL5 and less conserved in UCHL1 and UCHL3 (Fig. 3). Being the second most interconnected residue in the network analysis, L14 is connected to the AC1 through the proximity to F15 and L49 (Figs. 2 and 5a). Indeed, the F15L mutation also resulted in a significant increase in intensity of I47- $\delta 1$ and a significant loss of DUB activity (Fig. 5b, c). Furthermore, systematic mutagenesis of the phenylalanines in the AC1 resulted in significant functional losses, except for the F50L mutation (Fig. 5b). According to the methyl NMR spectral comparison, these phenylalanine mutations minimally perturbed the structure of BAP1-UCH without global unfolding, evidenced by the limited methyl CSPs induced by these mutations (Supplementary Fig. 9). These phenylalanines, except F50, are likely crucial for stabilizing the conformation of the substrate interaction sites, including the distal substrate binding site and the substrate binding cleft (Supplementary Fig. 10).

Surrounded by many aromatic side chains, L49 of BAP1-UCH may undergo considerable conformational rearrangement upon binding to ubiquitin. A cascade of side chain rearrangements is observed when comparing the apo and ubiquitin-bound crystal structures of UCHL1, which explains the slow k_{cat} value of Ub-AMC hydrolysis compared to that of UCHL3³². To evaluate the conformational changes in BAP1-UCH upon ubiquitin binding, we used a chemically modified ubiquitin variant, ubiquitin(Δ G76)-NH α -chloroacetyl (Ub(Δ G76)-Cl), to form a

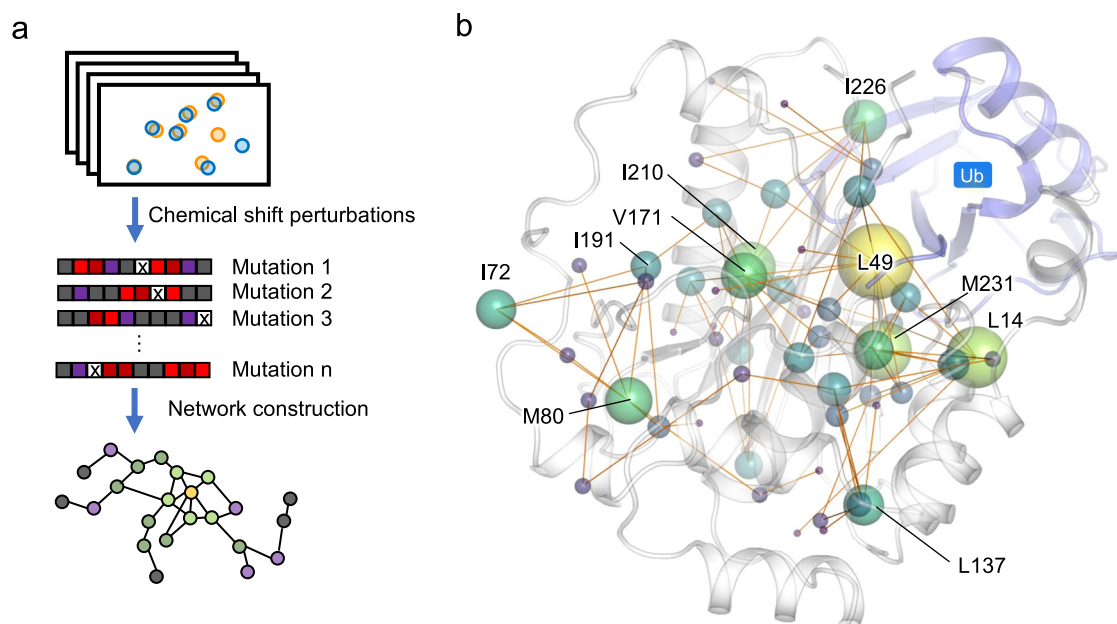


Fig. 2 | Network analysis of BAP1-UCH based on methyl NMR CSPs. a Schematic workflow of the network analysis. The methyl CSPs of an array of point mutations are compiled as inputs for network construction. **b** The methyl CSP network derived from 44 mutants. The sizes and color of the nodes—ramped from indigo to green to yellow—are proportional to the number of edges (orange lines)

connecting the individual nodes. L49 is the most connected node in this network. The nodes are shown in spheres at either C β positions or, in the case of methyl probes, at the corresponding methyl carbons. The atomic coordinates were extracted from the cryo-EM structure (PDB entry 8H1T <https://doi.org/10.2210/pdb8H1T/pdb>). The perturbation networks are visualized by PyMOL.

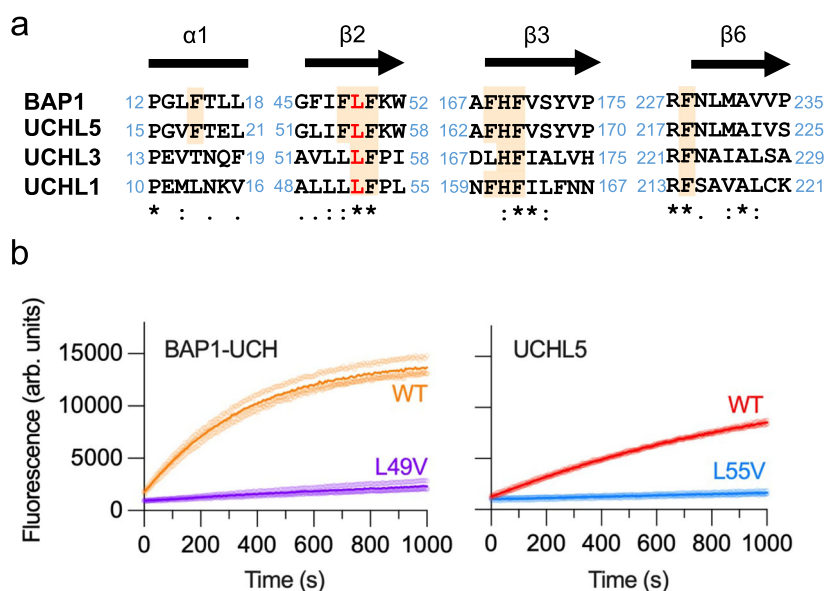


Fig. 3 | Loss of DUB activity by conservative L-to-V mutations in BAP1 and UCHL5. a Sequence alignment of four human UCHs highlights the conservation of L49 (red) and the phenylalanine cluster (orange square). **b** L49V mutation obliterated the DUB activity of BAP1 and the same was observed for the L55V

mutation in UCHL5. The DUB activity assay was performed in technical triplicates. The mean values are shown in solid lines, and the raw data points are shown in semitransparent open circles with matching colors.

suicide complex with [U- ^{13} C] BAP1-UCH through the covalent bond formation between the C-terminal modifier of Ub(Δ G76)-Cl and the thiol group of the catalytic C91⁴¹ (Methods). The resulting ^{13} C- ^{1} H SOFAT-HMQC spectrum showed clear up-field shifts for the methyl groups of L49 and M231 compared to the apo BAP1-UCH, indicative of ring current effect generated upon ubiquitin binding (Fig. 5d, e and Supplementary Fig. 11). However, the cryo-EM structures of ubiquitin-bound BAP1 have the side chain methyl groups of L49 positioned on the side of the phenyl ring of F170, which is expected to show a down-

field shift, contradicting the experimental observation (Supplementary Fig. 12).

Upon ubiquitin binding, we propose that this hydrophobic core undergoes a coordinated rearrangement involving L49, F170, F15, and M231. Homology models based on apo and ubiquitin-bound UCHL5 structures indicate that ubiquitin engagement promotes formation of stabilizing CH- π interactions between L49 and F170 and between M231 and F15, together with a π - π interaction between F170 and F15 (Fig. 5d). In UCHL5, the equivalent residue (L55) undergoes a χ 1

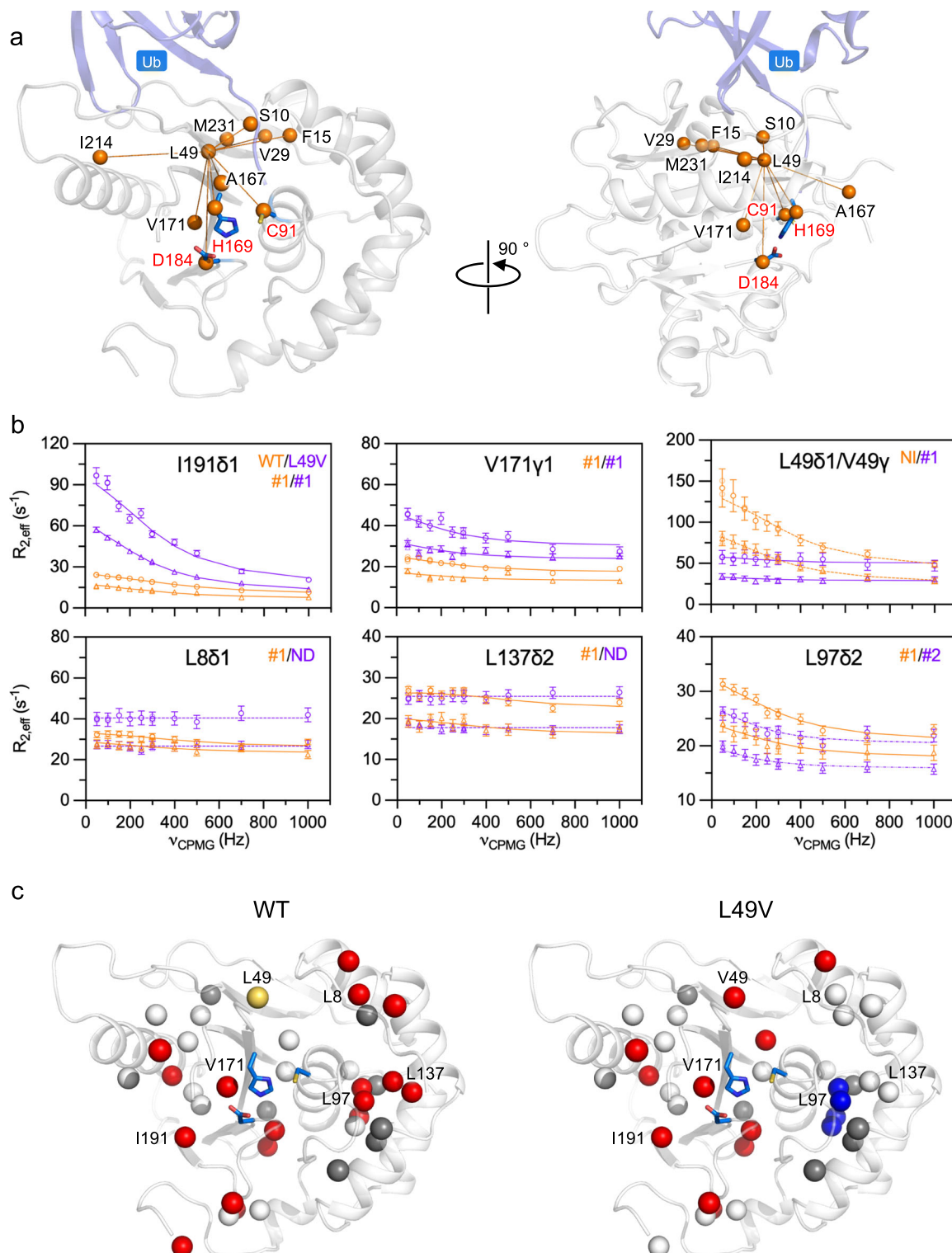


Fig. 4 | Alteration of dynamics coupling network within BAP1-UCH probed by CPMG relaxation dispersion experiments. **a** Structural mapping of the key dynamics network nodes reveals connections with functional motifs pertinent to the DUB activity, highlighted in semitransparent circles with different colors as indicated. **b** Representative CPMG dispersion profiles of WT (orange) and L49V (purple) recorded at the proton Larmor frequency of 600 MHz (open triangle) and 850 MHz (open circle) at 298 K with the fitting results shown in solid lines with matching colors. The estimated errors were generated from the Monte Carlo

simulations by Relax. **c** Structural mapping of the cluster of residues (red and blue spheres) that could be global-fit to a single two-site exchange model. The resulting parameters—exchange rate (k_{ex}) and minor population (p_B)—are tabulated below. In the case of WT, a single cluster (red spheres) was established. For L49V, an additional cluster (blue spheres) was needed to account for most of the CPMG relaxation dispersion data. Residues not included in the cluster analysis are shown in gray spheres. L49 cannot be included in the global fit for WT; it is highlighted in a yellow sphere. The methyl groups that exhibited no dispersion were shown in white.

Table 1 | Global fitting results of the CPMG relaxation dispersion data of WT and L49V

	WT		L49V	
	k_{ex} (s ⁻¹)	p_B (%)	k_{ex} (s ⁻¹)	p_B (%)
Cluster 1	1824 ± 97	3.2 ± 0.1	1610 ± 76	12.1 ± 1.1
Cluster 2			1212 ± 271	3.9 ± 1.0

rotameric change (~108°), supporting a rotamer-driven reorganization mechanism. We propose that an analogous rotameric shift in BAP1-UCH repositions F170 away from the catalytic cleft, thereby partially “unlocking” the hydrophobic barrier and permitting productive substrate positioning at C91. The L49V mutation shortens the aliphatic side chain by a single carbon atom yet abolishes DUB activity; the equivalent L55V mutation in UCHL5 showed the same effect (Fig. 3). Within the proposed mechanism, L49 is required to establish optimal CH–π geometry with F170. The reduced side-chain length in L49V disrupts this interaction and destabilizes the concerted rotameric transition of the F170–F15 aromatic pair. As a consequence, the hydrophobic core in front of C91 fails to reorganize efficiently upon ubiquitin binding, impairing allosteric coupling between substrate engagement and catalytic activation. Consistent with this model, the F170L mutation produces a similarly strong loss of activity, indicating that the integrity of the L49–F170 interaction is functionally critical.

We performed μs-timescale MD simulations using the cryo-EM structure of ubiquitin-bound (holo form) BAP1-UCH as the starting structure to further validate the homology model. In terms of the per-residue root-mean-square fluctuations (RMSF), the overall flexibility of WT and L49V in their apo forms is indistinguishable within the statistical uncertainty of the simulations (Supplementary Fig. 13). Nonetheless, when delved into the side chain rotameric states and inter-side chain distances within the allosteric network center, i.e., L49 and the phenylalanine cluster, a general agreement between the homology model and the χ¹/2 angle distributions can be reached (Supplementary Fig. 14). Specifically, the χ² angle of F170 sampled by the MD simulations of the apo and holo forms of WT was consistent with the homology model despite starting from the cryo-EM structure that is nearly perpendicular to the homology model. Importantly, the inter-side chain distances sampled in the WT apo form were essentially identical to those of the homology model. By contrast, the holo form exhibited more heterogeneous distance distributions likely due to the steric hindrance imposed by the insertion of the C-terminal tail of ubiquitin to the catalytic site. Nevertheless, the close methyl carbon-to-centroid distances necessary to favor the CH–π (3.5–4 Å) were regularly sampled (Supplementary Fig. 14b, d), in line with the observed upfield shifts for L49δ1/δ2 and M231ε (Fig. 5e). Introducing the L49V mutation in the holo form reduced the methyl carbon-to-centroid (CH–π) distances between V49 and F170 from 4.6 to 3.8 Å (Supplementary Fig. 14d, e). This shorter distance may stabilize the CH–π interaction, disrupt the balance of the CH–π and π–π interaction network, and hinder ubiquitin C-terminal tail access to the catalytic site, effectively halting the gating mechanism (Supplementary Fig. 14e). Additional experiments are needed to validate these MD simulation and homology modeling findings.

To further probe more subtle changes in side chain behavior, we analyzed rotamer populations by computing probability density functions for all χ¹ and χ² torsions of residues belonging to rigid regions of the protein (defined as residues with ⟨RMSF⟩ + SD < 0.35 nm in both systems). In the apo state, 56 out of 235 amino acids showed perturbed rotameric states upon mutation, identified as those for which the L49V versus WT probability density functions exhibited a Kullback–Leibler (KL) divergence greater than 0.1 (Supplementary Fig. 15). These residues are distributed throughout the protein,

forming a visually connected region that includes two of the three catalytic residues, H169 and D184. The affected set includes several aromatic residues: F22, Y44, F46, F50, W52, F82, H84, H94, F122, Y129, F168, H169, F170, Y173, F181, Y189, H193, and W202 (Supplementary Fig. 16).

Comparison between the KL divergence and the CSP-derived allosteric network showed qualitative agreement in that the nodes I191 and L137 distant from L49 in the network also exhibit significant KL divergence in the neighboring residues (Fig. 2b and Supplementary Fig. 15). Although the underlying conformational dynamics occur on the sub-microsecond timescale, our simulations supported the existence of long-range modifications in side chain dynamics induced by the L49V mutation. Notably, the perturbed residues identified by MD either overlap with or are direct neighbors of those detected by CPMG NMR measurements, further supporting their functional relevance.

To assess functional relevance, we selected residue W52—the highest-ranking site identified by KL divergence analysis (Supplementary Fig. 16)—for further investigation. Notably, the W52R variant has been associated with hereditary cancer-predisposing syndrome according to the ClinVar database. Accordingly, we created BAP1-UCH variants containing the W52R and W52A mutations and observed that both exhibited a 50% reduction in DUB activity (Supplementary Fig. 17), similar to the reductions seen with F15L, F48L, and F228H in Fig. 5b. Note that the functional significance of W52 could not be readily predicted based on sequence or the atomic model alone (Fig. 5a). The concordance between MD simulations and experimental DUB activity thus highlights the reliability of the predictive capability offered by the MD simulation approach.

Many high-frequency cancer mutations are reported within the AC2 of β-sheet rich hydrophobic core, e.g., S172, Y173, E182, D184, P190, and H193 (Fig. 5f). We showed herein that many of these cancer mutations resulted in increased aggregation propensity that prohibits the production of recombinant mutants for structural and functional characterizations, suggesting that these loss of function due to severe aggregation of these mutants may be responsible for oncogenesis (Supplementary Fig. 18). Intriguingly, the introduction of a clinical mutation S172I completely abolished the DUB activity, while the H193Q mutation only partially affected the DUB activity (Fig. 5g). Differential scanning calorimeter (DSC) analysis of the BAP1-UCH mutants showed that L49V slightly stabilized BAP1-UCH; H193Q reduced the melting temperature (T_m) of BAP1-UCH by almost 10 °C while the changes in the enthalpy of unfolding (ΔH) were marginal; by contrast, S172I had less impact on the T_m of BAP1-UCH but the onset of thermal melting began well below body temperature, indicating that S172I is highly destabilizing (Fig. 5h and Supplementary Table 5), reminiscent of the effects of other high-frequency cancer mutations, N78S, F81V and G128R¹⁶. Detailed ¹³C-¹H CSP analysis reveals long-range structural perturbations induced by the H193Q mutation, including L49 and I226 that are located more than 20 Å away from the mutation site (Supplementary Fig. 19). I226 is located at the ubiquitin binding site, the perturbation of which may be responsible for the partial loss of DUB activity. The recombinant protein expression yield of H193Q was 6-times lower than that of WT with a significantly lower solubility, making it unsuitable for quantitative relaxation dynamics analysis. Nonetheless, the CSP structural mapping clearly illustrates the long-range and reciprocal perturbations along the allosteric network that we have established in this study, thereby lending support to our model that disease-associated mutations along the allosteric network play important roles in the crosstalk and functional regulation.

Discussion

Hundreds of cancer mutations have been documented within the BAP1-UCH domain, but the molecular basis of how these mutations contribute to the onset of oncogenesis is not well understood, except

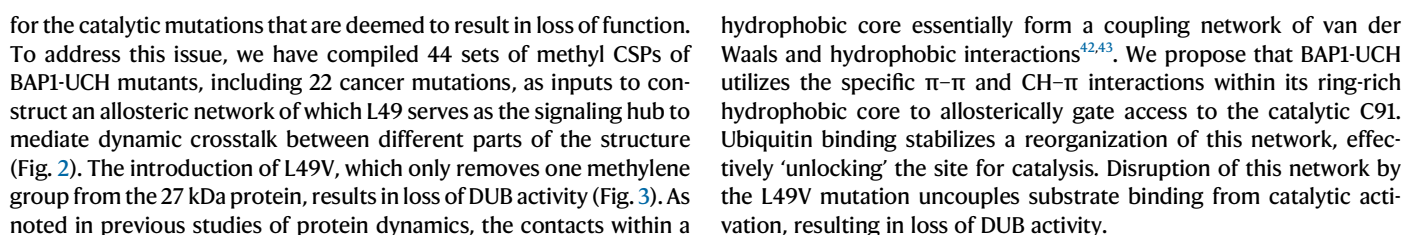


Fig. 5 | Structure-activity relationships of aromatic clusters within BAP1-UCH.

a The BAP1-UCH contains two aromatic clusters (ACs) in the central β -sheet, with aromatic side chains depicted as orange sticks and nearby methyl groups as red spheres. L49 is surrounded by several phenylalanines in AC1. **b** Mutational analysis reveals reduced DUB activity for most AC1 mutations, with F168L and F170L losing most of their DUB activity. The F228H mutation was used instead of F228L due to solubility issues. DUB activities of the individual mutants measured in technical triplicates are shown in solid lines and transparent open circles for the mean values and raw data points, respectively. **c** Methyl CSPs from L49V and F15L mutations indicate spatial connectivity among L49, I47, F15, and F170. Comparisons were made between WT and L49V using $[U\text{-}^2\text{H}, ^{13}\text{C}_m/\text{H}_m\text{-Ile-}\delta 1/\text{Leu-}\delta 1/2/\text{Val-}\gamma 1/2]$ labeled proteins; in the case of the comparison between F15L and WT, $[U\text{-}^{13}\text{C}]$ WT and F15L were used. **d** UCHL5-based homology modeling illustrates coordinated side chain conformational changes within the BAP1-UCH catalytic site, highlighting a 108° rotameric shift of L49 upon ubiquitin binding. Residue identities from UCHL5 and

BAP1-UCH are annotated in green and black. Distances for π - π and CH- π interactions were measured using PyMOL, with the C-terminus of ubiquitin shown in transparent purple. **e** Methyl NMR spectra of WT BAP1-UCH in both apo (orange) and Ub($\Delta 76$)-Cl-bound (purple) forms show upfield shifts for M231- ϵ and L49- $\delta 1/\delta 2$, consistent with ring current effects predicted by the model in **(d)**, as confirmed by ^{13}C -edited NOESY. **f** Cancer-associated mutations are mapped within the β -sheet rich hydrophobic core centered on $\beta 2$ - $\beta 5$, with $\text{C}\alpha$ atoms sized according to reported case numbers. Mutations that are soluble, insoluble, or untested are shown in blue, dark gray, and white, respectively. **g** Functional assays indicate that H193Q partially reduces DUB activity, while S172I results in a complete loss of function, following the same scheme in **(b)**. **h** DSC profiles of L49V, S172I, and H193Q mutants all show two thermal transitions shown in dotted lines and dashed lines after fitting to DSC profiles to the sum of two Gaussians; the WT profile is shown in gray area for comparison.

If this model is correct, perturbation of the L49-centered network should not only alter local side-chain geometry but also modify the dynamic coupling between distal regions of the protein and the catalytic site. Consistent with this prediction, methyl relaxation dispersion analysis revealed pronounced decoupling of internal dynamics on the μs – ms timescale in the L49V mutant (Fig. 4). Rather than simply increasing global flexibility, the mutation redistributes conformational entropy within the BAP1-UCH domain, weakening correlated motions that normally connect the L49 hub to the catalytic center. A similar entropy redistribution phenomenon was previously observed by hydrogen–deuterium exchange mass spectrometry (HDX-MS) in several cancer-associated BAP1-UCH mutants¹⁶. At the atomic level, such redistribution of conformational entropy has been well characterized by NMR CPMG relaxation dispersion analyses^{44,45}, supporting the interpretation that the L49V mutation disrupts dynamic allosteric communication rather than merely destabilizing the protein.

Remodeling of allosteric networks by mutation has been documented in several enzyme systems, including *E. coli* dihydrofolate reductase (ecDHFR), where loop mutations redistribute conformational populations and alter catalytic efficiency as revealed by room-temperature crystallography⁴⁶. In that case, the mutations were proposed to increase sampling of conformations that are suboptimal for transition-state formation. A conceptually similar framework may apply to BAP1-UCH. Here, we do not assign a specific static structure to the low-populated excited state detected by methyl CPMG relaxation dispersion. Rather, we propose that this state reflects a transient rearrangement of the L49-centered aromatic–aliphatic network that modulates catalytic-site accessibility and dynamic coupling within the hydrophobic core. In WT, this excited state is weakly populated ($p_B \approx 3\%$) and remains dynamically coherent with the catalytic center. The L49V mutation increases the population of this exchange process ($\sim 12\%$) and fragments the correlated dynamics into distinct kinetic clusters (Fig. 4, Table 1 and Supplementary Fig. 8), indicating redistribution of conformational entropy rather than stabilization of a discrete inactive structure. We suggest that the L49V mutation shifts the conformational equilibrium toward substates that are dynamically less effective at coupling ubiquitin binding to catalytic activation. In this model, L49 contributes to stabilization of the catalytically competent ensemble through contiguous CH- π and π - π interactions involving L49, F170, F15, and M231 within the AC1 as an integral component of the hydrophobic core (Fig. 5d). The model is supported by the upfield methyl chemical shifts observed in the covalent ubiquitin complex, together with homology modeling based on UCHL5 and μs -timescale MD simulations; the L49V mutation triggers redistribution of conformational populations and dynamic coupling underlies the loss of DUB activity (Fig. 5 and Supplementary Fig. 14). However, all our efforts to directly test this allosteric network—either by applying inhibitors aimed at reversing the dynamic decoupling or by identifying

compensatory mutations capable of restoring the network—were unsuccessful and may require future investigation.

Along the allosteric network that we have established in this study lie many cancer-associated mutations. L49V is identified in breast invasive carcinoma and kidney renal clear cell carcinoma³⁹ and has been suggested to be a risk factor for defective ferroptosis in cell line-based studies¹³. A recently identified Kury-Isidor syndrome (KURIS) involves a L49P mutation, associated with neurological developmental disorders^{47,48}. Several F170 mutations are reported in multiple clinical cases, including F170L, F170I, F170V, and F170C^{27,39,49}. Similar to L49V, F170L results in loss of DUB activity; other forms of F170 mutations may also confer similar functional losses. In addition to F170L, we showed that mutating any of the six phenylalanines within the AC1, except F50L, can negatively impact the DUB activity of BAP1-UCH (Fig. 5b). Specifically, F168L also resulted in a functional loss similar to that of L49V and F170L. Such an aromatic ring cluster is also present in UCHL5, and the L55V mutation, analogous to the L49V mutation in BAP1-UCH, also resulted in the loss of DUB activity of UCHL5 (Fig. 3).

Within the β -sheet rich hydrophobic core centered around $\beta 2$ - $\beta 5$. Y173C, E182D, and H193Q mutations are identified in uveal melanoma^{23,39}. S172I, Y173C, and P190H are high-frequency mutation sites found in many cancer types, including breast cancer, gastric adenocarcinoma, metastatic cancer, and cholangiocarcinoma³⁹. Attempts to produce recombinant BAP1-UCH harboring the Y173C, E182D and P190H/L mutations were unsuccessful due to misfolding, aggregation or low expression levels, indicating that these mutations are highly destabilizing (Supplementary Fig. 18). Indeed, the highly destabilized S172I shows the complete loss of DUB activity, whereas the less destabilized H193Q retains partial DUB activity (Fig. 5g, h).

In conclusion, this study provides compelling evidence that the functional integrity of BAP1-UCH is governed by a delicate network of allosteric interactions, underpinned by an intricate web of side chain dynamics and conserved aromatic residues. Our integrated NMR and mutational analyses reveal that even subtle perturbations, such as cancer-associated point mutations within the hydrophobic core, can destabilize the core architecture and impair DUB activity. Despite the relatively low occurrence of the L49V mutation in the cancer database, our network analysis has revealed the pivotal role of L49 in mediating the dynamic coupling between different structural elements within BAP1-UCH, and the functional importance of this particular residue is also present in its paralog, UCHL5. These findings not only deepen our understanding of the molecular mechanisms behind BAP1-UCH dysfunction in oncogenesis but also highlight potential avenues for therapeutic intervention targeting allosteric sites critical for structural communication and enzymatic regulation. For instance, NMR relaxation dynamics have been used to identify a cryptic drug binding site in Bcl-xL; a phenylalanine-to-tryptophan mutation can shift the conformational equilibrium of Bcl-xL towards an open conformation more

favorable for ligand binding⁵⁰. Conceptually, it may be possible to develop small molecules or protein binders to restore the dynamics decoupling caused by the L49V mutation in BAP1-UCH thereby restoring the DUB activity and, therefore, mitigate loss of function-associated oncogenesis.

Methods

Protein cloning, expression, and purification

The human BAP1-UCH domain (residues 1–238) was cloned into the pET28a vector as described previously¹⁶. The BAP1-UCH sequence was fused to an N-terminal thioredoxin (Trx-tag), with a hexahistidine tag (His₆-tag) on the N-terminus for affinity purification. Between the N-terminus of BAP1-UCH and the Trx-tag is a tobacco etch virus (TEV) protease cleavage site for tag removal. The human UCHL5 UCH domain (residues 1–240) was cloned into a modified pGEX4t-3 vector, modified to have a TEV protease cleavage site, for the removal of the N-terminal glutathione S-transferase (GST-tag). Site-directed mutagenesis was introduced by PCR and confirmed by DNA sequencing (Mission Biotech, Taiwan). The details of the primer design were described in Supplementary Data 1.

The expression plasmids of BAP1-UCH, UCHL5-UCH, and those of the mutants were transformed into an *E. coli* BL21 (DE3) strain for recombinant protein overexpression. The transformed cells were grown in lysogeny broth (LB) at 37 °C until the cell density (OD₆₀₀) reached 0.6–0.8, at which point, the over-expression of the recombinant proteins was induced by the addition of 0.05 mM (final concentration) isopropylthio-β-galactoside (IPTG) followed by further culturing at 16 °C for 18–20 h. The cell cultures were harvested by centrifugation (JA-14 and JLA-8.1, Beckman, USA) at 6000 rpm, which equates to 5524 g for JA-14 and 8983 g for JLA-8.1, for 20 min at 4 °C. The cell pellets were flash frozen in liquid nitrogen and stored at -80 °C until further use. Details of the BAP1-UCH mutants used in the NMR experiments and those of the insoluble mutants are listed in Supplementary Table 1. The UCHL5 WT and L55V mutation were expressed by 0.5 mM IPTG induction at 16 °C for 18–20 h. The stable isotope-labeled BAP1-UCH variants were over-expressed by *E. coli* BL21 (DE3) in the M9 media, and the appropriate precursors for selectively methyl-labeling were added 0.5–1 h before IPTG induction.

For [¹³C] labeling, ¹³C-labeled D-glucose (Cambridge Isotope Laboratory, USA) was used as the sole carbon in the M9 media. For selectively methyl-labeling under a perdeuterated background, the transformed cells were cultured in perdeuterated M9 media, containing 100% D₂O and ²H/¹²C-labeled D-glucose (Cambridge Isotope Laboratory, USA) with additional precursors for different methyl labeling patterns. The selective labeling of the Ile-δ1, Leu-δ1/2, Val-γ1/2, and Ala-β methyl groups was achieved by using an in vivo ILVA methyl labeling kit (CDLM-8806-KIT, Cambridge Isotope Laboratory, USA), containing α-ketobutyric acid (CDLM-7318-PK; Cambridge Isotope Laboratory, USA) and α-ketoisovaleric acid (CDLM-7317-PK; Cambridge Isotope Laboratory, USA) to yield [U-²H, ¹³C_m/H_m-Ile-δ1/Leu-δ1/2/Val-γ1/2/Ala-β]. The stereospecific (proS) labeling of Leu-δ2, and Val-γ2 was achieved by using the labeling kit QLAM-A^βI^{δ1}LV^{proS} (NMR-Bio, France) following manufacturer instructions to yield [U-²H, ¹³C_m/H_m-Ile-δ1/Leu-δ2/Val-γ2/Ala-β].

The cell pellets harboring over-expressed recombinant proteins were resuspended in a Tris-based lysis buffer (50 mM Tris (pH 8.0), 200 mM NaCl, 5 mM β-ME, 5% (v/v) glycerol, 0.01% (v/v) TritonX-100, 0.3 mg/mL lysozyme, 0.001% Benzonase) and lysed by a NanoLyzer N2 (NanoLyzer, Taiwan) at 18,000 psi at 4 °C. The cell debris was removed by centrifugation using a JLA-25.5 rotor (Beckman, USA) at 20,000 rpm, which equates to 48,300 g, for 30 min at 4 °C. The cell lysate containing target recombinant proteins was mixed with 1–2 mL nickel-nitrilotriacetic acid (Ni-NTA) resin (cOmplete His-Tag Purification Resin, Roche, Germany) for 1 h at 4 °C and then loaded onto a PD-10 column (Cytiva, USA) for gravity affinity chromatography. The Ni-

NTA resin was washed using buffer A (50 mM Tris-HCl (pH 8.0), 200 mM NaCl, 5 mM β-ME, 5% (v/v) glycerol), followed by another wash using buffer B (50 mM Tris-HCl (pH 8.0), 1000 mM NaCl, 5 mM β-ME, 5% (v/v) glycerol), and another wash using buffer A. The recombinant protein was eluted by elution buffer C (50 mM Tris-HCl (pH 8.0), 200 mM NaCl, 5 mM β-ME, 5% (v/v) glycerol, 300 mM imidazole) and dialyzed in buffer A with TEV protease for 16 h at 4 °C. The Trx-tag was removed by the Ni-NTA resin and followed by SEC purification using a HiLoad 16/600 Superdex 75 column (Cytiva, USA) in a SEC buffer, containing PBS (10 mM Na₂HPO₄ (pH 7.4), 1.8 mM KH₂PO₄, 2.7 mM KCl, 137 mM NaCl) supplemented with 5 mM β-ME. The SEC buffer was exchanged to specific NMR buffers according to experimental requirements.

To purify recombinant UCHL5, the cell pellet was resuspended in a phosphate-based lysis buffer (50 mM sodium phosphate buffer, pH 8.0, 300 mM NaCl, 1 mM DTT, 0.3 mg/mL lysozyme, 0.0005% Benzonase). The recombinant UCHL5 was purified using the Glutathione Sepharose™ 4B resin (Cytiva, USA) with a wash buffer D (50 mM sodium phosphate (pH 8.0), 300 mM NaCl, 1 mM DTT) and an elution buffer E (50 mM sodium phosphate (pH 8.0), 300 mM NaCl, 1 mM DTT, 10 mM glutathione reduced form). The GST-tag was removed by TEV protease digestion in the dialysis buffer F (PBS, pH 7.4, 1 mM DTT, 0.5 mM EDTA). The recombinant UCHL5 variants (WT and L55V) were subsequently purified using a HiLoad 16/600 Superdex 75 column in the SEC buffer G (PBS (pH 7.4), 1 mM Tris(2-carboxyethyl)phosphine (TCEP), and 0.5 mM ethylenediaminetetraacetic acid (EDTA)).

Preparation of BAP1-UCH covalently bound to Ub(ΔG76)-Cl

Ub(ΔG76)-Cl was prepared following a previously published protocol⁴¹, with minor modifications. Briefly, a fusion construct encoding Ub(ΔG76) linked to the intein GyrA was synthesized and subcloned into the pRSFDuet-1 expression vector, incorporating an N-terminal hexahistidine tag and a TEV protease cleavage site (ENLYFQGS). The fusion protein, Ub(ΔG76)-GyrA, was expressed and purified via nickel-affinity chromatography using 350 mM imidazole elution, followed by dialysis and cleavage with TEV protease in buffer containing 25 mM HEPES (pH 7.0) and 200 mM NaCl. To generate the C-terminal hydrazide intermediate, Ub(ΔG76)-GyrA (75 μM) was incubated with 100 mM MESNa and 120 mM hydrazine (pH 7.5) at 37 °C overnight. The resulting Ub(ΔG76)-hydrazide was purified by size-exclusion chromatography on a HiLoad 16/60 Superdex 75 column using an ÄKTA FPLC system. Fractions containing pure Ub(ΔG76)-hydrazide were pooled, concentrated, and stored at -80 °C. For chloroacetylation, 100 μM Ub(ΔG76)-hydrazide was buffer-exchanged into 100 mM citric acid (pH 3.0), followed by the addition of 20 mM chloroacetic anhydride. The reaction was carried out at 37 °C for 15 min. The resulting Ub(ΔG76)-Cl product was subsequently buffer-exchanged into 25 mM Tris-HCl (pH 7.6) containing 200 mM NaCl to remove excess reagent. The identities of both Ub(ΔG76)-hydrazide and Ub(ΔG76)-Cl were confirmed by MALDI-TOF mass spectrometry.

The Ub-BAP1-UCH complex was formed through the reaction of [¹³C] BAP1-UCH with Ub(ΔG76)-Cl. The BAP1-UCH and Ub(ΔG76)-Cl reaction was set at a ratio of 1:2 and mixed at 4 °C for 1 h. The complex was purified by SEC in PBS buffer and exchanged into the NMR buffer for data collection.

NMR spectroscopy for methyl group assignments

All NMR experiments were performed at 298 K on a Bruker NEO 600 spectrometer equipped with a TCI cryo-probe, a Bruker Avance III 600 spectrometer equipped with a TXI cryo-probe, or a Bruker Avance III 850 spectrometer equipped with a TCI cryo-probe. The spectrometers were operated using Topspin 3.2 on Avance III 600 and Avance III 850, and Topspin 4.1.3 on NEO 600. For mutation screening, [¹³C] BAP1-UCH WT and mutants were prepared in the MOPS buffer (50 mM MOPS (pH 7.0), 50 mM NaCl, 10 (v/v)% D₂O). Relaxation-optimized

^{13}C - ^1H SOFAST-HMQC^{51,52} spectra were collected for the BAP1-UCH variants for chemical shift perturbation analyses. For methyl NMR assignments, $[\text{U-}^{13}\text{C}]$ BAP1-UCH WT and the covalent complex of ubiquitin-bound $[\text{U-}^{13}\text{C}]$ BAP1-UCH WT were prepared in deuterated PBS (10 mM Na_2HPO_4 , 1.8 mM KH_2PO_4 , 2.7 mM KCl, 137 mM NaCl, 100 (v/v)% D_2O , pD 7.0) for the collection of ^{13}C -NOESY-HSQC (with a mixing time of 150 ms and a recycling delay of 1 s) and ^{13}C - ^1H SOFAST-HMQC spectra. $[\text{U-}^{13}\text{C}]$ BAP1-UCH L49V was prepared in deuterated PBS supplemented with 2 (v/v)% $[\text{2H}_5]$ glycerol (Cambridge Isotope Laboratory, USA). Additionally, $[\text{U-}^2\text{H}, ^{13}\text{C}_\text{m}/\text{H}_\text{m}\text{-Ile-}\delta 1/\text{Leu-}\delta 1/2/\text{Val-}\gamma 1/2/\text{Ala-}\beta]$ BAP1-UCH WT, $[\text{U-}^2\text{H}, ^{13}\text{C}_\text{m}/\text{H}_\text{m}\text{-Ile-}\delta 1/\text{Leu-}\delta 2/\text{Val-}\gamma 2/\text{Ala-}\beta]$ BAP1-UCH WT, and $[\text{U-}^2\text{H}, ^{13}\text{C}_\text{m}/\text{H}_\text{m}\text{-Ile-}\delta 1/\text{Leu-}\delta 1/2/\text{Val-}\gamma 1/2/\text{Ala-}\beta]$ BAP1-UCH L49V were prepared in the MOPS buffer supplied with 2 (v/v)% $[\text{2H}_5]$ glycerol for the collection of ^{13}C -edited 3D SOFAST-NOESY-HMQC⁵³ (with a mixing time of 500 ms and a recycling delay of 300 ms) and ^{13}C - ^1H -SOFAST-HMQC. All NMR spectra were processed by NMRPipe (v2021.237.09.07)⁵⁴ and analyzed by POKY⁵⁵. The 3D NOESY spectra of $[\text{U-}^{13}\text{C}]$ BAP1-UCH WT and the complex of Ub- $[\text{U-}^{13}\text{C}]$ BAP1-UCH WT were collected by Topspin default non-uniform sampling (NUS) scheme with a sampling density of 40%, and the NUS datasets were reconstructed by SMILE⁵⁶. The NOESY data of $[\text{U-}^{13}\text{C}]$ BAP1-UCH L49V were collected (with a mixing time of 150 ms and a recycling delay of 1 s) by hms1ST NUS scheme in Topspin at a sampling density of 30%, and the NUS datasets were reconstructed by hms1ST⁵⁷. The selectively methyl- labeled, including stereo-specifically labeled samples, were prepared in the MOPS buffer supplied with 2 (v/v)% $[\text{2H}_5]$ glycerol for traditional sampling of NOESY experiments (with a mixing time of 500 ms and a recycling delay of 300 ms). The methyl resonance assignments were accomplished by comparative analysis of the disappearance of mutated peak resonances, CSPs, through-space ^1H - ^1H NOE connectivities based on the homology models of BAP1 cryo-EM structures^{3,4}. The NMR assignments have been deposited in the Biological Magnetic Resonance Bank (BMRB), and the accession numbers are listed in Supplementary Data 2.

Network analysis of methyl chemical shift perturbations induced by mutagenesis

The individual methyl CSPs upon point mutations were calculated by the following equation:

$$\text{CSP(ppm)} = \sqrt{\left(\frac{\Delta\delta_{\text{C}}}{18}\right)^2 + \left(\frac{\Delta\delta_{\text{H}}}{2.6}\right)^2}, \quad (1)$$

where $\Delta\delta_{\text{C}}$ and $\Delta\delta_{\text{H}}$ are the chemical shift differences between WT and mutant *j*. The chemical shifts were normalized by the spectral width of 18 ppm and 2.6 ppm for the ^{13}C and ^1H , respectively. Residues that exhibited CSPs over two standard deviations above the mean CSP were considered significantly perturbed residues upon the given mutation *j*. To avoid the selection of nodes in the mutation with low CSP values (such as R59Q and L152I), the threshold was set at 0.01 ppm. For leucines and valines, only one of the two methyl groups was selected to be included in the network analysis dataset. A set of in-house Python scripts was generated based on the NetworkX function for network construction and visualization using PyMOL (2.4.2) (see **Code availability**).

CPMG relaxation dispersion dynamics analysis

CPMG relaxation dispersion experiments were collected at 298 K on a Bruker NEO 600 spectrometer and a Bruker NEO 850 spectrometer as described previously⁵⁸. The spectrometers were operated using Topspin 4.1.3 on NEO 600 and Topspin 4.3.0 on NEO 850. $[\text{U-}^2\text{H}, ^{13}\text{C}_\text{m}/\text{H}_\text{m}\text{-Ile-}\delta 1/\text{Leu-}\delta 1/2/\text{Val-}\gamma 1/2]$ BAP1-UCH WT and L49V were prepared in PBS (pD 7.0) in 100 (v/v)% D_2O supplemented with 2 (v/v)% $[\text{U-}^2\text{H}]$ glycerol. The protein concentrations of WT and L49V were 170 μM and 320 μM , respectively. The methyl TROSY multiple quantum relaxation

dispersion experiments were acquired with ν_{CPMG} of 50*, 100*, 150*, 200, 250, 300*, 400, 500, 700, and 1000 Hz and a total time of 20 ms (T_{relax})⁵⁹. Asterisks indicate the spectra that were recorded in technical duplicates; the CPMG experiments were repeated at 50, 150, and 300 Hz for WT and at 50, 100, and 300 Hz for L49V. The effective decay rate ($R_{2,\text{eff}}$) was calculated by the following formula:

$$R_{2,\text{eff}} = \left(-\frac{1}{T}\right) \ln \frac{I_{\nu_{\text{CPMG}}}}{I_0}, \quad (2)$$

where $I_{\nu_{\text{CPMG}}}$ and I_0 were the peak intensities with and without CPMG intervals. The T_{relax} was set to 20 ms. The relax dispersion curves were analyzed by Relax (version 5.0)⁶⁰ with no dispersion (No Rex) and NS MMQ 2-site models within the NMRbox virtual computing environment⁶¹.

DUB activity assay

The DUB assay was conducted using Ub-AMC (Cat#M3010; UBPBio, USA) as a model substrate as previously described^{16,62}. In brief, recombinant BAP1-UCH variants were diluted to a final concentration of 0.83 nM in the reaction buffer A (50 mM Tris-HCl (pH 8.5), 5 mM DTT, 0.5 mg/mL BSA, 0.5 mM EDTA). The recombinant UCHL5 variants were diluted to a final concentration of 0.5 nM in the reaction buffer B (50 mM Tris-HCl (pH 7.6), 5 mM DTT, 0.5 mg/mL BSA, 0.5 mM EDTA). The Ub-AMC was diluted to a final concentration of 1.5 μM in the reaction buffer A or B. The recombinant proteins were pre-incubated at 30 °C for 30 min before being mixed with Ub-AMC to monitor the DUB activity of BAP1-UCH and UCHL5 in 384-well microplates (Greiner bio-one, USA) at 30 °C. The AMC fluorescence was monitored by an Infinite® M1000 PRO plate reader (TECAN, Switzerland) for 1000 s with an interval of 5 s with excitation at 380 nm and emission at 460 nm. The kinetic traces were exported for visualization and analysis by Prism 10 (GraphPad, USA).

Size-exclusion chromatography-coupled small-angle X-ray scattering (SAXS)

The SAXS data of BAP1-UCH WT and L49V were collected at the Bio-SAXS beamline 13 A of Taiwan Photon Source (TPS) at the National Synchrotron Radiation Research Center (NSRRC), Taiwan. The Bio-SAXS beamline 13 A integrates the SAXS/UV-Vis-absorption measurement with a high-performance liquid chromatography (HPLC) system. 90 μL of BAP1-UCH variant at a concentration of 12 mg/mL was injected into the HPLC system and separated by BioSEC-3 (300 Å, 4.6×300 mm) (Agilent, USA) at a flow rate of 0.35 mL/min. The flow rate was reduced to 0.1 mL/min before the SAXS data collection to extend the duration of SAXS data collection. The exposure time of each frame was set at 2 s. The SAXS data were collected with a momentum transfer *q* ranging from 0.006 to 2.455 Å⁻¹ for a wavelength of 0.8265 Å, 15 keV, using an integrated detecting system comprising an Eiger X 9M SAXS detector (Dectris, Switzerland) and an Eiger X 1M WAXS detector (Dectris, Switzerland). An in-house LabVIEW-based software accomplished the data reduction, solvent subtraction, and merging of the SAXS data⁶³. The SAXS data were further processed and analyzed using PRIMUS in the ATSAS 3.2.1 software package to estimate the R_g and D_{max} value based on the Guinier approximation and the pairwise distance distribution function (PDDF, $P(r)$)⁶⁴. The data were plotted using Prism 10.

Differential scanning calorimetry (DSC)

The thermal unfolding experiments of BAP1-UCH variants were performed by Automatic MicroCal PEAQ-DSC (Malvern, UK). The protein concentration was set to 0.5 mg/mL in PBS. The scan rate was set at 200 °C/h over 10–90 °C. The DSC data were processed by the built-in software in MicroCal PEAQ-DSC (Malvern, UK). The baseline-corrected DSC profiles were exported to Prism 10 and fit to a sum of two

Gaussians to determine the two transition temperatures, T_{m1} and T_{m2} . The enthalpy of unfolding (ΔH) is defined by the area under the curve of each Gaussian distribution.

Molecular dynamics (MD) simulations

BAP1-UCH molecular dynamics simulations were performed using GROMACS 2025⁶⁵. The initial configuration was prepared by extracting residues 3–238 from chain L (and M in case of the Ub-bound holo structure) of the cryo-EM structure (PDB ID 8H1T <https://doi.org/10.2210/pdb8H1T/pdb>³). The missing loop was modeled using Modeller⁶⁶. The L49V mutation was introduced in PyMOL⁶⁷. Protonation states of ionizable residues were assigned assuming standard states at pH 7.0 (Asp/Glu deprotonated, Lys/Arg protonated), with histidines protonated according to their local hydrogen-bonding environment. The resulting protein conformations were parametrized with the DES-Amber force field⁶⁸ and the TIP4P-D water model⁶⁹. Each system was placed in a dodecahedral box (volume: 424.5 nm³ for the APO and 539 nm³ for the HOLO simulations) containing 12,808 water molecules (16,152 for the HOLO case) and 150 mM Na⁺/Cl[−], with additional ions added to ensure overall charge neutrality. Short-range electrostatic and Lennard–Jones interactions were truncated at 1.0 nm, and long-range electrostatics were computed using the particle mesh Ewald (PME) method⁷⁰. Covalent bonds involving hydrogen atoms were constrained using LINCS⁷¹, allowing a 2 fs integration time step. Energy minimization was followed by a 2 ns equilibration phase performed in the NPT ensemble at 298 K and 1 atm, using the velocity-rescale thermostat and the cell-rescale barostat⁷², with positional restraints applied to heavy atoms of the protein. Temperature and volume quickly reached stable plateaus within the simulated time (see Supplementary Fig. 20a, b). Production simulations were carried out in the NPT ensemble under the same temperature and pressure conditions, without positional restraints. For each system, three independent replicas were performed, each 2.2 μ s in length (total simulation time: 6.6 μ s per system, ~20 μ s in total), with velocities reinitialized at the start of each replica. The first 100 ns of each trajectory were discarded as additional equilibration prior to analysis (a RMSD plot for each system and replica is shown in Supplementary Fig. 20c).

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The methyl resonance assignments of BAP1-UCH variants have been deposited in the Biological Magnetic Resonance Bank (BMRB) under the accession numbers tabulated in Supplementary Data 2. The SAXS/WAXS data of BAP1-UCH WT and L49V have been deposited in the Small Angle Scattering Biological Data Bank (SASBDB; <https://www.sasbdb.org>) under accession numbers SASDXD6 and SASDXE6, respectively. The MD simulation trajectories are available through <https://doi.org/10.5281/zenodo.18777587>; also included are the input files for MD simulations, side chain rotamer histograms data, the script to perform the KL calculation, the RMSF raw data and the scripts to calculate the distances between the ring/methyl pseudo atoms. The plasmids encoding the BAP1-UCH variants are available upon request. The PDB code of the previously published structure used in this study is 8H1T <https://doi.org/10.2210/pdb8H1T/pdb>. The raw data underlying all Figures and Supplementary Figures, including the NMR CPMG relaxation dispersion data, are provided as a Source Data file. Source Data are provided as a Source Data file. Source data are provided with this paper.

Code availability

In-house Python scripts for perturbation network analysis are available at Github (<https://github.com/CHLai2025/13CMethylNetworkAnalysis.git>) and Zenodo <https://doi.org/10.5281/zenodo.20152904>.

References

- Louie, B. H. & Kurzrock, R. BAP1: not just a BRCA1-associated protein. *Cancer Treat. Rev.* **90**, 102091 (2020).
- Kwon, J., Lee, D. & Lee, S. A. BAP1 as a guardian of genome stability: implications in human cancer. *Exp. Mol. Med.* **55**, 745–754 (2023).
- Ge, W. et al. Basis of the H2AK119 specificity of the Polycomb repressive deubiquitinase. *Nature* **616**, 176–182 (2023).
- Thomas, J. F. et al. Structural basis of histone H2A lysine 119 deubiquitination by polycomb repressive deubiquitinase BAP1/ASXL1. *Sci. Adv.* **9**, eadg9832 (2023).
- Foglizzo, M. et al. A bidentate Polycomb Repressive-Deubiquitinase complex is required for efficient activity on nucleosomes. *Nat. Commun.* **9**, 3932 (2018).
- Yu, H. et al. Tumor suppressor and deubiquitinase BAP1 promotes DNA double-strand break repair. *Proc. Natl. Acad. Sci. USA*. **111**, 285–290 (2014).
- Ismail, I. H. et al. Germline mutations in BAP1 impair its function in DNA double-strand break repair. *Cancer Res.* **74**, 4282–4294 (2014).
- Lee, S. A. et al. BAP1 promotes the repair of UV-induced DNA damage via PARP1-mediated recruitment to damage sites and control of activity and stability. *Cell Death Differ.* **29**, 2381–2398 (2022).
- Lee, H. S., Lee, S. A., Hur, S. K., Seo, J. W. & Kwon, J. Stabilization and targeting of INO80 to replication forks by BAP1 during normal DNA synthesis. *Nat. Commun.* **5**, 5128 (2014).
- Klusmann, I. et al. Chromatin modifiers Mdm2 and RNF2 prevent RNA:DNA hybrids that impair DNA replication. *Proc. Natl. Acad. Sci. USA*. **115**, E11311–E11320 (2018).
- Daou, S. et al. The BAP1/ASXL2 Histone H2A deubiquitinase complex regulates cell proliferation and is disrupted in cancer. *J. Biol. Chem.* **290**, 28643–28663 (2015).
- Arenzana, T. L. et al. Tumor suppressor BAP1 is essential for thymic development and proliferative responses of T lymphocytes. *Sci. Immunol.* **3**, 1953 (2018).
- Zhang, Y. et al. BAP1 links metabolic regulation of ferroptosis to tumour suppression. *Nat. Cell Biol.* **20**, 1181–1192 (2018).
- He, M. et al. Intrinsic apoptosis shapes the tumor spectrum linked to inactivation of the deubiquitinase BAP1. *Science* **364**, 283–285 (2019).
- Bononi, A. et al. BAP1 regulates IP3R3-mediated Ca²⁺ flux to mitochondria suppressing cell transformation. *Nature* **546**, 549–553 (2017).
- Puri, S. et al. Impacts of cancer-associated mutations on the structure-activity relationship of BAP1. *J. Mol. Biol.* **434**, 167553 (2022).
- Bhattacharya, S., Hanpude, P. & Maiti, T. K. Cancer associated missense mutations in BAP1 catalytic domain induce amyloidogenic aggregation: a new insight in enzymatic inactivation. *Sci. Rep.* **5**, 18462 (2015).
- Rai, K., Pilarski, R., Cebulla, C. M. & Abdel-Rahman, M. H. Comprehensive review of BAP1 tumor predisposition syndrome with report of two new cases. *Clin. Genet.* **89**, 285–294 (2016).
- Walpole, S. et al. Comprehensive study of the clinical phenotype of germline BAP1 variant-carrying families worldwide. *J. Natl. Cancer Inst.* **110**, 1328–1341 (2018).
- Lalloo, F. et al. Clinical practice guidelines for the diagnosis and surveillance of BAP1 tumour predisposition syndrome. *Eur. J. Hum. Genet.* **31**, 1261–1269 (2023).

21. Star, P. et al. Germline BAP1-positive patients: the dilemmas of cancer surveillance and a proposed interdisciplinary consensus monitoring strategy. *Eur. J. Cancer* **92**, 48–53 (2018).
22. Pilarski, R. et al. Expanding the clinical phenotype of hereditary BAP1 cancer predisposition syndrome, reporting three new cases. *Genes Chromosomes Cancer* **53**, 177–182 (2014).
23. Klebe, S. et al. BAP1 hereditary cancer predisposition syndrome: a case report and review of literature. *Biomark. Res.* **3**, 14 (2015).
24. Hong, J. H. et al. Functional characterisation guides classification of novel BAP1 germline variants. *NPJ Genom. Med.* **5**, 50 (2020).
25. Harbour, J. W. et al. Frequent mutation of BAP1 in metastasizing uveal melanomas. *Science* **330**, 1410–1413 (2010).
26. Bott, M. et al. The nuclear deubiquitinase BAP1 is commonly inactivated by somatic mutations and 3p21.1 losses in malignant pleural mesothelioma. *Nat. Genet.* **43**, 668–672 (2011).
27. Pena-Llopis, S. et al. BAP1 loss defines a new class of renal cell carcinoma. *Nat. Genet.* **44**, 751–759 (2012).
28. Sahtoe, D. D., van Dijk, W. J., Ekkebus, R., Ovaa, H. & Sixma, T. K. BAP1/ASXL1 recruitment and activation for H2A deubiquitination. *Nat. Commun.* **7**, 10292 (2016).
29. Yang, T. J. et al. Tumor suppressor BAP1 nuclear import is governed by transportin-1. *J. Cell Biol.* **221**, e202201094 (2022).
30. Mashtalir, N. et al. Autodeubiquitination protects the tumor suppressor BAP1 from cytoplasmic sequestration mediated by the atypical ubiquitin ligase UBE2O. *Mol. Cell* **54**, 392–406 (2014).
31. Campagne, A. et al. BAP1 complex promotes transcription by opposing PRC1-mediated H2A ubiquitylation. *Nat. Commun.* **10**, 348 (2019).
32. Das, C. et al. Structural basis for conformational plasticity of the Parkinson's disease-associated ubiquitin hydrolase UCH-L1. *Proc. Natl. Acad. Sci. USA* **103**, 4675–4680 (2006).
33. Boudreaux, D. A., Maiti, T. K., Davies, C. W. & Das, C. Ubiquitin vinyl methyl ester binding orients the misaligned active site of the ubiquitin hydrolase UCHL1 into productive conformation. *Proc. Natl. Acad. Sci. USA* **107**, 9117–9122 (2010).
34. Johnston, S. C., Larsen, C. N., Cook, W. J., Wilkinson, K. D. & Hill, C. P. Crystal structure of a deubiquitinating enzyme (human UCH-L3) at 1.8 Å resolution. *EMBO J* **16**, 3787–3796 (1997).
35. Misaghi, S. et al. Structure of the ubiquitin hydrolase UCH-L3 complexed with a suicide substrate. *J. Biol. Chem.* **280**, 1512–1520 (2005).
36. Nishio, K. et al. Crystal structure of the de-ubiquitinating enzyme UCH37 (human UCH-L5) catalytic domain. *Biochem. Biophys. Res. Commun.* **390**, 855–860 (2009).
37. Sahtoe, D. D. et al. Mechanism of UCH-L5 activation and inhibition by DEUBAD domains in RPN13 and INO80G. *Mol. Cell* **57**, 887–900 (2015).
38. Puri, S. & Hsu, S.-T. D. Functional dynamics of human ubiquitin C-terminal hydrolases. *Front. Biophys.* **2**, <https://doi.org/10.3389/frbis.2024.1479898> (2024).
39. Sondka, Z. et al. COSMIC: a curated database of somatic variants and clinical data for cancer. *Nucleic Acids Res.* **52**, D1210–D1217 (2024).
40. Kenny, S. et al. Altered protein dynamics and a more reactive catalytic cysteine in a neurodegeneration-associated UCHL1 mutant. *J. Mol. Biol.* **436**, 168438 (2024).
41. Farnung, J., Tolmachova, K. A. & Bode, J. W. Installation of electrophiles onto the C-terminus of recombinant ubiquitin and ubiquitin-like proteins. *Chem. Sci.* **14**, 121–129 (2022).
42. Vondrasek, J., Bendova, L., Klusak, V. & Hobza, P. Unexpectedly strong energy stabilization inside the hydrophobic core of small protein rubredoxin mediated by aromatic residues: correlated ab initio quantum chemical calculations. *J. Am. Chem. Soc.* **127**, 2615–2619 (2005).
43. Kim, J. et al. A dynamic hydrophobic core orchestrates allostery in protein kinases. *Sci. Adv.* **3**, e1600663 (2017).
44. Tzeng, S. R. & Kalodimos, C. G. Protein activity regulation by conformational entropy. *Nature* **488**, 236–240 (2012).
45. Capdevila, D. A., Braymer, J. J., Edmonds, K. A., Wu, H. & Giedroc, D. P. Entropy redistribution controls allostery in a metalloregulatory protein. *Proc. Natl. Acad. Sci. USA* **114**, 4424–4429 (2017).
46. van den Bedem, H., Bhabha, G., Yang, K., Wright, P. E. & Fraser, J. S. Automated identification of functional dynamic contact networks from X-ray crystallography. *Nat. Methods* **10**, 896–902 (2013).
47. Kury, S. et al. Rare germline heterozygous missense variants in BRCA1-associated protein 1, BAP1, cause a syndromic neurodevelopmental disorder. *Am. J. Hum. Genet.* **109**, 361–372 (2022).
48. West, E. C. et al. BRCA1-associated protein 1: tumor predisposition syndrome and Kury-Isidor syndrome, from genotype-phenotype correlation to clinical management. *Clin. Genet.* **105**, 589–595 (2024).
49. Mori, T. et al. Somatic alteration and depleted nuclear expression of BAP1 in human esophageal squamous cell carcinoma. *Cancer Sci* **106**, 1118–1129 (2015).
50. Mizukoshi, Y. et al. Targeting the cryptic sites: NMR-based strategy to improve protein druggability by controlling the conformational equilibrium. *Sci. Adv.* **6**, eabd0480 (2020).
51. Schanda, P., Kupce, E. & Brutscher, B. SOFAST-HMQC experiments for recording two-dimensional heteronuclear correlation spectra of proteins within a few seconds. *J. Biomol. NMR* **33**, 199–211 (2005).
52. Hsu, S.-T. D., Cabrita, L. D., Fucini, P., Christodoulou, J. & Dobson, C. M. Probing side-chain dynamics of a ribosome-bound nascent chain using methyl NMR spectroscopy. *J. Am. Chem. Soc.* **131**, 8366–8367 (2009).
53. Rossi, P., Xia, Y., Khanra, N., Veglia, G. & Kalodimos, C. G. ¹⁵N and ¹³C- SOFAST-HMQC editing enhances 3D-NOESY sensitivity in highly deuterated, selectively [¹H,¹³C]-labeled proteins. *J. Biomol. NMR* **66**, 259–271 (2016).
54. Delaglio, F. et al. NMRPipe: a multidimensional spectral processing system based on UNIX pipes. *J. Biomol. NMR* **6**, 277–293 (1995).
55. Lee, W., Rahimi, M., Lee, Y. & Chiu, A. POKY: a software suite for multidimensional NMR and 3D structure calculation of biomolecules. *Bioinformatics* **37**, 3041–3042 (2021).
56. Ying, J., Delaglio, F., Torchia, D. A. & Bax, A. Sparse multidimensional iterative lineshape-enhanced (SMILE) reconstruction of both non-uniformly sampled and conventional NMR data. *J. Biomol. NMR* **68**, 101–118 (2017).
57. Hyberts, S. G., Milbradt, A. G., Wagner, A. B., Arthanari, H. & Wagner, G. Application of iterative soft thresholding for fast reconstruction of NMR data non-uniformly sampled with multidimensional Poisson Gap scheduling. *J. Biomol. NMR* **52**, 315–327 (2012).
58. Lou, Y. C. et al. Structure and dynamics of polymyxin-resistance-associated response regulator PmrA in complex with promoter DNA. *Nat. Commun.* **6**, 8838 (2015).
59. Korzhnev, D. M., Kloiber, K., Kanelis, V., Tugarinov, V. & Kay, L. E. Probing slow dynamics in high molecular weight proteins by methyl-TROSY NMR spectroscopy: application to a 723-residue enzyme. *J. Am. Chem. Soc.* **126**, 3964–3973 (2004).
60. Bieri, M., d'Auvergne, E. J. & Gooley, P. R. relaxGUI: a new software for fast and simple NMR relaxation data analysis and calculation of ps-ns and μs motion of proteins. *J. Biomol. NMR* **50**, 147–155 (2011).
61. Maciejewski, M. W. et al. NMRbox: A resource for biomolecular NMR computation. *Biophys. J.* **112**, 1529–1534 (2017).
62. Boudreaux, D. A., Chaney, J., Maiti, T. K. & Das, C. Contribution of active site glutamine to rate enhancement in ubiquitin C-terminal hydrolases. *FEBS J.* **279**, 1106–1118 (2012).

63. Shih, O. et al. Performance of the new biological small- and wide-angle X-ray scattering beamline 13A at the Taiwan Photon Source. *J. Appl. Crystallogr.* **55**, 340–352 (2022).
64. Manalastas-Cantos, K. et al. ATSAS 3.0: expanded functionality and new tools for small-angle scattering data analysis. *J. Appl. Crystallogr.* **54**, 343–355 (2021).
65. Abraham, M. J. et al. GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. *SoftwareX* **1–2**, 19–25 (2015).
66. Webb, B. & Sali, A. Comparative protein structure modeling using MODELLER. *Curr. Protoc. Protein Sci.* **86**, 29.1–29.37 (2016).
67. The PyMOL Molecular Graphics System 1.8 (2015).
68. Piana, S., Robustelli, P., Tan, D., Chen, S. & Shaw, D. E. Development of a force field for the simulation of single-chain proteins and protein-protein complexes. *J. Chem. Theory Comput.* **16**, 2494–2507 (2020).
69. Piana, S., Donchev, A. G., Robustelli, P. & Shaw, D. E. Water dispersion interactions strongly influence simulated structural properties of disordered protein states. *J. Phys. Chem. B* **119**, 5113–5123 (2015).
70. Essmann, U. et al. A smooth particle mesh Ewald method. *J. Chem. Phys.* **103**, 8577–8593 (1995).
71. Hess, B. P.-L. I. N. C. S. A parallel linear constraint solver for molecular simulation. *J. Chem. Theory Comput.* **4**, 116–122 (2008).
72. Bussi, G., Donadio, D. & Parrinello, M. Canonical sampling through velocity rescaling. *J. Chem. Phys.* **126**, 014101 (2007).

Acknowledgements

We thank the Academia Sinica High-field NMR Center (HFNMRC) and Biophysics Core Facility (BCF) for the technical support in NMR and DSC data collection, respectively; HFNMRC and BCF are funded by the Academia Sinica Core Facility and Innovative Instrument Projects AS-CFII-111-214 and AS-CFII-111-201, respectively. We are also grateful for the technical and instrumental support provided by the Medicinal Chemistry and Analytical Core Facility-NMR, funded by Academia Sinica Core Facility and Innovative Instrument Project (AS-NBRPCF-111-201). We thank the staff of the BioSAXS beamline TPS BL13A at the NSRRC, Taiwan, for supporting the SAXS data collection. We also thank the Protein Core and the Chemical Synthesis Core of the Institute of Biological Chemistry, Academia Sinica, for supporting the recombinant protein production and the synthesis of Ub(Δ G76)-Cl, respectively. This study made use of NMRbox: National Center for Biomolecular NMR Data Processing and Analysis, a Biomedical Technology Research Resource (BTRR), which is supported by NIH grant P41GM111135 (NIGMS). The preparation of Python scripts was supported by ChatGPT 4o and Claude 3.5 sonnet. Code generated with these tools was checked by the authors before use.

Author contributions

S.-T.D.H. conceived the experiments. C.-H.L. prepared all the BAP1-UCH variants, collected, processed, and analyzed the NMR data, performed the DUB activity assay, and analyzed DSC data. Y.-C.L., C.-F.C. and S.-T.D.H. supported the NMR data collection and analysis. W.-L.L. and K.-P.W. prepared the Ub(Δ G76)-Cl sample. M.K.S. supported recombinant protein production. Y.-S.W. collected and analyzed the SAXS data. C.C.

carried out the MD simulations. C.C., C.-H.L. and S.-T.D.H. analyzed the MD simulations. S.-T.D.H. and C.-H.L. wrote and revised the manuscript with inputs from all contributing authors. S.-T.D.H. supervised the project and obtained funding.

Funding

This work is supported by intramural fund, an Investigator Award (AS-IV-114-L04) to STDH, and a Career Development Award (AS-CDA-110-L03) to KPW. STDH is supported by the National Science and Technology Council (NSTC), Taiwan (110-2113-M-001-050-MY3, 113-2123-M-001-010 and 114-2123-M-001-008-). CHL is supported by the postdoctoral fellowships from NSTC (110-2811-M-001-583-, 113-2811-M-001-111 and 114-2811-M-001-106); MKS is also supported by the postdoctoral fellowships from NSTC (113-2811-M-001-110 and 114-2811-M-001-116). CC acknowledges CINECA for an award under the ISCRA initiative, for the availability of high-performance computing resources and support.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41467-026-73600-4>.

Correspondence and requests for materials should be addressed to Shang-Te Danny Hsu.

Peer review information *Nature Communications* thanks the anonymous reviewers for their contribution to the peer review of this work. A peer review file is available.

Reprints and permissions information is available at <http://www.nature.com/reprints>

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Open Access This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

© The Author(s) 2026