Supplementary Information

Table S1. Affinity measurement for some complexes cited in the current review. The '.' symbol represents a covalent bond whereas '/' represents a non-covalent interaction for a given complex. NMR: Nuclear Magnetic Resonance; SPR: Surface plasmon resonance; ITC: Isothermal Titration Calorimetry.

Complex	Kd	Method
hMms2/Ubc13	2 µM	Sedimentation [1]
hMms2/Ub	$98 \pm 5 \ \mu M$	NMR [2]
hMms2.Ubc13/Ub	$28 \pm 6 \ \mu M$	NMR [2]
hMms2/Ubc13	$49 \pm 7 \text{ nM}$	ITC [2]
HECT-Nedd4/Ub	11 μM	SPR [3]
HECT-Rsp5/Ub	$90\pm6.4~\mu M$	Fluorescence [4]
AMSH/Ub	$19 \pm 3 \ \mu M$	ITC [5]
AMSH/Lys63-Ub ₂	$19 \pm 4 \ \mu M$	ITC [5]
Sst2/Ub	$10.2 \pm 0.6 \ \mu M$	ITC [6]
Ataxin-3/Ub site1	$58 \pm 68 \ \mu M$	NMR [7]
Ataxin-3/Ub site2	$58 \pm 32 \ \mu M$	NMR [7]
Ataxin-3 ^{UIM12} /Ub	$97.0 \pm 16.9 \ \mu M$	NMR [8]
Ataxin-3 ^{UIM12} /Ub2	$Kd1 = 10 \ \mu M; Kd2 = 100-300 \ \mu M$	NMR [8]
STAM2 ^{VHS-UIM/} Lys63-Ub ₂	$Kd1 = 35 \pm 10 \ \mu M; \ Kd2 = 2 \pm 2 \ \mu M$	NMR [9]
$Vps36^{NZF2}/Lys63-Ub_2$	$0.38\pm0.10~\mu M$	Fluorescence [10]
Vps36 ^{NZF2} /Ub	$92 \pm 49 \ \mu M$	Fluorescence [10]
ALIX ^V /Ub	$119 \pm 9 \ \mu M$	Fluorescence [11]
p62 ^{UBA} -monomer/p62 ^{UBA} -dimer	3 µM	NMR [12]
$Nbr1^{UBA}/Lys63-Ub_2$	$4.9\pm2.0\;\mu M$	ITC [13]
Nbr1 ^{UBA} /Lys63-Ub ₂	$10.2 \pm 1.1 \ \mu M$	ITC [13]

Table S2. The different structures cited in the current review along with the method used. The '.' symbol represents a covalent bond whereas '/' represents a non-covalent interaction for a given complex.

Structure	Organism	PDB Code	Method
Mms2/Ubc13	human	1J7D	X-ray [1]
Mms2/Ubc13	yeast	1JAT	X-ray [14]
Mms2/Ubiquitin	human	1ZGU	NMR [15]
Mms2/Ubc13.Ub	yeast	2GMI	X-ray [16]
Nedd4L/UbcH5B.Ub	human	3JW0	X-ray [17]
Nedd4.Ub	human	4BBN	X-ray [18]
Rsp5.Ub/Sna3	yeast	4LCD	X-ray [19]
Nedd4/Ub	human	2XBB	X-ray [3]
Rsp5/Ub	yeast	30LM	X- ray [4]
SSt2/Ub	yeast	4K1R	X-ray [6]
AMSH-LP/Lys63-Ub ₂	human	2ZNV	X-ray [20]
AMSH	human	3RZU	X-ray [21]
AMSH-LP	human	2ZNR	X-ray [20]
Ataxin-3 ^{Josephin} /Ub	human	2JRI	NMR [7]
Ataxin-3 ^{UIM12} /Ub	human	2KLZ	NMR [8]

Structure	Organism	PDB Code	Method
Rap80 ^{UIM1-UIM2} /Lys63-Ub ₂	human	2RR9	NMR [22]
$Rap80^{UIM1-UIM2}/LYS63\text{-}Ub_2$	human	3A1Q	X-ray [22]
$TAB2^{NZF}/Lys63-Ub_2$	mouse	3A9J	X-ray [23]
$TAB2^{NZF}/Lys63-Ub_2$	human	2WWZ	X-ray [10]
$Alix^V$ open	human	4JJY	X-ray [24]
$Alix^V$ closed	human	20EV	X-ray [25]
NEMO/Lys63-Ub ₂	mouse	3JSV	X-ray [26]
NEMO/Met1-Ub ₂	mouse	2ZVO	X-ray [27]

Table II. Cont.

References

- Moraes, T.F.; Edwards, R.A.; McKenna, S.; Pastushok, L.; Xiao, W.; Glover, J.N.; Ellison, M.J. Crystal structure of the human ubiquitin conjugating enzyme complex, HMMS2-HUBC13. *Nat. Struct. Biol.* 2001, *8*, 669–673.
- McKenna, S.; Moraes, T.; Pastushok, L.; Ptak, C.; Xiao, W.; Spyracopoulos, L.; Ellison, M.J. An NMR-based model of the ubiquitin-bound human ubiquitin conjugation complex MMS2.Ubc13. The structural basis for lysine 63 chain catalysis. *J. Biol. Chem.* 2003, 278, 13151–13158.
- Maspero, E.; Mari, S.; Valentini, E.; Musacchio, A.; Fish, A.; Pasqualato, S.; Polo, S. Structure of the hect: Ubiquitin complex and its role in ubiquitin chain elongation. *EMBO Rep.* 2011, 12, 342–349.
- 4. Kim, H.C.; Steffen, A.M.; Oldham, M.L.; Chen, J.; Huibregtse, J.M. Structure and function of a hect domain ubiquitin-binding site. *EMBO Rep.* **2011**, *12*, 334–341.
- 5. Davies, C.W.; Paul, L.N.; Das, C. Mechanism of recruitment and activation of the endosomeassociated deubiquitinase AMSH. *Biochemistry* **2013**, *52*, 7818–7829.
- Shrestha, R.K.; Ronau, J.A.; Davies, C.W.; Guenette, R.G.; Strieter, E.R.; Paul, L.N.; Das, C. Insights into the mechanism of deubiquitination by jamm deubiquitinases from cocrystal structures of the enzyme with the substrate and product. *Biochemistry* 2014, *53*, 3199–3217.
- Nicastro, G.; Todi, S.V.; Karaca, E.; Bonvin, A.M.; Paulson, H.L.; Pastore, A. Understanding the role of the josephin domain in the polyub binding and cleavage properties of Ataxin-3. *PLoS One* 2010, *5*, e12430.
- 8. Song, A.X.; Zhou, C.J.; Peng, Y.; Gao, X.C.; Zhou, Z.R.; Fu, Q.S.; Hong, J.; Lin, D.H.; Hu, H.Y. Structural transformation of the tandem ubiquitin-interacting motifs in Ataxin-3 and their cooperative interactions with ubiquitin chains. *PLoS One* **2010**, *5*, e13202.
- Lange, A.; Ismail, M.B.; Riviere, G.; Hologne, M.; Lacabanne, D.; Guilliere, F.; Lancelin, J.M.; Krimm, I.; Walker, O. Competitive binding of ubpy and ubiquitin to the STAM2 SH3 domain revealed by NMR. *FEBS Lett.* 2012, 586, 3379–3384.
- 10. Kulathu, Y.; Akutsu, M.; Bremm, A.; Hofmann, K.; Komander, D. Two-sided ubiquitin binding explains specificity of the TAB2 NZF domain. *Nat. Struct. Mol. Biol.* **2009**, *16*, 1328–1330.

- Keren-Kaplan, T.; Attali, I.; Estrin, M.; Kuo, L.S.; Farkash, E.; Jerabek-Willemsen, M.; Blutraich, N.; Artzi, S.; Peri, A.; Freed, E.O.; *et al.* Structure-based in silico identification of ubiquitin-binding domains provides insights into the Alix-v: Ubiquitin complex and retrovirus budding. *EMBO J* 2013, *32*, 538–551.
- Isogai, S.; Morimoto, D.; Arita, K.; Unzai, S.; Tenno, T.; Hasegawa, J.; Sou, Y.S.; Komatsu, M.; Tanaka, K.; Shirakawa, M.; *et al.* Crystal structure of the ubiquitin-associated (uba) domain of p62 and its interaction with ubiquitin. *J. Biol. Chem.* 2011, 286, 31864–31874.
- 13. Walinda, E.; Morimoto, D.; Sugase, K.; Konuma, T.; Tochio, H.; Shirakawa, M. Solution structure of the ubiquitin-associated (UBA) domain of human autophagy receptor nbr1 and its interaction with ubiquitin and polyubiquitin. *J. Biol. Chem.* **2014**.
- VanDemark, A.P.; Hofmann, R.M.; Tsui, C.; Pickart, C.M.; Wolberger, C. Molecular insights into polyubiquitin chain assembly: Crystal structure of the mms2/ubc13 heterodimer. *Cell* 2001, 105, 711–720.
- Lewis, M.J.; Saltibus, L.F.; Hau, D.D.; Xiao, W.; Spyracopoulos, L. Structural basis for noncovalent interaction between ubiquitin and the ubiquitin conjugating enzyme variant human MMS2. *J. Biomol. NMR* 2006, *34*, 89–100.
- Eddins, M.J.; Carlile, C.M.; Gomez, K.M.; Pickart, C.M.; Wolberger, C. MMS2-UBC13 covalently bound to ubiquitin reveals the structural basis of linkage-specific polyubiquitin chain formation. *Nat. Struct. Mol. Biol.* 2006, *13*, 915–920.
- Kamadurai, H.B.; Souphron, J.; Scott, D.C.; Duda, D.M.; Miller, D.J.; Stringer, D.; Piper, R.C.; Schulman, B.A. Insights into ubiquitin transfer cascades from a structure of a UBCH5B approximately ubiquitin-hect(nedd4l) complex. *Mol. Cell.* 2009, *36*, 1095–1102.
- Maspero, E.; Valentini, E.; Mari, S.; Cecatiello, V.; Soffientini, P.; Pasqualato, S.; Polo, S. Structure of a ubiquitin-loaded hect ligase reveals the molecular basis for catalytic priming. *Nat. Struct. Mol. Biol.* 2013, 20, 696–701.
- Kamadurai, H.B.; Qiu, Y.; Deng, A.; Harrison, J.S.; Macdonald, C.; Actis, M.; Rodrigues, P.; Miller, D.J.; Souphron, J.; Lewis, S.M.; *et al.* Mechanism of ubiquitin ligation and lysine prioritization by a hect E3. *Elife* 2013, *2*, e00828.
- Sato, Y.; Yoshikawa, A.; Yamagata, A.; Mimura, H.; Yamashita, M.; Ookata, K.; Nureki, O.; Iwai, K.; Komada, M.; Fukai, S. Structural basis for specific cleavage of lys 63-linked polyubiquitin chains. *Nature* 2008, 455, 358–362.
- Davies, C.W.; Paul, L.N.; Kim, M.I.; Das, C. Structural and thermodynamic comparison of the catalytic domain of AMSH and AMSH-LP: Nearly identical fold but different stability. *J. Mol. Biol.* 2011, *413*, 416–429.
- Sekiyama, N.; Jee, J.; Isogai, S.; Akagi, K.; Huang, T.H.; Ariyoshi, M.; Tochio, H.; Shirakawa, M. NMR analysis of lys63-linked polyubiquitin recognition by the tandem ubiquitin-interacting motifs of Rap80. *J. Biomol. NMR* 2012, *52*, 339–350.
- Sato, Y.; Yoshikawa, A.; Yamashita, M.; Yamagata, A.; Fukai, S. Structural basis for specific recognition of lys 63-linked polyubiquitin chains by NZF domains of TAB2 and TAB3. *EMBO J.* 2009, *28*, 3903–3909.

- Pashkova, N.; Gakhar, L.; Winistorfer, S.C.; Sunshine, A.B.; Rich, M.; Dunham, M.J.; Yu, L.; Piper, R.C. The yeast alix homolog BRO1 functions as a ubiquitin receptor for protein sorting into multivesicular endosomes. *Dev. Cell.* 2013, 25, 520–533.
- 25. Fisher, R.D.; Chung, H.Y.; Zhai, Q.; Robinson, H.; Sundquist, W.I.; Hill, C.P. Structural and biochemical studies of Alix/Aip1 and its role in retrovirus budding. *Cell* **2007**, *128*, 841–852.
- 26. Yoshikawa, A.; Sato, Y.; Yamashita, M.; Mimura, H.; Yamagata, A.; Fukai, S. Crystal structure of the nemo ubiquitin-binding domain in complex with lys 63-linked di-ubiquitin. *FEBS Lett.* **2009**, *583*, 3317–3322.
- Rahighi, S.; Ikeda, F.; Kawasaki, M.; Akutsu, M.; Suzuki, N.; Kato, R.; Kensche, T.; Uejima, T.; Bloor, S.; Komander, D.; *et al.* Specific recognition of linear ubiquitin chains by nemo is important for NF-κB activation. *Cell* **2009**, *136*, 1098–1109.