

Supporting online material

MATERIALS AND METHODS

Molecular identification of the calypso gene

We previously mapped the *calypso* mutations to the right arm of chromosome 2 (2R) (S5). Complementation tests with a set of chromosomal deficiencies uncovering much of chromosome arm 2R allowed us to locate the *calypso* mutations to a short interval in the 52F-53A region. In particular, *calypso*¹ failed to complement *Df(2R)Jp4*, *Df(2R)Jp5*, *Df(2R)Jp6*, *Df(2R)Jp7*, *Df(2R)Jp8* or *Df(2R)Exel6063* but complemented all other tested deficiencies on chromosome arm 2R. *calypso*² also failed to complement *Df(2R)Jp4* and *Df(2R)Exel6063*. Because *Df(2R)Jp4* complemented mutations in *Khc8* (kindly provided by Pernille Rørth, Heidelberg), the *calypso* mutations were thus mapped to a chromosomal interval extending from the centromere-proximal end of *Df(2R)Exel6063* and the *Khc* locus. Moreover, we found that *calypso*¹ and *calypso*² both complemented *csul*^{RM} (kindly provided by Bernhard Mechler, Heidelberg), a viable but female-sterile P-element insertion and *csul*^{RL}, a small chromosomal deletion that removes *csul*, *Khc*, and *fidipidine*. Sequencing of the CG8445 open reading frame on the *FRT40 FRT42 y⁺ calypso*¹ and *FRT40 FRT42 y⁺ calypso*² chromosomes revealed a C to T transition that was present in both alleles and changed the CAA codon for Gln41 into a TAA termination codon; in contrast, the wild-type CAA codon for Gln41 was present on the isogenic *FRT40 FRT42 y⁺* chromosome on which these mutations had been induced by ethylmethanesulphonate (EMS) (S5). We note that even though *calypso*¹ and *calypso*² contain the same Gln41>stop mutation, the two alleles had been isolated in two independently performed mutagenesis experiments (S5). No other mutations were detected in the CG8445 open reading frame but we note that the *FRT40 FRT42 y⁺*, *FRT40 FRT42 y⁺ calypso*¹ and *FRT40 FRT42 y⁺ calypso*² chromosomes all contain a TCA codon (serine) at residue 17 of the CG8445 open reading frame, whereas the database genomic sequence and the SM6b balancer chromosome contain a GCA codon for alanine at this position.

Molecular analysis of Asx^{27J6}

Sequencing of the *Asx* open reading frame on the *FRT40 FRT42 y⁺ Asx^{27J6}* chromosome (S5) revealed a base change that changes **the XXX amino acid codon for XXX into a XXX termination codon.**

Antibodies

The following previously described antibodies were used in this study: anti-Ubx (S6), anti-Abd-B (S7), anti-H2A (Millipore, 07-146), anti-H2B (Millipore, 07-371), E6C5 (Millipore,

05-678), anti-Ubiquitin P4D1 (Santa Cruz Biotechnology, sc-8017), anti-Flag (Sigma, F7425), anti-HA (Sigma, H3663).

For this study, antibodies against Calypso₁₋₄₇₁ and against Asx₂₁₀₋₃₃₆ were raised in rabbits. In both cases, the epitopes for antibody production were expressed as 6xHis-tagged fusion proteins in *E. coli* and purified under denaturing conditions.

ChIP assays

X-ChIP from *Drosophila* larval imaginal discs and qPCR analysis to determine protein binding at specific chromosomal locations were performed as described (S8) with the following modifications: Washes of immunoprecipitated material on protein A-sepharose beads were performed for 10 min at 4 °C with 1 ml of the following buffers: 5 washes with RIPA buffer (350 mM NaCl (Asx-ChIP)/ 140 mM NaCl (Calypso-ChIP), 10 mM Tris-HCl pH 8.0, 1 mM EDTA, 1 % Triton X-100, 0.1 % SDS, 0.1 % sodium deoxycholate, 1 mM PMSF), followed by one wash with LiCl buffer (250 mM LiCl, 10 mM Tris-HCl pH 8.0, 1 mM EDTA, 0.5 % NP-40, 0.5 % sodium deoxycholate) and 2 washes with TE (10 mM Tris-HCl pH 8.0, 1 mM EDTA); each time, wash solution was removed after centrifugation for 2 min at 3000 g at 4 °C.

Quantitative PCR (qPCR) to determine binding at specific chromosomal locations

qPCR analysis was done as described (S8), using the following primers:

No.	Forward primer (5' to 3')	Reverse primer (5' to 3')	Position
1 (<i>Ubx</i>)	TGGGATTGCGATAGTGTGGTC	CGCAGCCATTATGAAACCTCCT	-37.7 kb
2 (<i>Ubx</i>)	ATTTGGTCCGCGAGTCGAGTGCATT	CCACATGCACATCCTGGATGCCAAT	-32.5 kb
3 (<i>Ubx</i>)	GCAGCATAAAACCGAAAGGA	CGCCAAACATTCAGAGGATAG	-30.9 kb
4 (<i>Ubx</i>)	TAGTCTTATCTGTATCTCGCTCTTA	CAGAACCAAAGTGCCGATAACTC	-29.8 kb
5 (<i>Ubx</i>)	AAGGCGAAAGAGAGCACCAA	CGTTTTAAGTGCGACTGAG	-29.6 kb
6 (<i>Ubx</i>)	GCACGCACTAAACCCATAA	TCCACCTCTCTCCTCTCTC	-29.0 kb
7 (<i>Ubx</i>)	GGTCAAAGGCCATACAATTCCA	ATCTGTGAGAATGCGGCATCTAA	-16.0 kb
8 (<i>Ubx</i>)	ATCGGTAGCTTGTTCGACGA	GGCTACTTGACAGGTGTGAGC	-2.4 kb
9 (<i>Ubx</i>)	TCCAATCCGTTGCCATCGAACGAAT	TTAGGCCGAGTCGAGTGAGTTGAGT	0 kb
10 (<i>Ubx</i>)	AATTGGTTTCCAGGGATCTGC	ATCCAAAGGAGGCAAAGGAAC	+0.8 kb
11 (<i>Ubx</i>)	ATGATATCTCGTCTGGCACTAC	AGACATCCAGCAAACCTGCGATA	+8.0 kb
12 (<i>Ubx</i>)	GCCGTGGAGCAGTTCAAAGTA	TCGTTGGTCTGTCTCTTAATT	+26.8 kb
13 (<i>Ubx</i>)	CCATAAGAAATGCCACTTTGC	CTCTCACTCTCACTGTGAT	+31.5 kb
14 (<i>Ubx</i>)	GTCCTGGCCAAGGCAAATATT	CGAAAGGAGAACGGAGAATGG	+34.4 kb
15 (<i>Ubx</i>)	GGCATCTTCCAGTTTTGAGT	ACCAGCATTCGTCACTATTTCG	+70.6 kb
16 (<i>Ubx</i>)	GCCGAGGGTCAGAGAGTTTA	CTGCATCCGACCACTTACCT	+77.4 kb
17 (<i>Abd-B</i>)	GGAATACCGCACTGTCGTAGG	GCAGCCATCATGGATGTGAA	+72.3 kb
18 (<i>Scr</i>)	GAAGTGCGCCACGTTCAAT	TCCTCTCTCTCGCACTCGTT	+0.2 kb
19 (ctrl.)	TCAAGCCGAACCTCTAAAAAT	AACGCCAACAAACAGAAAATG	-12.5 kb
20 (ctrl.)	CCGAACATGAGAGATGGAAAA	AAAGTGCCGACAATGCAGTTA	-3.1 kb
21 (ctrl.)	CAGTTGATGGGATGAATTTGG	TGCTGTGGTTCTATCCAAAC	+12.4 kb

Tandem affinity purification (TAP) of Calypso complexes

The α -tubulin1-TAP-calypso transgene in the *Drosophila* transformation vector pCaSpeR had the following structure: A 2.6-kb fragment of the α -tubulin1 gene containing promoter and 5' untranslated region sequences (S9), followed by the N-terminal TAP-tag was linked to a calypso cDNA fragment that contained the whole Calypso open reading frame (detailed plasmid map available upon request). Rescue function of the α -tubulin1-TAP-calypso transgene was tested by introducing the 3 independent transgene insertions into a calypso²/Df(2R)Exel6063 mutant background; in all cases we obtained w; calypso²/Df(2R)Exel6063; α -tubulin1-TAP-calypso/+ animals that were wild-type in appearance and fully viable and fertile.

TAP was performed on embryonic nuclear extracts prepared from wild-type or α -tubulin1-TAP-calypso transgenic embryos, following previously described protocols (S10).

Mass spectrometry, capillary LC-MS/MS analysis and protein identification

Silver stained protein bands were excised and trypsin digested as previously described (S11). The samples were loaded and separated on a nano-flow 1D-plus Eksigent (Eksigent, Dublin, CA) HPLC system coupled to a qStar Pulsar i quadrupole time-of-flight MS (Applied Biosystems, Darmstadt, Germany). The peptides were separated by a linear gradient which started at 100% mobile phase A and increased the mobile-phase composition to 50% B (0.5% acetic acid in 98% acetonitrile) over a span of 45 (single protein band) or 120 (complex samples) minutes at a constant flow rate of 200 nl/min. The mass spectrometer was operated in data-dependent positive-ion mode. MS spectra were acquired over m/z range from 350 to 1300 for 1 second and one subsequent MS/MS spectra from 60 to 1800 m/z for 1.5 seconds (S12).

MS/MS data was extracted using the AnalystQS software (Applied Biosystems, Darmstadt, Germany). Peptides were identified by searching the peak-list against the NCBI database using the MASCOT (Matrix Science, London, UK) algorithm. Peptide tolerance was limited to 50 ppm, and peptides with a score below 15 were excluded. Protein identifications were accepted if a single peptide with an individual MASCOT score above 45 or at least two peptides with a summed MASCOT score of more than 40 was identified.

A detailed list of peptide sequences obtained from mass spectrometry analysis of the protein bands shown in Fig. 1 is shown in Table S1. Peptides identified by LC-MS/MS analysis of total purified material are also shown in Table S1.

Constructs for baculovirus production and purification of recombinant proteins

Baculoviruses expressing Flag-Esc and Flag-dRing have been described previously (S13, S14). For this study we generated baculovirus vectors (pFastBac) that encode the following proteins: Flag-Calypso and Flag-Calypso^{C131S}, both containing the entire Calypso₁₋₄₇₁ open reading frame; HA-Asx, containing the entire Asx₁₋₁₆₆₈ open reading frame and HA-Asx₂₋₃₃₇. Detailed plasmid maps for all constructs are available upon request.

Flag-affinity purification of proteins and complexes was carried out as described (S8) with the only modification that protein complexes were not reconstituted by co-infecting Sf9 cells with multiple viruses but were reconstituted by infecting cells with individual viruses and then mixing cell lysates and incubating them for 2-3 hours at 4°C under mild agitation prior to Flag-affinity purification.

Deubiquitination assay on Ub-AMC

25 pmol ubiquitin-amidomethylcoumarin (Ub-AMC) (BostonBiochem, U-550) were coincubated with 10 pmol of protein or protein complex in 1x assay buffer (25 mM Hepes pH 7.5, 0.25 mM EDTA, 0.05% Chaps, 10% DMSO, 10 mM DTT) (S15) at 25°C. Fluorescence spectroscopy measurements were done 1x/ 20 seconds at Ex = 380 nm and Em = 436 nm.

Generation of H2A-ub1-containing mononucleosomes

As template for assembly of mononucleosomes we used PCR to generate a 288 bp long 5'-biotinylated DNA fragment that included 87 bp of pUC19 DNA followed by a unique EcoRV restriction site and a 201 bp long '601' nucleosome positioning sequence (S16) (full fragment sequence available upon request).

Recombinant *Xenopus* or *Drosophila* octamers were prepared as described (S17) and mononucleosomes were assembled from the 5'-biotinylated 288 bp DNA fragment by stepwise salt dialysis, as follows. Octamers and DNA were mixed in a ratio of 1.0 µg octamers : 1.0 µg DNA (288 bp) in TE buffer containing 2M NaCl (2M NaCl, 10mM Tris-HCl pH 8.0, 0.1 mM EDTA pH 8.0). The mixture was incubated at room temperature for 30 minutes, then stepwise dialyzed against TE buffer containing 1.2 M NaCl, 1.0 M NaCl, 0.8 M NaCl, and 0.6 M NaCl at 4°C for two hours each. The final dialysis step was carried out overnight against buffer C (50 mM Tris-HCl pH 7.5, 100 mM NaCl, 10 mM MgCl₂, 1 µM ZnCl₂, 1 mM DTT) (S18). Nucleosomal H2A was ubiquitinated using recombinant human E1, recombinant human E2 (UbcH5c) and recombinant partial human E3 complex (Ring1b₁₋₁₅₉•Bmi1₁₋₁₀₉) as described before (S18) (Recombinant E1, E2, and E3 enzymes were a kind gift of Gretel Buchwald and Titia Sixma, Netherlands Cancer Institute, Amsterdam). For

purification of ubiquitinated nucleosomes, the reaction mixture was incubated with Streptavidin-coupled Dynabeads (Dynabeads M-280 Streptavidin, Invitrogen) for 1 hour at room temperature, followed by removal of the supernatant and washes of the bead-bound nucleosomes with buffer C (4 x 2-minute washes). Nucleosomes were released from beads by cleavage with the restriction enzyme EcoRV for 1 hour at 37°C. The supernatant containing H2Aub1-mononucleosomes was then used for deubiquitination assays.

Generation of H2B-ub1-containing mononucleosomes

Xenopus octamers containing H2B-ub1 were chemically generated as described (S19). Mononucleosome assembly was performed by stepwise salt dialysis as described above and the nucleosomes were directly used for deubiquitination assays.

Deubiquitination assays on H2A-ub1- or H2B-ub1-containing mononucleosomes

Flag-Calypso, Flag-Calypso^{C131S}, Flag-Calypso•HA-Asx₂₋₃₃₇ or Flag-Calypso^{C131S}•HA-Asx₂₋₃₃₇ were mixed on ice with H2Aub1- or H2Bub1-containing mononucleosomes in an enzyme-to-substrate ratio of 1:1 (40 pmol Calypso and 20 pmol H2Aub1 or H2Bub1-containing nucleosome) in 50 ul deubiquitination buffer (150 mM NaCl, 50 mM Tris-HCl pH 7.5, 10 mM MgCl₂, 1 μM ZnCl₂, 1 mM DTT) and reactions were incubated at 25°C, 12.5 ul aliquots (i.e. containing 5 pmol nucleosomes) were removed at indicated time points and reactions were stopped by addition of SDS sample loading buffer and incubation at 95°C for 5 min.

Deubiquitination assays on K48- and K63-linked ubiquitin polymers

Flag-Calypso, Flag-Calypso^{C131S}, Flag-Calypso•HA-Asx₂₋₃₃₇ or Flag-Calypso^{C131S}•HA-Asx₂₋₃₃₇ were mixed on ice with K48- or K63-linked ubiquitin polymers (6-mers, Boston Biochem, UC-317 and UC-217) in a molar enzyme to substrate ratio of roughly 1:1 where the molarity of substrate is calculated according to the number of cleavable bonds within the ubiquitin polymers. The reaction was carried out for 40 min at 25°C in 50 ul deubiquitination buffer (150 mM NaCl, 50 mM Tris-HCl pH 7.5, 10 mM MgCl₂, 1 mM ZnCl₂, 1 mM DTT) and was stopped by addition of SDS sample loading buffer and incubation at 95°C for 5 min.

Assessment of antibody E6C5 specificity for detection of H2A-ub1

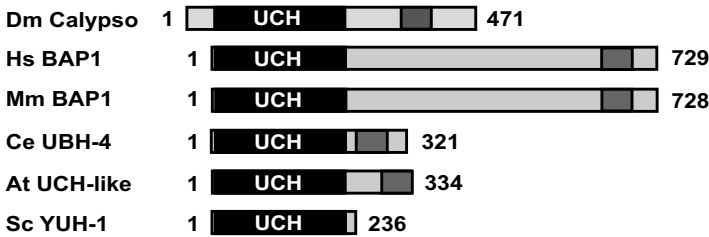
For the western blot analyses shown in Fig. 3E, H2A-ub1-containing *Drosophila* mononucleosomes were generated as described above. K48-linked ubiquitin pentamers (Boston Biochem, UC-216) and K63-linked ubiquitin pentamers (Boston Biochem, UC-316) were used as control but were both also efficiently recognized by the E6C5 antibody.

Immunostaining of Drosophila embryos and imaginal discs

Staining of *Drosophila* embryos and larval imaginal discs was performed following standard protocols. Induction of homozygous mutant cell clones in imaginal discs of *Drosophila* larvae and the rescue experiments with *hsp70-calypso* transgenes shown in Fig. 4 were performed as previously described (S20).

Figure S1

A



B

Dm Calypso	45	GWLELESDPGLFLLLLKDFGCHDVQVE-EVYDLQ---KP-----IESP-YGFIFLFRWIEERRARRKIVETTAEIFVKDEE	115
Hs BAP1	4	GWLELESDPGLFLLLVDFGVKGVQVE-EIYDLQ---SK-----CQGFVYGFIFLFWKIEERRSRKRVSTLVDDTSVIDDD	75
Mm BAP1	4	GWLELESDPGLFLLLVDFGVKGVQVE-EIYDLQ---SK-----CQGFVYGFIFLFWKIEERRSRKRVSTLVDDTSVIDDD	75
Ce UBH-4	6	SWCLIESDPGVFTEMLRFGVDGLQVE-ELYSLD-DDKA-----MTRPTYGLIFLFWKWR--QGETTGIP-----S	67
At UCH-like	2	SWLPVESDPGIFTEIIQQMQVKGQVE-ELYSLDFNSLD-----EIRPVYGLILLYKWRPEEKENRVVIT-----E	66
Sc YUH-1	7	AVVPIESNPEVFTNFAHKLGLKNEWAYFDIYSLT---EPELLAFLPRPVKAIVLLFPINEDRKSSTSQQI-----T	74
↓			
Dm Calypso	116	AISSIFFAQVVPNSCATHALLSVLLNCNENNIQL--GDTLSR-LKHTKGMSPENKGLAIGNTPELACAHNSHAMPOARRRL	195
Hs BAP1	76	IVNNMFFAHQLIPNSCATHALLSVLLNC--SSVDL--GPTLSR-MKDFTKGFSPESKGYAIGNAPELAKAHNSHARPEPRHLP	153
Mm BAP1	76	IVNNMFFAHQLIPNSCATHALLSVLLNC--SNVDL--GPTLSR-MKDFTKGFSPESKGYAIGNAPELAKAHNSHARPEPRHLP	153
Ce UBH-4	68	DKQNIFFAHQTIQNACATQALINLLMNV--EDTDVKGILNINLQ-YKEFAIDLDPNTRGHCLNSSEEIRTVHNSFSR-QTLFEL	146
At UCH-like	67	PNPNFFASQIINNACATQAILSVLMNS--SSIDI--GSELSE-LKQFAKEFPPELKLAINNNEAIRAAHNTFARPDSSIM	144
Sc YUH-1	75	SSYDVIWFKQSVKNACGLYAILHLSLSN---NQSLLEPGSDLDNFKLSQSDTSSSKNRFDDVTTDQFVLNVIKENVQTFSTGQS	154
↓ ↓			
Dm Calypso	196	ERTG-AGVSSCRFTGEAFHFVSFVPIGQFLFDGLK-PYPMNHGGWEDSE---DWTDFRRVMAERLGLA	261
Hs BAP1	154	EKQN--GLSAVR-TMEAFHFVSYPITGRFLFDGLK-VYPIDHGPWGEDE---EWTDKARRVIMERIGLA	217
Mm BAP1	154	EKQN--GLSAVR-TMEAFHFVSYPITGRFLFDGLK-VYPIDHGPWGEDE---EWTDKARRVIMERIGLA	217
Ce UBH-4	147	DIKG-----GESEDNYHFVTVYPIGNKVYELDGLR-ELPLEVAEFQKEQ---DWIEAIKPVIQORMQKY	206
At UCH-like	144	EDEELAAAKNLDEDDVYHYISYLPVDGILYELDGLK-EGPISLQCLGEPGEGIEWLRMVQPVVQEQIDRY	214
Sc YUH-1	155	EAPE-----ATADTNLHYITYVEENGGIFELDGRNLSGPLYLGLK--SDPTATDLIE--QELVRVRVASY	214

C

Dm Calypso	345	TEIAINEQHLDENRRHMFKVDASRRRTHNYDKFICTFLSMLAHQGVLGELVQSQHLLPSKKVSGQGAA-NRISKQ - 35 AA - KTPGRRRKGRNKCRRK	471
Hs BAP1	640	AEIANYEACLKEEVEKRRKFKIDDQRRRTHNYDEFICTFISMLAQEGMLANLVEQNISVRRR---QGVSIGRLHQ-----RKPDRRKRSRPYKAKRQ	729
Mm BAP1	639	AEIANYEACLKEEVEKRRKFKIDDQRRRTHNYDEFICTFISMLAQEGMLANLVEQNISVRRR---QGVSIGRLHQ-----RKPDRRKRSRPYKAKRQ	728
Ce UBH-4	243	EQIADLNKAIADEYKMEMYRKENRRRHHNYTPFVIELMKILAKEGKLVGLVDNAYQAAKEKSKLNTDITKLELK-----RKQ	321
At UCH-like	277	IGLETVSQKIVMEEKSKNWKENMRKHHNYVPLFNFELKILADKKLKLIAKHH-----P	334

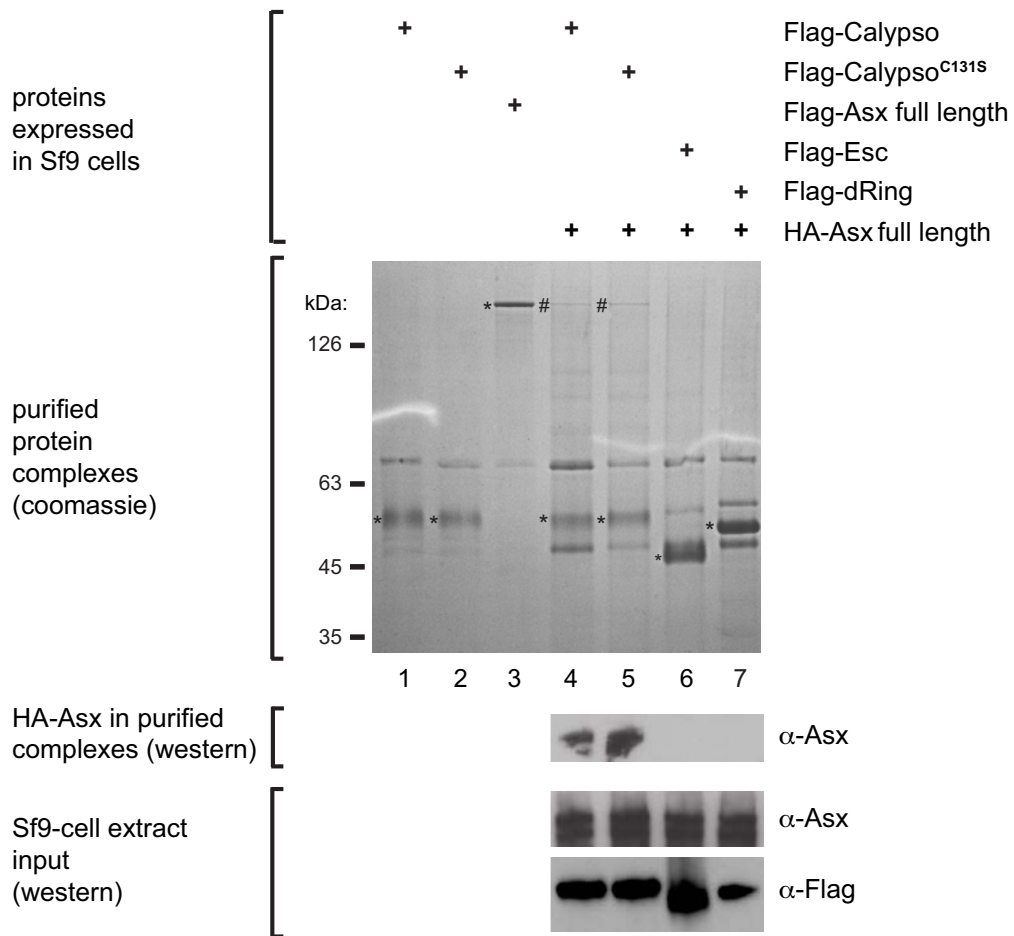
Comparison of Calypso with other UCH family members

(A) The *Drosophila melanogaster* (Dm) Calypso (CG8445) protein and its orthologues in *Homo sapiens* (Hs), *Mus musculus* (Mm), *Caenorhabditis elegans* (Ce), and *Arabidopsis thaliana* (At) all contain a C-terminal domain (C) that is not present in other UCH family members or in YUH-1, the only UCH family member in *Saccharomyces cerevisiae* (Sc).

(B) MAFFT alignment of the UCH domains of the proteins shown in (A). The three conserved residues that are required for catalytic activity (S1,S2) are marked with arrows.

(C) MAFFT alignment of the C-terminal domain marked in (A).

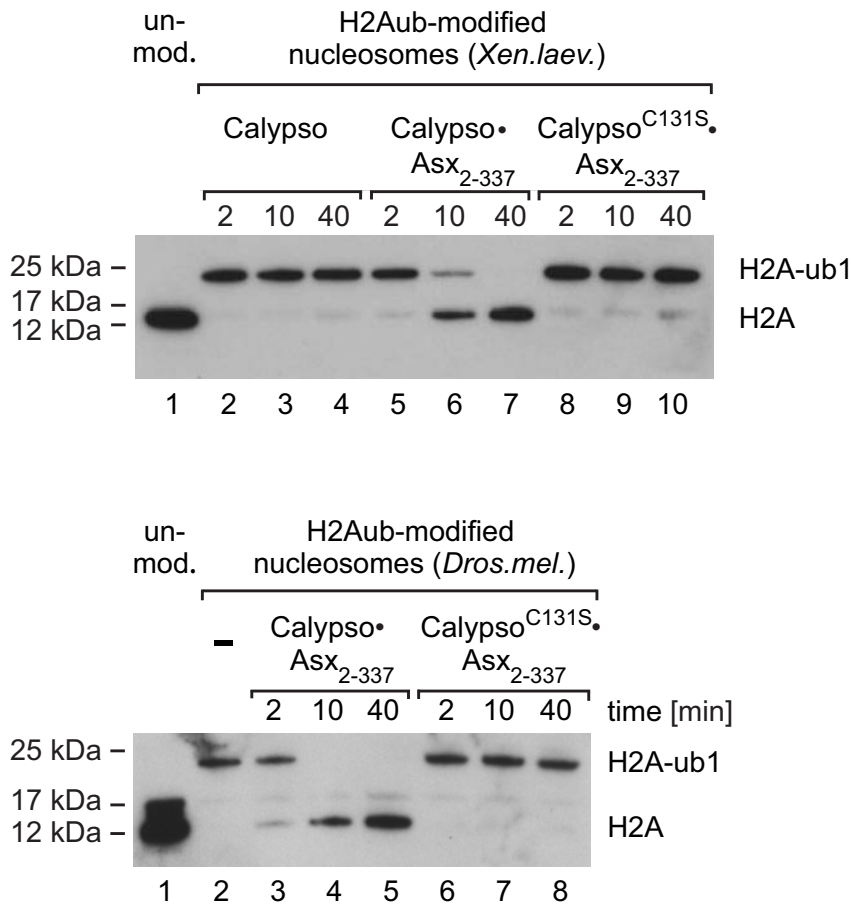
Figure S2



Reconstitution of PR-DUB with full-length Calypso and Asx proteins

Proteins were extracted by Flag-affinity purification from cell lysates containing the indicated Flag-tagged proteins and HA-Asx₁₋₁₆₆₈. Proteins were visualized by coomassie staining or western blot analysis, as indicated. Input material for experiments in lanes 4-7 was probed by western blotting to ensure that comparable amounts of proteins were present in cell lysates. On the coomassie-stained gel, Flag-tagged proteins are marked with an asterisk and HA-Asx₁₋₁₆₆₈ is marked with #.

Figure S3

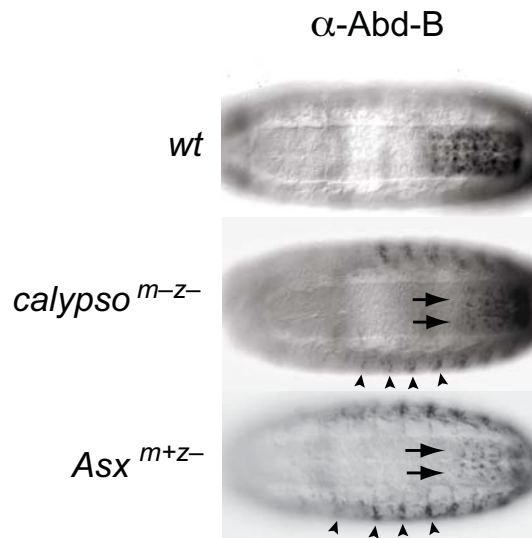


Calypso requires Asx as a cofactor for H2A-deubiquitination

(A) 20 pmol *Xenopus* mononucleosomes containing H2A-ub1 were incubated with 40 pmol Calypso (lanes 2-4), 40 pmol Calypso-Asx₂₋₃₃₇ or 40 pmol Calypso^{C131S}-Asx₂₋₃₃₇ and deubiquitination was monitored at indicated time points by western blot analysis with anti-H2A antibody (5 pmol nucleosome/lane). Nucleosomal H2A-ub1 was rapidly deubiquitinated by Calypso-Asx₂₋₃₃₇ (lanes 5-7) but not by Calypso alone (lanes 2-4) or by Calypso^{C131S}-Asx₂₋₃₃₇ (lanes 8-10).

(B) Calypso-Asx₂₋₃₃₇ also rapidly deubiquitinated H2A-ub1 in nucleosomes that had been reconstituted using *Drosophila* histone octamers. Reactions contained 15 pmol nucleosomes and 30 pmol of enzyme and deubiquitination was monitored at the same time points as in (A) by western blot analysis with anti-H2A antibody (5 pmol nucleosome/lane). Comparison of (A) and (B) shows that deubiquitination of H2A-ub1 occurs with similar kinetics on *Xenopus* and *Drosophila* nucleosomes.

Figure S4



Phenotype of *calypso* and *Asx* mutant *Drosophila* embryos

Ventral views of stage 14-16 embryos stained with antibody against the HOX protein Abd-B. (Top) wildtype (*wt*) embryo; (middle), *calypso*² homozygous embryo derived from females with *calypso*² mutant germ cells and therefore lacking maternal and zygotic *calypso*⁺ product (*calypso*^{*m-z-*}); (bottom) *Asx*^{27J6} homozygous embryo derived from *Asx*^{27J6} heterozygous mothers (*Asx*^{*m+z-*}). *calypso* and *Asx* mutant embryos both show misexpression of Abd-B in the epidermis (arrowheads, compare with *wt*) but in the central nervous system, they also show a partial loss of Abd-B expression (arrows, compare with *wt*), reminiscent of embryos mutant for the trithorax group genes *trithorax* or *ash1* (S3). Misexpression of Abd-B in the epidermis is not as widespread as in embryos mutant for other PcG genes (S3, S4). In embryos, the requirement for PR-DUB in PcG repression may thus be less pronounced than in larvae.

Supplemental table S1

Sequenced peptides identifying protein bands (shown in figure 1). Protein identifications were accepted if the following minimal conditions were met: Presence of one peptide with a mascot score of at least 45 or presence of two peptides with a summed score of 40.

Peptides identified	Peptide seq.	MS/MS
Asx (CG8787)		
AAAAAAAAAAAAAAAAAAAAQAQATSSYPSAISPGSK	+	+
ACLEWR	+	+
DGKIDLETPDSILASTNLR	+	+
EASELEQPSSSASGGSPSEAIR	+	+
ERLSEGEFTPENQLK	+	+
EVLASIPGFSVKPR	+	+
HFEPFWGEK		+
IDLETPDSILASTNLR	+	+
IIVKPIPPEK	+	+
KPMAPSEEEAAVSTAPAPPTR	+	+
KQAPTTIATINLDDDLDELPSTSK	+	+
LSASCLNNEFFAR	+	+
LSEGEFTPENQLK	+	+
LTAAQIEQTK	+	+
LTAAQIEQTKDGG		+
QAPTTIATINLDDDLDELPSTSK		+
SEPKPPATSQQKPLQQATCDNETELK		+
TTVAVAVADK		+
AGTSQASTMREVLASIPGFSVKPR		+
Calypso (CG8445)		
ADEQGEGSNGDSQRPDTPPTLLEPSAFT		+
GLAIGNTPELACAHNSHAMPQAR	+	+
LGIATGEQDIR	+	+
LLKADEQGEGSNGDSQRPDTPPTLLEPSAFT		+
NLDTEIAINEQHLADENDR	+	+
NLDTEIAINEQHLADENDRR	+	+
TNQAIVSGTLQK		+
DLQSLLK	+	+
FNLMAVVPDRR	+	+

List of other proteins in the purified material that are not visible as additional bands in figure 1:

Peptides identified	Peptide seq.	MS/MS
Protein on ecdysone puffs		
AAAPAAVASPAAAATSADASPSPAK		+

VETGVLKPGTVVVFAPANITTEVK	+	
Ribosomal protein S9 HIDFSLK IGVLDESR IIGEYGLR IPSVFSK LFQGNALLR		+ + + + +
Ubiquitin MQIFVK TITLEVEPSDTIENVK T LSDYNIQK T LSDYNIQKESTLHLVLR	+ +	+ + + +
Tubulin alpha-4 chain DVNAAVSAIK ENIAVLER SIFVDLEPTVIDDVR TKEELTAGSSASVGHDT SANDAR	+ + + +	
Nonmuscle myosin-II heavy chain AAKEELQALSK DLLAKEEGAE EK IANLEEQL ENEGKER LNKDIEALER		+ + + +
Ribosomal protein L13.e GFTLEELK GPVLP I KNEQPAVVEFR LILFPINEK TIGIAVDR		+ + + +
Ribosomal protein S6 DIPGLTDTTIPR LITPVVLQR LNVSY PATGCQK LYNLSKEDDVR	+ +	+ + + +
Actin, muscle HQGVMVGMGQKDSYVGDEA QSK SYELPDGQVITIGNER VAPEEHPVLLTEAPLNPK EITALAPSTMK	+ + +	+ + + +
Ribosomal protein L12 CVGGEV GATSSLAPK HPHDVIDELNEGSIEVPAE IGPLGLSPK		+ + +
Protein lava lamp DAELQDANLVSK RAKLIER	+ +	
Ribosomal protein L8 SLDFAER TSGNYATVIAHNQDTK	+ +	
Ribosomal protein L27 SLNYNHLMPTR		+

YTAHDISFEK		+
Nonmuscle myosin heavy chain VEEQLENEGKER		+
Beta-1 tubulin INVYYNEASGGK		+
H1 histone GGSSLLAIK		+
Mevalonate kinase NTKALVSGVSQR	+	
Ribosomal protein L4 QAYAVSELAGHQTSAESWGTGR	+	
Ribosomal protein L10Ab VCILGDQQHCDEAK		+
Ribosomal protein S17 GLQLTQPNTNNFGR		+
Ribosomal protein L15 GLQSIAEER		+
Ribosomal protein DL11 VLEQLTGQQPVFSK		+
Ribosomal protein L23a DYDALDIANK		+
Ribosomal protein S19 IANQIVFK		+
Ribosomal protein L7a NFGIGQNVQPK		+

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