Appendix

The UBA domain of Ubc1 (Ube2K) Facilitates the Assembly of K48/K63-Branched Ubiquitin Chains

Table S1. Plasmids

name	insert	backbone	origin
Ubc1 (recombinant expression in *E. coli*)			
pTX449	GST-Ubc1 (GST cleavable with HRV3C)	pGex-6p1	this study
pTX457	GST-Ubc1-ΔUBA (Ubc1,1-150)	pGex-6p1	this study
pTX432	GST-UBA (Ubc1,151-215)	pGex-6p1	this study
pTX454	GST-Ubc1 LRV (QGF179-181)	pGex-6p1	this study
pTX451	GST-Ubc1 K93R	pGex-6p1	this study
pTX459	GST-Ubc1-ΔUBA K93R	pGex-6p1	this study
pLP034	GST-Ubc1 EEAA (E211,E212)	pGex-6P1	this study
pLP106	GST-Ubc1 K93R, EEAA (E211,E212)	pGex-6P1	this study
pLP007	GST-Ubc1(K93R)-3xGS-CUE-GS-6xHis	pGex-6p1	this study
pLP008	GST-Ubc1(K93R)-6xGS-CUE-GS-6xHis	pGex-6p1	this study
pLP009	GST-Ubc1(K93R)-12xGS-CUE-GS-6xHis	pGex-6p1	this study
pLP010	GST-Ubc1(K93R)-3xGS-UBA(Dsk2)	pGex-6p1	this study
pLP011	GST-Ubc1(K93R)-6xGS-UBA(Dsk2)	pGex-6p1	this study
pLP012	GST-Ubc1(K93R)-12xGS-UBA(Dsk2)	pGex-6p1	this study
Ubiquitin (recombinant expression in *E. coli*)			
pMD10	hUb (human ubiquitin, codon optimised for *E. Coli*)	pETM60	von Delbrück *et al,* 2016
pMD16	hUb K48R	pETM60	von Delbrück *et al,* 2016
pMD18	hUb K63R	pETM60	von Delbrück *et al,* 2016
pLP109	hUb K48R, K63R	pETM60	this study
pMD14	hUb R42A	pETM60	von Delbrück *et al,* 2016
pLP107	hUb R42A, K48R	pETM60	this study
pMD11	hUb-6xHis	pETM60	von Delbrück *et al,* 2016
pMD17	hUb-6xHis K48R	pETM60	von Delbrück *et al,* 2016
pMD15	hUb-6xHis R42A	pETM60	von Delbrück *et al,* 2016
pLP108	hUb-6xHis R54A	pETM60	this study
pLP079	linUb2-6xHis	pRSF Duet	this study
pLP110	linUb2-6xHis K48R(proximal)	pRSF Duet	this study
pLP111	linUb2-6xHis K48R(distal)	pRSF Duet	this study
pMD12	human ubiquitin S20C (*E. coli* codon optimized)	pETM60	von Delbrück *et al,* 2016
pMD13	hUb S20C - 6xHis	PETM60	von Delbrück *et al,* 2016
Vectors for ubiquitin expression in *S. cerevisiae*			
pGR295	10xHis-Ub	-	Provided by Gwenael Rabut
pLP048	10xHis-Ub R54A	pGR295	this study
pLP105	10xHis-Ub R54A, K63R	pGR295	this study
pLP069	Ubc1(URA) deletion-cassette	pBluescript	this study
Other enzymes and proteins			
pMD28	GST-Ubc13	pGEX4T1	Mansour *et al,* 2015
pMD29	GST-Uev1a	pGEX6p1	Mansour *et al,* 2015
pMD27	Ube2K 6xHis	pET28	gift from Prof. R. Klevit, (Christensen *et al,* 2007)
pLP054	Ube2K K97R, 6xHis	pET28	this study
pLP061	GST-Ubc20	pGex-6p1	this study
pTX481	hUbe1-6xHis	pET21d	Berndsen *et al,* 2011
pTX249	Ubc7	pGEX6p1	Bagola *et al,* 2013
pMD26	Cdc34	pGEX6p1	von Delbrück *et al,* 2016
pSH006	GST-Dsk2-UBA (aa241-374)	pGex-6p1	Bagola *et al,* 2013
pTX410	Cue1 (ΔTM,24-203) pGex-6p1	pGex-6p1	Bagola *et al,* 2013
pTX410	Cue1 (ΔTM,24-203) pGex-6p1	pGex-6p1	Bagola *et al,* 2013

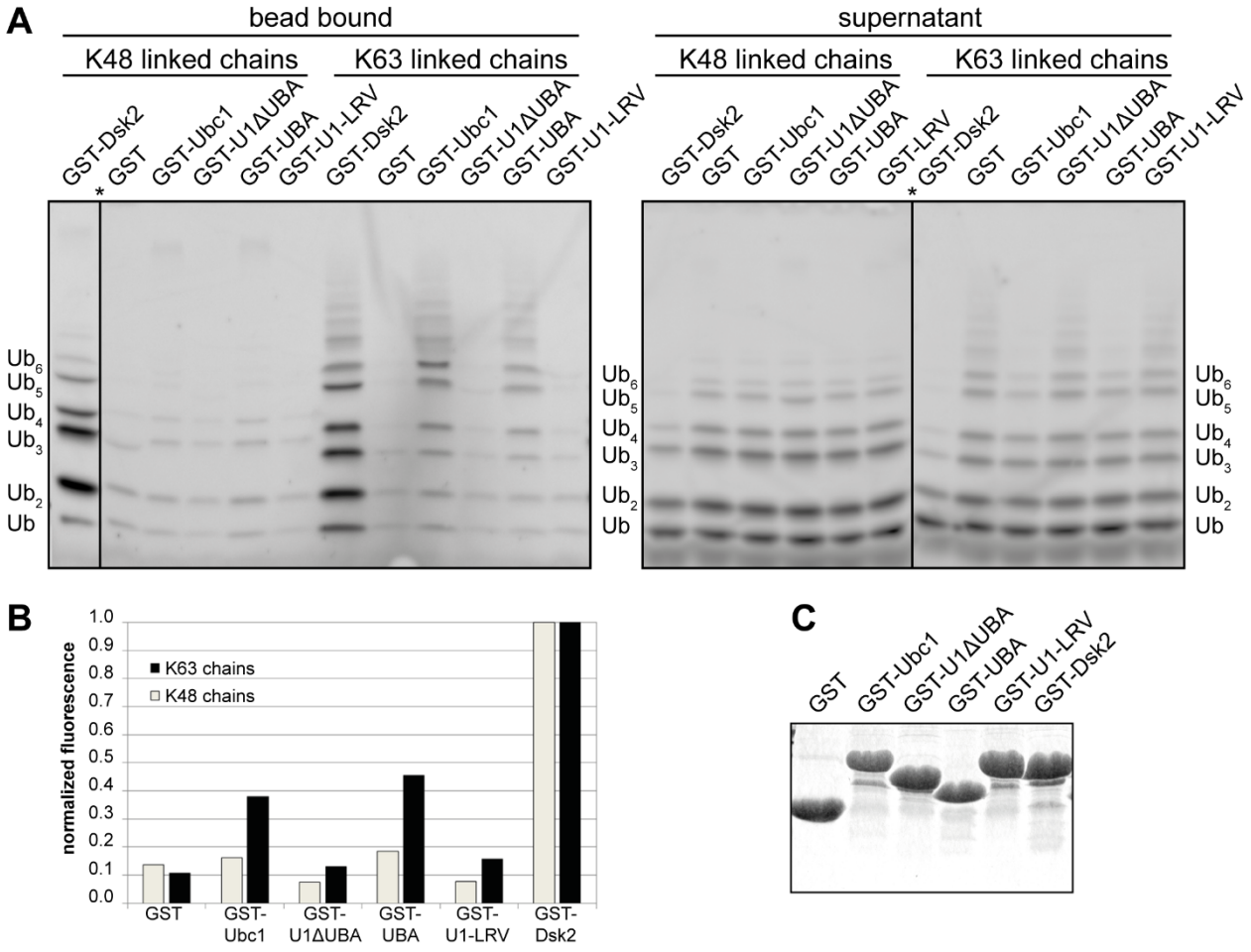

Figure S1. The UBA domain of Ubc1 mediates preferential binding to K63-linked chains over K48-linked chains. A) *In vitro* binding experiments were performed with fluorescently labeled K48-linked chains or K63-linked chains in presence of various GST fusion proteins immobilized on GSH resin. The UBA domain of Dsk2 (GST-Dks2, aa241-374) was used as positive binding control, because it was previously reported to interact unspecifically with differently linked Ub chains. (Raasi *et al,* 2005) GST expressed from an empty pGex6p1-vector was used as negative control. Ubc1 expression vectors fused to GST harbored either the full length enzyme (GST-Ubc1), only the catalytic core domain (GST-Ubc1-ΔUBA, aa1-150), the UBA domain (GST-UBA, aa151-215) or a Ubc1 variant with amino acid substitutions, which were previously reported to disrupt Ub binding (Wilson *et al,* 2009) (GST-LRV, aa179-181 QGF to LRV). Ub chains associated with resin fraction ("bead bound" as shown in Fig 1A) were separated from unbound Ub chains ("supernatant") and analyzed by SDS-PAGE and fluorescence scan. Asterisk indicates that lanes from the same gel were cropped and moved. (B) Total fluorescence per lane was quantified. Fluorescence in bead bound fraction over total fluorescence in both fractions was calculated and normalized to Dsk2-UBA. (C) Input controls of immobilized GST fusion proteins were analyzed by SDS-PAGE and Coomassie staining.

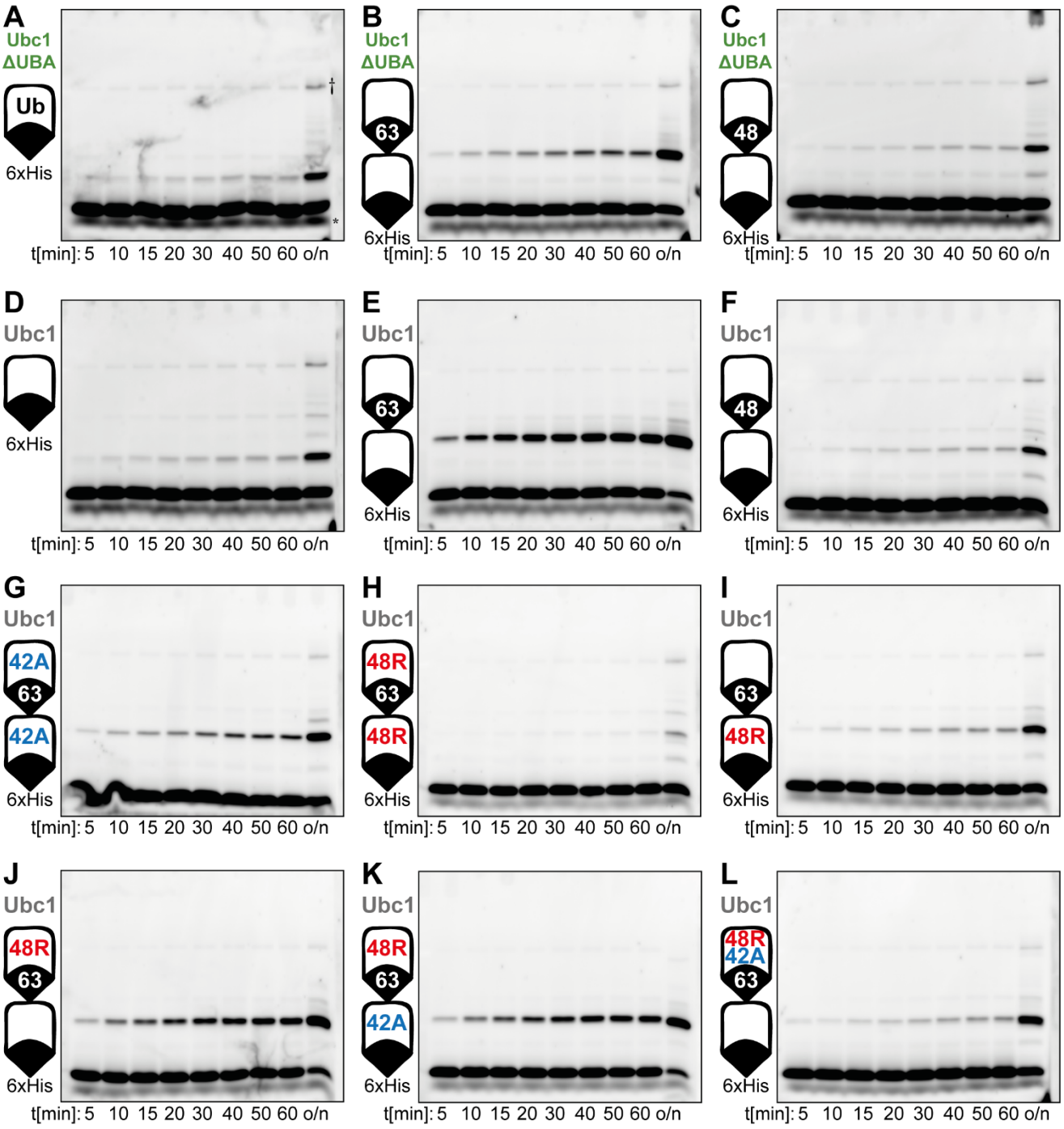

Figure S2. Representative SDS-PAGE and fluorescence scans of single turnover ubiquitination experiments for kinetic analysis described in Fig 2. Top row shows reactions with Ubc1(K93R)-ΔUBA (aa1-150), while the remaining reactions were performed in presence of full-length Ubc1(K93R). Asterisk (*) indicates signal from free dye. Cross (†) indicates E1 enzyme modified with fluorescent Ub. C-terminally capped (6xHis-tagged) acceptor Ub was (A,D) monoubiquitin, (B,E) K63-linked diubiquitin (K63-Ub_2) or (C,F) K48-Ub_2. Moreover, mutated acceptor K63-Ub_2 was used with (G) R42A in both moieties, (H) K48R in both moieties, (I) K48R in the proximal moiety, (J) K48R in the distal moiety, (K) K48R in the distal moiety plus R42A in the proximal moiety or (L) K48R plus R42A in the distal moiety.

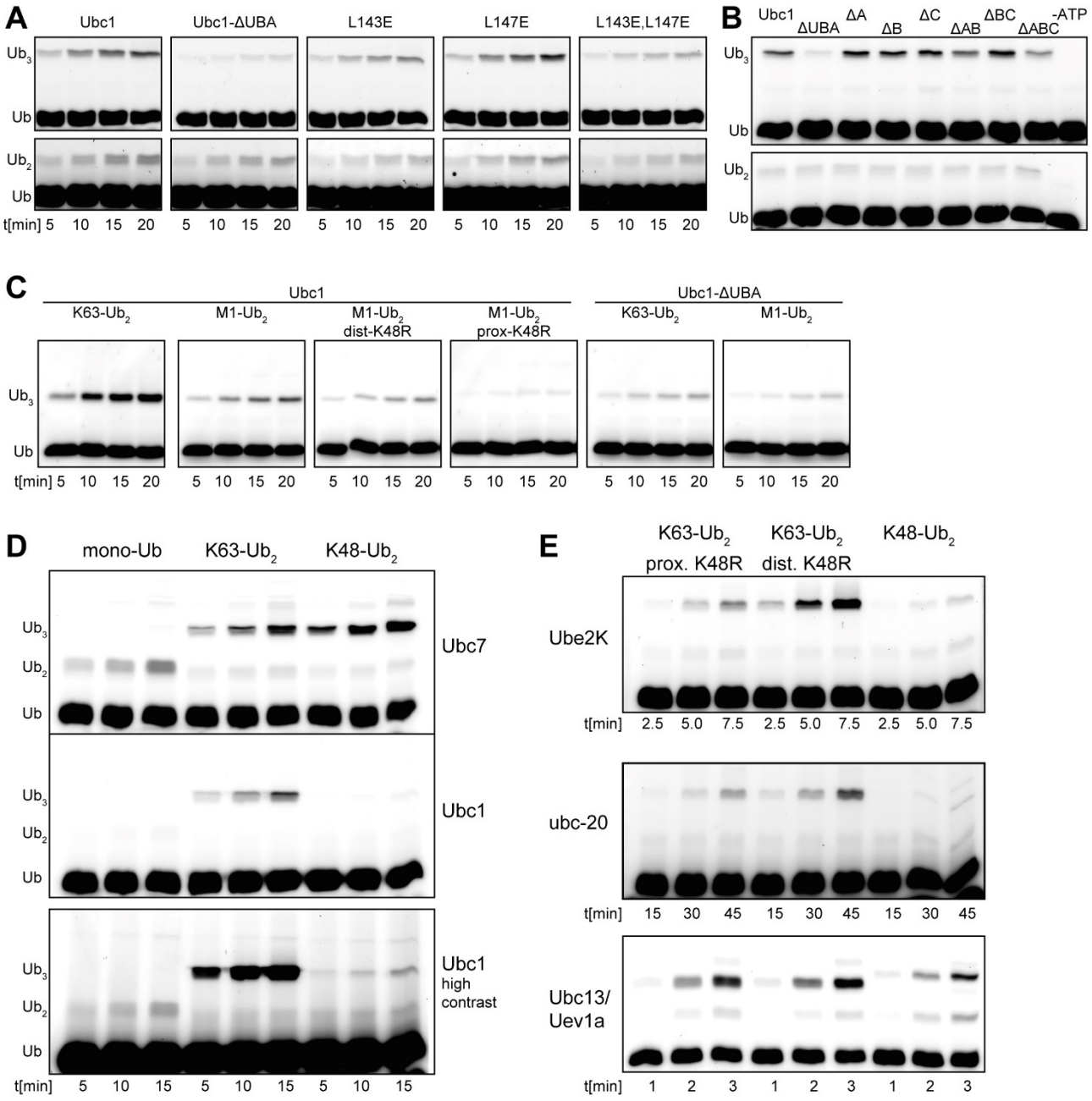

Figure S3. Representative SDS-PAGE and fluorescence scans for single turnover ubiquitination experiments Fig 3, Fig 5 and Fig 6. Images show scans for (A) Fig 3B, (B) Fig 3C, (C) Fig 3D, (D) Fig 5A and (E) Fig 5B-C. (A) Reactions with K63-Ub_2 as acceptor are shown in the top panels and reactions with acceptor monoubiquitin in the lower panels. (B) Reactions were analyzed after 5 min for K63-Ub_2 as acceptor (top) or after 20 min for monoubiquitin as acceptor (bottom). Ubc1 constructs as explained in Fig 3C and a control without ATP with wildtype Ubc1 ("-ATP"). (C) Acceptor Ub and Ubc1 constructs are indicated above the scans. (D) Top panel displays reactions with Ubc7. Reactions with Ubc1 at the same contrast level are shown in the mid panel and with higher contrast at the bottom. (E) Acceptor Ub is indicated above the scans and enzymes used on the left.

Appendix References

Bagola K, von Delbrück M, Dittmar G, Scheffner M, Ziv I, Glickman MH, Ciechanover A, Sommer T (2013) Ubiquitin binding by a CUE domain regulates ubiquitin chain formation by ERAD E3 ligases. *Mol Cell* 50: 528–539

Berndsen CE, Wolberger C (2011) A spectrophotometric assay for conjugation of ubiquitin and ubiquitin-like proteins. *Anal Biochem* 418: 102–110

Christensen DE, Brzovic PS, Klevit RE (2007) E2–BRCA1 RING interactions dictate synthesis of mono- or specific polyubiquitin chain linkages. *Nat Struct Mol Biol* 14: 941–948

Mansour W, Nakasone MA, von Delbrück M, Yu Z, Krutauz D, Reis N, Kleifeld O, Sommer T, Fushman D, Glickman MH (2015) Disassembly of Lys 11 and mixed linkage polyubiquitin conjugates provides insights into function of proteasomal deubiquitinases Rpn11 and Ubp6. *J Biol Chem* 290: 4688–4704