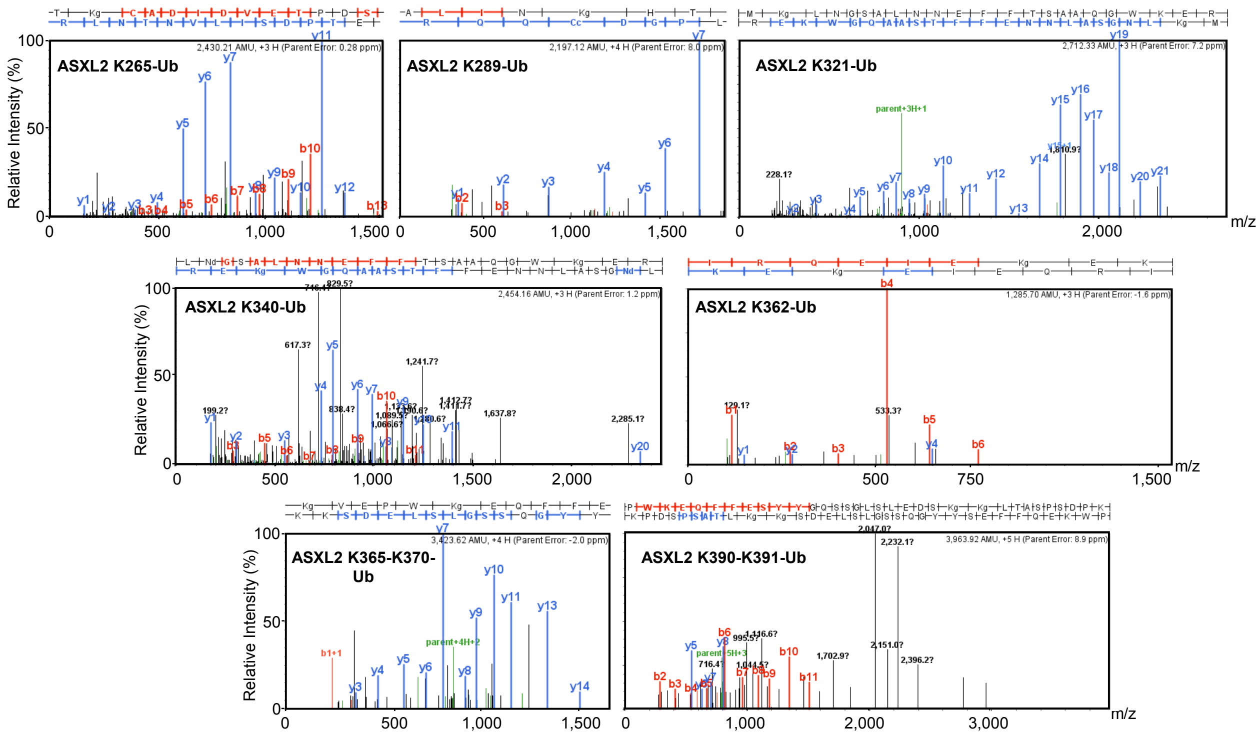
Supplementary Information

Monoubiquitination of ASXLs controls the deubiquitinase activity of the tumor suppressor BAP1

Daou et al.

a



b

Proteins	Ub Sites	This study	PhosphoSitePlus records (https://www.phosphosite.org/)
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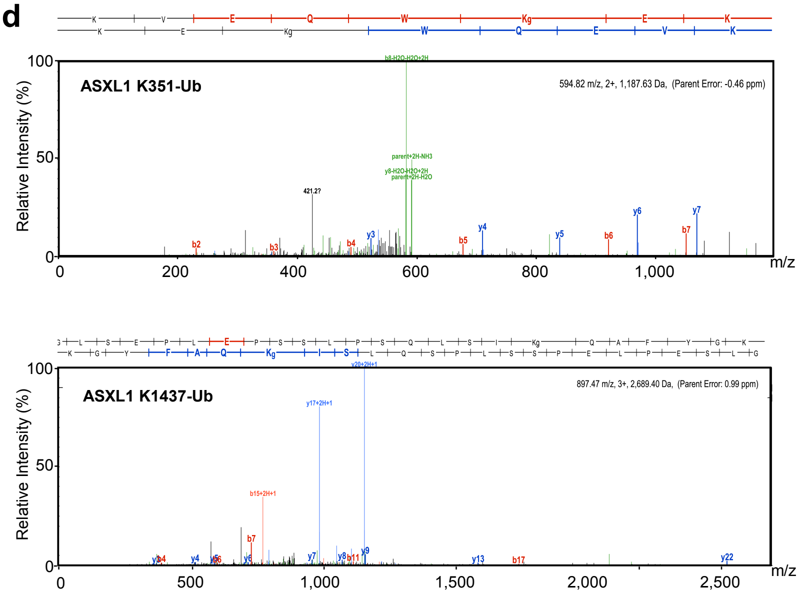
ASXL2	K289	ALINKHTFSVLPGDCQQR	
ASXL2	K321	MKLNGSALNNEFFTSAAQGWKER	
ASXL2	K340	LNGSAMNNEFFTSAAQGWKER	
ASXL2	K362	IRQEIEKEK	
ASXL2	K365 K370	KVEPWKEQFFESYYGQSSGLSLEDSKK	24 104
ASXL2	K390 K391	PWKEQFFESYYGQSSGLSLEDSKKLTASPSDPK	
ASXL2	K1245		3
ASXL2	K1259		2
ASXL1	K351	KKVEQWKEKF	55
ASXL1	K353		1
ASXL1	K362		3
ASXL1	K367		2
ASXL1	K1371		1
ASXL1	K1379		2
ASXL1	K1419		2
ASXL1	K1437	GLSEPLEPSSLPSQLSIKQAFYGKL	3
ASXL1	K1446		1
ASXL3	K350	EKKTEPWKEKFFERF	2
ASXL3	K352		1

d



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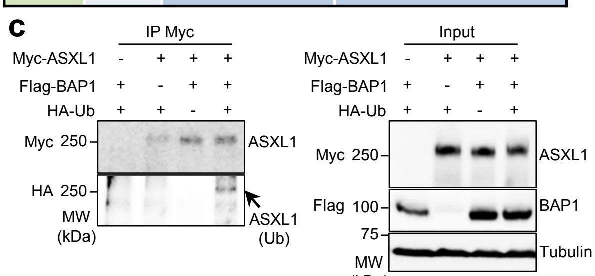

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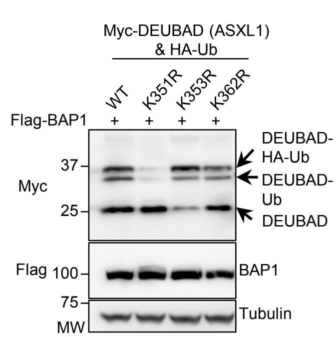

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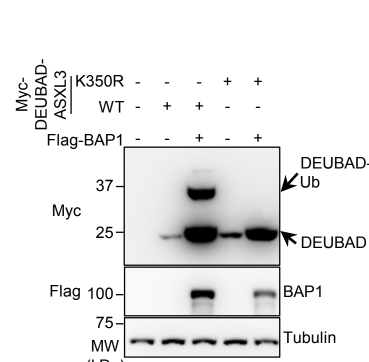
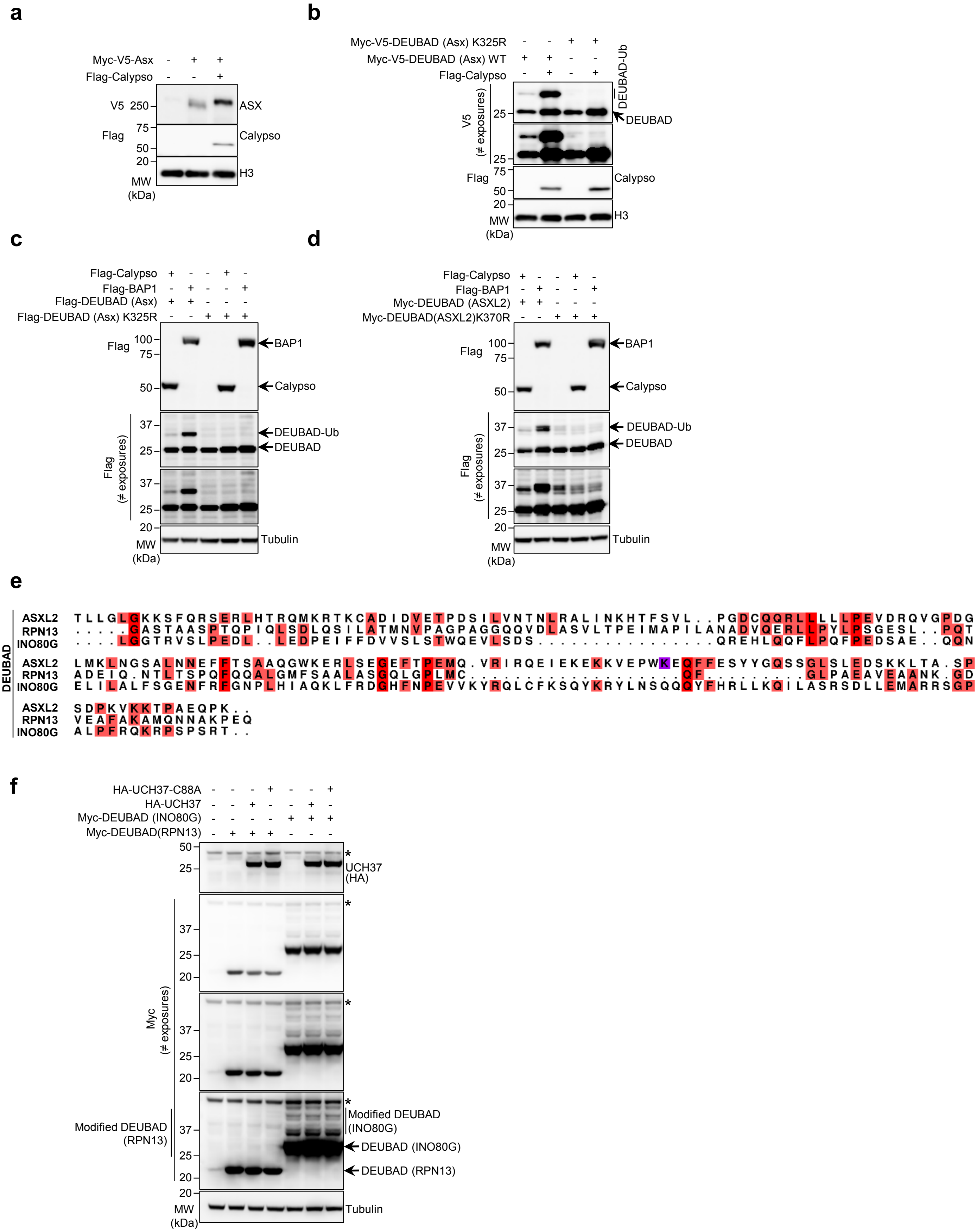


Supplementary Figure 1

Supplementary Figure 1. Characterization of DEUBAD monoubiquitination.

a Identification of ubiquitination sites in ASXL2. HEK293T cells were transfected with Flag-ASXL2 expression vector. Flag purified ASXL2 from total extracts was analyzed by mass spectrometry (MS). MS/MS spectra of ASXL2 ubiquitinated lysine residues are indicated. The modified lysine residues by the C-terminal Gly-Gly fragment of ubiquitin are indicated as Kg. n=1 biological sample. **b** Summary of ASXL1, ASXL2 and ASXL3 ubiquitination sites identified in this study and others. Number of records of the corresponding ubiquitination sites were extracted from PhosphoSitePlus. **c** Myc-ASXL1 was co-transfected in HEK293T cells with and without Flag-BAP1 or HA-Ub and immunoprecipitation with Myc antibody was performed to analyze ASXL1 ubiquitination as indicated. n=3 independent biological replicates. **d** Identification of ubiquitination sites in ASXL1. HEK293T cells were transfected with expression vector for Flag-ASXL1. ASXL1 was purified for MS analysis. MS/MS spectra indicating Ub remnant on K351 and K1437. The modified lysine residues within the peptide were indicated as described above. n=1 biological sample. **e** Lysine 351 is the BAP1-dependent monoubiquitination site of ASXL1. HEK293T cells were transfected as indicated with Myc-DEUBAD constructs for ASXL1 and cell lysates were used for western blotting. n=3 independent biological replicates. **f** ASXL3 is ubiquitinated on its DEUBAD on K350 in BAP1-dependent manner. HEK293T cells were transfected with Myc-DEUBAD (ASXL3) or Myc-DEUBAD (ASXL3) K350R with or without Flag-BAP1 and DEUBAD monoubiquitination was analyzed as indicated. Note that ASXL3 DEUBAD is highly stabilized in the presence of BAP1. n=2 biological sample. Tubulin was used as a loading control for panels **c, e** and **f**.

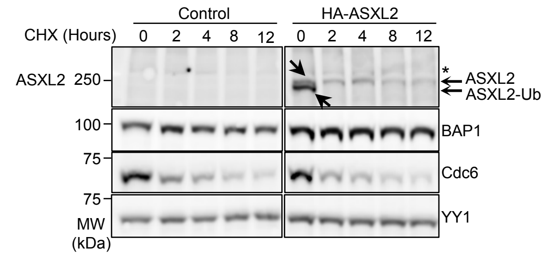


Supplementary Figure 2

Supplementary Figure 2. Monoubiquitination of DEUBAD is conserved in *Drosophila*.

a *Drosophila* Asx is monoubiquitinated in Calypso-dependent-manner. Myc-V5-Asx was transfected with and without Flag-Calypso in S2 cells. Note the Asx band upper shift suggestive of its modification in the presence of Calypso. n=3 biological replicates. **b** *Drosophila* DEUBAD K325 is monoubiquitinated in Calypso-dependent-manner. Myc-V5-DEUBAD (Asx) and its K325R mutant constructs were co-transfected with and without Calypso expression constructs in S2 cells. The cells were then harvested for western blotting analysis. n=3 biological replicates. **c** *Drosophila* DEUBAD is ubiquitinated in human cells**.** HEK293T cells were transfected with either Flag-BAP1 or Flag-Calypso along with Flag-DEUBAD (Asx) or Flag-DEUBAD (Asx) K325R and harvested for immunoblotting analysis as indicated. n=2 biological replicates. **d** Calypso promotes DEUBAD ubiquitination in human cells. HEK293T cells were transfected with either Flag-BAP1 or Flag-Calypso along with Myc-DEUBAD (ASXL2) or Myc-DEUBAD (ASXL2) K370R and harvested for immunoblotting analysis as indicated. n=2 biological replicates. **e** K370 of ASXL2 DEUBAD is not conserved in RPN13 or INO80G DEUBADs. Multiple sequence alignment of the DEUBAD domains of ASXL2, RPN13 and INO80G. The K370 of ASXL2, including its peptide sequence context, is not found in RPN13 or INO80G DEUBADs. The K370 is highlighted in purple. **f** UCH37 does not promotes RPN13 or INO80G DEUBAD monoubiquitination. HEK293T cells were transfected with either Myc-DEUBAD (RPN13) or Myc-DEUBAD (INO80G) with and without Flag-UCH37 or its catalytic dead mutant (C88A). Cells were harvested for immunoblotting analysis as indicated. The star indicates a non-specific band. n=3 biological replicates. Tubulin was used as a loading control for panels **c, d** and **f**. Histone H3 was used as a loading control for panels **a, b**.

a



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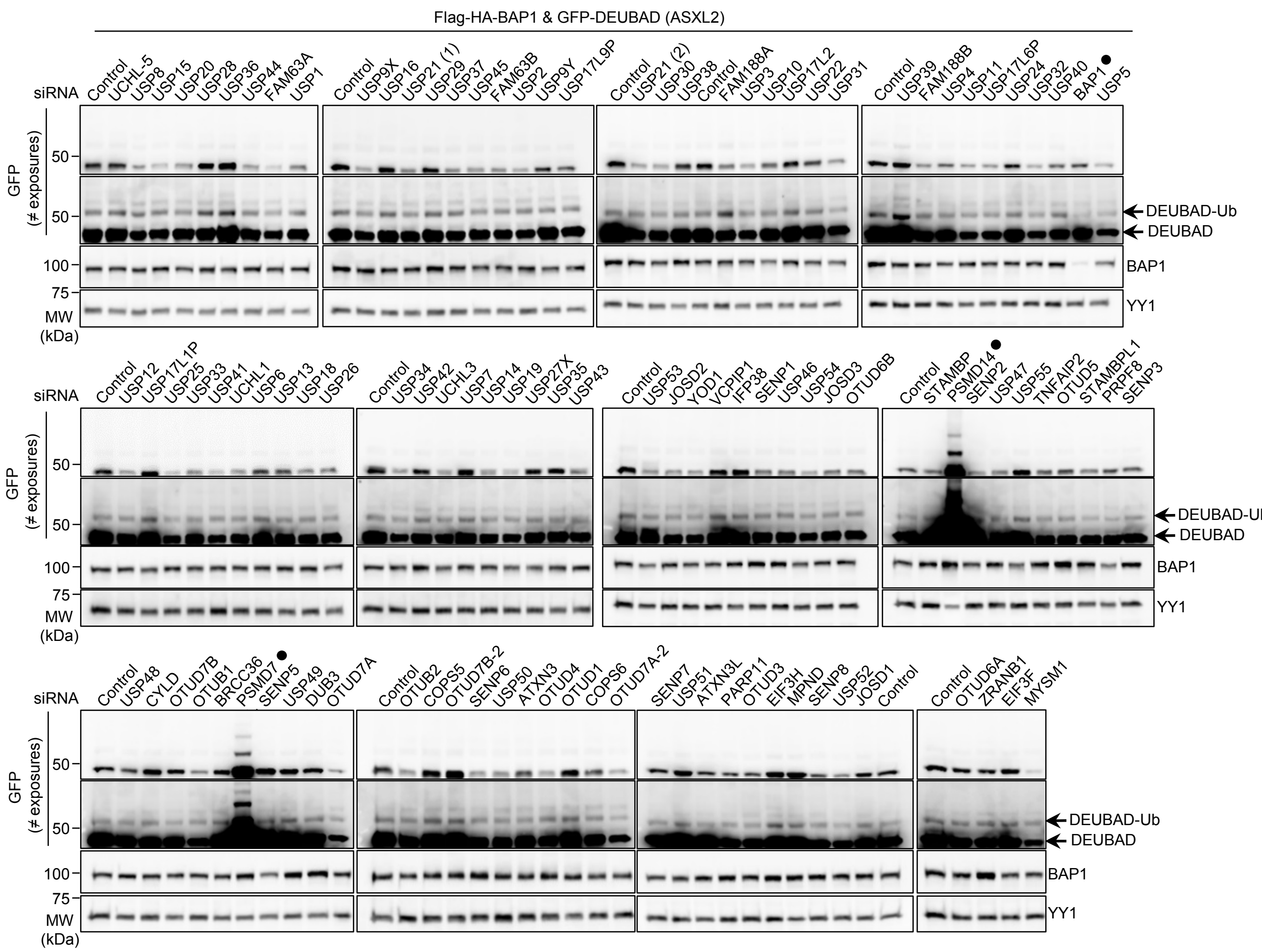
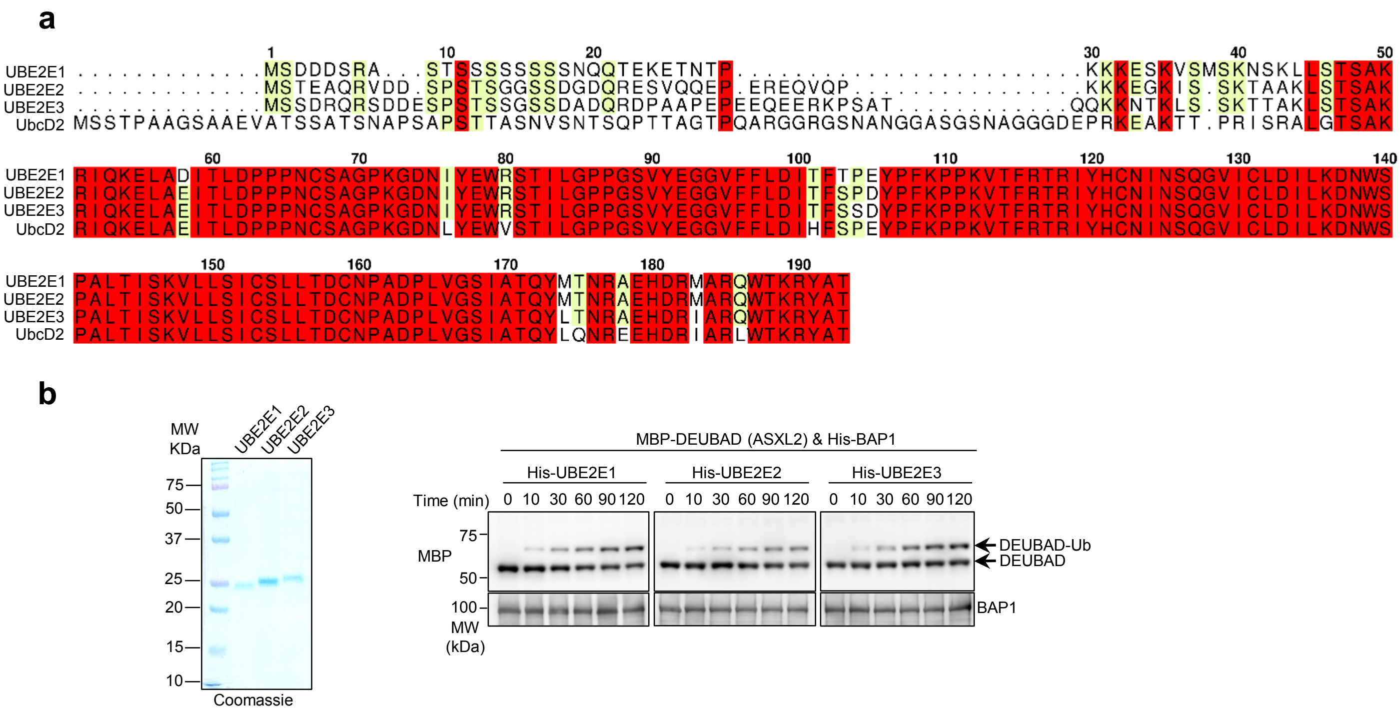


Supplementary Figure 3

Supplementary Figure 3. ASXL2 stability is regulated by the proteasome and deubiquitination.

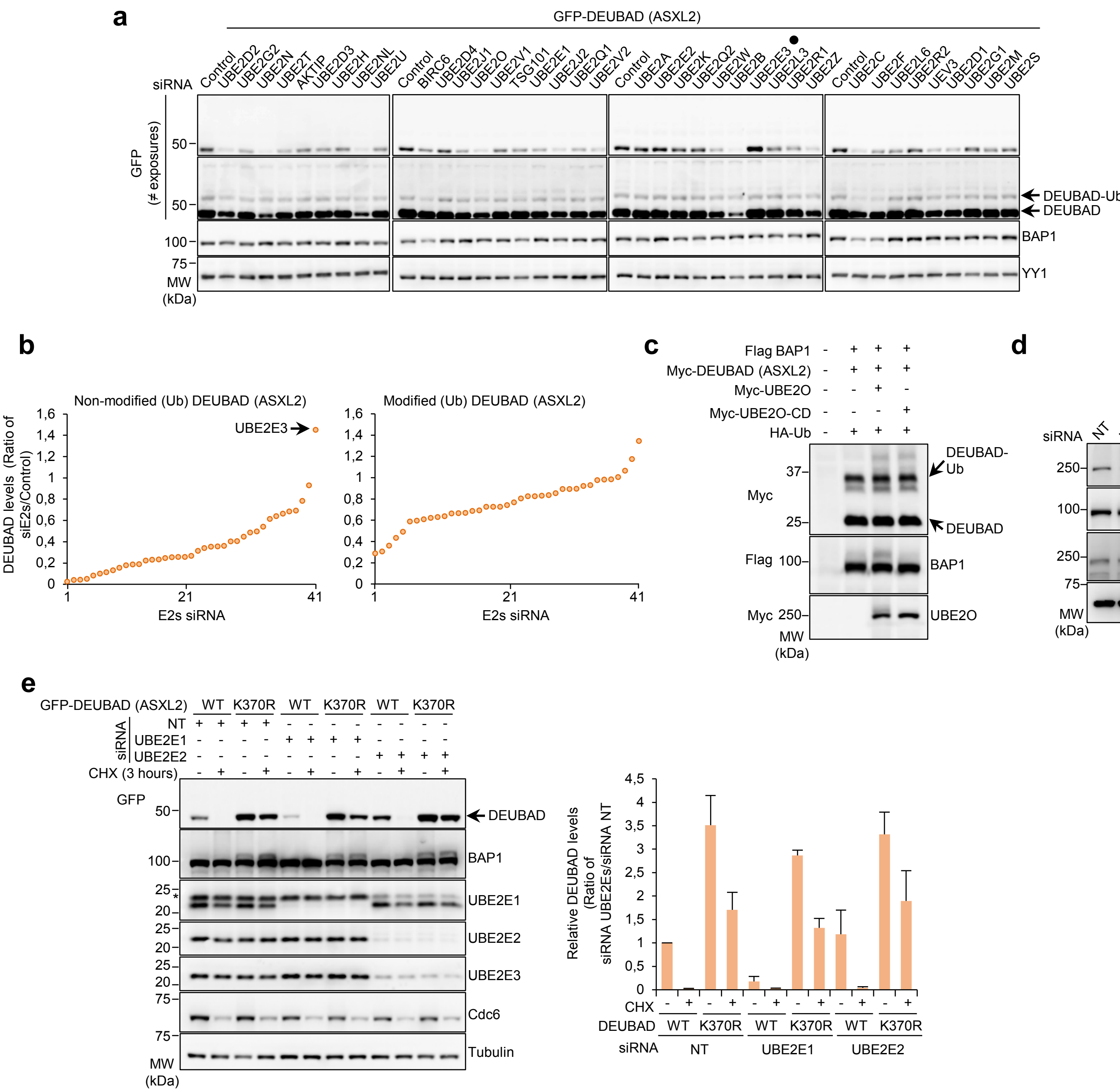
a Monoubiquitinated ASXL2 is very stable comparatively to the unmodified form. U-2 OS cells stably expressing control empty vector or HA-ASXL2 were treated with cycloheximide (CHX) for the indicated times and harvested for immunoblotting analysis. The arrows indicate the non-modified and the monoubiquitinated form of ASXL2. The star indicates a non-specific band. n=2 biological replicates. **b** DUB siRNA screen identifies potential regulators of DEUBAD ubiquitination. U-2 OS cells stably expressing Flag-HA-BAP1 and GFP-DEUBAD (ASXL2) were transfected with an siRNA library for human deubiquitinases assembled from individual siRNAs provided by Sigma (see **Supplementary Table 1**). Cell extracts were used for immunoblotting. The black dots indicate the siRNA for BAP1, PSMD14 and PSMD7. n=1 biological replicate. YY1 was used as a loading control for panels **a** and **b**.



Supplementary Figure 4

Supplementary Figure 4. UBE2E Ub-conjugating enzymes catalyze DEUBAD monoubiquitination.

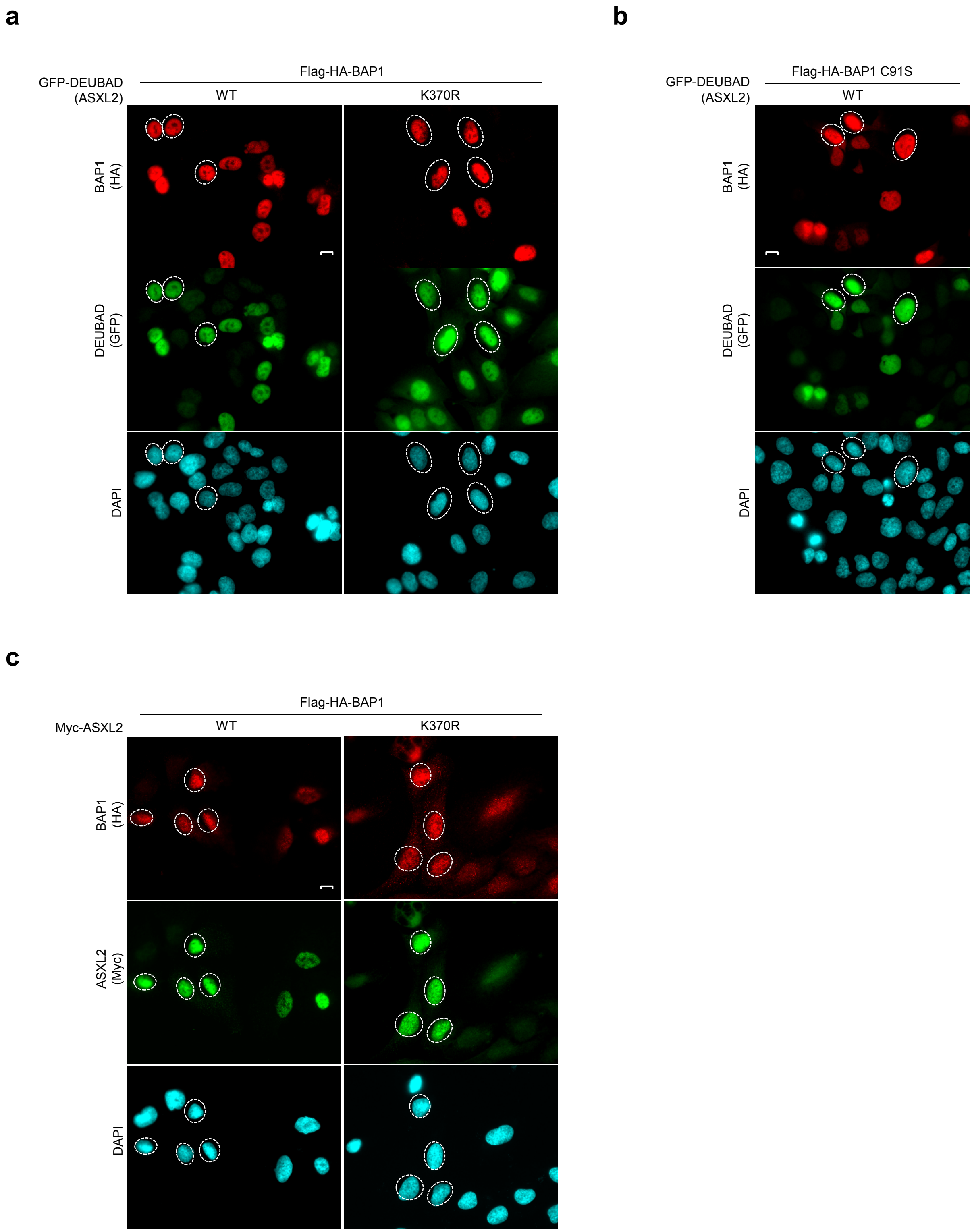
a Alignment of human UBE2E1, UBE2E2 and UBE2E3 sequences with *Drosophila* UbcD2. **b** UBE2E1, UBE2E2 and UBE2E3 catalyze DEUBAD ubiquitination *in vitro***.** Left panel, analysis of UBE2E1, UBE2E2 and UBE2E3 proteins using coomassie blue staining. His-tagged human UBE2E2 and UBE2E3 proteins were purified from bacteria and recombinant His-UBE2E1 was from Boston Biochem. Right panel**,** bacteria purified His-BAP1/MBP-DEUBAD (ASXL2) complex was incubated with recombinant His-tagged UBE2E1 or UBE2E2 or UBE2E3 enzymes for *in vitro* ubiquitination assays for the indicated times. The reactions were stopped at the indicated time points and analyzed by immunoblotting. n=2 biological replicates.



Supplementary Figure 5

Supplementary Figure 5. UBE2E3 regulates DEUBAD stability.

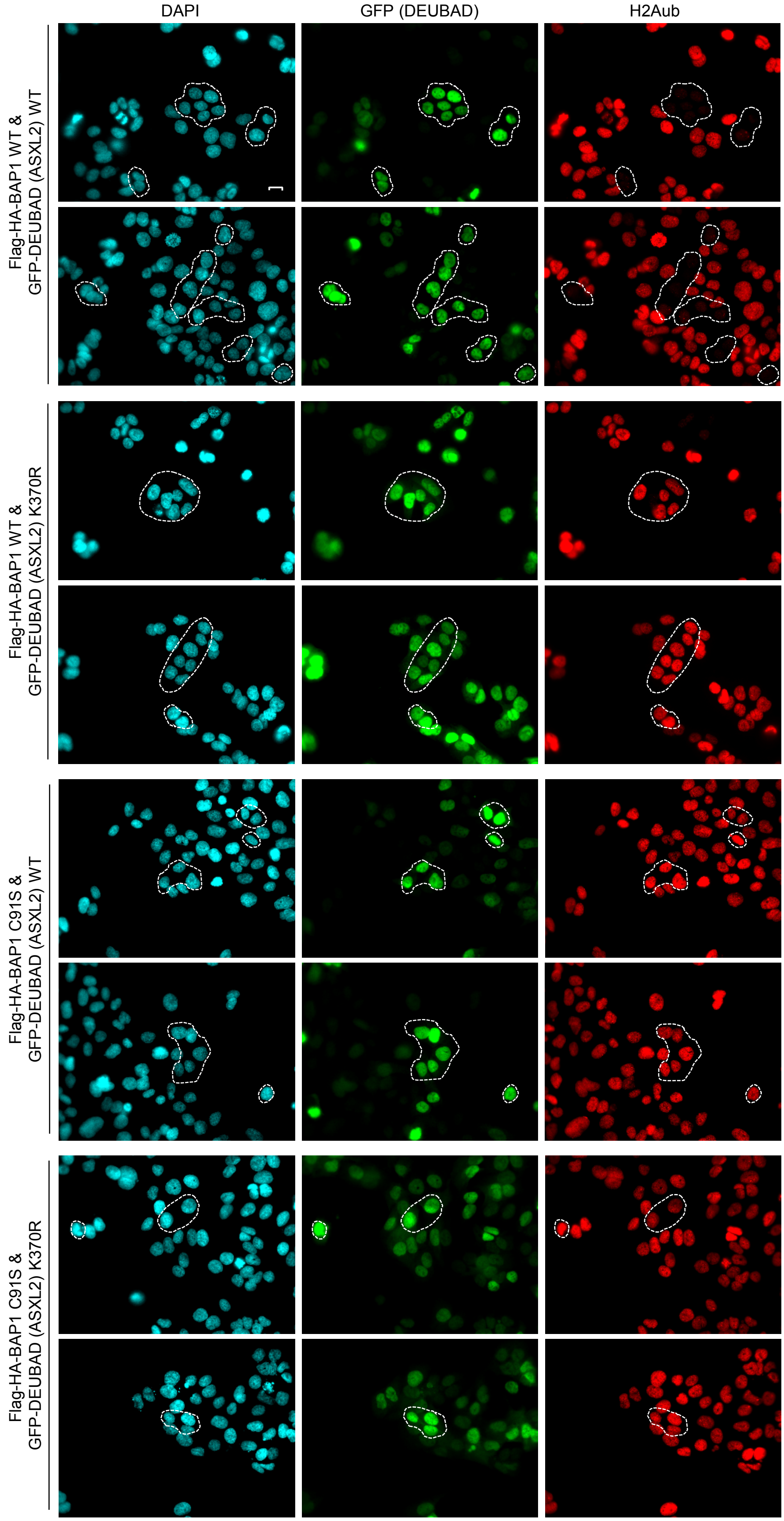
a E2 ubiquitin-conjugating siRNA screen identifies potential regulators of DEUBAD ubiquitination. U-2 OS cells stably expressing GFP-DEUBAD (ASXL2) were transfected with siRNA library for all human E2s and DEUBAD protein levels were analyzed by western blotting. n=1 biological replicate. **b** UBE2E3 is the only E2 whose depletion significantly increases the levels of the non-modified form of DEUBAD (ASXL2). Densitometry analysis of the indicated protein bands in E2s siRNAs versus siRNA control. **c** UBE2O overexpression does not affect DEUBAD ubiquitination. HEK293T cells were transfected with expression vectors for Myc-DEUBAD (ASXL2), Flag-BAP1 and HA-Ub with or without Myc-UBE2O or Myc-UBE2O catalytic dead (CD). Cells were harvested for immunoblotting analysis. n=2 biological replicates. **d** UBE2O depletion does not affect ASXL2 stability. U-2 OS cells were transfected with Non-target (NT) control siRNA as well as UBE2O siRNA and were harvested for immunoblotting analysis as indicated. n=3 biological replicates. **e** Effects of UBE2E1 or UBE2E2 depletion on DEUBAD stability. U-2 OS cells stably expressing either GFP-DEUBAD (ASXL2) or GFP-DEUBAD (ASXL2) K370R were transfected with Non-target siRNA control or siRNA for UBE2E1 or UBE2E2 and then treated with CHX for the indicated times. Cell lysates were used for immunoblotting. Densitometry analysis of DEUBAD protein levels was conducted and presented for each siRNA condition. The star indicates UBE2E2 band detected with UBE2E1 antibody. Error bars represent **s.d** (mean ± SD). n=3 biological replicates. YY1 was used as a loading control for panel **a**. Tubulin was used as a loading control for panels **d** and **e**.



Supplementary Figure 6

Supplementary Figure 6. Expression levels of DEUBAD (ASXL2) and ASXL2 or their corresponding mutants in the presence of BAP1.

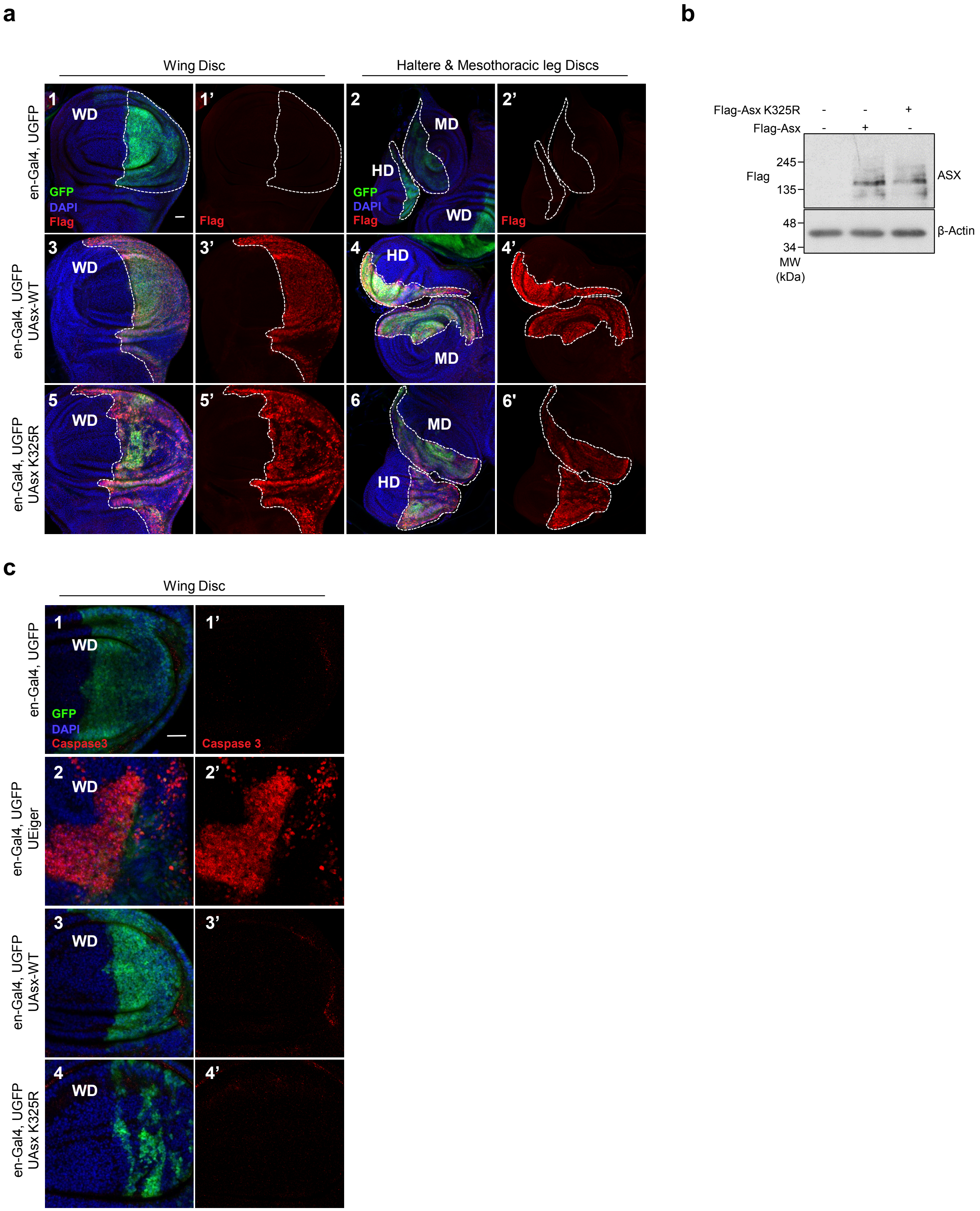
a-c U-2 OS cells stably expressing Flag-HA-BAP1 or Flag-HA-BAP1 C91S were transduced with lentivirus expressing constructs for either GFP-DEUBAD (ASXL2) or GFP-DEUBAD (ASXL2) K370R (**a**, **b**) or Myc-ASXL2 WT or Myc-ASXL2 K370R (**c**) and harvested for immunostaining analysis. The encircled cells co-express the different forms of BAP1 with the corresponding forms of ASXL2 proteins. GFP DEUBAD (ASXL2) (green), Myc-ASXL2 (green), HA-BAP1 (red), DAPI (blue). Note that DEUBAD or ASXL2 and BAP1 are generally co-stabilized when co-expressed together. Scale bars: 15 μm. n=3 biological replicates.



Supplementary Figure 7

Supplementary Figure 7. DEUBAD monoubiquitination promotes BAP1 DUB activity

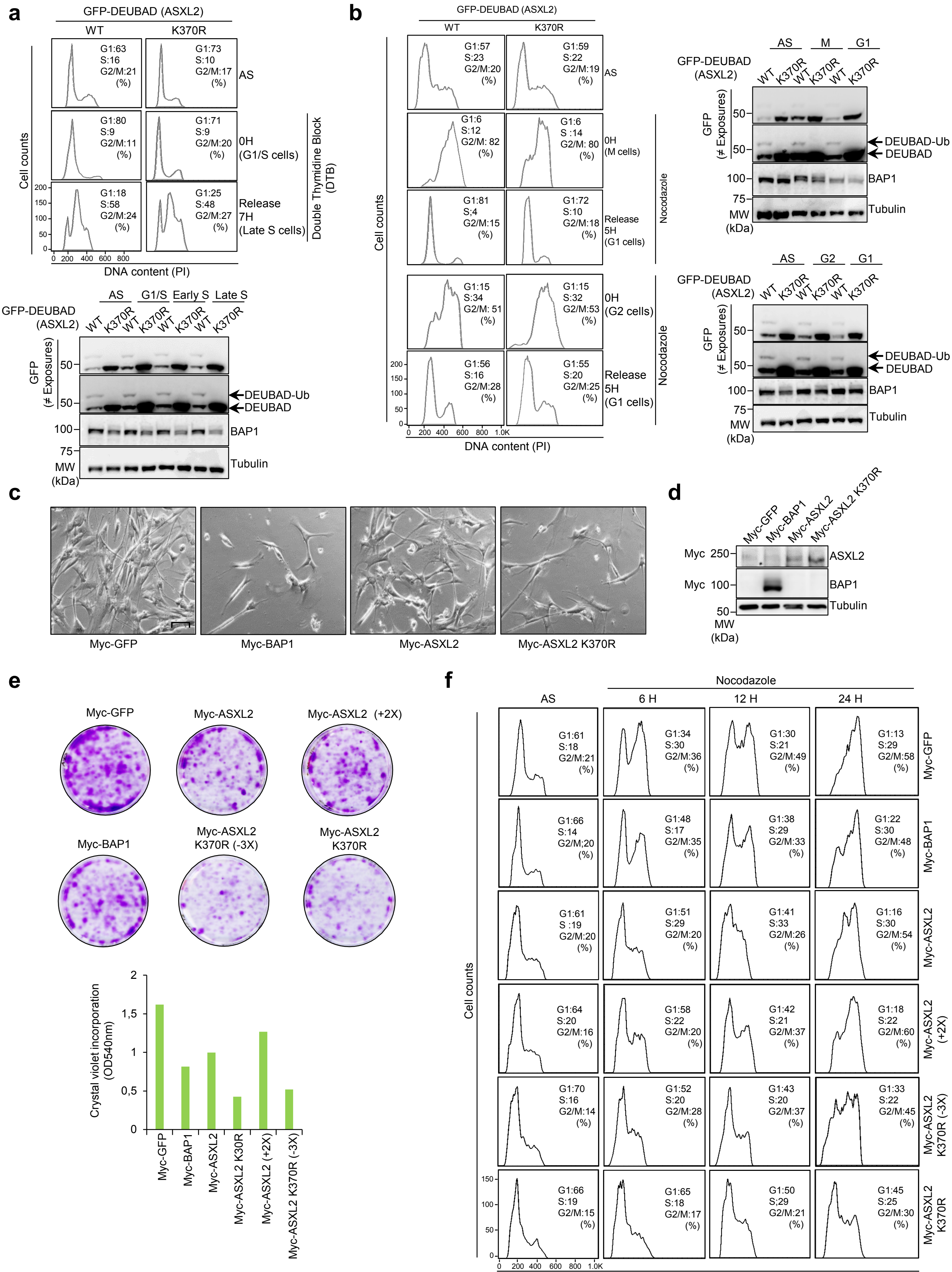
U-2 OS cells stably expressing Flag-HA-BAP1 or Flag-HA-BAP1 C91S were transduced with lentivirus expressing constructs for either GFP-DEUBAD (ASXL2) or GFP-DEUBAD (ASXL2) K370R and harvested for H2Aub levels analysis by immunostaining. GFP DEUBAD (ASXL2) (green), GFP DEUBAD (ASXL2) K370R (green), H2Aub (red), DAPI (blue). Selected cells showing either decrease or no change of H2Aub levels were encircled. Scale bars: 20 μm. n=3 biological replicates.



Supplementary Figure 8

Supplementary Figure 8. *Drosophila* Asx WT or K325R are expressed at similar levels in Drosophila tissues

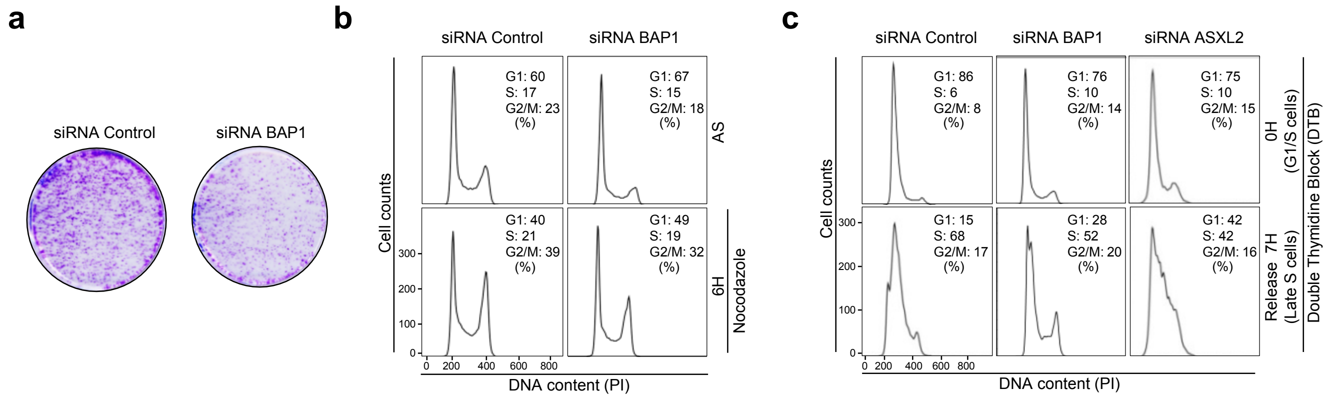
a Immunostaining showing the expression levels of Asx WT or Asx K325R (Flag) during larval disc development. Imaginal discs were stained with anti-Flag (red) for Asx expression and DAPI (blue) for nuclei. GFP staining indicates the expression pattern of *en-Gal4.* The genotypes are indicated*.* WD: Wing imaginal disc, HD: Haltere imaginal disc; MD: Mesothoracic leg imaginal disc. Scale bars: 20 μm. n=3 biological replicates**. b** Immunoblot showing the expression levels of Asx WT or Asx K325R (Flag) in whole larva extracts. Ubiquitous expression was achieved through the use of the ubiquitin-Gal4 driver. Actin was used as a loading control. **c** Overexpression of Asx (WT or K325R) does not induce apoptosis during wing development. Apoptotic cells were stained for cleaved caspase-3. Expression of Eiger was conducted as a positive control for cleaved caspase-3 staining. The genotypes are indicated. GFP and DAPI staining were performed as indicated above. Scale bars: 20 μm. n=2 biological replicates.



Supplementary Figure 9

Supplementary Figure 9. Expression of ASXL2 K370R reduces mammalian cell proliferation

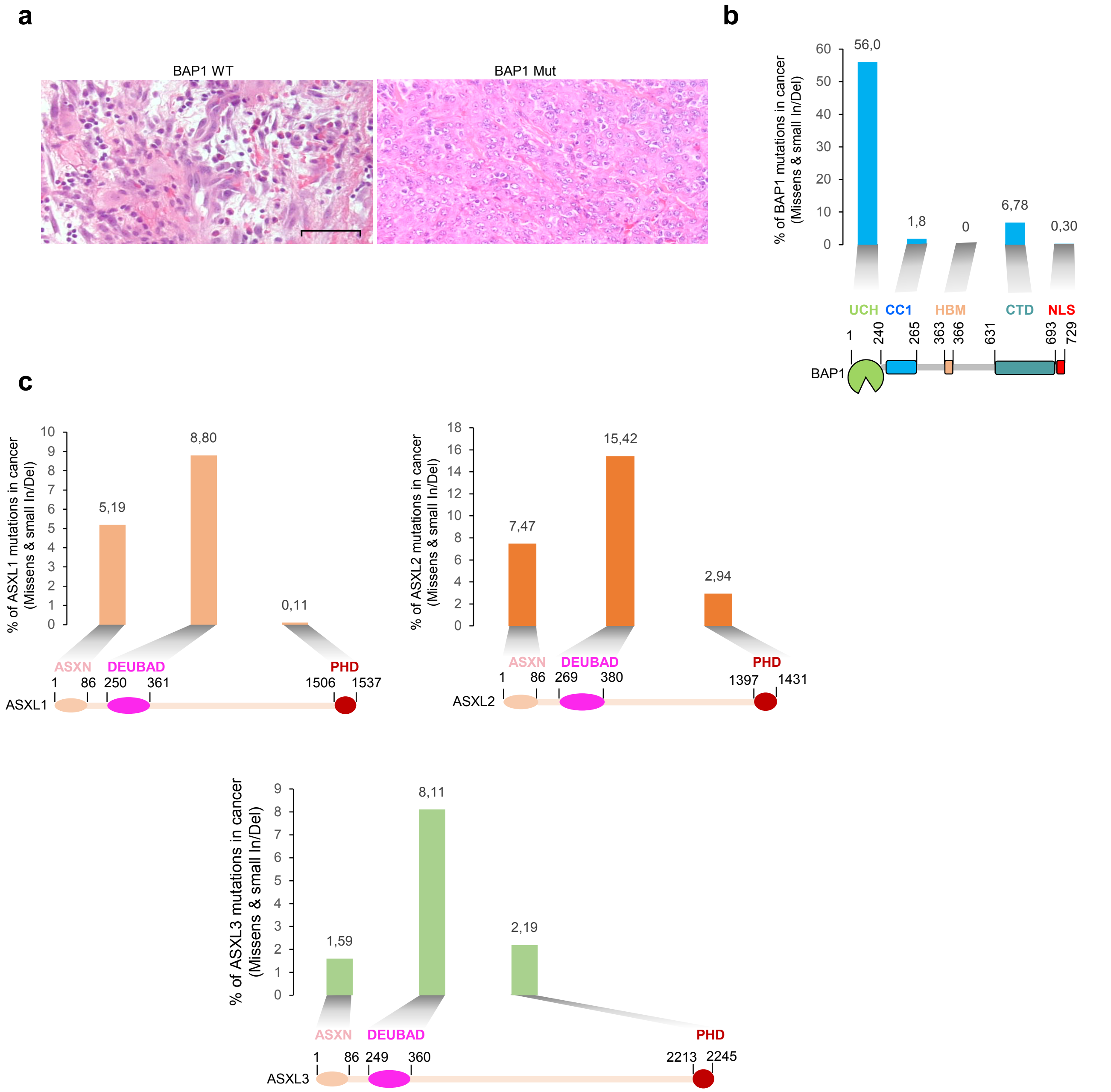
a-b DEUBAD of ASXL2 is constitutively monoubiquitinated during cell cycle progression. U-2 OS cells stably expressing Flag-HA-BAP1 were transduced with either GFP-DEUBAD (ASXL2) or GFP-DEUBAD (ASXL2) K370R and synchronized in different phases of the cell cycle. Cells were arrested in G1/S by double thymidine block then released for 7 hours and cell cycle progression was analyzed by FACS (**a**). Cells were also treated with nocodazole to induce M arrest. Cells were harvested at the indicated times post-release for FACS analysis (**b**). DEUBAD monoubiquitination levels and BAP1 protein levels were evaluated by immunoblotting. n=2 biological replicates. **c-d** Human primary fibroblasts (LF1) were transduced with lentiviral control or BAP1 or ASXL2 or ASXL2 K370R lentiviral expression vectors. Equal numbers of cells were either plated and phase contrast pictures were taken **(c)** or harvested for protein expression analysis **(d)**. n=3 biological replicates. **e-f** 3T3L1 mouse preadipocytes cells were transduced with different amounts of BAP1, ASXL2 WT or K370R lentivirus particles and equal numbers of cells were either plated for crystal violet staining **(e)** or treated with nocodazole and collected for FACS analysis as indicated **(f).** Note that (+2X) refers to transduction of cells with twice the amount of viruses culture media we normally use for Myc-ASXL2, and (-3X) refers to transduction of the cells with three times less the amount of viruses culture media we normally use for ASXL2 K370R. This adjustment was conducted to correct for the expression levels usually higher for ASXL2 K370R. As: Asynchronous cell population. n=2 biological replicates. Tubulin was used as a loading control for panels **a, b** and **d**.



Supplementary Figure 10

Supplementary Figure 10. BAP1 depletion reduces cell proliferation.

a-c U-2 OS cells were transfected with NT siRNA control or siRNA for BAP1. Equal number of cells were plated for colony formation assay (**a**) or treated with nocodazole for FACS analysis (**b**). Equal number of U-2 OS cells transfected with NT siRNA control or siRNA for BAP1 or ASXL2 were subjected to double thymidine block as indicated, and cell cycle progression was analyzed by FACS following release from S-phase block (**c**). n=3 biological replicates.

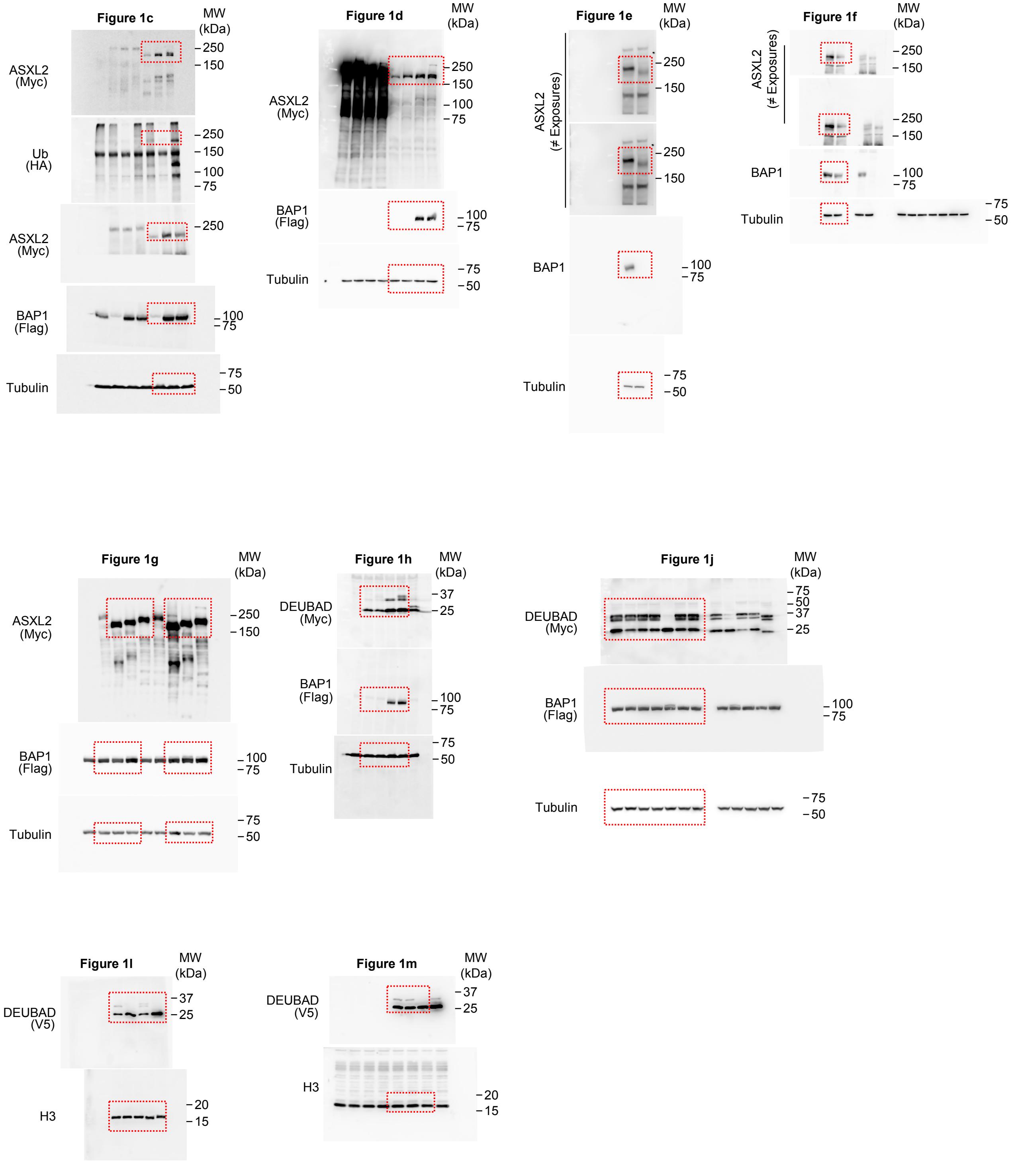


Supplementary Figure 11

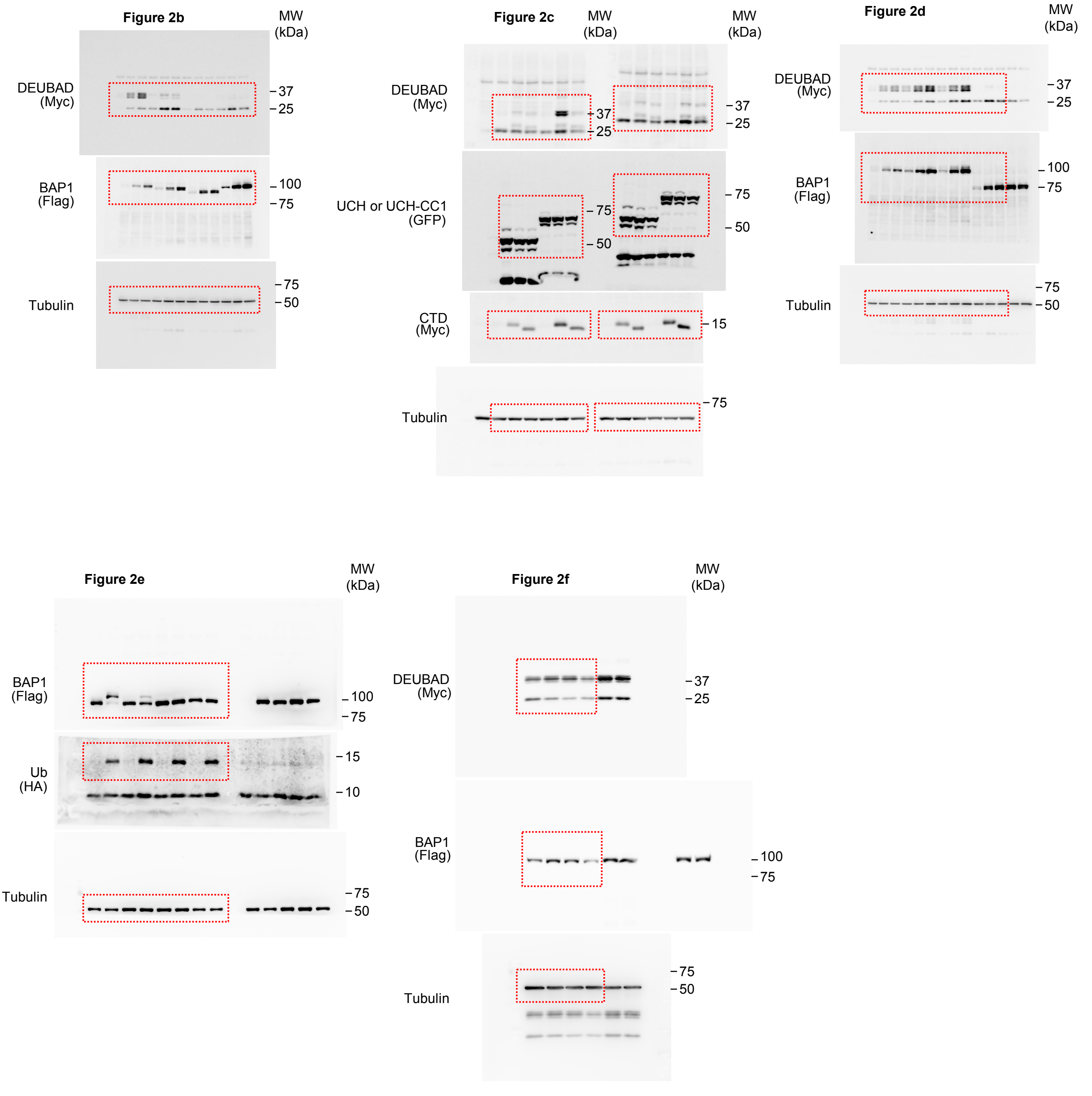
Supplementary Figure 11. Cancer-associated mutations of BAP1 and ASXLs.

a Haemotoxylin and Eosin staining of mesothelioma biopsies corresponding to **Figure 9j**. Images were taken at 100X magnification. Scale bars: 100 µm. **b-c** Schematic representation of BAP1 (**b**) and ASXLs (**c**) showing cancer mutations frequencies among their functional domains. Data integrating multiple cancer types was compiled from C-Bioportal database. Note that only missense mutations and small in-frame insertions/deletions were considered.

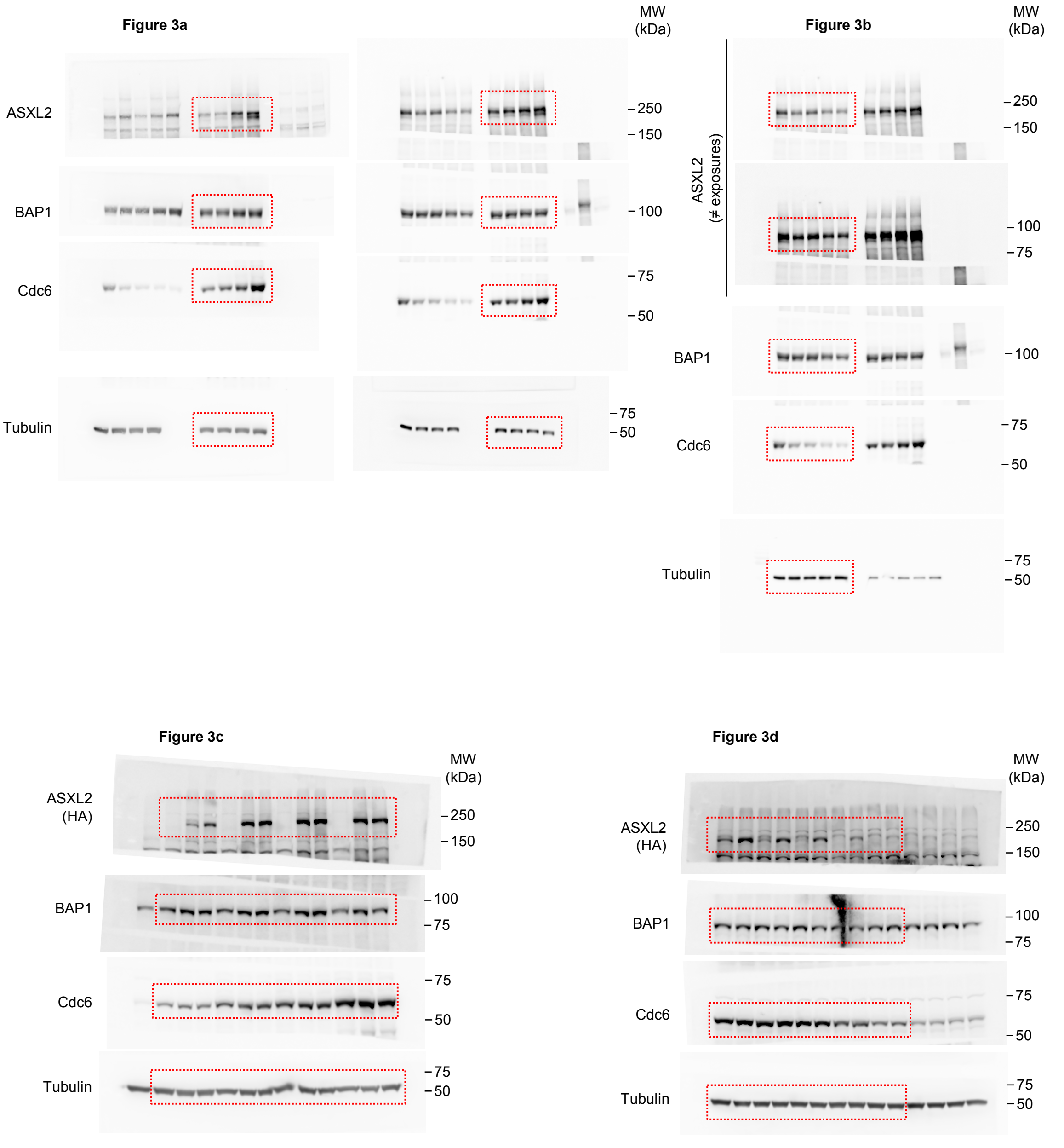
Supplementary Figure 12. Uncropped immunoblots used for the indicated proteins in the **Figure 1**



Supplementary Figure 13. Uncropped immunoblots used for the indicated proteins in the **Figure 2**



Supplementary Figure 14. Uncropped immunoblots used for the indicated proteins in the **Figure 3**



Supplementary Figure 15. Uncropped immunoblots used for the indicated proteins in the **Figure 3**

Figure 3e

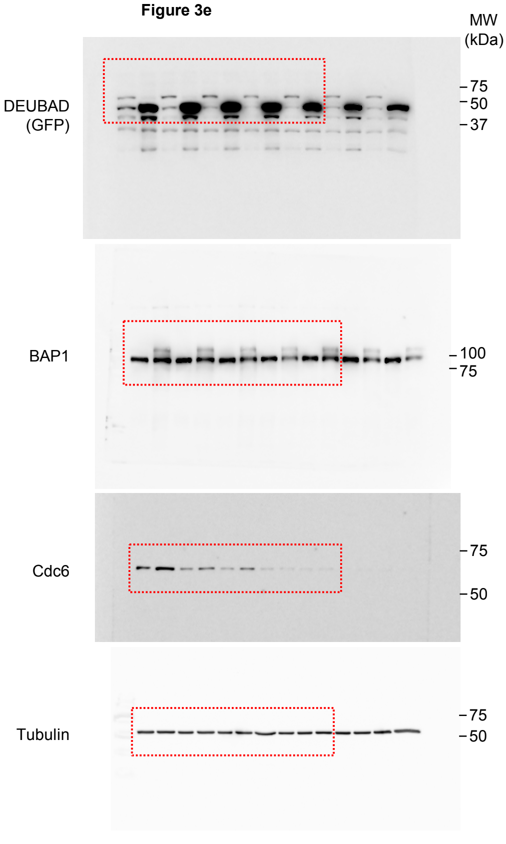


Figure 3f

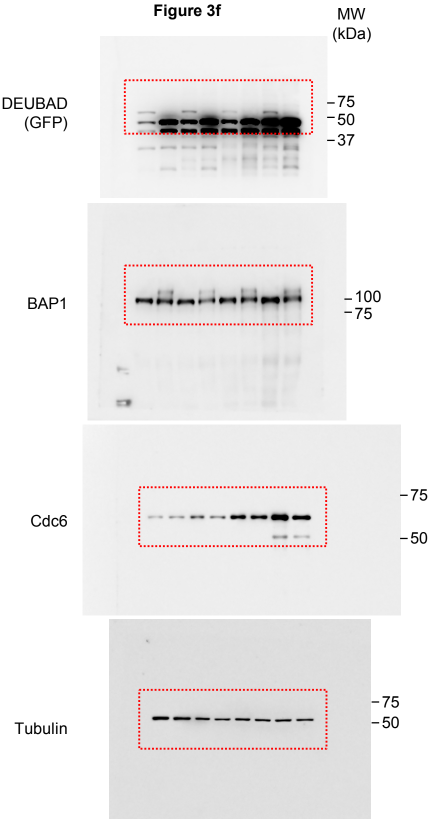


Figure 3g

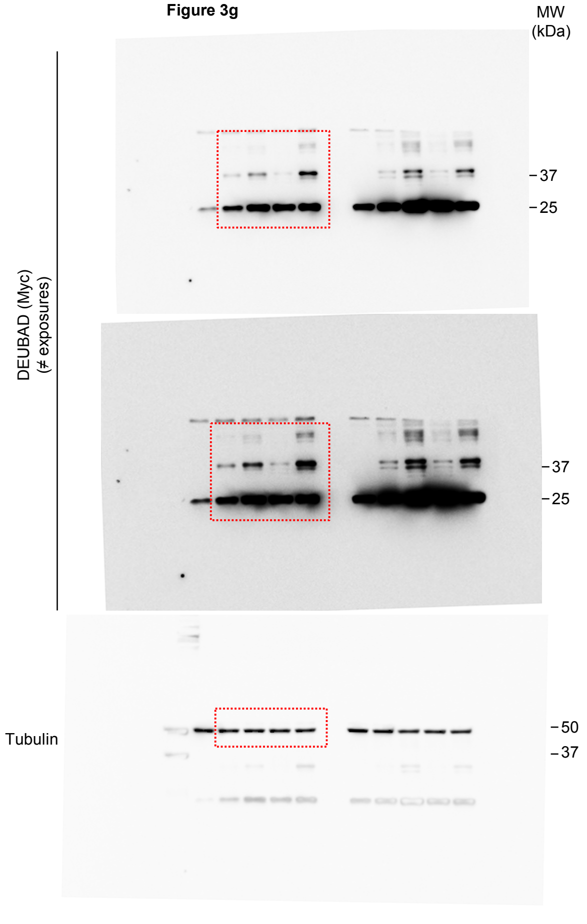
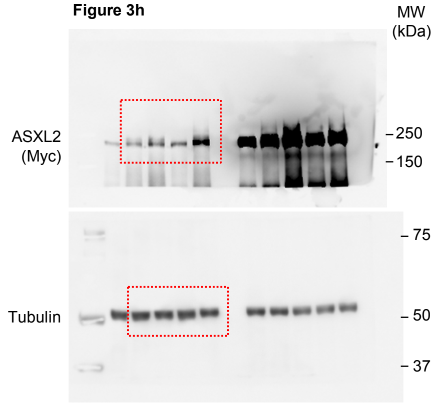
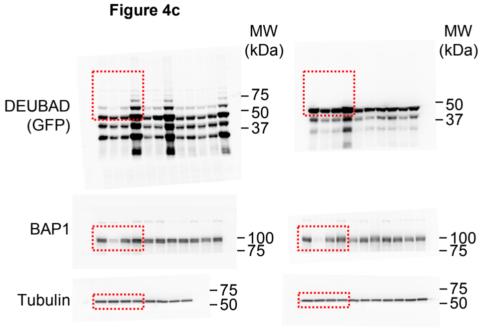


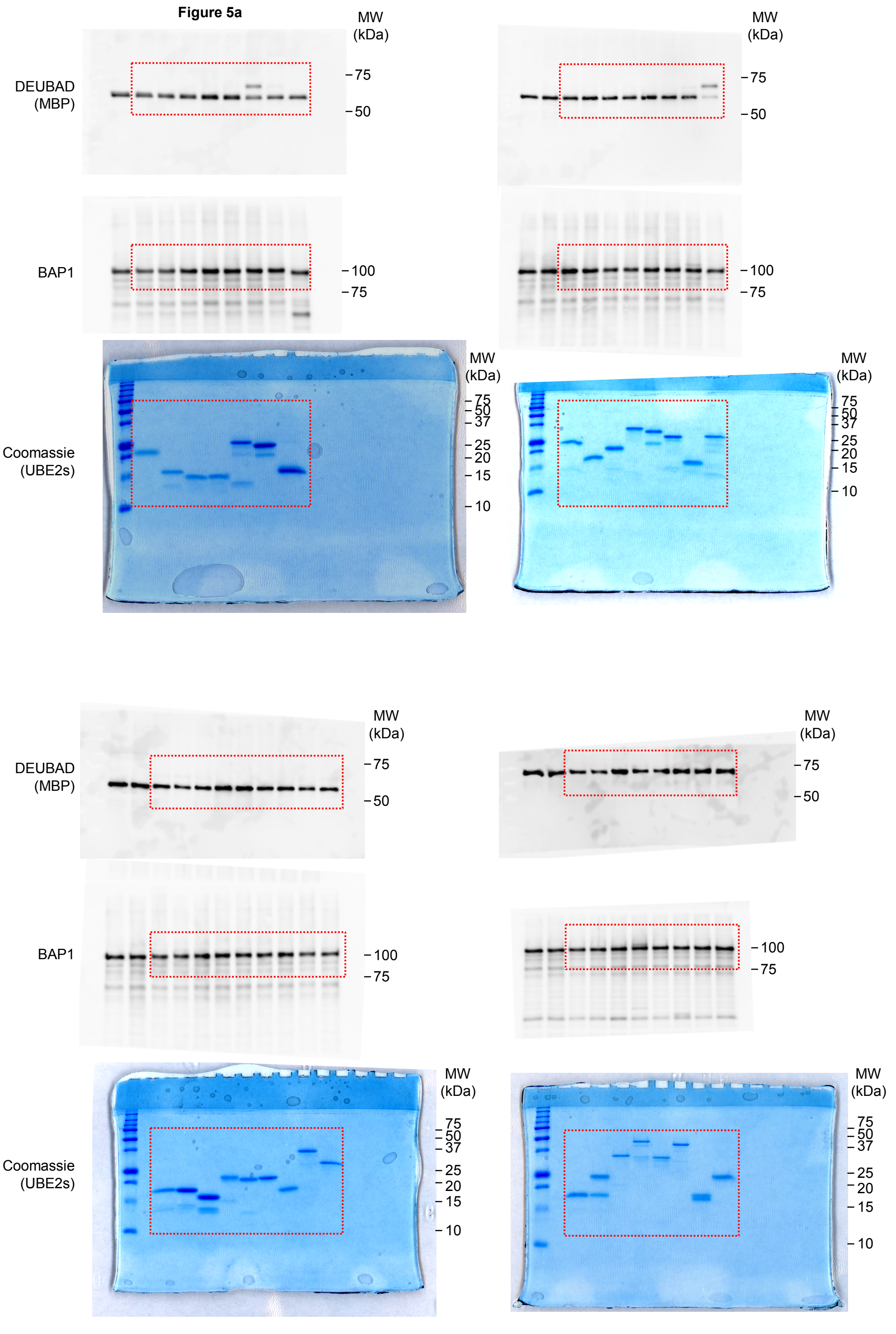
Figure 3h



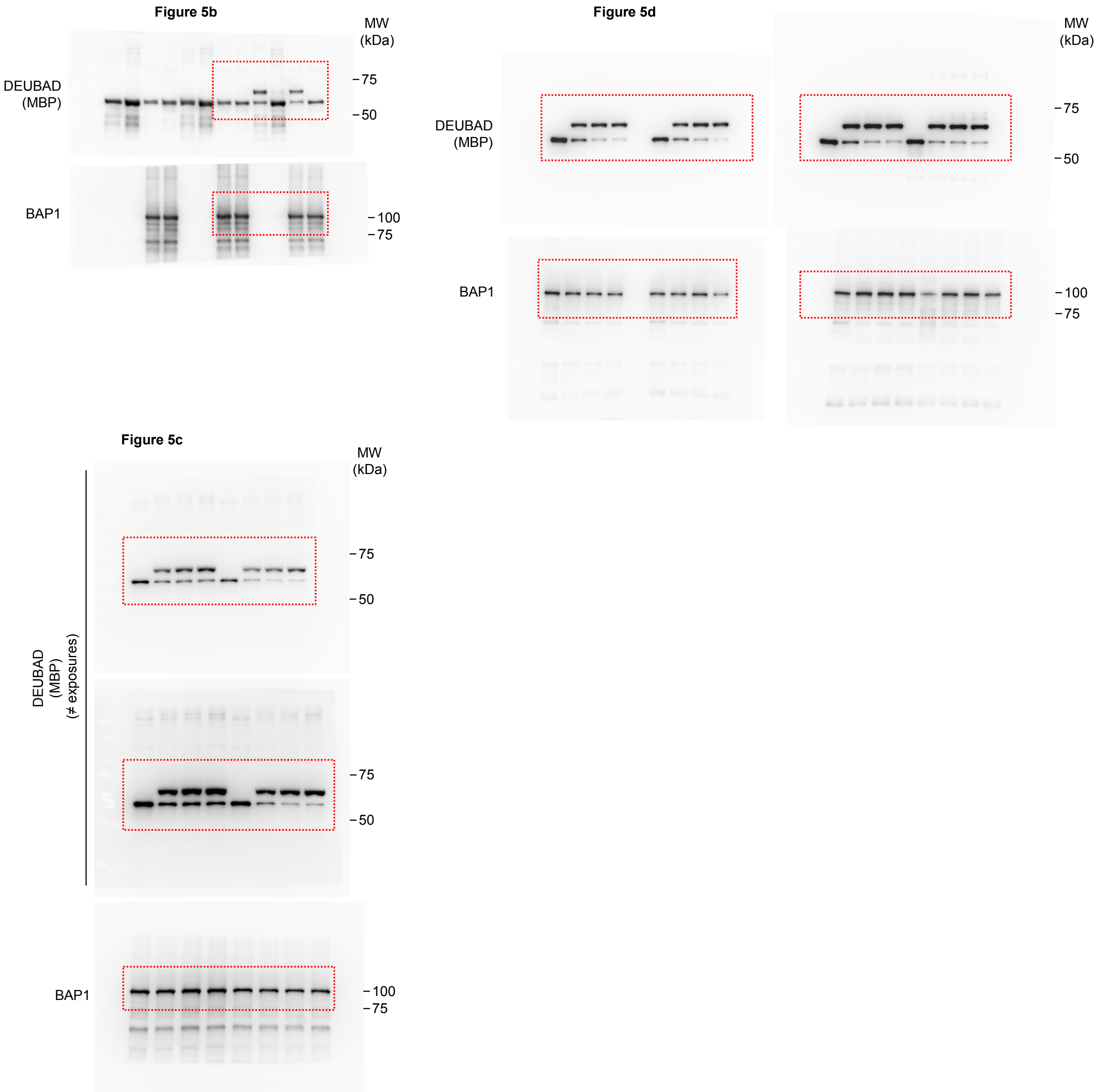
Supplementary Figure 16. Uncropped immunoblots used for the indicated proteins in the **Figure 4**



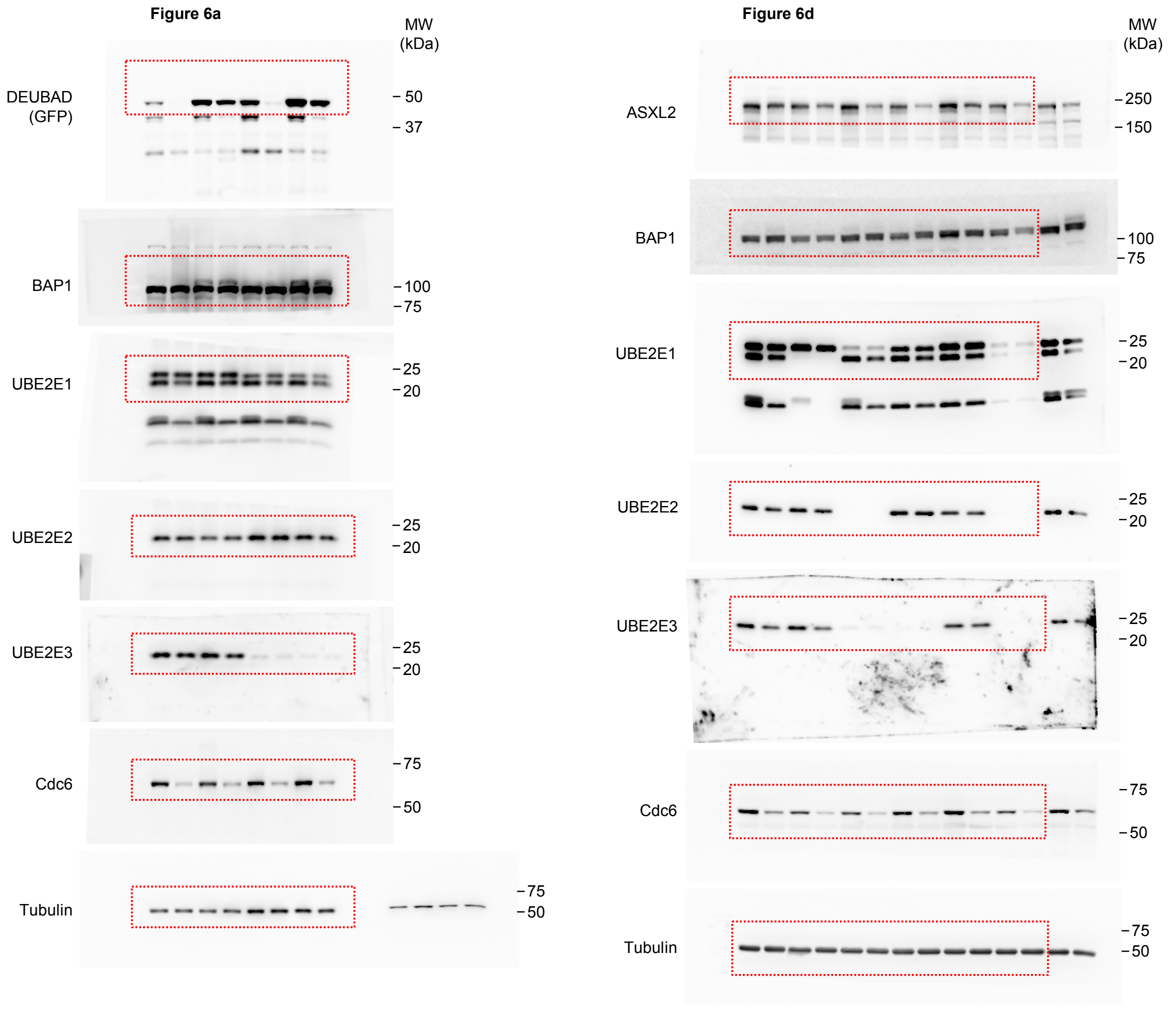
Supplementary Figure 17. Uncropped immunoblots used for the indicated proteins in the **Figure 5**



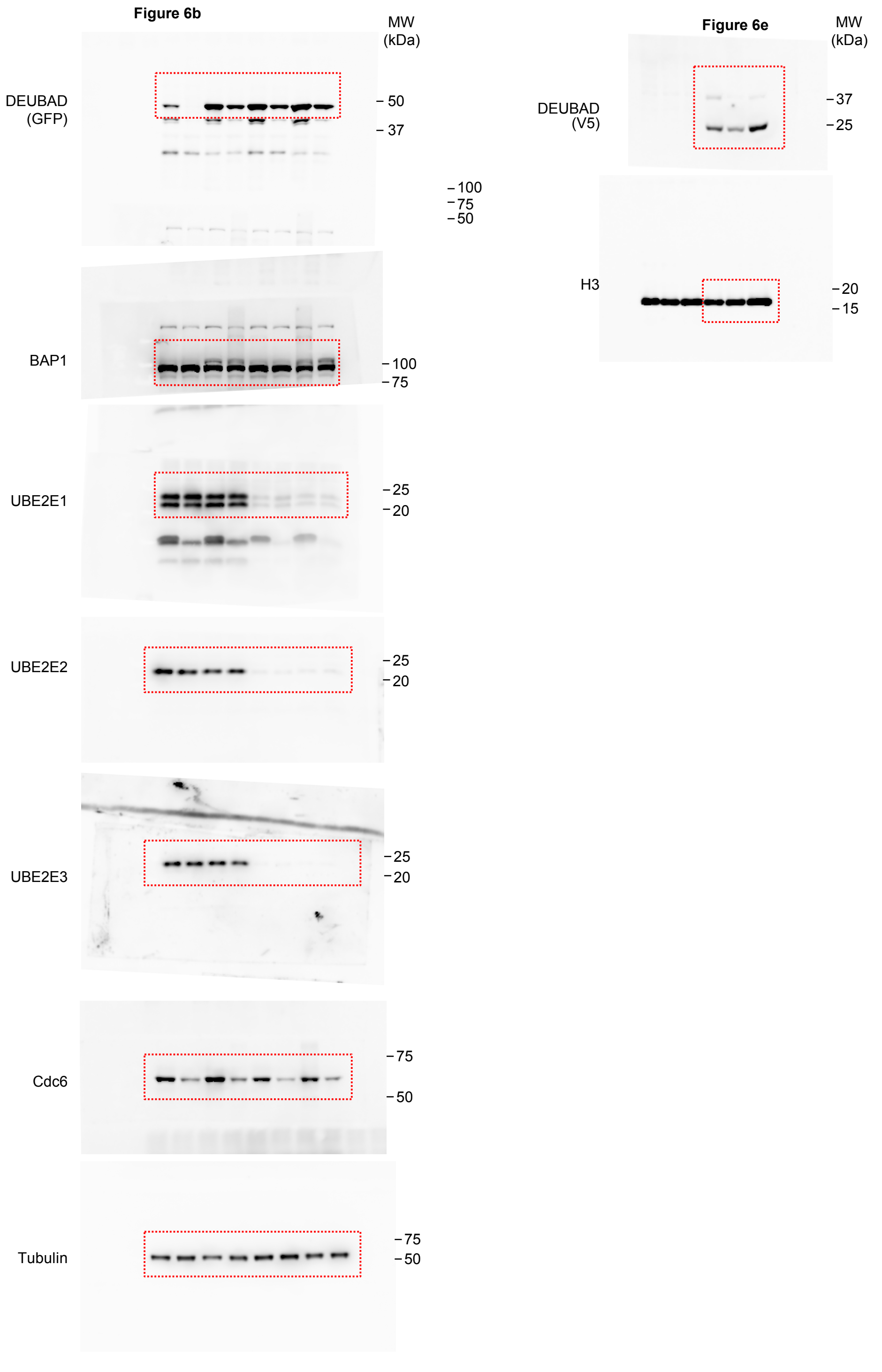
Supplementary Figure 18. Uncropped immunoblots used for the indicated proteins in the **Figure 5**



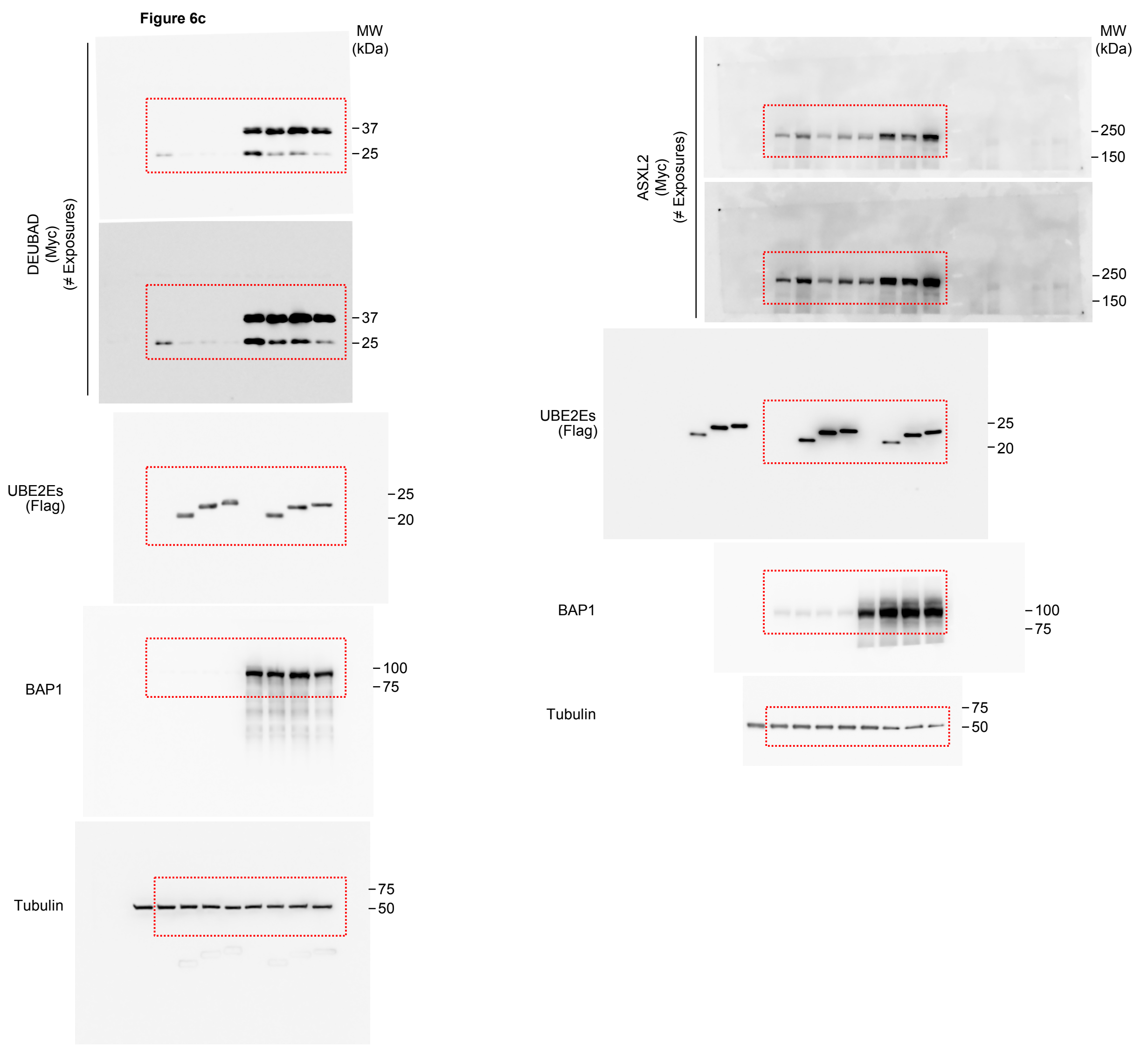
Supplementary Figure 19. Uncropped immunoblots used for the indicated proteins in the **Figure 6**



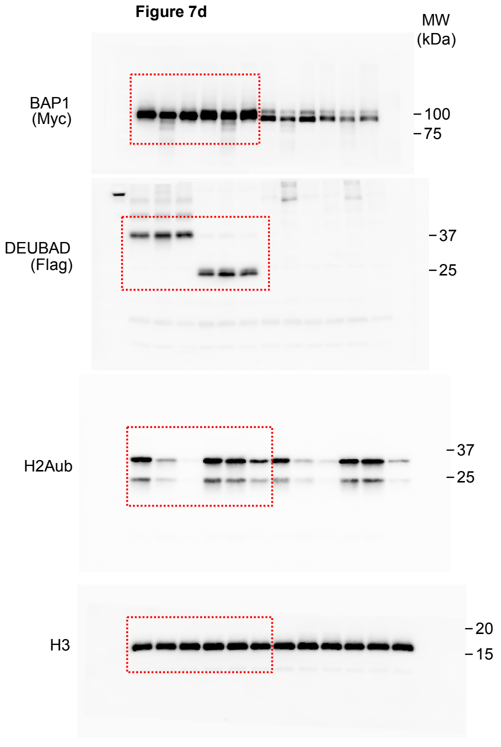
Supplementary Figure 20. Uncropped immunoblots used for the indicated proteins in the **Figure 6**

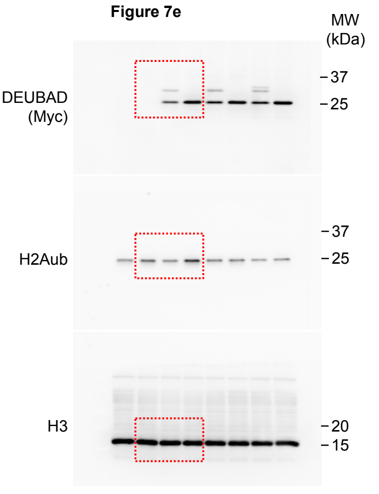


Supplementary Figure 21. Uncropped immunoblots used for the indicated proteins in the **Figure 6**



Supplementary Figure 22. Uncropped immunoblots used for the indicated proteins in the **Figure 7**





Supplementary Figure 23. Uncropped immunoblots used for the indicated proteins in the **Figure 9**

Figure 9a

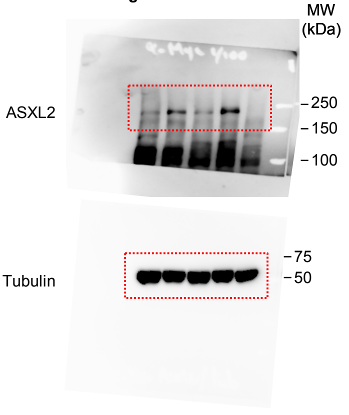
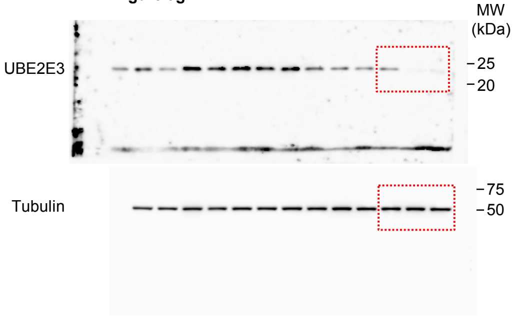
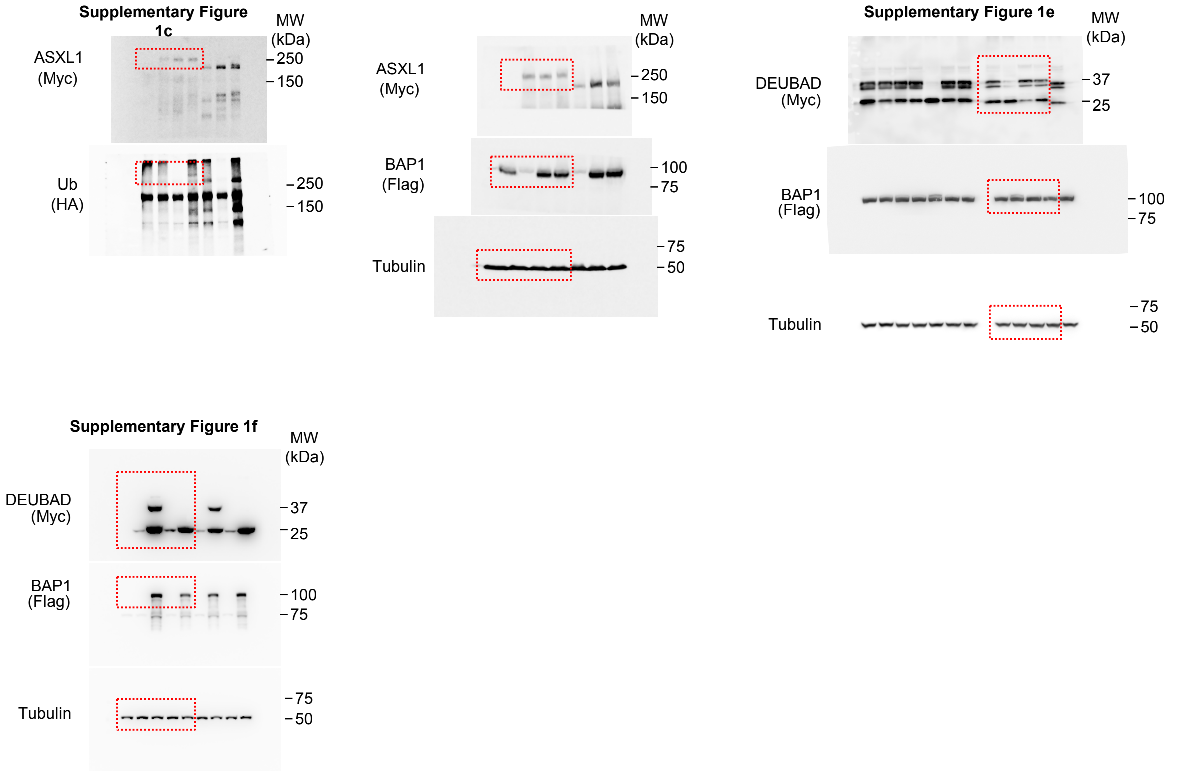


Figure 9g

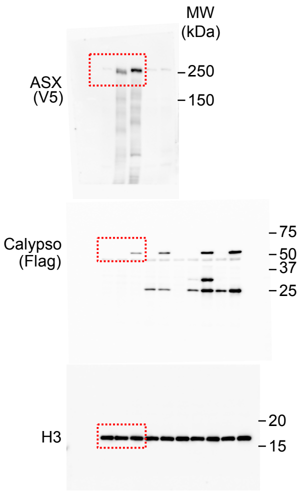


Supplementary Figure 24. Uncropped immunoblots used for the indicated proteins in the **Supplementary Figure 1**

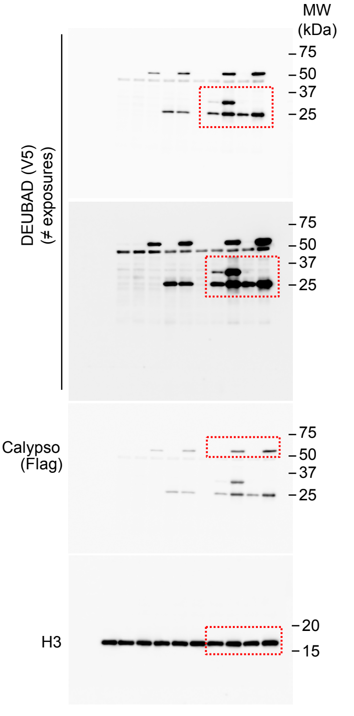


Supplementary Figure 25. Uncropped immunoblots used for the indicated proteins in the **Supplementary Figure 2**

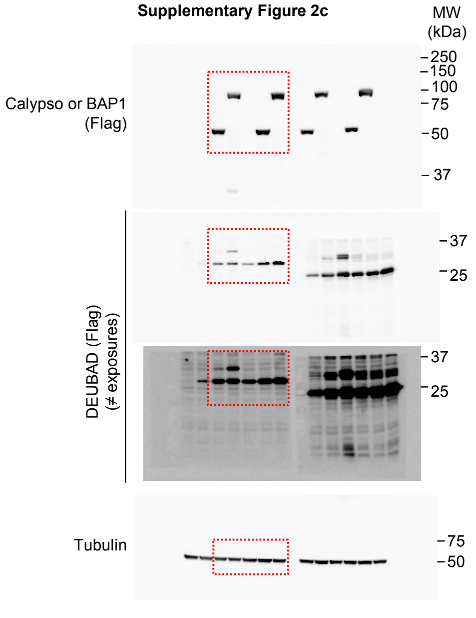
Supplementary Figure 2a



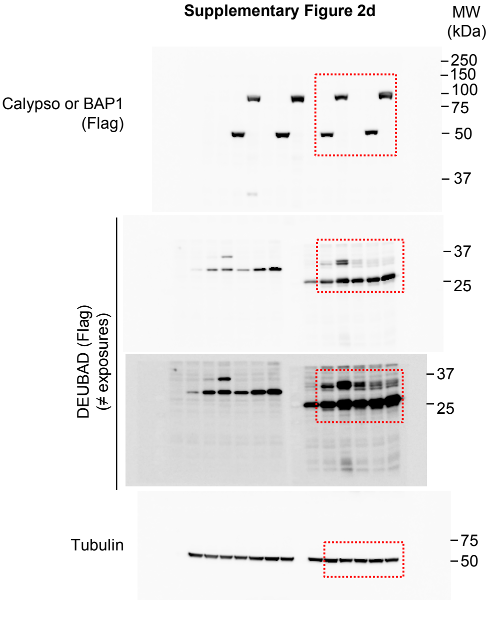
Supplementary Figure 2b



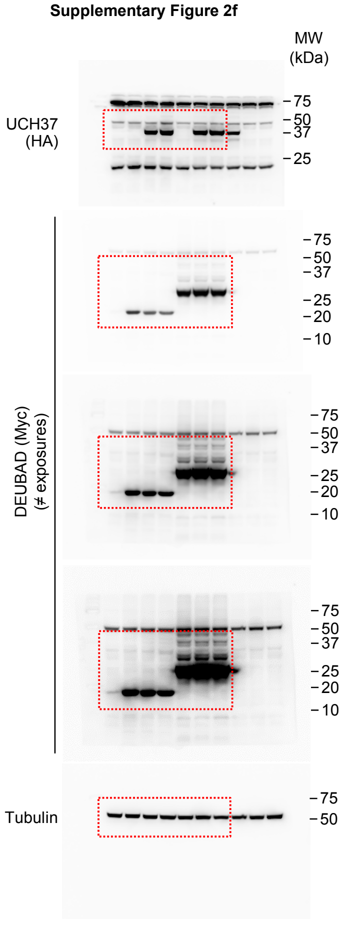
Supplementary Figure 2c



Supplementary Figure 2d

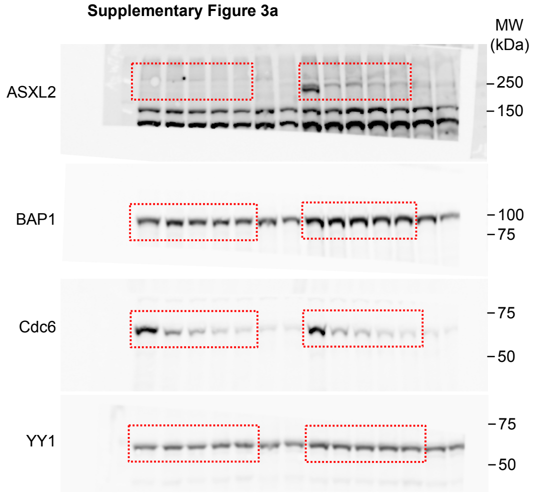


Supplementary Figure 26. Uncropped immunoblots used for the indicated proteins in the **Supplementary Figure 2**

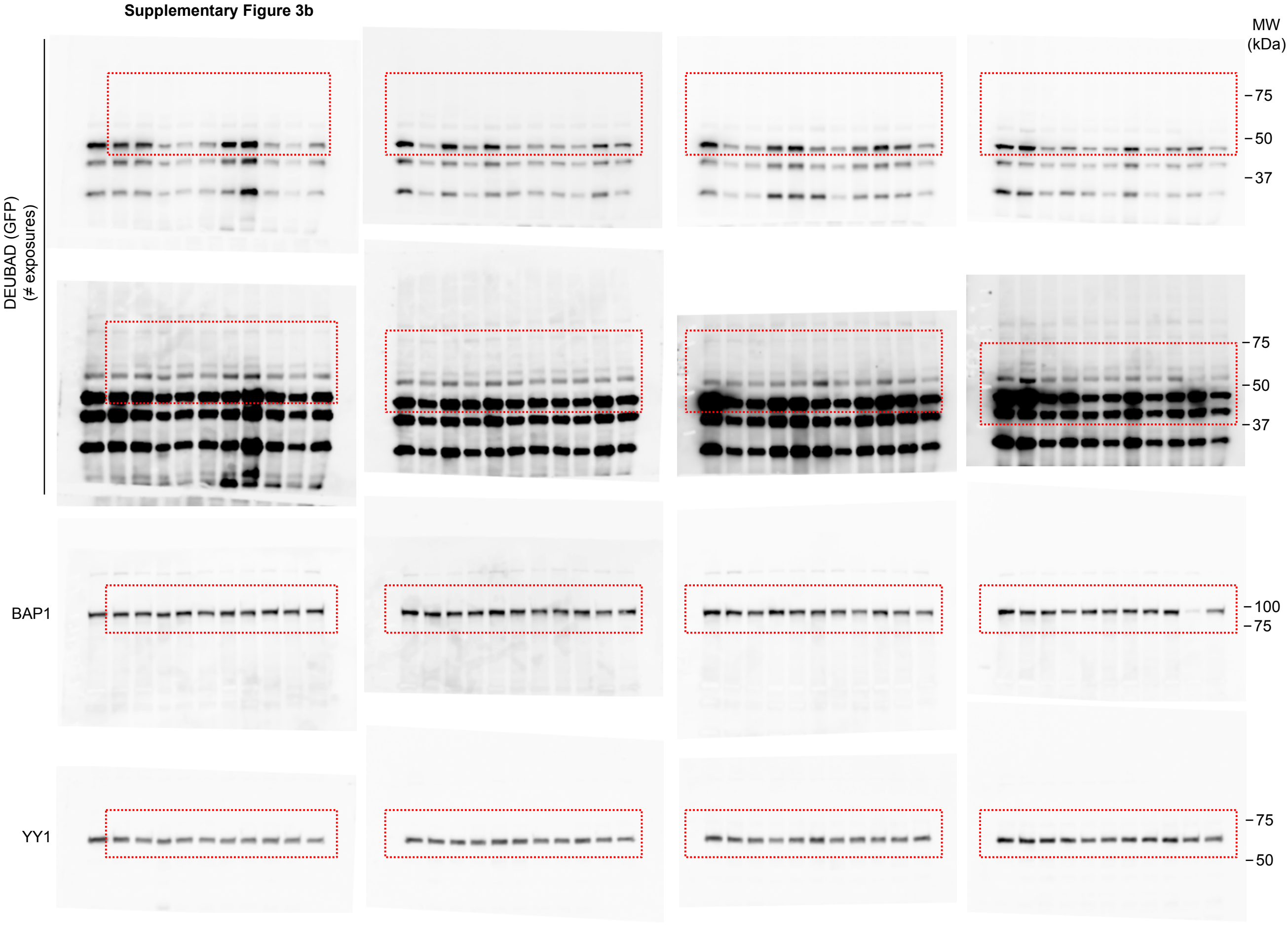


Supplementary Figure 27. Uncropped immunoblots used for the indicated proteins in the **Supplementary Figure 3**

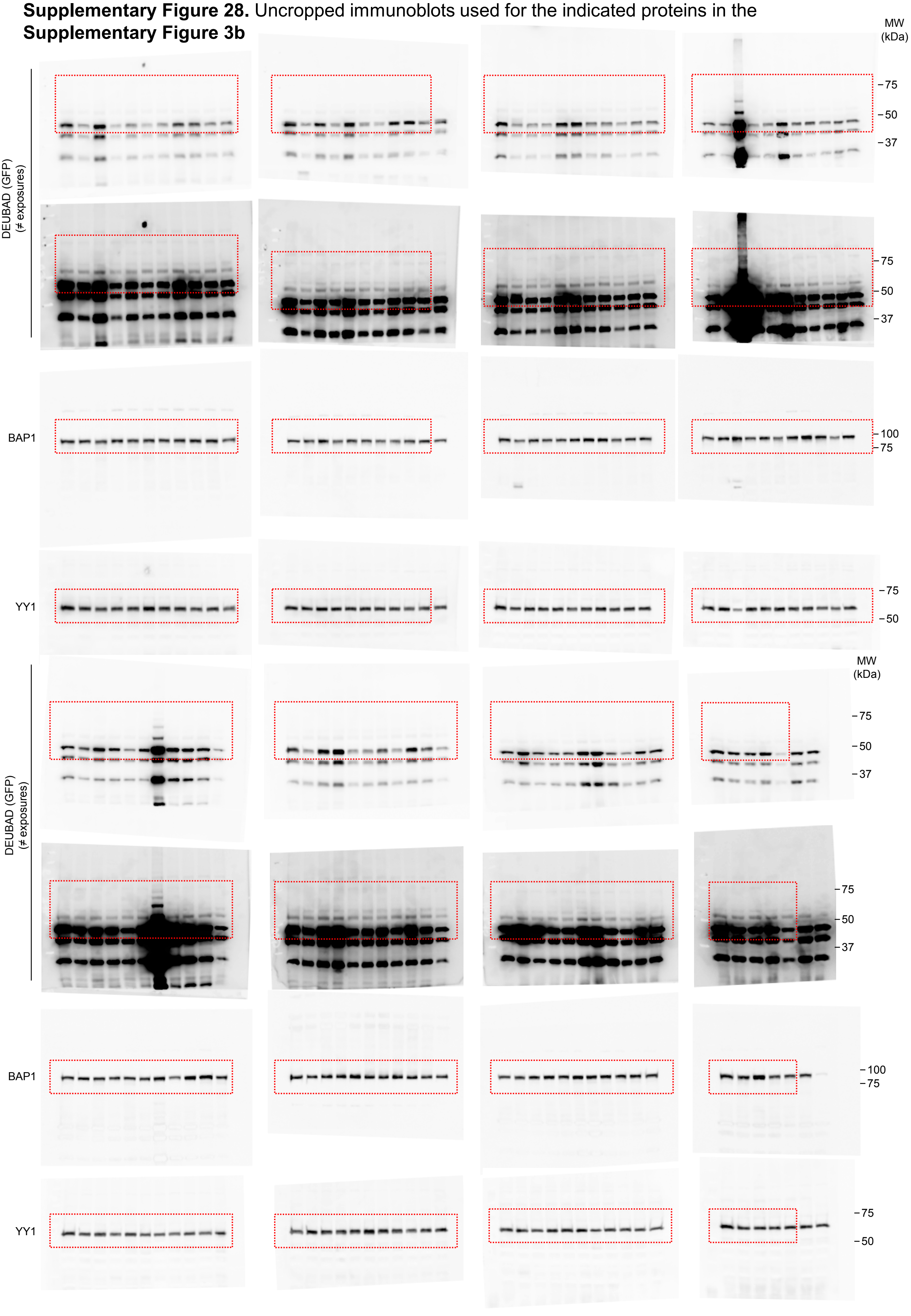
Supplementary Figure 3a



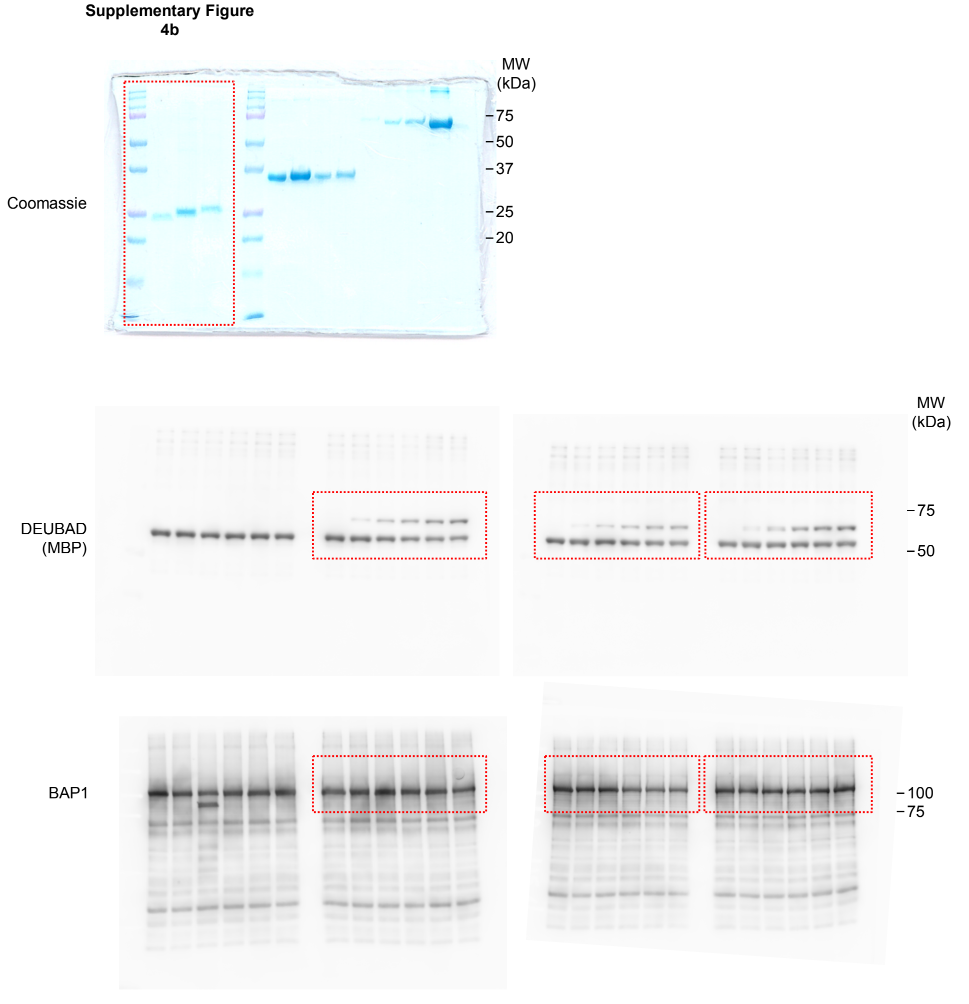
Supplementary Figure 3b



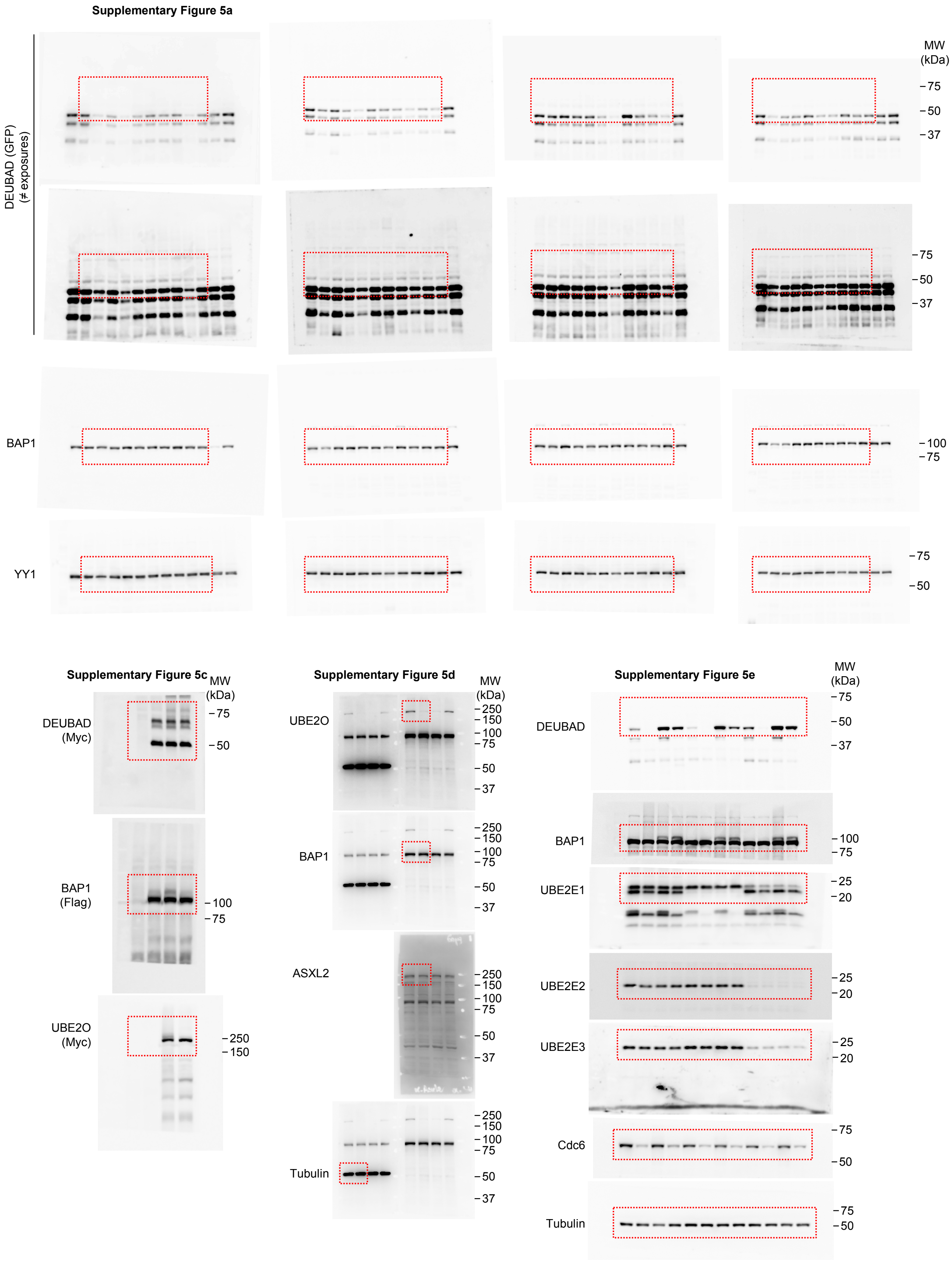
Supplementary Figure 28. Uncropped immunoblots used for the indicated proteins in the **Supplementary Figure 3b**



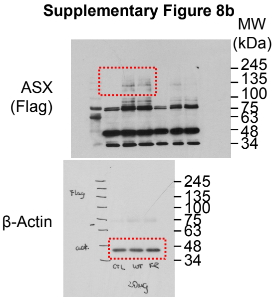
Supplementary Figure 29. Uncropped immunoblots used for the indicated proteins in the **Supplementary Figure 4**



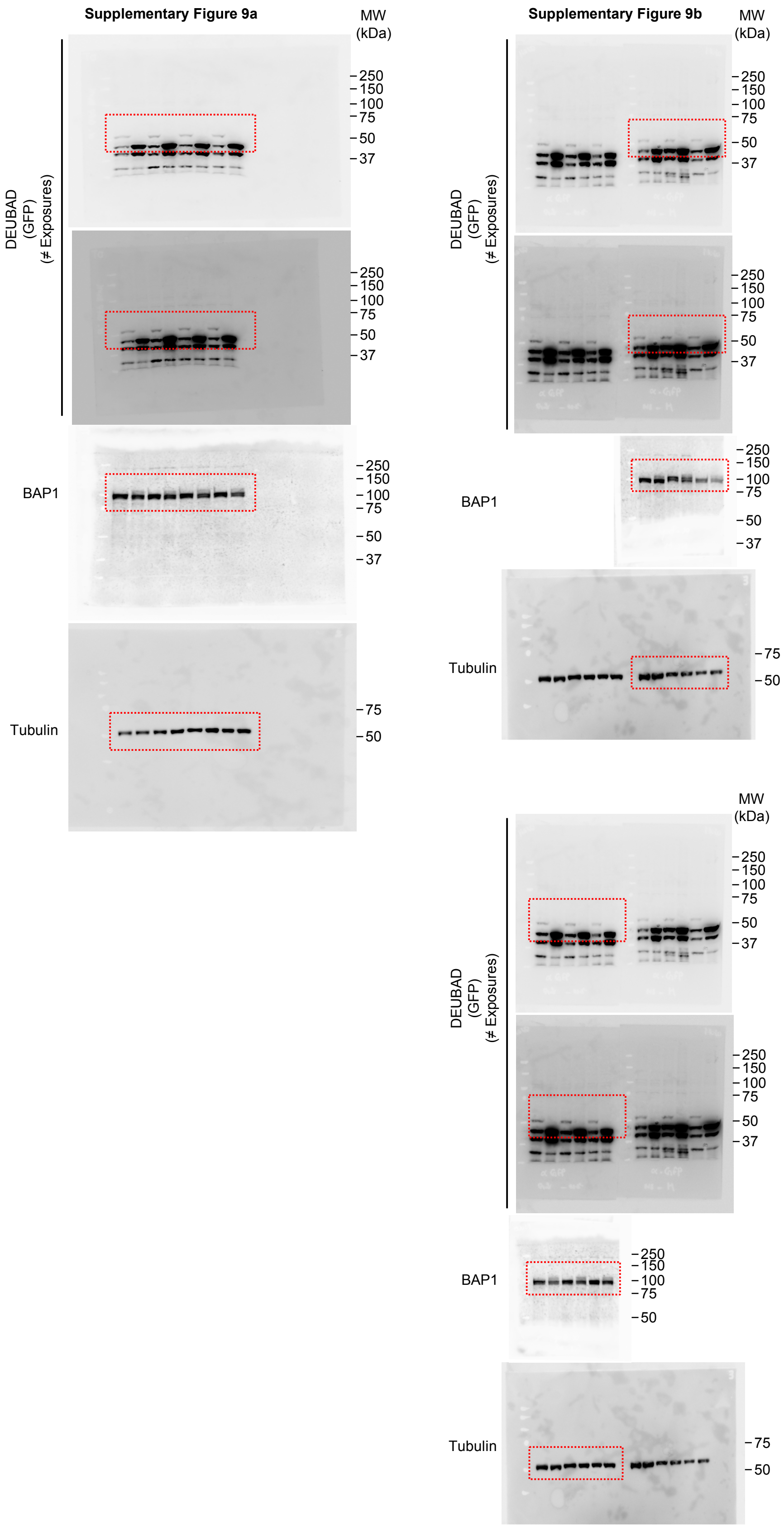
Supplementary Figure 30. Uncropped immunoblots used for the indicated proteins in the **Supplementary Figure 5**



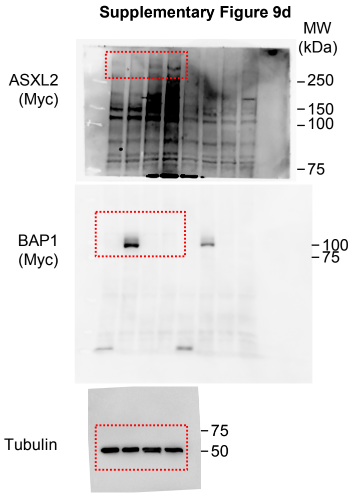
Supplementary Figure 31. Uncropped immunoblots used for the indicated proteins in the **Supplementary Figure 8**



Supplementary Figure 32. Uncropped immunoblots used for the indicated proteins in the **Supplementary Figure 9**



Supplementary Figure 33. Uncropped immunoblots used for the indicated proteins in the **Supplementary Figure 9**



Supplementary Figure 34. Uncropped FACS plots used in the **Figure 9**

Figure 9b

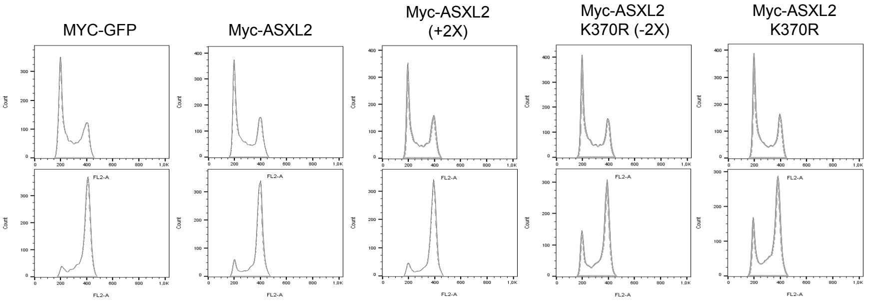


Figure 9e

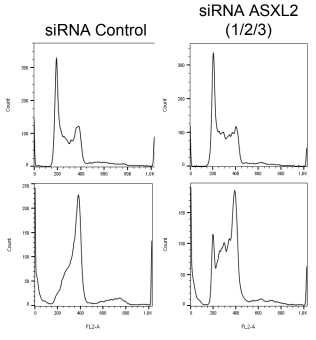
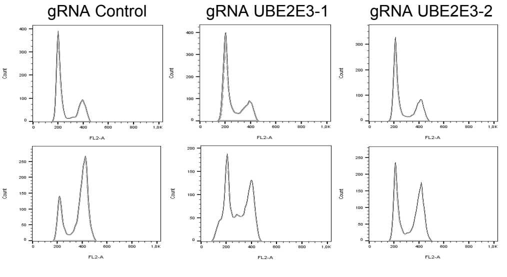
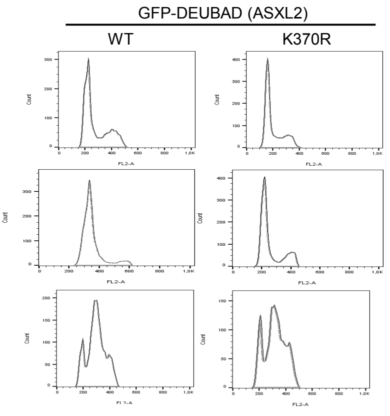


Figure 9i

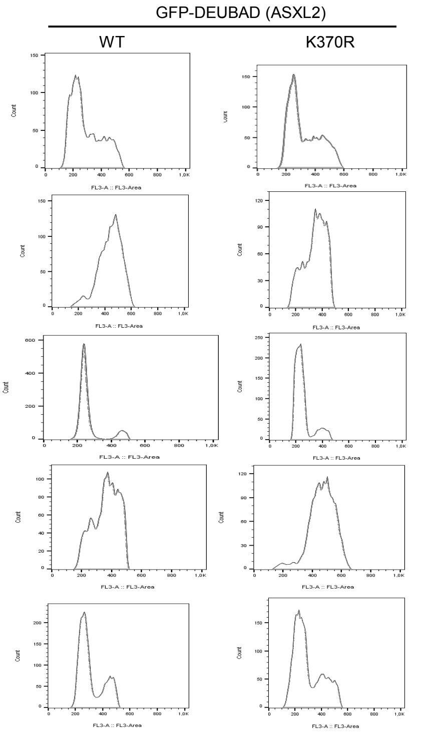


Supplementary Figure 35. Uncropped FACS plots used in the **Supplementary Figure 9**

Supplementary Figure 9a

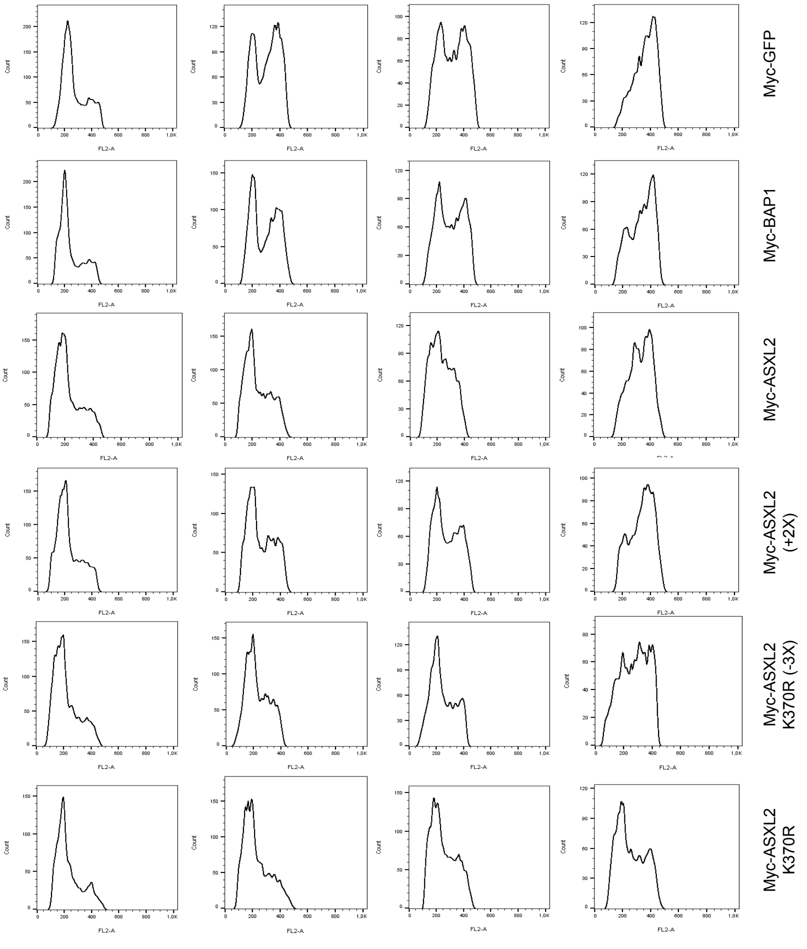


Supplementary Figure 9b



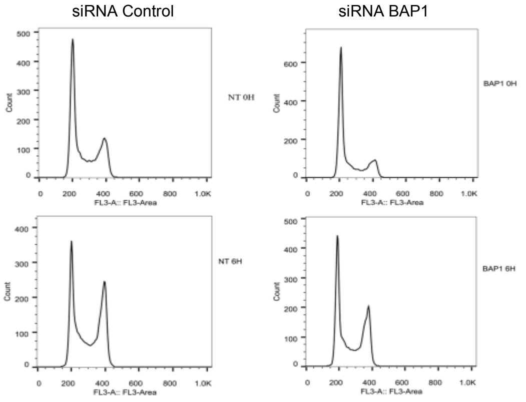
Supplementary Figure 36. Uncropped FACS plots used in the **Supplementary Figure 9**

Supplementary Figure 9f

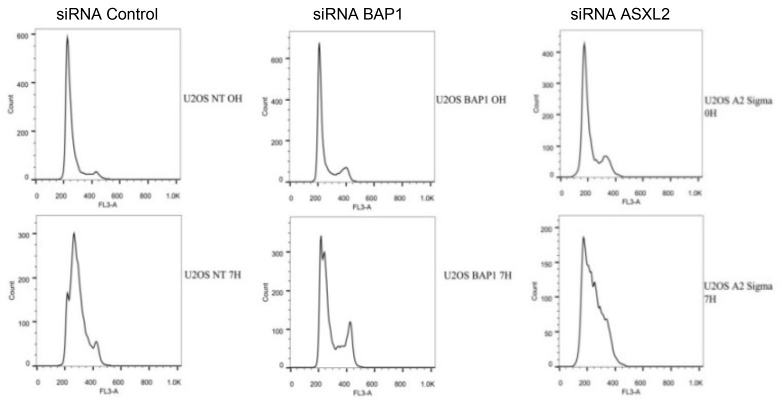


Supplementary Figure 37. Uncropped FACS plots used in the **Supplementary Figure 10**

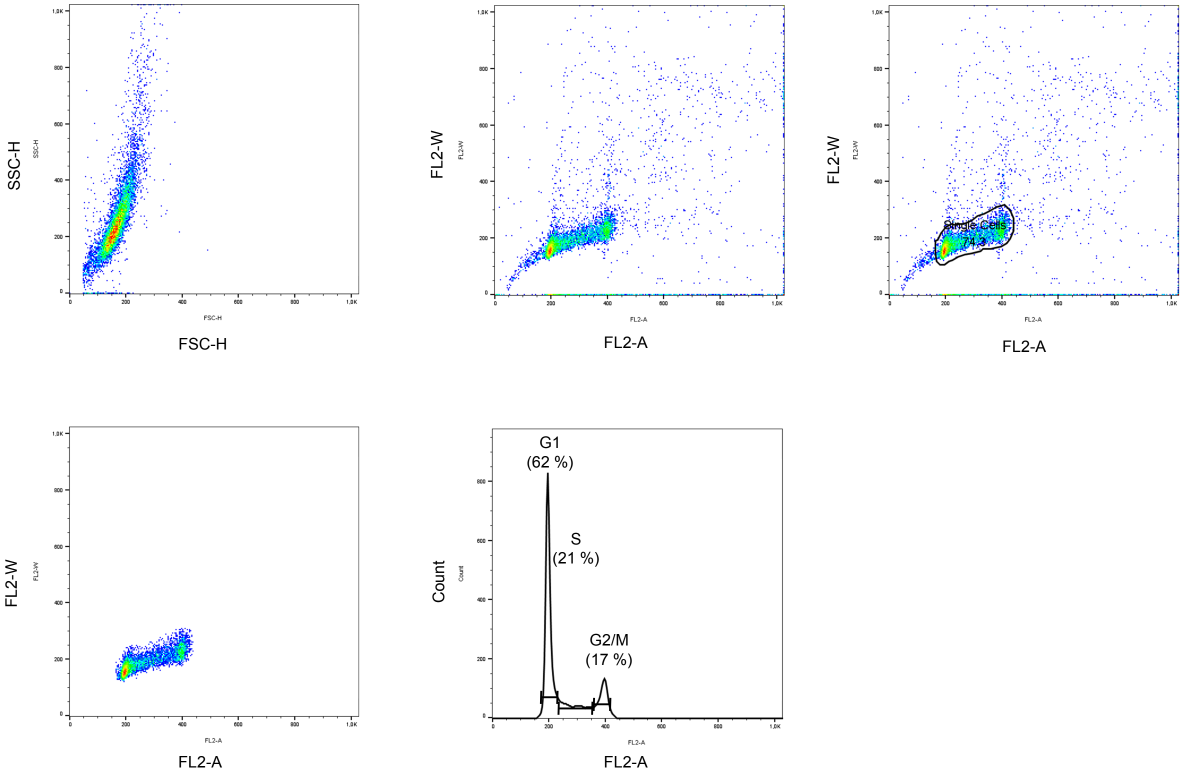
Supplementary Figure 10b



Supplementary Figure 10c



Supplementary Figure 38. Exemple of gating procedure related to **Figures 9b**, **9e**, **9i**, and **Supplementary Figures 9a**, **9b**, **9f**, **10b**, **10c**



Supplementary Table 1 : DUB siRNA library

Target	siRNA_1	Name	siRNA_2	Name
FAM63A	CAGACUUGGUAAUGUCCUU	SASI_Hs01_00094199	GGGUGAACUUAGCGUCUUU	SASI_Hs01_00094200
FAM63B	CCAUCAUCACCCAGAAUGA	SASI_Hs02_00308987	CUCAGAAUUUCAUCUUCGA	SASI_Hs01_00060574
FAM188A	CACAGAUCGCUCUCCUUCA	SASI_Hs01_00110290	CCUUGAUAGAUCCUGUAUA	SASI_Hs01_00110291
FAM188B	CUCAUCACCUCAUCACCGA	SASI_Hs01_00139144	CUCUACUUGCCUGGUGGUA	SASI_Hs01_00139145
BAP1	CGUGAUUGAUGAUGAUAUU	SASI_Hs01_00105395	CCAUCAACGUCUUGGCUGA	SASI_Hs01_00105396
UCHL1	GGACAAGAAGUUAGUCCUA	SASI_Hs01_00178415	GGCCAAUAAUCAAGACAAA	SASI_Hs01_00178416
UCHL3	CUGAUUCAUGCUAUUGCAA	SASI_Hs01_00200423	GCAUCUCUAUGAAUUAGAU	SASI_Hs01_00200424
UCHL5	CAGUUAUGUUCCUGUUAAU	SASI_Hs01_00142742	GAAGCAUAAUUAUCUGCCU	SASI_Hs01_00142743
USP1	GUAUACUUCAGGUAUUAUA	SASI_Hs01_00204271	CCAUACAAACAUUGGUAAA	SASI_Hs01_00204272
USP2	GACCUAAGUCCAACCCUGA	SASI_Hs01_00149958	CUAAGAGACCUGGACUUAA	SASI_Hs02_00337104
USP3	CGGAUAAACUUUAAUACCU	SASI_Hs01_00023593	CAUUACACAGCAUACGCAA	SASI_Hs01_00023595
USP4	CACUACACUGCAUAUGCGA	SASI_Hs01_00113975	GAGAAUCACAGGUUGAGGA	SASI_Hs01_00113976
USP5	CUGUCAAGCUGGGCACCAU	SASI_Hs02_00319396	CUAAUGAAGUGUUCCGCUU	SASI_Hs02_00319397
USP6	GUGUUGAUGCCAAUAACCA	SASI_Hs01_00167628	GAGAAUGGGAGACAUAUAA	SASI_Hs01_00167629
USP7	GACGUUUCGAAUAGAGGAA	SASI_Hs01_00079539	GACUUUGAGAACAGGCGAA	SASI_Hs01_00079540
USP8	CCUUUGACAAGAGCACGAA	SASI_Hs02_00339089	GAGAAUGGGACCACUGAAU	SASI_Hs01_00136039
USP9X	GUCGUUACAGCUAGUAUUU	SASI_Hs01_00026227	CUGUGAUUCAGCAACUCUA	SASI_Hs02_00308595
USP9Y	GUAGUGAUUUACACGAUGA	SASI_Hs01_00081909	CUUACUAAGAGCCACACUA	SASI_Hs02_00338066
USP10	GUCAUUGAACCCAGUGACA	SASI_Hs01_00213007	GUUCUAAUGUGGAGGCGGA	SASI_Hs01_00213008
USP11	CAGAGAUGAAGAAGCGUUA	SASI_Hs01_00148685	GAUUCUAUUGGCCUAGUAU	SASI_Hs01_00148686
USP12	GAAACUCUGUGCAGUGAAU	SASI_Hs01_00167303	CAUCAGAUAUCUCAAAGAA	SASI_Hs01_00167305
USP13	CUGAAUACUUGGUAGUGCA	SASI_Hs01_00108438	GAGCUAUUUGCAUUCAUCA	SASI_Hs01_00108439
USP14	CAAUAAUUGUGGAUACUAU	SASI_Hs01_00089059	GAUAUUGGCUCCAAUAAUU	SASI_Hs01_00089060
USP15	CUCUUGAGAAUGUGCCGAU	SASI_Hs01_00059894	CACAAUAGAUACAAUUGAA	SASI_Hs01_00059895
USP16	CAUCUUUGGUGGUGAACUA	SASI_Hs01_00210886	GAGAAACUUCGAGAUGCGA	SASI_Hs02_00304059
USP17L9P	GAAAUUCCUUCAAGAGCAA	SASI_Hs02_00324631	GGAAAUUCCUUCAAGAGCA	SASI_Hs02_00324632
USP17L2	CUAUCAUUGCGGUCUUUGU	SASI_Hs02_00372940	CAACAAACUUGCCAAGAAU	SASI_Hs02_00372941
USP17L6P	GCCUAUCAUUGUGGUGUUU	SASI_Hs02_00494866	CAGGCAACAAGAUUGCCAA	SASI_Hs02_00494867
USP17L1P	CUAUCAUUGCGGUCUUUGU	SASI_Hs02_00517496	GCAACAAACUUGCCAAGAA	SASI_Hs02_00517497
USP18	GCUUCAAUGACUCCAAUAU	SASI_Hs01_00221412	GUCAUUACUGUGUCUACAU	SASI_Hs01_00221413
USP19	CACAAGAUGAGGAAUGACU	SASI_Hs01_00130241	GCAAGUUCUGCAUUGGUCA	SASI_Hs01_00130242
USP20	GGACUUAUCACUGCCCAUU	SASI_Hs01_00033438	GGUUCUACGUGUCCCGCGA	SASI_Hs01_00033439
USP21-Iso-1	CCAACUUAGCCCGUUCCAA	SASI_Hs01_00193567	GACAAGAUGGCUCAUCACA	SASI_Hs01_00193568
USP21-Iso-2	CCAACUUAGCCCGUUCCAA	SASI_Hs01_00177787	GACAAGAUGGCUCAUCACA	SASI_Hs01_00177788
USP22	CAAAGCAGCUCACUAUGAA	SASI_Hs02_00347438	CUGAUCAACCUUGGGAACA	SASI_Hs02_00347439
USP24	GCACAAUACUGUGACCGUA	SASI_Hs02_00347524	GAAACUCAGGGUUGAUACU	SASI_Hs02_00347525
USP25	CUAUGGUUCCGGUCCCAAA	SASI_Hs02_00344762	GAAAGAUUACCUCACGGUA	SASI_Hs01_00191397
USP26	CUACAGAAGUCUAACAGGA	SASI_Hs01_00043149	CCAUCUUGGGAAGACUCUA	SASI_Hs01_00043150
USP27X	GAUACUGAGAGAUUUCUUU	SASI_Hs02_00394607	CCUGUAUUACGGAGGUAUA	SASI_Hs02_00394608

USP28	GACCUUACUCAUGAUAACA	SASI_Hs01_00077918	GACACUAUUGGGCCUAUAU	SASI_Hs01_00077919
USP29	GAAAGAAGCUCUCAUUGAA	SASI_Hs01_00032719	GAAUAACGAGCAAGUUUAU	SASI_Hs01_00032720
USP30	CACGAAUUAUUCCAUGUCA	SASI_Hs01_00155679	CUAGUCAACACAACCCUAA	SASI_Hs01_00155680
USP31	GCAUUCAGGUGUGUCCAUU	SASI_Hs01_00020641	GAGUCAUCCCUUUCAAGUA	SASI_Hs01_00020642
USP32	GAUAAUCAGCCAUUAGUAA	SASI_Hs01_00086230	GGAACUAUGUUAUACGGGA	SASI_Hs01_00086231
USP33	CACAGAUCCUUCCAUCAAA	SASI_Hs01_00180288	GAAGUGUUAUUUCAGACAU	SASI_Hs01_00180289
USP34	GGAUCUAGCAAUGAGGUUA	SASI_Hs01_00089169	GAUCUUAGGGCUGAAGUAA	SASI_Hs02_00346129
USP35	GCAAGAUUGGUCUCAUCAA	SASI_Hs02_00353962	CUGUUAAGAAGUUCAGCAU	SASI_Hs02_00353963
USP36	CUAAGACGGUGAAGCUGAA	SASI_Hs02_00357787	CGUAUAUGUCCCAGAAUAA	SASI_Hs01_00194136
USP37	CAGCUAAGUCAUAACAUUA	SASI_Hs01_00018875	CUUGUCUAUUGACAAAGUA	SASI_Hs02_00354323
USP38	GAGAGAUAGUCCCAGUGCA	SASI_Hs01_00189218	GUGAAACUUCUUUACAGGA	SASI_Hs02_00360065
USP39	CAUAUGAUGGUACCACUUA	SASI_Hs01_00034674	CAAUGAUUAUGCCAACGCU	SASI_Hs01_00034675
USP40	CACUGAAAGAACUUCUGAU	SASI_Hs01_00145783	CACAUGUCUUUCCAGCUAA	SASI_Hs02_00351527
USP41	GUACGUGCAUCCUUGUGUA	custom made by Sigma	UUGUUCAGGGCUCAUCAGU	custom made by Sigma
USP42	CAGUCUACCUCGAACGCAU	SASI_Hs01_00078970	GUUAAUAGGUCCUCAGUGA	SASI_Hs01_00078971
USP43	GUGAUCUUGGUUGAACUGU	SASI_Hs02_00367061	GUGAAAGGCAGAAGCAUUA	SASI_Hs02_00367062
USP44	GAAGGAUACUAAUGGGUAA	SASI_Hs02_00309548	CAACAAAUCAAAUACCAUA	SASI_Hs02_00309549
USP45	CCAGUUUACAUCUAUGGAA	SASI_Hs02_00315256	CACUACACUGCUUAUGUGA	SASI_Hs02_00315257
USP46	GUCUCAAUGGUCUGGCUGU	SASI_Hs01_00080807	GGUAUCACUCCGAGUCUCA	SASI_Hs01_00080808
USP47	GCAUAUAUGCUGAUCUAUA	SASI_Hs01_00112225	CAUGCAAGUUUCUGCUAGA	SASI_Hs01_00112227
USP48	CUUUAUGUCUCUAUUGGAA	SASI_Hs01_00185218	CUACUUAUGUCCAAGCACU	SASI_Hs01_00185219
USP49	CUGAAACACUUUGAGGAGA	SASI_Hs01_00055585	CUCAGAAGUGGUGCUGCUU	SASI_Hs02_00352112
USP50	CACUACACUGCUUUCUGCA	SASI_Hs02_00373416	GGAAAUAUCCUAAAUACAA	SASI_Hs02_00373417
USP51	CAUAGUGUUUCUACCACCA	SASI_Hs02_00372924	CAAAGCUACCAGGAGUCUA	SASI_Hs02_00372925
USP52	CUGUCUACCUGUUCCAUAU	SASI_Hs02_00329594	CAGUUUGACAUGAAUUGGA	SASI_Hs02_00329595
USP53	GAACCAAGUUUAGAAGUGA	SASI_Hs01_00234222	CAGCUAAGUUAAGUCACAU	SASI_Hs01_00234224
USP54	GACUUAGCAGAAGAUGUUA	SASI_Hs02_00366408	CCAAGUAUUGUUAAGCCAA	SASI_Hs02_00366409
USP55	CCAUAUUCAUGUUGCACUU	SASI_Hs02_00340306	GUAUCGAGCAAAUAAUCAU	SASI_Hs01_00128479
CYLD	GAACGAUGUAGAAUAUUAU	SASI_Hs02_00309208	GAACAGAUUCCACUCUUUA	SASI_Hs02_00309209
DUB3	CUAUCAUUGCGGUCUUUGU	SASI_Hs02_00372940	CAACAAACUUGCCAAGAAU	SASI_Hs02_00372941
ATXN3	GAAGAAUUAGCACAACUAA	SASI_Hs01_00043300	CAAAUGAUGGCUCAGGAAU	SASI_Hs01_00043301
ATXN3L	CAUUAUCAUCUAUGGGAUA	SASI_Hs02_00379672	CAAUUAACAUGGAUUUCAU	SASI_Hs02_00379673
JOSD1	CGGGAUACGCUGCAAGAGA	SASI_Hs02_00346483	CACUUCAGACCAAAGGCUA	SASI_Hs01_00072064
JOSD2	GGUGGACGGUGUCUACUAC	SASI_Hs01_00128488	GCAACUAUGAUGUCAAUGU	SASI_Hs01_00128489
JOSD3	GACAUCUGAUGCUGUGGAA	SASI_Hs01_00119698	GAUAAAUCAGGAAUAGAUU	SASI_Hs01_00119700
TNFAIP2	GGAAGAAAUACACAUAUUU	SASI_Hs01_00033556	GGAUGUUACCAGGACAUUU	SASI_Hs01_00033557
OTUD7B	CAACUAAUUGGCUCAUCAA	SASI_Hs01_00089029	CUGAUCAAGCUUGCCUCAA	SASI_Hs01_00089030
OTUD7A	GUCCUAGCCCAUAUAUUAA	SASI_Hs01_00055782	GACUUGAUCGAGCAGGCAA	SASI_Hs01_00055783
OTUD4	UAAUUUAUCGGGAACCAAA	SASI_Hs02_00323440	GUUUCUCCUUCACAAGUAA	SASI_Hs02_00323441
PARP11	CACAAUCAAACACAUGAAU	SASI_Hs01_00173656	GUGUUCAGUUAGCAGUGAA	SASI_Hs02_00353319
OTUD6A	CACAAUCAAACACAUGAAU	SASI_Hs01_00173656	GUGUUCAGUUAGCAGUGAA	SASI_Hs02_00353319

YOD1	GAAAUAUGUGUAGUGGAUA	SASI_Hs01_00052848	GUAACUUCCCUGAUCCAGA	SASI_Hs01_00052849
OTUD6B	GAAGUCAGACCGCUGAGUA	SASI_Hs01_00222466	CUACUAAGGAGAAUAAGAU	SASI_Hs02_00348712
OTUD5	CACUAUAAUUCAGUGGUGA	SASI_Hs01_00052828	GGACUUUACCACCUACAUU	SASI_Hs01_00052829
OTUB1	GCAAGUUCUUCGAGCACUU	SASI_Hs01_00080525	CCUGGAAGUUCCUUAGGGA	SASI_Hs02_00350395
OTUB2	CAGAGUGCCUCGGACCACA	SASI_Hs01_00010701	GACAUCAAAGACUUCUGCA	SASI_Hs01_00010702
OTUD1	CUGAUUUCUCACAGUGUAA	SASI_Hs01_00270248	GAUGUUUCAUGAUAGCUUU	SASI_Hs01_00270249
OTUD3	GAAAUCAGGGCUUAAAUGA	SASI_Hs02_00347304	GUAGUGAUUCAUCAACUUA	SASI_Hs02_00347305
ZRANB1	GAACUUGAAGUAGACUUUA	SASI_Hs01_00155779	GAAUCGUCCUUCUGCCUUU	SASI_Hs02_00350242
VCPIP1	GUAACUGCCUUUCAGGGAA	SASI_Hs02_00357704	GAUGUAUGGUCUUAUGCAA	SASI_Hs01_00165636
STAMBP	CCAAAGAAUAUACAGAAUA	SASI_Hs01_00182263	CAAAGUGCUGGGUCUGAUU	SASI_Hs01_00182264
STAMBPL1	GAGUUAGCCCGAGGUCAAA	SASI_Hs01_00241436	CAAUUCCUUGCUGAAUGUA	SASI_Hs01_00241437
BRCC36	GUCAGAAUUGUUCACAUUC	SASI_Hs01_00177040	CAUCUUACGACGUUCUGAU	SASI_Hs01_00177042
COPS5	CCAUUUGUAGCAGUGGUGA	SASI_Hs02_00342404	GUGAAUCUUGGCGCCUUUA	SASI_Hs01_00209042
COPS6	GAUCUUCCUGUCAGCGUUU	SASI_Hs02_00342398	CACACUGGUUGGUCAACUU	SASI_Hs01_00117938
EIF3H	CAAGGAUCUCUCUCACUAA	SASI_Hs01_00072290	CAAAUAUCACCUUUGAGUA	SASI_Hs01_00072291
EIF3F	GAGGAUGUACUGUCUGGAA	SASI_Hs01_00246686	CCGAUGACUUUGAGACCAU	SASI_Hs02_00336223
IFP38	CAUAGUGUUGCAAUAUGCA	SASI_Hs01_00017774	CAUAUGAGCAUCAAAGCCU	SASI_Hs01_00017775
PSMD14	GGUCUUAGGACAUGAACCA	SASI_Hs02_00340316	GUGAUUGAUGUGUUUGCUA	SASI_Hs01_00024446
PRPF8	CACGUAUCAAGAUUGGACU	SASI_Hs01_00015918	GGAUUAUGAUGCGCCGAGA	SASI_Hs01_00015919
PSMD7	CGUGUUGUUGGUGUGCUUU	SASI_Hs02_00334491	CACUUGUUACGAGAUAUCA	SASI_Hs02_00334492
CEZANNE	CAACUAAUUGGCUCAUCAA	SASI_Hs01_00089029	CUGAUCAAGCUUGCCUCAA	SASI_Hs01_00089030
CEZANNE2	GUCCUAGCCCAUAUAUUAA	SASI_Hs01_00055782	GACUUGAUCGAGCAGGCAA	SASI_Hs01_00055783
MPND	CCGAGAUGCUGCUGGUGGA	SASI_Hs01_00162447	GGAGUGAGGUCGUGGGUUA	SASI_Hs01_00162448
MYSM1	CUUACGAGAUAUUGACACA	SASI_Hs02_00317976	GACAAGAUGGAUAAUAGAA	SASI_Hs02_00317977
SENP1	GAAACAGCCGAAGUCUUUA	SASI_Hs01_00206194	GACAUUUGGACCGAUCUUU	SASI_Hs01_00206195
SENP2	GGAAUAAGUGACUAUCCAA	SASI_Hs01_00048880	GCUGUAACAGAGAUGAUUU	SASI_Hs02_00354976
SENP3	GUGCAUUGGUCCCUCAUCU	SASI_Hs01_00092168	CUGAUGCCAGCAUCCUCAU	SASI_Hs01_00092171
SENP5	CUGGUAACCAAAGGAUAUA	SASI_Hs02_00366591	GUGCUAAGACCAAGUUCAA	SASI_Hs01_00030279
SENP6	CAUACAGAUGGCUUAAGCA	SASI_Hs01_00034500	CCAUUAUUAAGAACGUCAA	SASI_Hs01_00034502
SENP7	CAAUAGCAGUGAUUGUGGA	SASI_Hs02_00312204	CAGUAUUAUGCCUAGUAAU	SASI_Hs02_00312205
SENP8	CAGUUCAUCAAGUGCACUA	SASI_Hs01_00195113	CCAACUUAUUUGAACAUUU	SASI_Hs01_00195114
Control	UGGUUUACAUGUCGACUAA	Human Non-Target 1_s	UGGUUUACAUGUUGUGUGA	Human Non-Target 2_s
Control	UGGUUUACAUGUUUUCUGA	Human Non-Target 3_s	UGGUUUACAUGUUUUCCUA	Human Non-Target 4_s

Supplementary Table 2: E2 ubiquitin-conjugaiting siRNA library

Target	siRNA_1	Name	siRNA_2	Name	siRNA_3	Name
AKTIP	GUUUACAUCCCUGAUAACU	SASI_Hs01_00086240	GAAUUUACCUUGGUUGUGA	SASI_Hs01_00086241	GCGUAUUUAAGUUUACAGU	SASI_Hs01_00086242
BIRC6	GUUAUGAGCUGCUUGUAGA	SASI_Hs01_00172666	GCUACAACAGGACUGACUA	SASI_Hs02_00349133	GGUACAAUCACAUCUAGCA	SASI_Hs02_00349134
TSG101	CUCAAUGCCUUGAAACGAA	SASI_Hs01_00180607	CUAUUGAAGACACUAUCUU	SASI_Hs01_00180608	CUUAUAGAGGUAAUACAUA	SASI_Hs02_00341284
UBE2A	CUUCUACCCUCUCCUUAAA	SASI_Hs01_00056615	GUCCUUCACUUGUGUUGGA	SASI_Hs02_00335488	GUACCUGGGUUACUUGCAA	SASI_Hs01_00056617
UBE2B	CUGGAUGAACCGAAUCCUA	SASI_Hs01_00017234	CUCCAUCUAUUGAUAGUUU	SASI_Hs01_00017235	CUCUCCAUCUAUUGAUAGU	SASI_Hs01_00017236
UBE2C	GCUCUCCAUCCAGAGCCUU	SASI_Hs01_00219473	CCAACAUUGAUAGUCCCUU	SASI_Hs01_00219474	GGACCAUUCUGCUCUCCAU	SASI_Hs01_00219475
UBE2D1	CCUUAAGACUGUGAUGAGA	SASI_Hs01_00156911	GAUAUUGCACAAAUCUAUA	SASI_Hs01_00156912	CAUAUCAAGGUGGAGUCUU	SASI_Hs01_00156913
UBE2D2	CUGAGAUUGCUCGGAUCUA	SASI_Hs01_00228722	GAAAUACUGUACUUCUGUA	SASI_Hs01_00228723	CCAUUUGACUGCUUUCUAU	SASI_Hs01_00228724
UBE2D3	GAGAAUUGGUAUUGAAUUU	SASI_Hs01_00192688	GCUUUAUGCUCAUAACUGA	SASI_Hs01_00192689	GGAGAAUUGGUAUUGAAUU	SASI_Hs01_00192690
UBE2D4	GCAGCAUGUUGGUGCCAUU	SASI_Hs01_00206454	CUCAUCUGCUGCAGAACCA	SASI_Hs01_00206455	GGCUUUGUUCGCACCUCAU	SASI_Hs01_00206457
UBE2E1	GCUUGUAGUCUGUAAAUUU	SASI_Hs01_00147122	GGUAUCUUGCUACAGUAGA	SASI_Hs01_00147123	CUGAAAUGUAGUACAGAAA	SASI_Hs01_00147124
UBE2E2	GAAUGGAGGUCAACUAUAU	SASI_Hs01_00102037	GGUUACCUUCCGAACAAGA	SASI_Hs01_00102038	CGGCUUUAACUAUUUCUAA	SASI_Hs01_00102039
UBE2E3	GUGUAUAUGUUAUACUGAU	SASI_Hs01_00107201	GUAGAUUAGUUAUGUUUAA	SASI_Hs01_00107202	CAUCUGUCUGGACAUCCUU	SASI_Hs01_00107203
UBE2F	GAGUUGUCUGCUUACCUUA	SASI_Hs01_00199216	GAGAGAACAUUCAAUUGAU	SASI_Hs01_00199220	CGGAAUAAAGUGGAUGACU	SASI_Hs02_00362455
UBE2G1	CAACAUGAUCCGGAUAAUA	SASI_Hs01_00219877	GGAAGAAUUGUAAAGACCU	SASI_Hs01_00219879	CACCUAUUGCUGGACUUCU	SASI_Hs02_00335492
UBE2G2	CACCCUUUCUGGGUCCUUC	SASI_Hs01_00077052	CCAGAACGCUCCUUCUGAA	SASI_Hs01_00077053	CCCAGAACGCUCCUUCUGA	SASI_Hs01_00077054
UBE2H	CAGCUCUUGGAUGUGAAGA	SASI_Hs02_00335498	GACUUAAUGAAUUUGUAGU	SASI_Hs01_00024164	GAGAGUAAACAUGAGGUUA	SASI_Hs01_00024165
UBE2J1	GAAGUCAAUUCAUCUGGAA	SASI_Hs01_00027696	CCUUUAGAGGAUAACCUUU	SASI_Hs01_00027697	CUUAUGAGCUGUGACUCAA	SASI_Hs01_00027698
UBE2J2	GGUUUGCAGCCUUUGCUUA	SASI_Hs01_00095058	GAGACGUCGGACUUCACGA	SASI_Hs01_00095059	CUGAAGUCGUGGAGGAGAU	SASI_Hs01_00095060
UBE2K	GCAUAGAGAGCUGCUGAUA	SASI_Hs02_00325256	GGAAGAAACAUGCUACUUU	SASI_Hs02_00325257	GUAGAUCUUGUAGAUGAGA	SASI_Hs02_00325258
UBE2L3	CUAGUUCUGCUCUCUUUGU	SASI_Hs01_00049984	GGCUUAUUGUUCCUGACAA	SASI_Hs01_00049991	CUGACAACCCUCCAUAUGA	SASI_Hs01_00049985
UBE2L6	GAUUUAGGUGCUAAACCGU	SASI_Hs01_00205565	CACUGAUGGGAAUAUACAG	SASI_Hs01_00205566	CCCAUCAUCAGCAGUGAGA	SASI_Hs01_00205567
UBE2M	CCUGCAGUAUCUCUUCUUG	SASI_Hs01_00232487	CUCCAUAAUUUAUGGCCUG	SASI_Hs01_00232488	GGUGAAGUGUGAGACAAUG	SASI_Hs01_00232489
UBE2N	CAGACAUCUUCAGUCCUUU	SASI_Hs01_00012964	GAAGAAUAUGUUUAGAUAU	SASI_Hs01_00012965	CCUCUUUGUUUGCAUUUAA	SASI_Hs01_00012966
UBE2NL	GUAUUUAAAUUGAUCCAAU	SASI_Hs01_00199377	CCAAUCAUUAAGUGUGCAU	SASI_Hs01_00199378	CCAGACAUCUUCAGUUAUU	SASI_Hs01_00199379
UBE2O	GAGACUUCGUGGUAGAUAA	SASI_Hs01_00037229	CUGACAUCGUCAUCCGCAU	SASI_Hs01_00037230	CAUGGAUUACCAAGAGUUU	SASI_Hs01_00037231
UBE2Q1	CUGUAUGAUUGGAAUGUCA	SASI_Hs01_00241781	GUAUGAUUGGAAUGUCAAA	SASI_Hs01_00241782	CAUGGAACUUCUCACCAAA	SASI_Hs01_00241783
UBE2Q2	GGCUAAAUAUGUUGACUGU	SASI_Hs01_00128047	GGUGUUACCUGUUCUCUCA	SASI_Hs01_00128048	CUGUUGUAUGUUUGGACUA	SASI_Hs01_00128049
UBE2R1	CUCCUUAGACGACAGACUA	SASI_Hs02_00337425	GGACCAUUCUCCUGAGUGU	SASI_Hs01_00071093	GAAGGAUCGGGAGUACACA	SASI_Hs01_00071094
UBE2R2	GAGUAACCCUCCACAGAAU	SASI_Hs01_00058641	CUUCAGAUUCUUGACCAAA	SASI_Hs01_00058647	CACAGAAUGUCUGAAUUCU	SASI_Hs01_00058642
UBE2S	CCCGUCUGCUCACAGAGAU	SASI_Hs01_00070712	GCCUGCUCUUGGAGAACUA	SASI_Hs01_00070713	CGCCUGCUCUUGGAGAACU	SASI_Hs01_00070714
UBE2T	GCCAACACACCUUAUGAGA	SASI_Hs01_00129086	CUGCUCAUGUCAGAACCCA	SASI_Hs01_00129087	CCAACACACCUUAUGAGAA	SASI_Hs01_00129088
UBE2U	CUGCUAAGCCUGUAAGUGA	SASI_Hs01_00182543	CCAGAAUACUGGUUAAAGA	SASI_Hs01_00182544	GAAUACUGGUUAAAGAUGA	SASI_Hs02_00366264
UBE2V1	GCCUAAUGAUGUCUAAAGA	SASI_Hs01_00020112	CUAUUUAAAUUGAUUCCCA	SASI_Hs01_00020119	CCACUGUCCACGUAGUUGA	SASI_Hs01_00020121
UBE2V2	GUUGUACUUCAAGAGCUAA	SASI_Hs01_00031079	GUCAGUUAGAUUUGUAACA	SASI_Hs01_00031080	CUGACUACUAGUAGUAUCA	SASI_Hs01_00031082
UBE2W	CAUGUAACAAGAAUCCAAA	SASI_Hs02_00303787	CAAUCAGUUUGUCUUAGCA	SASI_Hs02_00303788	CAACUGUUUAUAUUUGGAU	SASI_Hs01_00231191
UBE2Z	CUGUUGACAUGACUAAGAU	SASI_Hs01_00048090	GAAUGUAUCCGGCACGAGA	SASI_Hs01_00048091	GAAAGUCUGCUUGAGUAUU	SASI_Hs01_00048092
UEV3	GAUGUAUCAGGGUAAUACA	SASI_Hs01_00049331	GUAUCAGGGUAAUACAUAU	SASI_Hs01_00049334	CCAAUUCGUUUCUGGAUUU	SASI_Hs01_00049335

Supplementary Table 3: Mesothelioma IHC analysis

Group	Specimen ID	Histology	UBE2E3	UBE2E1	UBE2E2	ASXL2
WT BAP1	MVB0020	epithelial	positive on normal meso cells	Negative on tumor cells, positive mostly nuclear blood vessels, muscles cells and lymphocytes	Positive in cytoplasm of tumor cells. Stains cytoplasm of inflammatory cells, muscle cells and blood vessels.	Focally Positive in cytoplasm of tumor cells. Stains cytoplasm of blood vessles and inflammatory cells.
WT BAP1	MVB0061	sarcomatoid	mostly positive on nuclear and cytoplasmic, area where tumor cells are negative	n/a damaged sample	Negative in tumor cells	Negative
WT BAP1	MVB0077	biphasic	positive, mostly cytoplasmic	Negative in tumor cells	Strongly positive in cytoplasm of tumor cells	Focally positive in cytoplasm of tumor cells.
WT BAP1	MVB0428	epithelial	focally positive on both nuclear and cytoplasmic	Negative in tumor cells	Positive cytoplasmic staining in tumor cells *	Positive in nucleus of tumor cells*
WT BAP1	MVB0436	epithelial	positive, mostly nuclear	Negative in tumor cells	Positive cytoplasmic in tumor cells	Focally positive in cytoplasm of tumor cells.
WT BAP1	MVB0453	high grade epithelial/sarc	positive, mostly nuclear	Negative in tumor cells	Positive cytoplasmic in tumor cells * pic of blood vessels	Positive in cytoplasm only *
WT BAP1	MVB0456	epithelial/biphasic	positive, nuclear	Negative in tumor cells	Positive in cytoplasm of tumor cells	Focally positive mostly in cytoplasm in tumor cells. In normal mesothelial cells, focally positive 40%
WT BAP1	MVB0469	biphasic	positive, mostly nuclear	Focally positive nucleus 30% in tumor cells, Positive in lymphocytes nucleus	Positive in cytoplasm of tumor cells 70%	Positive nuclear and cytoplasmic staining
WT BAP1	MVB0479	biphasic	positive, nuclear	Focally positive in nucleus of tumor cells 30%	Negative in tumor cells	Negative
WT BAP1	MVB0489	epithelial	positive, nuclear	Negative in tumor cells	Negative in tumor cells	Negative
BAP1 mutant	MVB0026	epithelial	positive, mostly nuclear	Focally positive nuclear 30% of tumor cells, Some tumor cells also have nuclear plus cytoplasmic staining. Nuclear staining is always much stronger than cyto. Negative tumor cells are negative in cyto and nucleus. Stains nuclei of lymphocytes	Positive Largely cytoplasmic staining in tumor cells.	Focally positive nuclear 30% tumor cells and weak cytoplasmic staining.
BAP1 mutant	MVB0029	epithelial	focally positive, weak mostly cytoplasmic	Negative in tumor cells. Focally, some tumor cells show cytoplasmic staining. lymphocytes show nuclear staining	focally, 5%of tumor cells show cytoplasmic staining, tumor cells largely negative	Negative
BAP1 mutant	MVB0389	epithelial	negative, mostly. Few areas of positivity	Negative in tumor cells	Focally positive in cytoplasm of tumor cells 20% *	Negative
BAP1 mutant	MVB0405	epithelial	negative, largely. Some nuclear, focal positivity	Negative in tumor cells with some focal rare cytoplasmic staining. Lymphocytes and blood vessels are positive in nuclei	Focally positive in cytoplasm 10% of tumor cells. Normal mesothelial cells positive in cytoplasm	Negative on tumor cells, shows some nuclear positivity on normal mesothelial
BAP1 mutant	MVB0409	epithelial	negative, mostly. Some weak focal nuclear staining	Negative in tumor cells	Focally positive in cytoplasm 20% of tumor cells	Positive in nucleus of tumor cells
BAP1 mutant	MVB0432	epithelial	negative. Some weak focal cytoplasmic staining	Negative in tumor cells	Focally Positive in cytoplasm of tumor cells 70%	Negative
BAP1 mutant	MVB0447	epithelial	negative	Negative in tumor cells	Negative in tumor cells	Negative
BAP1 mutant	MVB0451	epithelial	positive, nuclear	Focally positive in nucleus of tumor cells 10%	Focally positive in cytoplasm *	Negative
BAP1 mutant	MVB0471	epithelial	negative. Occassional weak focal cytoplasmic staining	Focally positive in nuclei of tumor cells 50%	Focally positive in cytoplasm of tumor cells 10%. Plasma cells positive in cytoplasm	Positive in nucleus of tumor cells 50%
BAP1 mutant	MVB0491	biphasic	focally positive ~50% in nucleus	Positive in nucleus and cytoplasm 50% of tumor cells	Positive in cytoplasm of tumor cells	Positive in nucleus and cytoplams of tumor cells 20%

Supplementary Table 4: List of Oligonucleotides used

Primer name	Sequence
ASXL2ΔASXN_Forward	GGCCGCACATATGGGGAAGAAGTCATTCCAGA
ASXL2ΔASXN_Reverse	GATCTCTGGAATGACTTCTTCCCCATATGTGC
ASXL2ΔPHD_Forward	GGCTCCGCGGCCGCCCCCTTCACCATGAGGGAAAAGGGACGTAGGAAG
ASXL2ΔPHD_Reverse	GCCTTACCTGGCGCGCCTCATACCTG GCTACCTGGTACAGCAGAGTT
DEUBAD(ASXL2) K289R_Forward	GCACTGATCAACAGGCACACATTTTCAGTCCTT
DEUBAD(ASXL2) K289R_Reverse	GAAAATGTGTGCCTGTTGATCAGTGCTCGCAGAT
DEUBAD(ASXL2) K340R_Forward	CCAAGGCTGGAGGGAAAGACTCTCAGAAGGTGA
DEUBAD(ASXL2) K340R_Reverse	AGAGTCTTTCCCTCCAGCCTTGGGCTGCTGAAG
DEUBAD(ASXL2) K365R_Forward	GAAGGAGAAAAGAGTGGAGCCATGGAAAGAACA
DEUBAD(ASXL2) K365R_Reverse	CATGGCTCCACTCTTTTCTCCTTCTCAATCTCTTG
DEUBAD(ASXL2) K370R_Forward	GGAGCCATGGAGAGAACAATTCTTTGAAAGCTAC
DEUBAD(ASXL2) K370R_Reverse	AGAATTGTTCTCTCCATGGCTCCACTTTTTTCTCC
DEUBAD(ASXL2) K390R_Forward	TGAAGATTCTAGGAAATTGACAGCTTCTCCCAGT
DEUBAD(ASXL2) K390R_Reverse	AGCTGTCAATTTCCTAGAATCTTCAAGGCTCAGGC
DEUBAD(ASXL2) K391R_Forward	GAAGATTCTAAGAGATTGACAGCTTCTCCCAGTGA
DEUBAD(ASXL2) K391R_Reverse	AGAAGCTGTCAATCTCTTAGAATCTTCAAGGCTC
DEUBAD(ASXL1) K351R_Forward	GGAACAATGGAGAGAAAAGTTCTTTGAAGACTAC
DEUBAD(ASXL1) K351R_Reverse	AGAACTTTTCTCTCCATTGTTCCACCTTCTTTTC
DEUBAD(ASXL1) K353R_Forward	CAATGGAAAGAAAGGTTCTTTGAAGACTACTATGG
DEUBAD(ASXL1) K353R_Reverse	CTTCAAAGAACCTTTCTTTCCATTGTTCCACCT
DEUBAD(ASXL1) K362R_Forward	CTATGGACAGAGGCTGGGTTTGACCAAAGAAGAG
DEUBAD(ASXL1) K362R_Reverse	GTCAAACCCAGCCTCTGTCCATAGTAGTCTTCAA
GFP_gRNA_Forward	caccgGGGCGAGGAGCTGTTCACCG
GFP_gRNA_Reverse	aaacCGGTGAACAGCTCCTCGCCCc
hUBE2E3_gRNA-1_Forward	caccgCTGGAGCGGCTGGGTCTCGC
hUBE2E3_gRNA-1_Reverse	aaacGCGAGACCCAGCCGCTCCAGc
hUBE2E3_gRNA-2_Forward	caccgGGTGCTGGGGCTCTCATCAT
hUBE2E3_gRNA-2_Reverse	aaacATGATGAGAGCCCCAGCACCc
dsRNAi_UbcD2_Forward(+T7)	taatacgactcactatagggTTCGCTTAGCAACCGAAAAC
dsRNAi_UbcD2_Reverse(+T7)	taatacgactcactatagggCGCATTACGCATTAAAATAGGC
dsRNAi_Calypso_Forward(+T7)	taatacgactcactatagggATGCCTTGTTCCACACATCC
dsRNAi_Calypso_Reverse(+T7)	taatacgactcactatagggGAAATGGCCTCCTCATCCTT
dsRNAi_Luc_Forward(+T7)	taatacgactcactatagggTGCTGGACAGCTTCATCAAC
dsRNAi_Luc_Reverse(+T7)	taatacgactcactatagggATGTCCTCCTCGATGTCTGG
Asx_K325R_Forward	GGATCCCTGGcgcCTGAAACACTTTGAGCCCTT
Asx_K325R_Reverse	AGTGTTTCAGGcgcCCAGGGATCCAACTTGTTCT
DEUBAD(Asx)_Forward	GCGGCCGCcATGAGCGTTAAACCGCGT
DEUBAD(Asx)_Reverse	GGCGCGCCCCTGCAGGTTAATTTTTACA
Flag_Calypso_Forward	actcGGATCCatgGACTACAAAGACGATGACGACAAGAACGCTGCTGGAGGAGGTAGTG
Flag_Calypso_Reverse	gagtGATATCggcgcgccCTACTTCCGTTTTCTGCACTTGTT

Supplementary Table 5: List of antibodies used

Antibody	Source	Catalogue number	Dilution
Mouse monoclonal anti-BAP1 (C4)	Santa Cruz	sc-28383	1:1000
Rabbit polyclonal anti-BAP1 (H300)	Santa Cruz	sc-28236	1:1000
Rabbit polyclonal anti-YY1 (H414)	Santa Cruz	sc-1703	1:1000
Mouse monoclonal anti-CDC6 (180)	Santa Cruz	sc-9964	1:1000
Mouse monoclonal anti-Tubulin (B-5-1-2)	Santa Cruz	sc-SC-23948	1:2000
Rabbit polyclonal anti-ASXL2	Bethyl Laboratories	A302-037A	1:2000
Mouse monoclonal anti-Flag (M2)	Sigma-Aldrich	F3165	1:2000
Rabbit polyclonal anti-GST	Sigma-Aldrich	G7781	1:20000
Mouse monoclonal anti-MYC	Covance	9E10	1:1000
Rabbit polyclonal anti-H2Aub K119	Cell signaling	D27C4	1:30000
Mouse monoclonal anti-MBP	Cell signaling	E8038	1:5000
Mouse anti-V5	Abcam	ab27671	1:2000
Mouse anti-Myc (home-made)	Mashtalir et al., 2014	N/A	1:1000
Mouse anti-HA (home-made)	Mashtalir et al., 2014	N/A	1:1000
Rabbit polyclonal anti-UBE2E1	Boston Biochem	A-630	1:1000
Rabbit polyclonal anti-UBE2E2	Bethyl Laboratories	A303-485A	1:2000
Mouse polyclonal anti-UBE2E3	Abcam	ab66257	1:2000
Mouse anti-Ubx	DSHB	FP3.38	1:200
Rabbit polyclonal anti-UBE2E1/UbcH6 (for IHC)	Novus Biologicals	NBP1-78983	1:600
Mouse monoclonal anti-UBE2E3 (7E8) (for IHC)	Novus Biologicals	NBP2-03819	1:200
Rabbit polyclonal anti-H3	Abcam	ab1791	1:50000
Rabbit polyclonal anti-UBE2O	Novus Biologicals	NBP1-32020	1:2000
Anti-mouse Alexa Fluor® 594	Life Technologies	A11005	1:1000
anti-mouse Alexa Fluor® 488	Life Technologies	A11029	1:1000
Anti-rabbit Alexa Fluor® 488	Life Technologies	A11008	1:1000
anti-rabbit Alexa Fluor® 594	Life Technologies	A11012	1:1000

Supplementary Table 6: List of commercial enzymes used for ubiquitination assays

Recombinant Enzyme	Source	Catalogue number
UBE1	Boston Biochem	E-305
UbcH5a/UBE2D1	Boston Biochem	E2-616
UbcH5b/UBE2D2	Boston Biochem	E2-622
His6-UbcH6/UBE2E1	Boston Biochem	E2-630
UbcH7/UBE2L3	Boston Biochem	E2-640
UbcH8/UBE2L6	Boston Biochem	E2-644
His6-UBE2E3	Boston Biochem	E2-678
His6-UbcH10/UBE2C	Boston Biochem	E2-650
His6-UBE2W Isoform 1	Boston Biochem	E2-740
His6-UBE2S	Boston Biochem	E2-690
His6-UBE2F	Boston Biochem	E2-690
UBE2K/E2-25K	Boston Biochem	E2-603
UBE2M/Ubc12	Boston Biochem	E2-656
His6-UbcH3/Cdc34	Boston Biochem	E2-610
His6-UBE2T	Boston Biochem	E2-695
His6-UBE2N/Ubc13	Boston Biochem	E2-660
His6-UBE2N (Ubc13)/UBE2V1 (Uev1) Complex	Boston Biochem	E2-664
His6 UBE2N/UBE2V2 Complex	Boston Biochem	E2-666
UbcH2/UBE2H	Boston Biochem	E2-607
His6-HR6A/UBE2A	Boston Biochem	E2-612
His6-HR6B/UBE2B	Boston Biochem	E2-613
UBE2I (Ubc9)	Boston Biochem	E2-645
His6-Uev1a/UBE2V1	Boston Biochem	E2-662
His6-Use1/UBE2Z	Boston Biochem	E2-677
His6-UBE2G2	Boston Biochem	E2-680
His6-UBE2G1/E2-17K	Boston Biochem	E2-700
His6-UBE2D4 (UbcH5D)	Boston Biochem	E2-705
His6-UBE2J2	Boston Biochem	E2-710
His6-UBE2R2/CDC34B	Boston Biochem	E2-715
His6-UBE2Q1-2	Boston Biochem	E2-720
His6-UBE2Q2-1	Boston Biochem	E2-745
His6-UBE2J1	Boston Biochem	E2-750

Supplementary Table 7: Characteristics of the human patient's samples used in the study

ID numbers	Age	Gender	Genotype (WT or BAP1 mutant)	MM Diagnosis (Primary vs. Metastatic)	Type of Therapy
MVB0020	31	Female	WT	Primary and metastatic	Surgery and chemotherapy
MVB0061	78	Male	WT	Primary	Surgery
MVB0077	84	Male	WT	Primary	Talc Pleurodesis
MVB0428	66	Female	WT	Primary	Surgery and chemotherapy
MVB0436	32	Male	WT	Primary	Surgery and chemotherapy
MVB0453	34	Female	WT	Primary	Surgery and chemotherapy
MVB0456	23	Female	WT	Primary	Surgery
MVB0469	46	Female	WT	Primary	Surgery and chemotherapy
MVB0479	73	Male	WT	Primary	Surgery and chemotherapy
MVB0489	43	Male	WT	Primary	Surgery and chemotherapy
MVB0026	85	Male	BAP1 mutant	Primary	Talc Pleurodesis and radiation
MVB0029	73	Male	BAP1 mutant	Primary and metastatic	Surgery
MVB0389	60	Male	BAP1 mutant	Primary	Surgery and chemotherapy
MVB0405	67	Male	BAP1 mutant	Primary	Surgery and radiation
MVB0409	63	Male	BAP1 mutant	Primary	Surgery and chemotherapy
MVB0432	77	Male	BAP1 mutant	Primary	Chemotherapy
MVB0447	51	Female	BAP1 mutant	Primary and metastatic	Surgery and chemotherapy
MVB0451	71	Male	BAP1 mutant	Primary	Chemotherapy
MVB0471	59	Male	BAP1 mutant	Primary and metastatic	Surgery and chemotherapy
MVB0491	68	Female	BAP1 mutant	Primary	Surgery, chemotherapy and talc pleurodesis