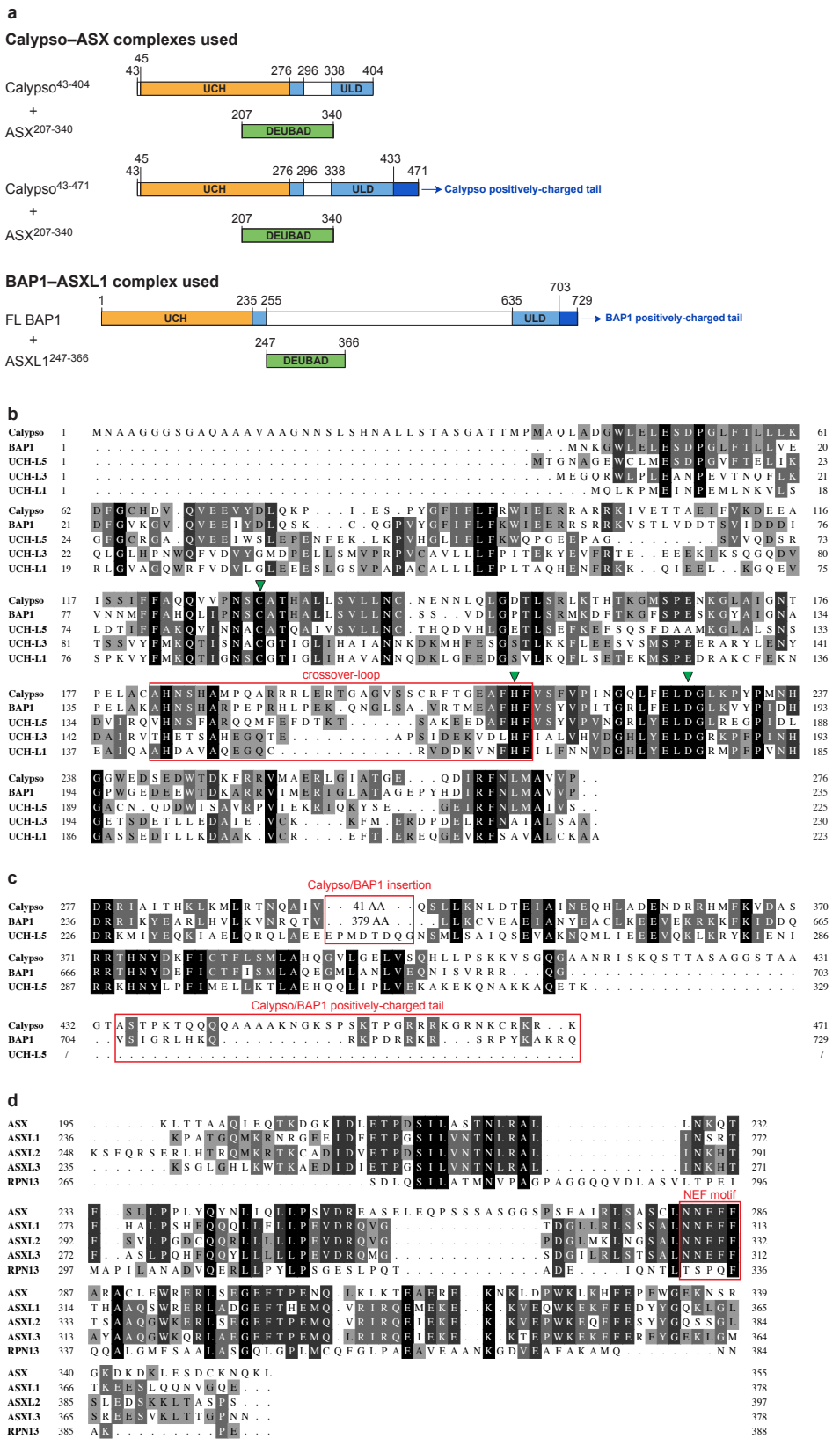
Supplementary Information

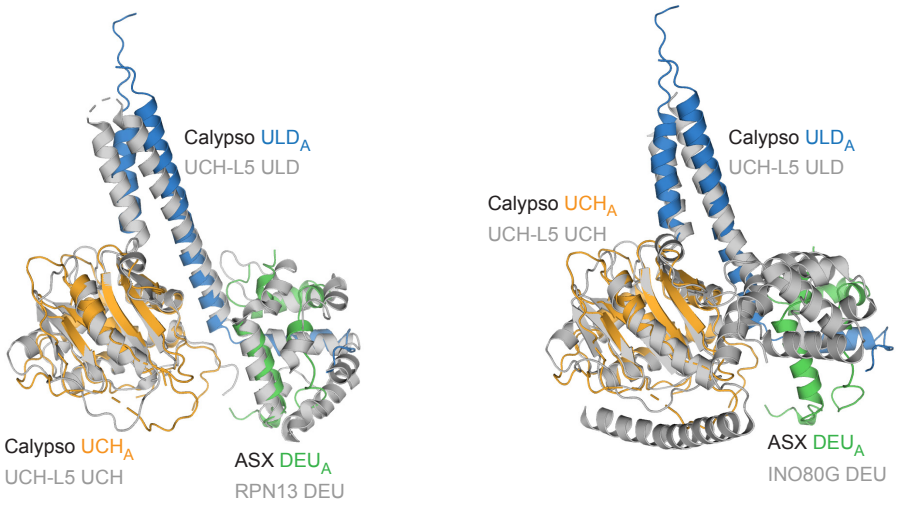
A bidentate Polycomb Repressive-Deubiquitinase complex is required for efficient activity on nucleosomes.

Foglizzo et al.

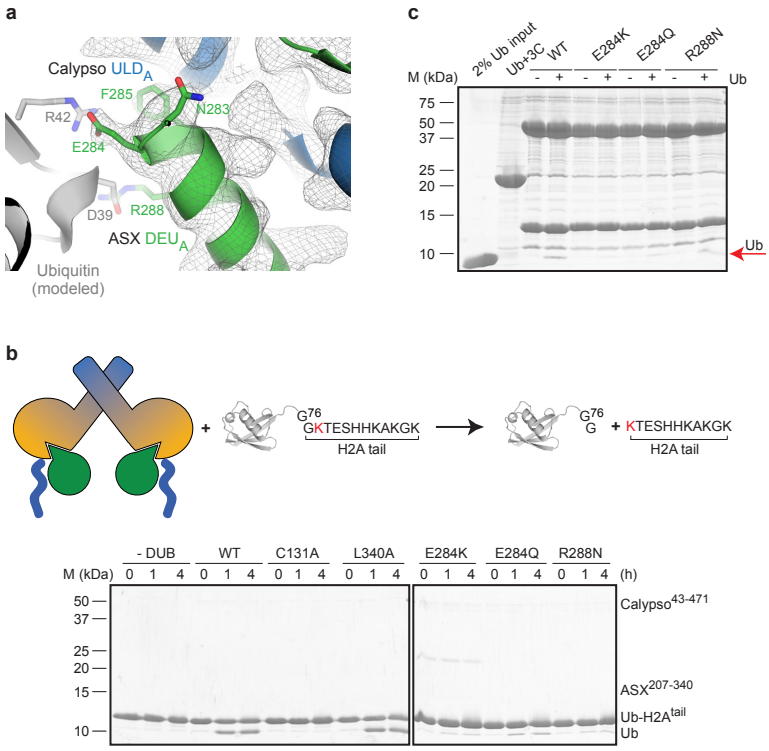


Supplementary Fig. 1| Comparison of Calypso and ASX with other family members. a, Schematic of the Calypso–ASX and BAP1–ASXL1 complexes used in this study. UCH = ubiquitin C-terminal hydrolase; ULD = UCH37-like domain; FL = full-length; ASX = additional sex combs; ASXL1 = ASX-like 1. **b**, Multiple sequence

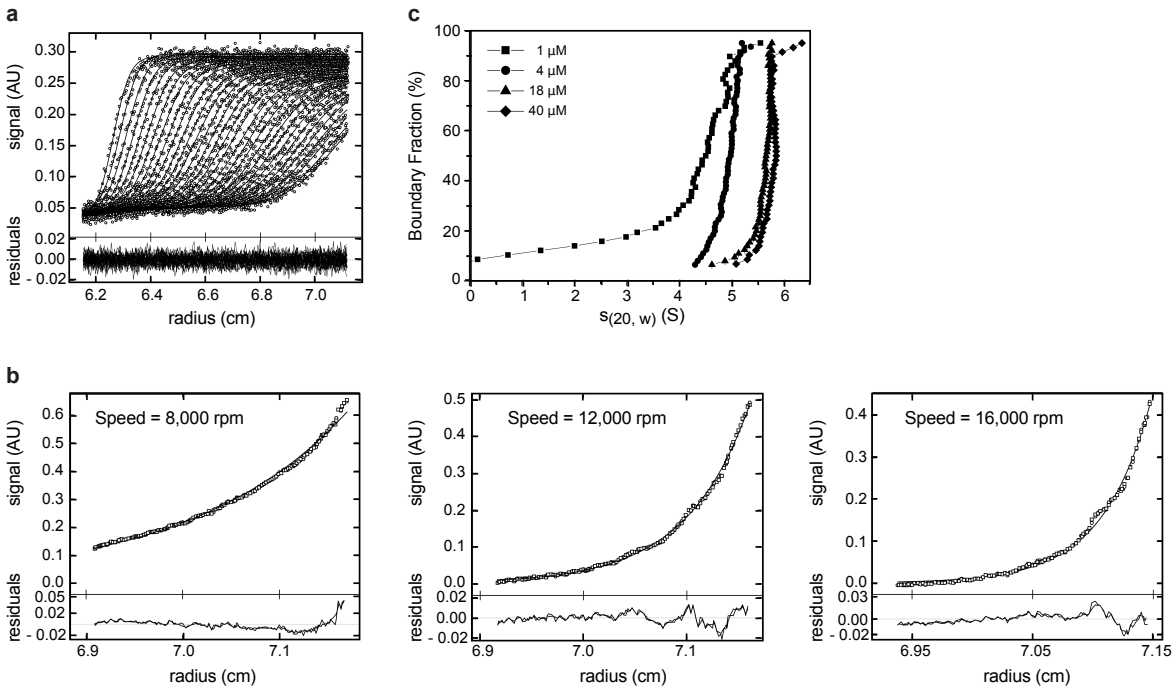
alignment of the UCH domains in Calypso, BAP1, UCH-L5, UCH-L3 and UCH-L1. Identity between the proteins is indicated with black shading; similarity with grey shading. The crossover-loop is highlighted with a red box; green arrows indicate the residues forming the active site triad. **c**, Multiple sequence alignment of the ULD domains in Calypso, BAP1 and UCH-L5. Identity and similarity between the proteins are indicated as in **b**. The Calypso/BAP1 insertion and the C-terminal positively-charged tail are highlighted with red boxes. **d**, Multiple sequence alignment of the Deubad domains in ASX, ASXL1, ASXL2, ASXL3 and Rpn13. Identity and similarity between the proteins are indicated as described in **b**. The NEF region is highlighted with a red box. Deubad = deubiquitinase adaptor domain; ASXL = ASX-like.



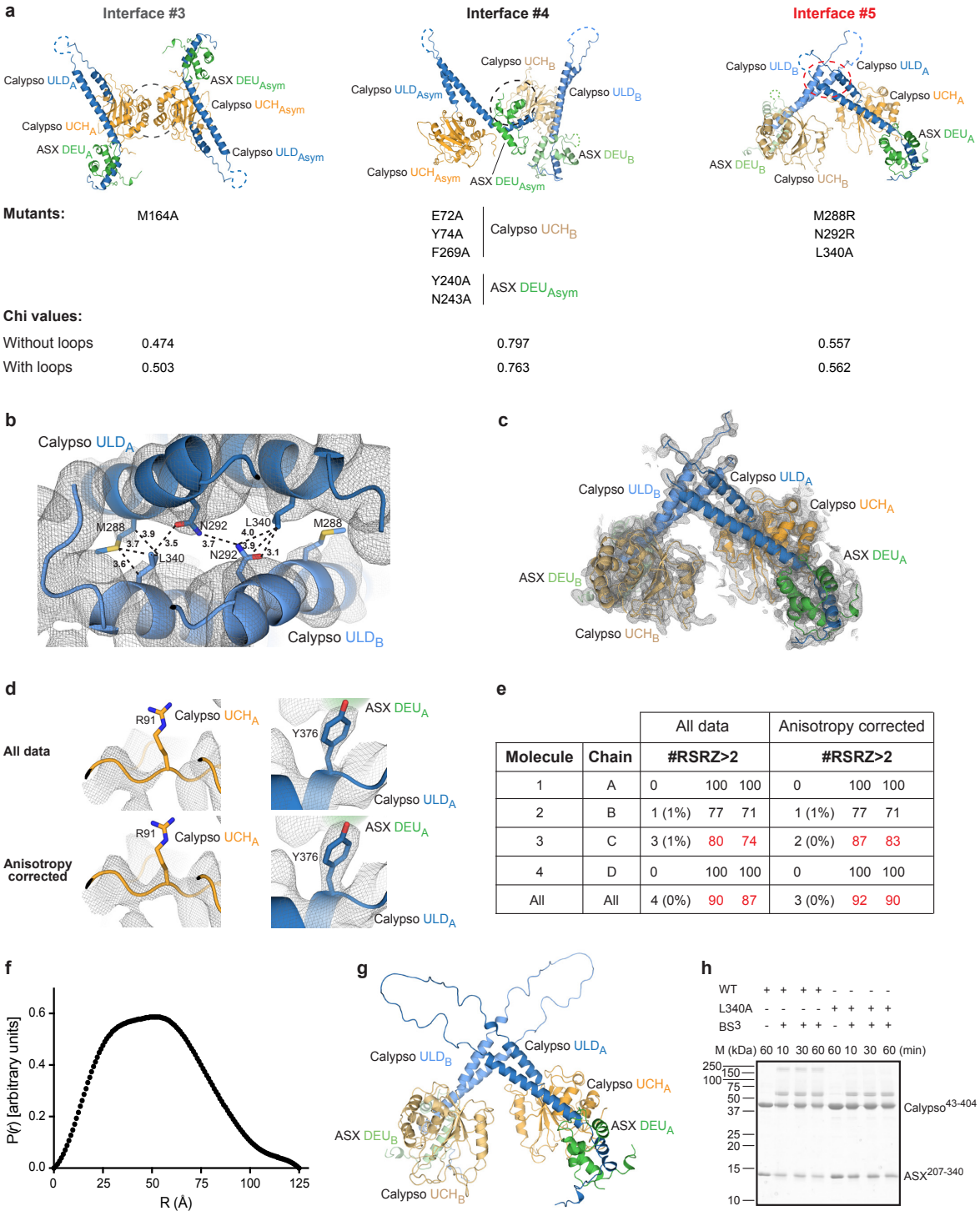
Supplementary Fig. 2| Comparison of the Calypso–ASX complex with the activated and inhibited UCH-L5 complexes. Overlay of Calypso–ASX onto the structures of the UCH-L5–RPN13 (PDB code: 4UEM) (*left*) and UCH-L5–INO80G (PDB code: 4UF5) (*right*) complexes (ref. [1]). The UCH and ULD domains of Calypso, and the Deubad domain of ASX, are shown in cartoon and colored as described in **Fig. 1b**. The corresponding domains of UCH-L5 and RPN13/INO80G are colored grey. Chain B of the Calypso–ASX structure has been removed for clarity. UCH = ubiquitin C-terminal hydrolase; ULD = UCH37-like domain; Deubad (DEU) = deubiquitinase adaptor domain; ASX = additional sex combs.



Supplementary Fig. 3| The *Drosophila* PR-DUB complex as a model for understanding the impact of cancer-derived missense mutations in the human PR-DUB. a, A 2Fo-Fc electron density map (contoured at 1 σ level) around the NEF region of the ASX Deubad domain. Residues in the NEF-motif of ASX, Arg288, and the putative interacting amino acids in the modeled ubiquitin are shown as sticks. ULD = UCH37-like domain; Deubad (DEU) = deubiquitinase adaptor domain; ASX = additional sex combs. **b**, Schematic representation of the Ubiquitin-H2A^{tail} hydrolysis assays performed in this study (*top*). The additional glycine residue following ubiquitin Gly76 provides a linker between the C-terminus of ubiquitin and the N-terminus of the Histone 2A tail. Deubiquitination assays comparing the ability of wild-type and mutant Calypso–ASX complexes to remove ubiquitin from Ubiquitin-H2A^{tail} (*bottom*). Reactions were incubated at 37°C for 4 h; samples were analysed using 14-20% SDS-PAGE gradient gels and visualised by staining with Coomassie Blue. C131A refers to a mutation in the active site Cysteine of Calypso. DUB = deubiquitinating enzyme; WT = wild-type; Ub = Ubiquitin; H2A = Histone 2A. **c**, His_6-fused wild-type and mutants Calypso–ASX complexes were bound to Ni^{2+}-NTA beads and tested in pull-down experiments with purified ubiquitin. The extent of ubiquitin binding was detected by Coomassie Blue staining.



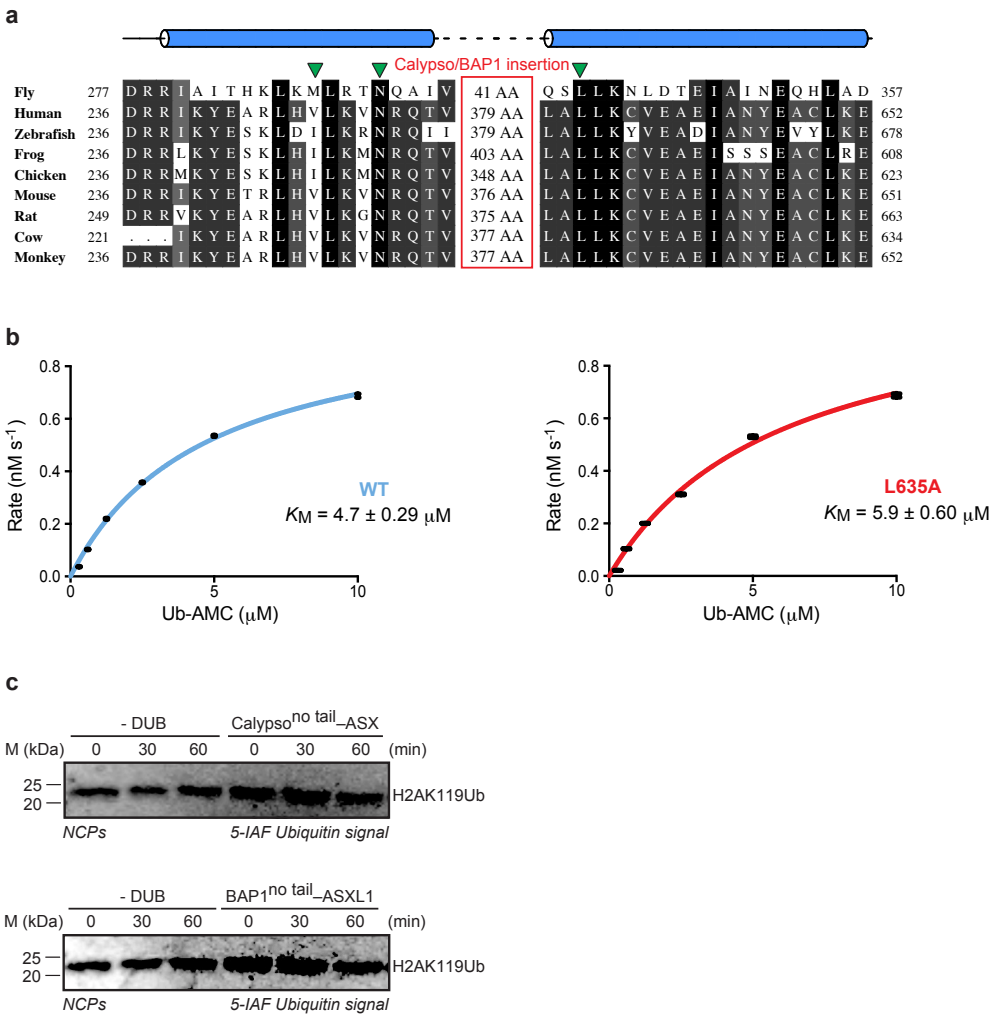
Supplementary Fig. 4| Characterisation of the Calypso–ASX oligomeric state by analytical ultracentrifugation. a, Raw sedimentation velocity data and residuals, corresponding to data shown in **Fig. 3b**, for Calypso–ASX at 40 μM. **b**, Sedimentation equilibrium data at three speeds, fitted to a single species model with a fitted mass of 112.9 kDa (+/- 0.3 kDa). Each panel shows two data sets, taken 1 hour apart, to demonstrate that the system had come to equilibrium. The global reduced chi-squared for the fit was 2.35 (n=857). **c**, van Holde-Weischet analysis of Calypso–ASX at concentrations of 1 μM (squares), 4 μM (circles), 18 μM (triangles) and 40 μM (diamonds).



		All data			Anisotropy corrected		
Molecule	**Chain**	**#RSRZ>2**			**#RSRZ>2**		
1	A	0	100	100	0	100	100
2	B	1 (1%)	77	71	1 (1%)	77	71
3	C	3 (1%)	80	74	2 (0%)	87	83
4	D	0	100	100	0	100	100
All	All	4 (0%)	90	87	3 (0%)	92	90

Supplementary Fig. 5| Structural, biophysical and biochemical characterisation of the 2:2 Calypso–ASX oligomer. a, Schematic of the possible hetero-tetrameric interfaces identified by PISA (ref. [2]), with the designed mutant(s) and the Chi values obtained from SEC-SAXS experiments listed below. UCH = ubiquitin C-terminal hydrolase; ULD = UCH37-like domain; Deubad (DEU) = deubiquitinase adaptor domain; ASX = additional sex combs. **b,** A composite omit map (contoured at 1 σ level) of the Calypso dimer interface, showing the packing of the two coiled-coil hairpins. Residues involved in dimerisation are shown in stick form. Main- and side-

chain contacts between residues are indicated by dashed lines, with distance measurements (numbers) shown in Å. **c**, A 2Fo-Fc electron density map (contoured at 1 σ level) of the Calypso–ASX crystal structure. The structure is shown in cartoon representation and colored as described in **Fig. 1b**. **d**, Composite omit maps (contoured at 1 σ level) for the full (*top*) and anisotropy corrected (*bottom*) datasets, showing regions in the structure that were improved after anisotropy correction. **e**, Table showing the number (and percentage) of RSRZ outliers, followed by percentile scores relative to all X-ray entries and entries with similar resolution. Percentile scores that improved after anisotropy correction are highlighted in red. **f**, iInteratomic distance distributions of the Calypso–ASX complex. **g**, Structure of the Calypso–ASX complex used for SAXS experiments, with loops that were not defined by electron density modeled. The structure is shown in cartoon representation and colored as described in **Fig. 1b**. **h**, Assays comparing the appearance of cross-linked species in wild-type and L340A Calypso–ASX complexes over time. Samples were resolved by reducing SDS-PAGE gel and stained with Coomassie Blue. WT = wild-type; BS^3 = bis(sulfosuccinimidyl)suberate.



Supplementary Fig. 6| Impact of PR-DUBs oligomerisation in nucleosome recruitment and activity. a, Multiple sequence alignment of the coiled-coil hairpin across nine different BAP1/Calypso-like DUBs. Identity between the proteins is indicated with black shading; similarity with grey shading. The BAP1/Calypso insertion is highlighted with a red box; green arrows indicate the residues targeted for mutations. Secondary structure elements are indicated. **b**, Michaelis-Menten analysis of wild-type and L635A BAP1–ASXL1 proteins cleavage of Ubiquitin-AMC. Error bars indicate ±SEM (*n* = 2 independent experiments). WT = wild-type. **c**, Deubiquitination assays comparing the ability of wild-type Calypso$^{no\ tail}$–ASX (*top*) and BAP1$^{no\ tail}$–ASXL1 (*bottom*) complexes to cleave ubiquitin from H2AK119Ub nucleosomes. Assays were visualised as described in **Fig. 5b**. DUB = deubiquitinating enzyme; ASX = additional sex combs; ASXL1 = ASX-like 1.

Fig. 4e

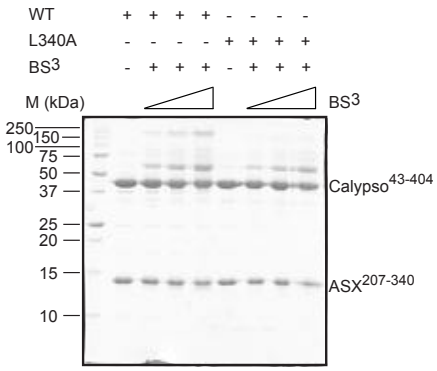
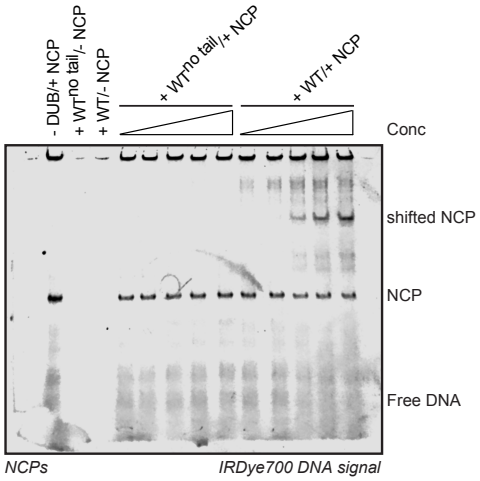


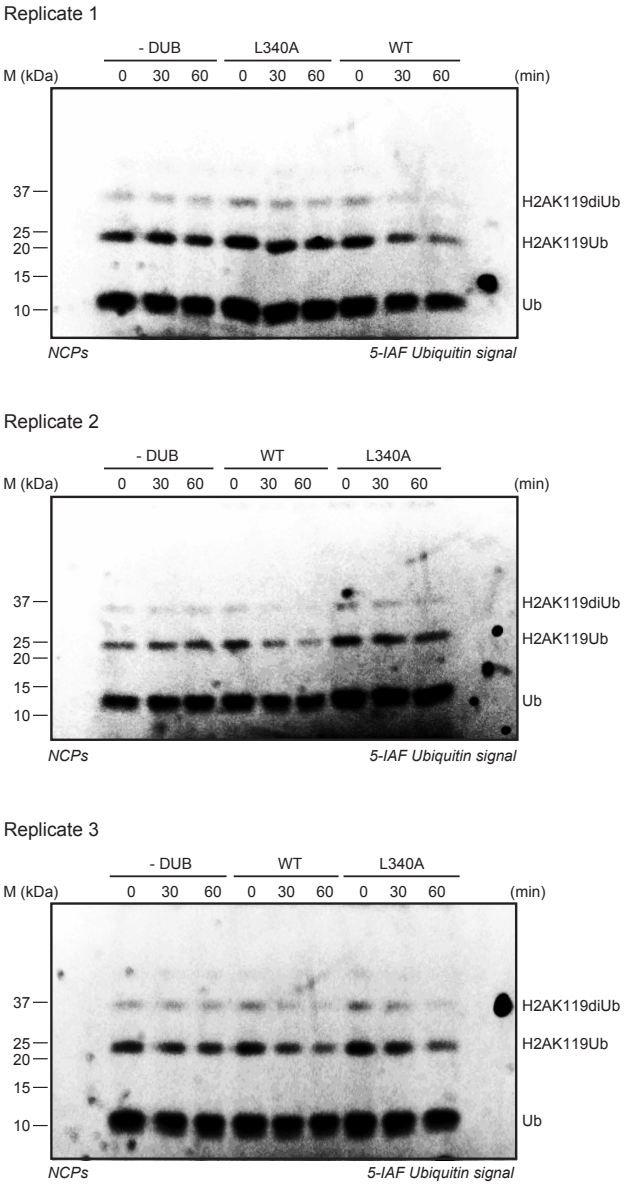
Fig. 6a



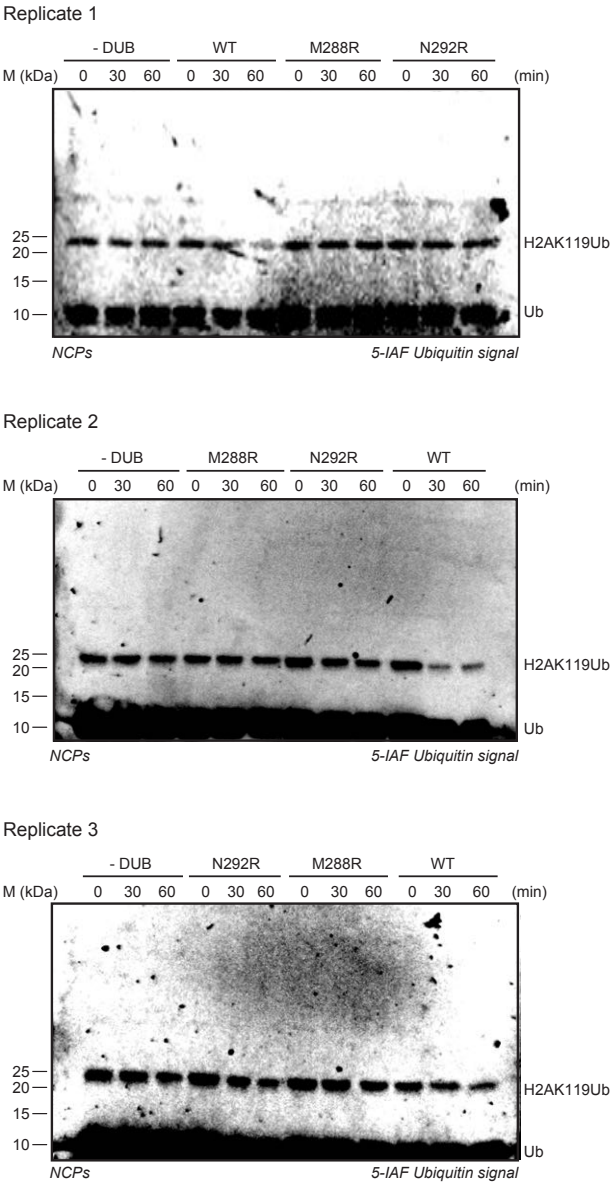
Supplementary Fig. 7| Uncropped gels for Fig. 4e and Fig. 6a.

Fig. 5b

Calypso–ASX WT and L340A assay replicates

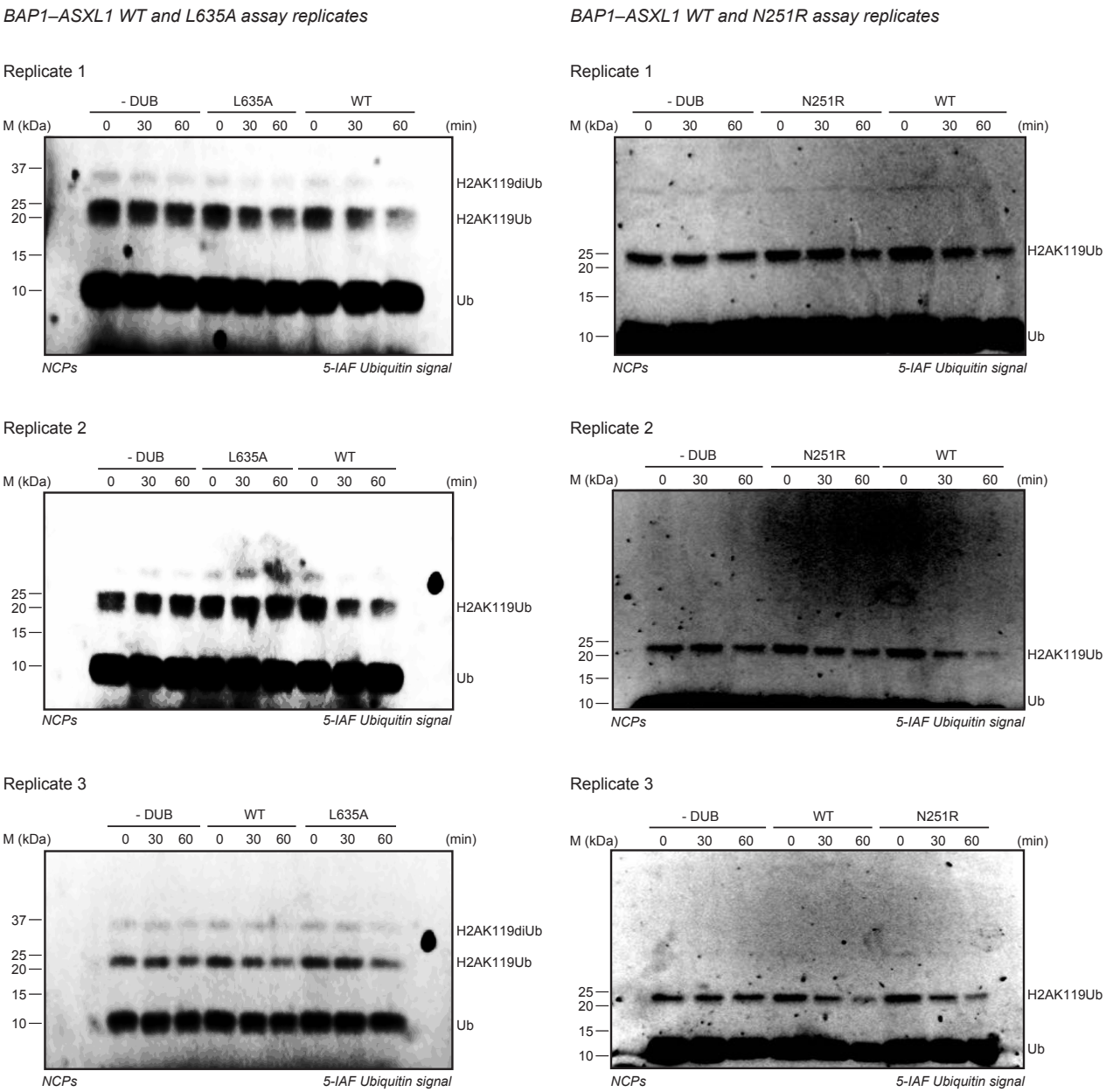


Calypso–ASX WT, M288R and N292R assay replicates

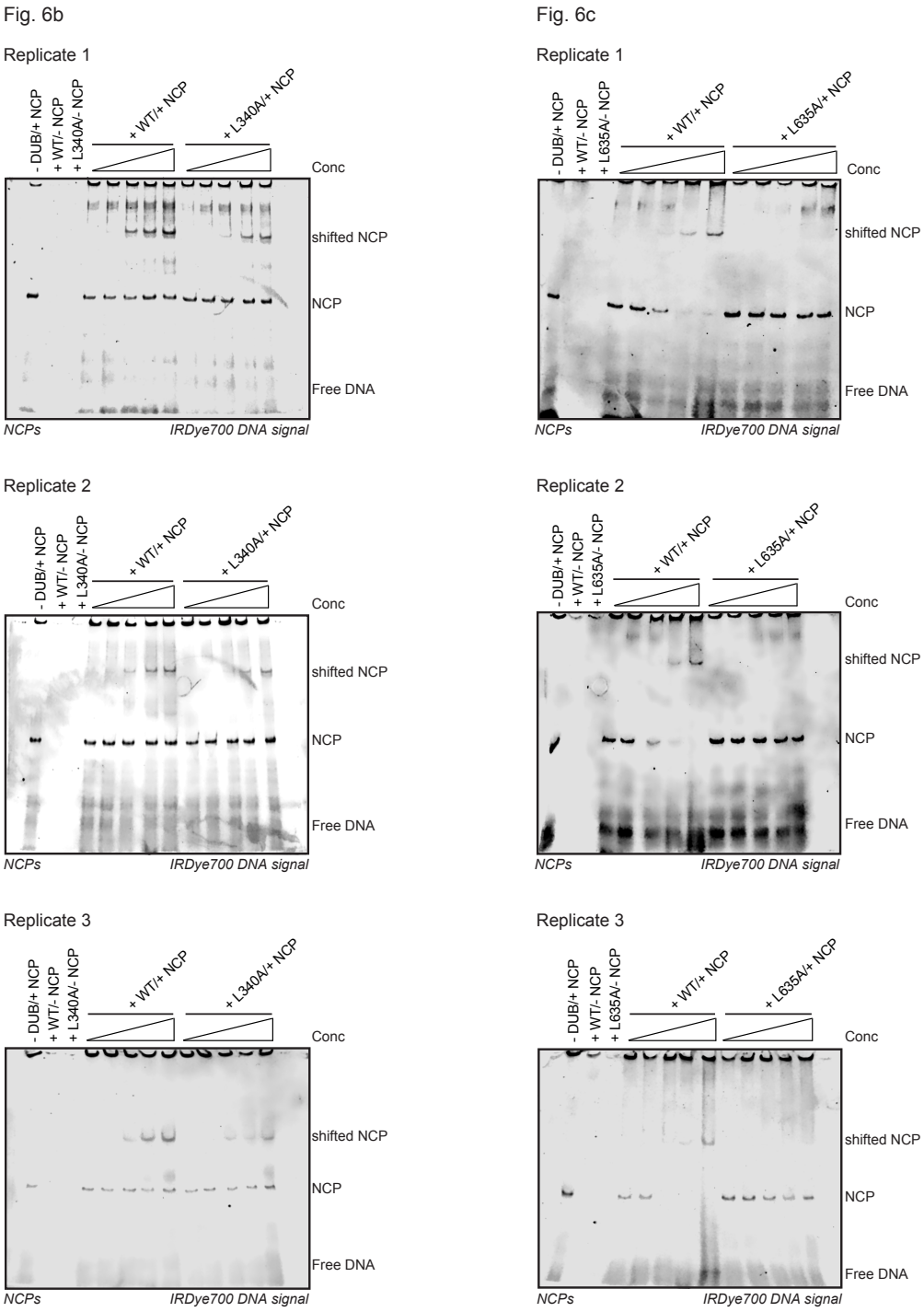


Supplementary Fig. 8| Uncropped gels for the replicate experiments shown in Fig. 5b. For each set of experiments, gels shown in **Fig. 5b** correspond to the first experimental replicate (Replicate 1).

Fig. 5c

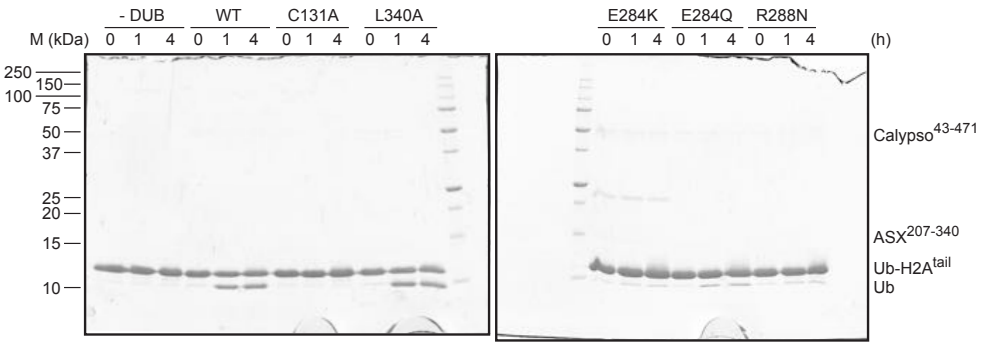


Supplementary Fig. 9| Uncropped gels for the replicate experiments shown in Fig. 5c. For each set of experiments, gels shown in **Fig. 5c** correspond to the first experimental replicate (Replicate 1).

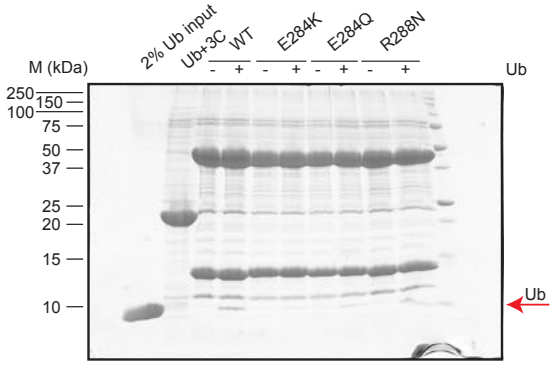


Supplementary Fig. 10| Uncropped gels for the replicate experiments shown in Fig. 6b,c. Gels shown in **Fig. 6b** and **Fig. 6c** correspond to the first experimental replicate (Replicate 1).

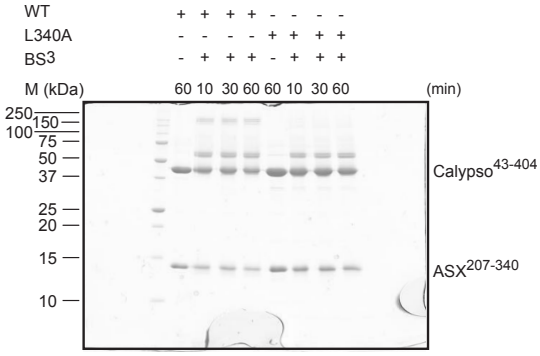
Supplementary Fig. 3b



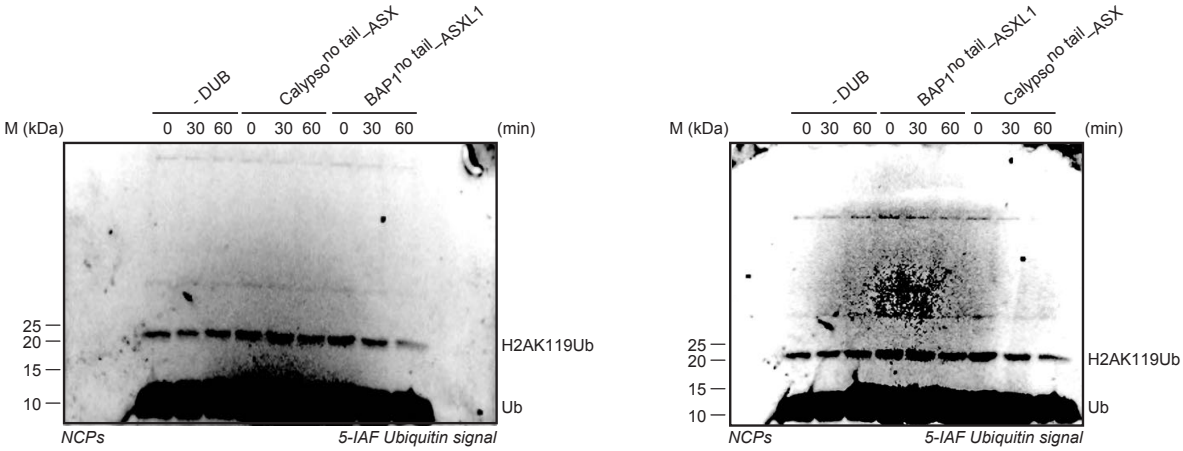
Supplementary Fig. 3c



Supplementary Fig. 5e



Supplementary Fig. 6c



Supplementary Fig. 11| Uncropped gels for Supplementary Figures.

Supplementary Table 1. X-ray data collection and refinement statistics

	Calypso–ASX complex (PDB: 6CGA)
Data collection	
Space group	$I12_1$
Cell dimensions	
a, *b*, *c* (Å)	150.17, 65.63, 162.76
α, β, γ (°)	90, 114.35, 90
Resolution (Å)	43.67-3.50 (3.83-3.50)[a]
R_{pim} (all I^+/I^-)	0.062 (0.714)
Total no. of reflections	50698 (11657)
Total no. of unique reflections	18300 (4333)
Mean I / σI	7.1 (1.4)
$CC_{1/2}$	0.998 (0.613)
Completeness (%)	98.8 (99.1)
Redundancy	2.8 (2.7)
Refinement	
Resolution (Å)	43.67-3.50
No. atoms	
Protein	5885
Water	0
Before anisotropic scaling	
No. reflections	18271
Completeness (%)	96.57
R_{work} / R_{free}	0.265/0.303
Average *B*-factor (Å^2)	
Protein	176.86
R.m.s. deviations	
Bond lengths (Å)	0.005
Bond angles (°)	1.06
After anisotropic scaling	
No. reflections	15404
Completeness (%)	83.1
R_{work} / R_{free}	0.244/0.294
Average *B*-factor (Å^2)	
Protein	132.17
R.m.s. deviations	
Bond lengths (Å)	0.002
Bond angles (°)	0.557

Diffraction data were obtained from one protein crystal.
R.m.s. deviations, root-mean-square deviations.
[a]Values in parentheses are for the highest-resolution shell.

Supplementary Table 2. Cancer-derived missense mutations in BAP1

Mutation ID in BAP1	# Instances	Corresponding residue in Calypso	Domain ID	References
G4C	1	G45	UCH	TCGA[a], 2014; TCGA, unpublished
L8R	1	L49	UCH	DFCI[b], 2015
S10N/T	3	S51	UCH	TCGA, 2013; DFCI, 2016; TCGA, unpublished
P12T/S	2	P53	UCH	JHU[c], 2014; MSK[d], 2017
G13D/V	2	G54	UCH	Novartis/Broad, 2012; MSKCC[d], 2017
L14F	2	L55	UCH	MSKCC, 2017
F15V	1	F56	UCH	DFCI/MSKCC, 2014
L17P	1	L58	UCH	TCGA, 2013; TCGA, unpublished
V19M	1	L60	UCH	Novartis/Broad, 2012
E20D	1	K61	UCH	MSKCC, 2017
V24G/D	2	C65	UCH	BGI[e], 2012; METABRIC[f], 2012; METABRIC, 2016
E30K	1	E71	UCH	TCGA, unpublished
E31K/V/A/G/D	8	E72	UCH	Novartis/Broad, 2012; TCGA, 2013; TCGA, 2014; U Tokyo[g], 2013; MSKCC, 2014; TCGA, unpublished
Y33D/C	2	Y74	UCH	Novartis/Broad, 2012; TCGA, unpublished
D34A/Y	2	D75	UCH	Inserm[h], 2015; MSKCC, 2017
L35F	1	L76	UCH	DFCI, 2015
G41C	1	S82	UCH	MSKCC, 2017
V43A	1	P83	UCH	DFCI, 2016
G45E/R	2	G85	UCH	TCGA, unpublished
I47M	2	I87	UCH	TCGA, 2014; MSKCC, 2017; TCGA, unpublished
L49R/V/P	4	L89	UCH	METABRIC, 2012; TCGA, 2012; TCGA, 2013; TCGA, 2015; METABRIC, 2016; TCGA, unpublished
K51I	1	R91	UCH	U Tokyo, 2013

Mutation ID in BAP1	# Instances	Corresponding residue in Calypso	Domain ID	References
E54K	1	E94	UCH	TCGA, 2013
R56C	3	R96	UCH	METABRIC, 2012; U Tokyo, 2014; METABRIC, 2016; MSKCC, 2017
R57Q	1	R97	UCH	MSKCC, 2017
R59Q/W	3	R99	UCH	MSKCC, 2017; TCGA, unpublished
R60Q	2	R100	UCH	Novartis/Broad, 2012
S63C	1	V103	UCH	MSKCC, 2017
D67G	1	A107	UCH	MSKCC, 2017
D68G	2	E108	UCH	MSKCC, 2017; TCGA, unpublished
V71M	1	V111	UCH	MSKCC, 2017
I72T/S	4	K112	UCH	MSK, 2017; MSKCC, 2017
D73Y	1	D113	UCH	JHU, 2014
D75G	1	E115	UCH	TCGA, 2013; TCGA, unpublished
I76T	1	A116	UCH	TCGA, unpublished
N78S	4	S118	UCH	TCGA, 2013; Singapore, 2014; TCGA, unpublished
N79S	1	S119	UCH	Novartis/Broad, 2012
F81L	1	F121	UCH	MSKCC, 2017
Q85H	1	Q125	UCH	NYU[i], 2015
S90C	1	S130	UCH	MSKCC, 2017
C91G/F/R/Y	6	C131	UCH (active site)	U Tokyo, 2013; NYU, 2015; MSKCC, 2017
T93A	1	T133	UCH	TCGA, 2015; TCGA, unpublished
H94D/R	2	H134	UCH	MSKCC, 2017
A95D/T	2	A135	UCH	Novartis/Broad, 2012; Robinson *et al*., 2015
V99M	3	V139	UCH	TCGA, 2014; MSKCC, 2017; TCGA, unpublished
N102I/D/S	3	N142	UCH	METABRIC, 2012; UCLA[j], 2014; METABRIC, 2016; MSKCC, 2017
S105I	1	E145	UCH	METABRIC, 2012; METABRIC, 2016

Mutation ID in BAP1	# Instances	Corresponding residue in Calypso	Domain ID	References
G109V	2	G151	UCH	TCGA, 2013; MSK, 2017; MSKCC, 2017; TCGA, unpublished
R114C	1	R156	UCH	MSKCC, 2017
F118V	1	H160	UCH	TCGA, unpublished
K120E	2	K162	UCH	METABRIC, 2012; METABRIC, 2016; MSK, 2017
S123T	1	S165	UCH	Broad, 2013
E125D/K	2	E167	UCH	MSKCC, 2017
G132D	1	G174	UCH	MSKCC, 2017
P135R/L	3	P177	UCH	Broad, 2012; TCGA, 2016; MSK, 2017; MSKCC, 2017
A138T/V	2	A180	UCH	TCGA, 2015; MSKCC, 2017
A140V	2	A182	UCH (crossover-loop)	TCGA, unpublished Broad, 2012; TCGA, 2016
H141N/Y	3	H183	UCH (crossover-loop)	MSKCC, 2017
S143N	1	S185	UCH (crossover-loop)	TCGA, unpublished
H144D	1	H186	UCH (crossover-loop)	MSK, 2017; MSKCC, 2017
A145G	1	A187	UCH (crossover-loop)	TCGA, 2013; TCGA, unpublished
R146K	2	M188	UCH (crossover-loop)	MSKCC, 2017
P149Q	1	A191	UCH (crossover-loop)	MSKCC, 2016
R150H	1	R192	UCH (crossover-loop)	DFCI, 2016
G158D	1	G201	UCH (crossover-loop)	MSK, 2018
R163W/Q	2	F207	UCH (crossover-loop)	TCGA, 2014; DFCI, 2016; TCGA, 2017; TCGA, unpublished
H169Y/Q	5	H213	UCH (active site and crossover-loop)	BGI, 2013; JHU, 2013; Singapore, 2014; MSKCC, 2017; TCGA, unpublished

Mutation ID in BAP1	# Instances	Corresponding residue in Calypso	Domain ID	References
F170V/C/L	5	F214	UCH (crossover-loop)	Broad/Cornell, 2012; TCGA, 2013; MSKCC, 2017; TCGA, unpublished
V171D	1	V215	UCH	BGI, 2012
S172N/I	3	S216	UCH	MSKCC, 2017
Y173C/S	6	F217	UCH	MSK, 2017; MSKCC, 2017; TCGA, unpublished
P175S/H	3	P219	UCH	Novartis/Broad, 2012; TCGA, 2014; MSKCC, 2017; TCGA, unpublished
G178V	1	G222	UCH	Novartis/Broad, 2012
R179W	3	Q223	UCH	METABRIC, 2012; TCGA, 2014; METABRIC, 2016; QCMG[k], 2016; TCGA, 2016; TCGA, unpublished
E182D	1	E226	UCH	MSKCC, 2017
D184A/H	2	D228	UCH (active site)	MSKCC, 2014; MSKCC, 2017
G185R	10	G229	UCH	IRC[l], 2014; MSKCC, 2017; TCGA, unpublished
L186Q	1	L230	UCH	MSKCC, 2017
Y189F	1	Y233	UCH	MSKCC, 2017
P190H	2	P234	UCH	TCGA, 2014; MSKCC, 2017; TCGA, unpublished
G194R	2	G238	UCH	MSK, 2016; TCGA, unpublished
W196G	1	W240	UCH	TCGA, unpublished
E200G	1	E244	UCH	TCGA, 2012
W202L	1	W246	UCH	TCGA, unpublished
T203A	1	T247	UCH	METABRIC, 2012; METABRIC, 2016
R208Q/W/G	4	R252	UCH	Novartis/Broad, 2012; MSKCC, 2017
I210T	1	M254	UCH	MSKCC, 2017
M211V	1	A255	UCH	TCGA, 2013; TCGA, unpublished
R213H	1	R257	UCH	MSKCC, 2017
I214M	1	L258	UCH	TCGA, unpublished
A217T	1	A261	UCH	MSKCC, 2017
E221K	2	-	UCH	DFCI/MSKCC, 2014 MSKCC, 2017

Mutation ID in BAP1	# Instances	Corresponding residue in Calypso	Domain ID	References
P222T	1	-	UCH	METABRIC, 2012; METABRIC, 2016
Y223F	1	-	UCH	MSK, 2017; MSKCC, 2017
D225N/H/Y	6	D266	UCH	Sanger, 2012; MSK, 2013; TCGA, 2014; MSKCC, 2017; TCGA, unpublished
R227H/C/P/L	13	R268	UCH	TCGA, 2011; TCGA, 2012; MSK, 2013; TCGA, 2016; MSK, 2017; MSKCC, 2017; TCGA, unpublished
N229H	1	N270	UCH	TCGA, 2013; TCGA, unpublished
L230Q	1	L271	UCH	U Tokyo, 2013
M231T/I/K	3	M272	UCH	BGI, 2013; MSKCC, 2017
R237C/H	2	R278	ULD	Broad, 2013; TCGA, 2015; DFCI, 2016
E242G/K	2	T283	ULD	TCGA, 2013; MSKCC, 2017
L245P	1	L286	ULD	TCGA, 2014; TCGA, unpublished
R252P	1	Q293	ULD	METABRIC, 2012; METABRIC, 2016
I263M	1	L303	BAP1/Calypso insertion	Novartis/Broad, 2012
R264T	1	-	BAP1/Calypso insertion	METABRIC, 2012; METABRIC, 2016
T266P	1	K305	BAP1/Calypso insertion	TCGA, unpublished
I271T	2	-	BAP1/Calypso insertion	MSK, 2016; TCGA, unpublished
N290S	1	Q318	BAP1/Calypso insertion	MSKCC, 2017
S292F	1	-	BAP1/Calypso insertion	MSK, 2017
P293L/S	4	P320	BAP1/Calypso insertion	METABRIC, 2012; METABRIC, 2016; MSK, 2017; MSKCC, 2017
N299S	1	-	BAP1/Calypso insertion	TCGA, 2012; TCGA, unpublished

Mutation ID in BAP1	# Instances	Corresponding residue in Calypso	Domain ID	References
A303P	1	-	BAP1/Calypso insertion	MSKCC, 2013
G307D	1	-	BAP1/Calypso insertion	Novartis/Broad, 2012
D311Y	1	-	BAP1/Calypso insertion	TCGA, unpublished
G312C	1	-	BAP1/Calypso insertion	Michigan, 2012
A316V	1	-	BAP1/Calypso insertion	DFCI, 2016
A321V/T	3	-	BAP1/Calypso insertion	METABRIC, 2012; METABRIC, 2016; MSKCC, 2017
A323S	1	-	BAP1/Calypso insertion	TCGA, unpublished
S325F	2	-	BAP1/Calypso insertion	MSK, 2017; MSKCC, 2017
P328S	1	-	BAP1/Calypso insertion	MSKCC, 2017
K331E	1	-	BAP1/Calypso insertion	METABRIC, 2012; METABRIC, 2016
P332L	1	-	BAP1/Calypso insertion	MSKCC, 2014
P338S	1	-	BAP1/Calypso insertion	TCGA, 2014; TCGA, unpublished
G340S	1	-	BAP1/Calypso insertion	TCGA, 2016
N344S	1	-	BAP1/Calypso insertion	Broad, 2013
G345V	1	-	BAP1/Calypso insertion	MSKCC, 2017
V354I/G	3	T325	BAP1/Calypso insertion	METABRIC, 2012; METABRIC, 2016; MSKCC, 2017
L357M	1	E328	BAP1/Calypso insertion	TCGA, 2013; TCGA, unpublished
L361V	2	F332	BAP1/Calypso insertion	Broad, 2012; TCGA, 2016
P370H	1	-	BAP1/Calypso insertion	TCGA, unpublished
Q372E	1	-	BAP1/Calypso insertion	METABRIC, 2012; METABRIC, 2016
E374Q	1	-	BAP1/Calypso insertion	Roger, 2016; UCLA, 2016
A379T	1	-	BAP1/Calypso insertion	MSKCC, 2017
R383H	1	-	BAP1/Calypso insertion	Novartis/Broad, 2012

Mutation ID in BAP1	# Instances	Corresponding residue in Calypso	Domain ID	References
R385Q	1	-	BAP1/Calypso insertion	JHU, 2014
E398D	1	-	BAP1/Calypso insertion	MSKCC, 2017
D399N	2	-	BAP1/Calypso insertion	TCGA, 2013; TCGA, 2015; TCGA, unpublished
Y401D	1	-	BAP1/Calypso insertion	Novartis/Broad, 2012
D404E	1	-	BAP1/Calypso insertion	METABRIC, 2012; METABRIC, 2016
V409L/A	2	-	BAP1/Calypso insertion	TCGA, 2013 MSKCC, 2017; TCGA, unpublished
N413S	1	-	BAP1/Calypso insertion	MSKCC, 2017
L416F	1	-	BAP1/Calypso insertion	TCGA, unpublished
R417M	2	-	BAP1/Calypso insertion	Novartis/Broad, 2012; TCGA, unpublished
G422R	1	-	BAP1/Calypso insertion	METABRIC, 2012; METABRIC, 2016
L429W	1	-	BAP1/Calypso insertion	Robinson *et al*., 2015
L437P	1	-	BAP1/Calypso insertion	Novartis/Broad, 2012
V439L	3	-	BAP1/Calypso insertion	JHU, 2013; MSKCC, 2017; TCGA, unpublished
N446S	1	-	BAP1/Calypso insertion	METABRIC, 2012; METABRIC, 2016
V447I	2	-	BAP1/Calypso insertion	METABRIC, 2012; Novartis/Broad, 2012; METABRIC, 2016
E450K	1	-	BAP1/Calypso insertion	MSK, 2017; MSKCC, 2017
K457R	1	-	BAP1/Calypso insertion	TCGA, unpublished
P462S	1	-	BAP1/Calypso insertion	MSKCC, 2017
L463V	1	-	BAP1/Calypso insertion	Novartis/Broad, 2012
I465M	1	-	BAP1/Calypso insertion	MSK, 2017; MSKCC, 2017
G472R	1	-	BAP1/Calypso insertion	MSKCC, 2017

Mutation ID in BAP1	# Instances	Corresponding residue in Calypso	Domain ID	References
S482L	1	-	BAP1/Calypso insertion	Novartis/Broad, 2012
P486L	1	-	BAP1/Calypso insertion	MSKCC, 2017
N490S	1	-	BAP1/Calypso insertion	MSKCC, 2017
S492N	1	-	BAP1/Calypso insertion	MSKCC, 2017
T495M	1	-	BAP1/Calypso insertion	TCGA, 2013; TCGA, unpublished
A496T	1	-	BAP1/Calypso insertion	TCGA, 2013; TCGA, unpublished
E498K/Q	4	-	BAP1/Calypso insertion	MSK, 2017; MSKCC, 2017
G500D	2	-	BAP1/Calypso insertion	Novartis/Broad, 2012; DFCI, 2016
A502P	1	-	BAP1/Calypso insertion	TCGA, unpublished
R508C	1	-	BAP1/Calypso insertion	Novartis/Broad, 2012
R512L/C	3	-	BAP1/Calypso insertion	Novartis/Broad, 2012; TCGA, 2012; DFCI, 2016; TCGA, unpublished
P516L	1	-	BAP1/Calypso insertion	Yale, 2012
T517M	4	-	BAP1/Calypso insertion	NCI[m], 2012; Novartis/Broad, 2012; Institut Curie, 2014; MSKCC, 2015
R518L	1	-	BAP1/Calypso insertion	MSKCC, 2017
P519L	1	-	BAP1/Calypso insertion	MSKCC, 2017
P522T	1	-	BAP1/Calypso insertion	Novartis/Broad, 2012
V523I	1	-	BAP1/Calypso insertion	Novartis/Broad, 2012
S525C	1	-	BAP1/Calypso insertion	BCCRC[n], 2012
H526Y	1	-	BAP1/Calypso insertion	MSKCC, 2017
L539Q	1	-	BAP1/Calypso insertion	TCGA, unpublished
R540H	1	-	BAP1/Calypso insertion	CPC GENE[o], 2017

Mutation ID in BAP1	# Instances	Corresponding residue in Calypso	Domain ID	References
V541G	1	-	BAP1/Calypso insertion	TCGA, 2014; TCGA, unpublished
Y546H	1	-	BAP1/Calypso insertion	TCGA, unpublished
R548C	2	-	BAP1/Calypso insertion	TCGA, 2013; TCGA, unpublished
P555T	1	-	BAP1/Calypso insertion	METABRIC, 2012; METABRIC, 2016
E566K	2	-	BAP1/Calypso insertion	METABRIC, 2012; TCGA, 2015; METABRIC, 2016; TCGA, unpublished
A574V	1	-	BAP1/Calypso insertion	MSK, 2017 MSKCC, 2017
G579R	1	-	BAP1/Calypso insertion	MSKCC, 2014
I586L	2	-	BAP1/Calypso insertion	Broad, 2011; TCGA, 2015; TCGA, unpublished
P588T	1	-	BAP1/Calypso insertion	Broad, 2013
K601E	1	-	BAP1/Calypso insertion	METABRIC, 2012; METABRIC, 2016
E602V	1	-	BAP1/Calypso insertion	METABRIC, 2012; METABRIC, 2016
V604M	1	-	BAP1/Calypso insertion	TCGA, 2014; TCGA, unpublished
T607M	2	-	BAP1/Calypso insertion	Broad, 2014; DFCI, 2016
R610K	1	-	BAP1/Calypso insertion	MSKCC, 2017
K612M	1	-	BAP1/Calypso insertion	Broad, 2012
T613M	1	-	BAP1/Calypso insertion	MSK, 2017; MSKCC, 2017
G619D	1	-	BAP1/Calypso insertion	TCGA, 2013; TCGA, unpublished
L622M	1	-	BAP1/Calypso insertion	TCGA, 2014; TCGA, 2016; TCGA, unpublished
P629S	1	-	BAP1/Calypso insertion	TCGA, unpublished
E631Q/V	4	-	BAP1/Calypso insertion	TCGA, 2014; MSK, 2017; MSKCC, 2017; TCGA, unpublished

Mutation ID in BAP1	# Instances	Corresponding residue in Calypso	Domain ID	References
L632P	1	-	BAP1/Calypso insertion	TCGA, 2016
V639L	1	L344	ULD	NCI, 2012
A641T	1	T346	ULD	MSKCC, 2017
E642D	1	E347	ULD	MSKCC, 2017
I643T	1	I348	ULD	MSKCC, 2017
A644V	1	A349	ULD	Novartis/Broad, 2012
C649Y	1	H354	ULD	METABRIC, 2012; METABRIC, 2016
E653K	1	E358	ULD	MSKCC, 2017
K656N	1	R361	ULD	TCGA, 2014; TCGA, unpublished
R657W	1	R362	ULD	MSKCC, 2017
K658N	1	H363	ULD	MSKCC, 2014
I662N	1	V367	ULD	TCGA, 2013
D663H	1	D368	ULD	TCGA, 2014; TCGA, unpublished
R667K	1	R372	ULD	MSKCC, 2017
I675F	1	I380	ULD	MSKCC, 2017
F678L	1	F383	ULD	MSKCC, 2017
E685V	1	Q390	ULD	TCGA, unpublished
G686C	1	G391	ULD	TCGA, 2013; TCGA, unpublished
I696T	3	L401	ULD	MSKCC, 2017
R699P/W	3	S404	ULD	METABRIC, 2012; METABRIC, 2016; MSKCC, 2017
R700Q	1	K405	ULD	TCGA, 2013; TCGA, unpublished
R701C	1	K406	ULD	MSKCC, 2017
R717Q/W	3	G458	ULD (C-ter. tail)	METABRIC, 2012; DFCI, 2016; METABRIC, 2016; MSKCC, 2017
R718W	1	R459	ULD (C-ter. tail)	MSKCC, 2017
P723A/S	3	C467	ULD (C-ter. tail)	MSKCC, 2014; MSK, 2017

Mutation ID in BAP1	# Instances	Corresponding residue in Calypso	Domain ID	References
K727M	1	-	ULD (C-ter. tail)	MSKCC, 2017
R728H	1	-	ULD (C-ter. tail)	MSKCC, 2017

[a]TCGA, The Cancer Genome Atlas. [b]DFCI, Dana-Farber Cancer Institute. [c]JHU, Johns Hopkins University. [d]MSK/MSKCC, Memorial Sloan Kettering Cancer Center. [e]BGI, Beijing Genomics Institute. [f]METABRIC, Molecular Taxonomy of Breast Cancer International Consortium. [g]U Tokyo, University of Tokyo. [h]Inserm, National Institute of Health and Medical Research. [i]NYU, New York University. [j]UCLA, University of California, Los Angeles. [k]QCMG, Queensland Centre for Medical Genomics. [l]IRC, Institute of Cancer Research. [m]NCI = National Cancer Institute. [n]BCCRC, BC Cancer Research Centre. [o]CPC GENE, Canadian Prostate Cancer Genome Network.

Supplementary Table 3. Cancer-derived missense mutations in the NEF-motifs of ASXL1 and ASXL2

Mutation ID in ASXL1	# Instances	Corresponding residue in ASX	References
H315R/N/Y	3	R288	TCGA[a], 2012; MSKCC[b], 2017; MSK[b], 2018
Mutation ID in ASXL2	**# Instances**	**Corresponding residue in ASX**	**References**
N329K	1	N283	MSKCC, 2017
E330K/Q	7	E284	METABRIC[c], 2012; TCGA, 2014 METABRIC, 2016; MSKCC, 2017; TCGA, unpublished
F331L	2	F285	TCGA, 2012; TCGA, 2013; TCGA, unpublished
S334L	1	R288	TCGA, 2015; TCGA, unpublished

[a]TCGA, The Cancer Genome Atlas. [b]MSK/MSKCC, Memorial Sloan Kettering Cancer Center. [c]METABRIC, Molecular Taxonomy of Breast Cancer International Consortium.

Supplementary Table 4. Crystallographic interfaces identified by PISA in the Calypso–ASX structure

Interface #	**Chain ID** (Structure 1)	**Domain ID** (Structure 1)	**Symmetry op.** (Structure 2)	**Chain ID** (Structure 2)	**Domain ID** (Structure 2)	**Interface area** (Å)	**ΔG** (kcal/mol)	**ΔG** P-value
1	D	ASX DEU_B	x, y, z	C	Calypso UCH/ULD_B	1420.6	-26.4	0.091
2	B	ASX DEU_A	x, y, z	A	Calypso UCH/ULD_A	1350.1	-23.5	0.111
3	A	Calypso UCH/ULD_A	-x, y, -z	A	Calypso UCH/ULD_A	599.4	-2.6	0.604
4	C	Calypso UCH/ULD_B	-x+1/2, y-1/2, -z+1/2	B	ASX DEU_A	517.6	-2.6	0.701
5	C	Calypso UCH/ULD_B	x, y, z	A	Calypso UCH/ULD_A	399.5	-8.3	0.085

Supplementary Table 5. SAXS data collection and analysis statistics

	Calypso–ASX complex
Data-collection parameters	
Instrument	Australian Synchrotron SAXS/WAXS beamline
Beam geometry	120 μm point source
Wavelength (Å)	1.033
Exposure time	2 sec exposures
Temperature (K)	285
q range ($Å^{-1}$)[a]	0.00644 to 0.250
Protein concentration	60 µL of 8 mg/ml protein *via* inline gel filtration chromatography in 0.5M NaCl, 20mM HEPES pH 7.5, 5% v/v glycerol, 0.2mM TCEP
Structural parameters	
I(0) (cm^{-1}) [from P(r)]	0.02506 ± 0.0001
R_g (Å) [from P(r)]	40.60 ± 0.24
D_{max} (Å)	125
I(0) (cm^{-1}) (from Guinier)	0.02505 ± 0.0002
R_g (Å) (from Guinier)	40.40 ± 0.49
Software employed	
Primary data reduction	Scatterbrain (Australian Synchrotron)
Data processing	PRIMUS, GNOM
Computation of model intensities	CRYSOL

[a]q is the magnitude of the scattering vector, which is related to the scattering angle (2θ) and the wavelength (λ) as follows: $q = (4\pi/\lambda)\sin\theta$

SUPPLEMENTARY REFERENCES

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2. Krissinel, E. & Henrick, K. Inference of macromolecular assemblies from crystalline state. *J Mol Biol* **372**, 774-97 (2007).