SUPPLEMENTARY INFORMATION

Manuscript:

"Differential inhibition of human and trypanosome ubiquitin E1s by TAK-243 offers possibilities for parasite selective inhibitors."

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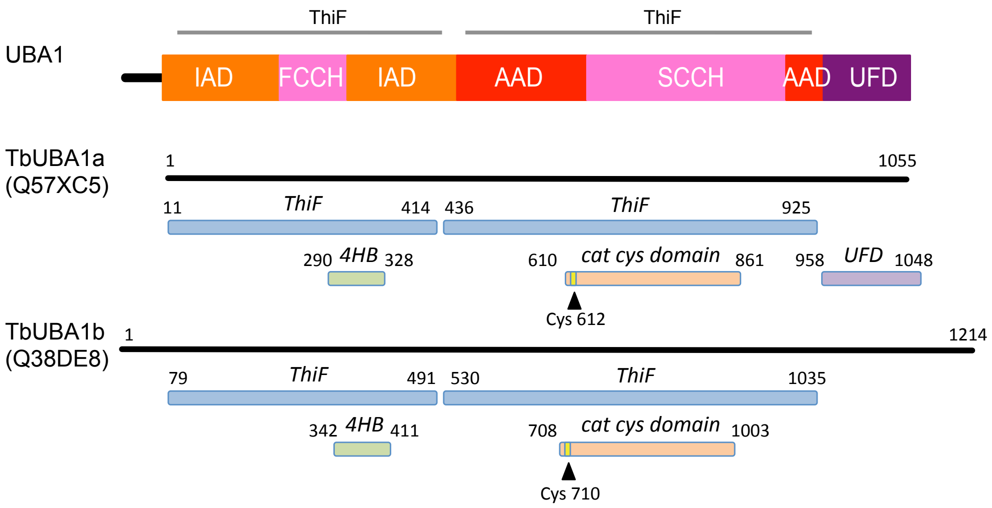
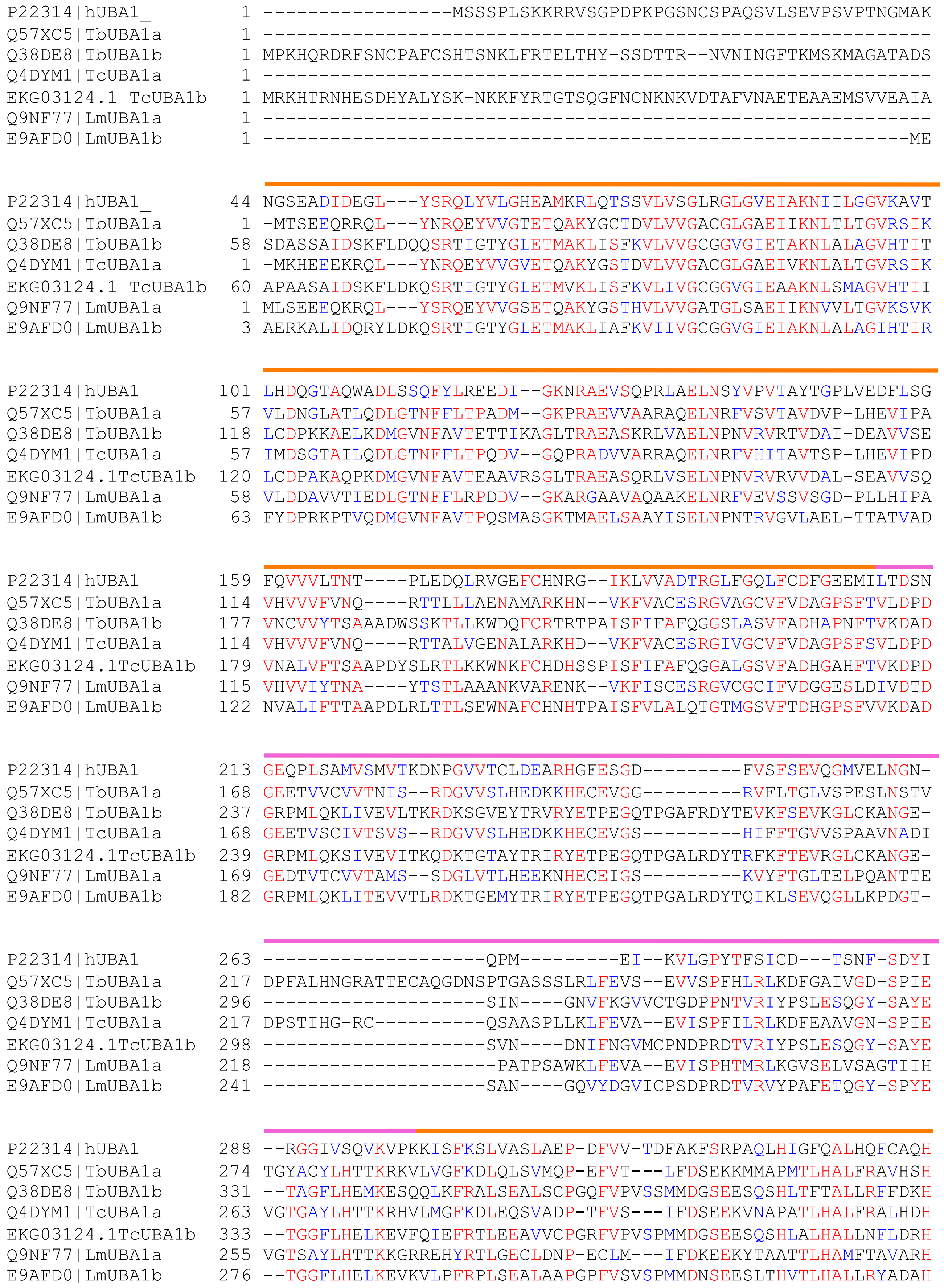


Figure S1. Domains in TbUBA1a and TbUBA1b as found on the UniProt website.

The domains are: the THIF-type NAD/FAD binding fold (ThiF, pf00899), the 4 helix bundle (4HB, pf16191), the UBA-E1-thiolCys (catalytic cys domain, pf10585) and the E1-Ubiquitin fold domain (UFD, pf09358).

Figure S2



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Q38DE8|TbUBA1b        1 MPKHQRDRFSNCPAFCSHTSNKLFRTELTHY-SSDTTR--NVNINGFTKMSKMAGATADS
Q4DYM1|TcUBA1a        1 ------------------------------------------------------------
EKG03124.1 TcUBA1b    1 MRKHTRNHESDHYALYSK-NKKFYRTGTSQGFNCNKNKVDTAFVNAETEAAEMSVVEAIA
Q9NF77|LmUBA1a        1 ------------------------------------------------------------
E9AFD0|LmUBA1b        1 ----------------------------------------------------------ME

P22314|hUBA1_        44 NGSEADIDEGL---YSRQLYVLGHEAMKRLQTSSVLVSGLRGLGVEIAKNIILGGVKAVT
Q57XC5|TbUBA1a        1 -MTSEEQRRQL---YNRQEYVVGTETQAKYGCTDVLVVGACGLGAEIIKNLTLTGVRSIK
Q38DE8|TbUBA1b       58 SDASSAIDSKFLDQQSRTIGTYGLETMAKLISFKVLVVGCGGVGIETAKNLALAGVHTIT
Q4DYM1|TcUBA1a        1 -MKHEEEKRQL---YNRQEYVVGVETQAKYGSTDVLVVGACGLGAEIVKNLALTGVRSIK
EKG03124.1 TcUBA1b   60 APAASAIDSKFLDKQSRTIGTYGLETMVKLISFKVLIVGCGGVGIEAAKNLSMAGVHTII
Q9NF77|LmUBA1a        1 MLSEEEQKRQL---YSRQEYVVGSETQAKYGSTHVLVVGATGLSAEIIKNVVLTGVKSVK
E9AFD0|LmUBA1b        3 AERKALIDQRYLDKQSRTIGTYGLETMAKLIAFKVIIVGCGGVGIEIAKNLALAGIHTIR

P22314|hUBA1        101 LHDQGTAQWADLSSQFYLREEDI--GKNRAEVSQPRLAELNSYVPVTAYTGPLVEDFLSG
Q57XC5|TbUBA1a       57 VLDNGLATLQDLGTNFFLTPADM--GKPRAEVVAARAQELNRFVSVTAVDVP-LHEVIPA
Q38DE8|TbUBA1b      118 LCDPKKAELKDMGVNFAVTETTIKAGLTRAEASKRLVAELNPNVRVRTVDAI-DEAVVSE
Q4DYM1|TcUBA1a       57 IMDSGTAILQDLGTNFFLTPQDV--GQPRADVVARRAQELNRFVHITAVTSP-LHEVIPD
EKG03124.1TcUBA1b   120 LCDPAKAQPKDMGVNFAVTEAAVRSGLTRAEASQRLVSELNPNVRVRVVDAL-SEAVVSQ
Q9NF77|LmUBA1a       58 VLDDAVVTIEDLGTNFFLRPDDV--GKARGAAVAQAAKELNRFVEVSSVSGD-PLLHIPA
E9AFD0|LmUBA1b       63 FYDPRKPTVQDMGVNFAVTPQSMASGKTMAELSAAYISELNPNTRVGVLAEL-TTATVAD

P22314|hUBA1        159 FQVVVLTNT----PLEDQLRVGEFCHNRG--IKLVVADTRGLFGQLFCDFGEEMILTDSN
Q57XC5|TbUBA1a      114 VHVVVFVNQ----RTTLLLAENAMARKHN--VKFVACESRGVAGCVFVDAGPSFTVLDPD
Q38DE8|TbUBA1b      177 VNCVVYTSAAADWSSKTLLKWDQFCRTRTPAISFIFAFQGGSLASVFADHAPNFTVKDAD
Q4DYM1|TcUBA1a      114 VHVVVFVNQ----RTTALVGENALARKHD--VKFVACESRGIVGCVFVDAGPSFSVLDPD
EKG03124.1TcUBA1b   179 VNALVFTSAAPDYSLRTLKKWNKFCHDHSSPISFIFAFQGGALGSVFADHGAHFTVKDPD
Q9NF77|LmUBA1a      115 VHVVIYTNA----YTSTLAAANKVARENK--VKFISCESRGVCGCIFVDGGESLDIVDTD
E9AFD0|LmUBA1b      122 NVALIFTTAAPDLRLTTLSEWNAFCHNHTPAISFVLALQTGTMGSVFTDHGPSFVVKDAD

P22314|hUBA1        213 GEQPLSAMVSMVTKDNPGVVTCLDEARHGFESGD---------FVSFSEVQGMVELNGN-
Q57XC5|TbUBA1a      168 GEETVVCVVTNIS--RDGVVSLHEDKKHECEVGG---------RVFLTGLVSPESLNSTV
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Q9NF77|LmUBA1a      169 GEDTVTCVVTAMS--SDGLVTLHEEKNHECEIGS---------KVYFTGLTELPQANTTE
E9AFD0|LmUBA1b      182 GRPMLQKLITEVVTLRDKTGEMYTRIRYETPEGQTPGALRDYTQIKLSEVQGLLKPDGT-

P22314|hUBA1        263 --------------------QPM---------EI--KVLGPYTFSICD---TSNF-SDYI
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Q38DE8|TbUBA1b      296 --------------------SIN----GNVFKGVVCTGDPPNTVRIYPSLESQGY-SAYE
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EKG03124.1TcUBA1b   298 --------------------SVN----DNIFNGVMCPNDPRDTVRIYPSLESQGY-SAYE
Q9NF77|LmUBA1a      218 ---------------------PATPSAWKLFEVA--EVISPHTMRLKGVSELVSAGTIIH
E9AFD0|LmUBA1b      241 --------------------SAN----GQVYDGVICPSDPRDTVRVYPAFETQGY-SPYE

P22314|hUBA1        288 --RGGIVSQVKVPKKISFKSLVASLAEP-DFVV-TDFAKFSRPAQLHIGFQALHQFCAQH
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Q38DE8|TbUBA1b      331 --TAGFLHEMKESQQLKFRALSEALSCPGQFVPVSSMMDGSEESQSHLTFTALLRFFDKH
Q4DYM1|TcUBA1a      263 VGTGAYLHTTKRHVLMGFKDLEQSVADP-TFVS---IFDSEEKVNAPATLHALFRALHDH
EKG03124.1TcUBA1b   333 --TGGFLHELKEVFQIEFRTLEEAVVCPGRFVPVSPMMDGSEESQSHLALHALLNFLDRH
Q9NF77|LmUBA1a      255 VGTSAYLHTTKKGRREHYRTLGECLDNP-ECLM---IFDKEEKYTAATTLHAMFTAVARH
E9AFD0|LmUBA1b      276 --TGGFLHELKEVKVLPFRPLSEALAAPGPFVSVSPMMDNSEESLTHVTLHALLRYADAH
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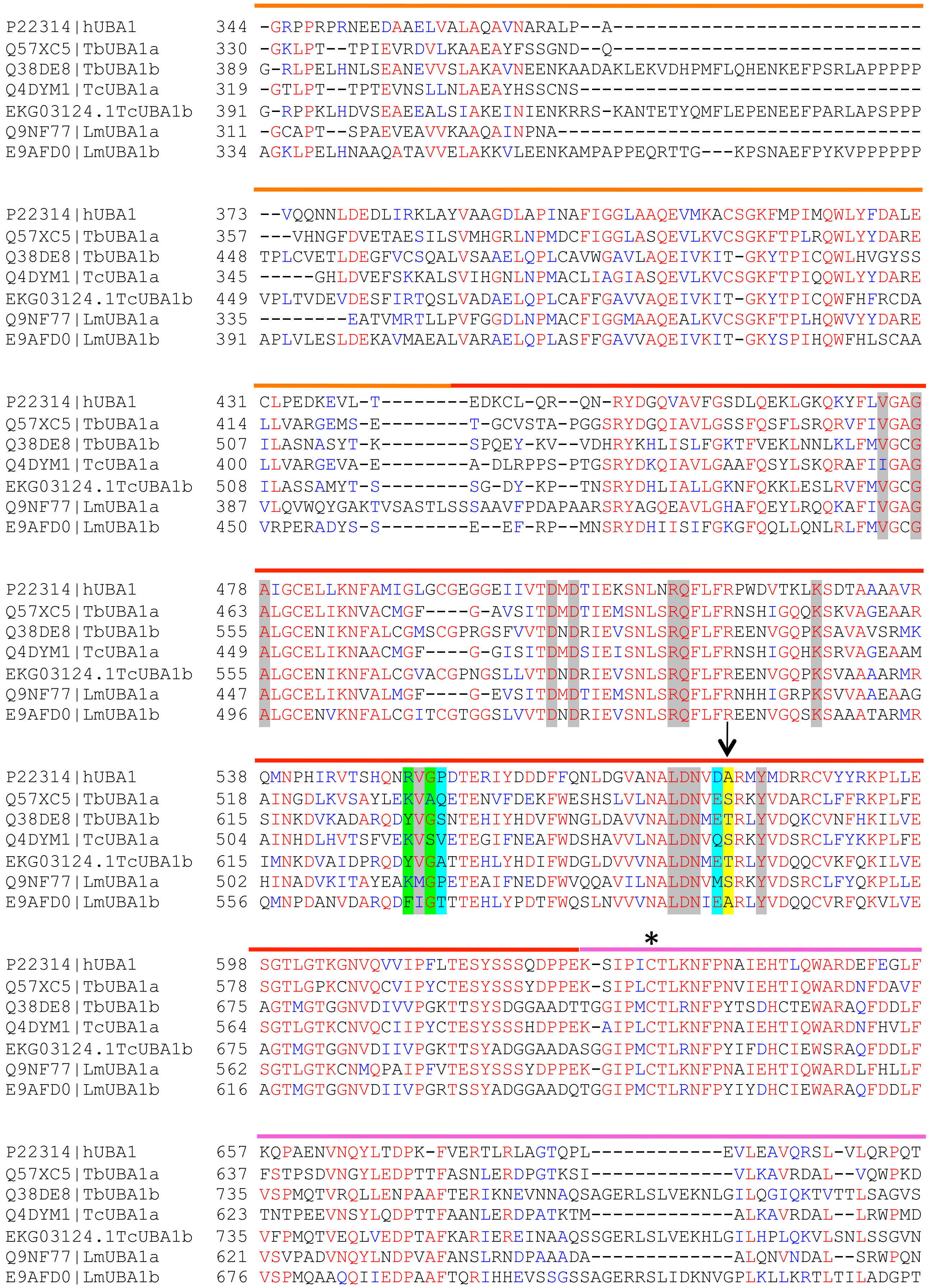
Figure S2 continued



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Q4DYM1|TcUBA1a      319 -GTLPT--TPTEVNSLLNLAEAYHSSCNS-------------------------------
EKG03124.1TcUBA1b   391 G-RPPKLHDVSEAEEALSIAKEINIENKRRS-KANTETYQMFLEPENEEFPARLAPSPPP
Q9NF77|LmUBA1a      311 -GCAPT--SPAEVEAVVKAAQAINPNA---------------------------------
E9AFD0|LmUBA1b      334 AGKLPELHNAAQATAVVELAKKVLEENKAMPAPPEQRTTG---KPSNAEFPYKVPPPPPP

P22314|hUBA1        373 --VQQNNLDEDLIRKLAYVAAGDLAPINAFIGGLAAQEVMKACSGKFMPIMQWLYFDALE
Q57XC5|TbUBA1a      357 ---VHNGFDVETAESILSVMHGRLNPMDCFIGGLASQEVLKVCSGKFTPLRQWLYYDARE
Q38DE8|TbUBA1b      448 TPLCVETLDEGFVCSQALVSAAELQPLCAVWGAVLAQEIVKIT-GKYTPICQWLHVGYSS
Q4DYM1|TcUBA1a      345 -----GHLDVEFSKKALSVIHGNLNPMACLIAGIASQEVLKVCSGKFTPIQQWLYYDARE
EKG03124.1TcUBA1b   449 VPLTVDEVDESFIRTQSLVADAELQPLCAFFGAVVAQEIVKIT-GKYTPICQWFHFRCDA
Q9NF77|LmUBA1a      335 --------EATVMRTLLPVFGGDLNPMACFIGGMAAQEALKVCSGKFTPLHQWVYYDARE
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P22314|hUBA1        431 CLPEDKEVL-T--------EDKCL-QR--QN-RYDGQVAVFGSDLQEKLGKQKYFLVGAG
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Q38DE8|TbUBA1b      507 ILASNASYT-K--------SPQEY-KV--VDHRYKHLISLFGKTFVEKLNNLKLFMVGCG
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Q57XC5|TbUBA1a      463 ALGCELIKNVACMGF----G-AVSITDMDTIEMSNLSRQFLFRNSHIGQQKSKVAGEAAR
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EKG03124.1TcUBA1b   555 ALGCENIKNFALCGVACGPNGSLLVTDNDRIEVSNLSRQFLFREENVGQPKSVAAAARMR
Q9NF77|LmUBA1a      447 ALGCELIKNVALMGF----G-EVSITDMDTIEMSNLSRQFLFRNHHIGRPKSVVAAEAAG
E9AFD0|LmUBA1b      496 ALGCENVKNFALCGITCGTGGSLVVTDNDRIEVSNLSRQFLFREENVGQSKSAAATARMR

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Q38DE8|TbUBA1b      615 SINKDVKADARQDYVGSNTEHIYHDVFWNGLDAVVNALDNMETRLYVDQKCVNFHKILVE
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EKG03124.1TcUBA1b   615 IMNKDVAIDPRQDYVGATTEHLYHDIFWDGLDVVVNALDNMETRLYVDQQCVKFQKILVE
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E9AFD0|LmUBA1b      556 QMNPDANVDARQDFIGTTTEHLYPDTFWQSLNVVVNALDNIEARLYVDQQCVRFQKVLVE

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EKG03124.1TcUBA1b   675 AGTMGTGGNVDIIVPGKTTSYADGGAADASGGIPMCTLRNFPYIFDHCIEWSRAQFDDLF
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P22314|hUBA1        657 KQPAENVNQYLTDPK-FVERTLRLAGTQPL------------EVLEAVQRSL-VLQRPQT
Q57XC5|TbUBA1a      637 FSTPSDVNGYLEDPTTFASNLERDPGTKSI-------------VLKAVRDAL--VQWPKD
Q38DE8|TbUBA1b      735 VSPMQTVRQLLENPAAFTERIKNEVNNAQSAGERLSLVEKNLGILQGIQKTVTTLSAGVS
Q4DYM1|TcUBA1a      623 TNTPEEVNSYLQDPTTFAANLERDPATKTM-------------ALKAVRDAL--LRWPMD
EKG03124.1TcUBA1b   735 VFPMQTVEQLVEDPTAFKARIEREINAAQSSGERLSLVEKHLGILHPLQKVLSNLSSGVN
Q9NF77|LmUBA1a      621 VSVPADVNQYLNDPVAFANSLRNDPAAADA-------------ALQNVNDAL--SRWPQN
E9AFD0|LmUBA1b      676 VSPMQAAQQIIEDPAAFTQRIHHEVSSGSSAGERRSLIDKNVGPLKLLKRTLTILADGPT
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Figure S2 continued

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Q38DE8|TbUBA1b     795 MEKCVQCAWETMFHLFRDRILDLQRSFPKDAKKKNGEKFWSGHRKYPTPLEVNIKALSSD
Q4DYM1|TcUBA1a     668 AADCVRIARRLYHEYFSDAFRQLLYNIPIDKRNENGELFWSGAKKPPTPQEFSP----DS
EKG03124.1TcUBA1b  795 MEKCFQCAWELMFYLFRDRIMDLQRSFPRDAKKKNGEDFWSGHRKYPTALNVDPKTIASN
Q9NF77|LmUBA1a     666 EQNCVRLARLLYQEHFNDGFRQLLHSIPLDKRNEDGQLFWGGAKKPPTPQEFDV----NS
E9AFD0|LmUBA1b     736 MDRCVALGWEQLFKMFRDRILDLQAAFPRGAKKKNGEDFWSGHRKYPTALQVTAADIATN

P22314|hUBA1       759 PLHLDYVMAAANLFAQTYGLTG--SQ---------------DRAAVATFLQSVQVPEFTP
Q57XC5|TbUBA1a     738 ELNVSFVYHCAKLLAQVYNLSAF-TL---------------SVKEVAELAMQVAVPGFVP
Q38DE8|TbUBA1b     855 PDVVEFLISAANLFACMYGIHPQKHEPRLNDPKKRWMQQYRTLDWLNGVMKNCTVPEYKP
Q4DYM1|TcUBA1a     724 ELSMSFVYHCAYLLACVYGLPPF-TL---------------SRADVARVAGETSVPEFVP
EKG03124.1TcUBA1b  855 KDAVEFLIAASNLFACMYGVHPPKHEPRFNDANNRWMQQYRSLEWINKIIEKREVPVYHP
Q9NF77|LmUBA1a     722 EQDTEFVYHCACLFAKVYQLPAF-SL---------------SKEETARLAAAVTVPDFVP
E9AFD0|LmUBA1b     796 PDAKNFLVAAINLYACMFGVHPPKHEARFNDEKNRWMQEYRTDAWIQAEVNKLPTPAYVA

P22314|hUBA1       802 KS------GVKIHVSDQELQSANASVDDSRLEELKATLPSPDKLPGFKMYPIDFEKDDDS
Q57XC5|TbUBA1a     782 RE---------ARFETNEAENKEGAAAQLVGDLTMQDLPPVSQFNSRRMNPLVFEKDDPN
Q38DE8|TbUBA1b     915 GSVEGLDDDLLQSMEKQEVSKDETTKEQTLNNLLSSVVALAQKCHNMNTVPLDFEKDDDD
Q4DYM1|TcUBA1a     768 RQ---------AVFATSEAEK-EESVANLAAEIGLQDLPPVSEFHGRRMVPEFFEKDDPT
EKG03124.1TcUBA1b  915 GAVEGLDDDILDAIQTHDGAKKEETKEEQLGQLLCNIMTLAGSCRGTKATPLDFEKDDDD
Q9NF77|LmUBA1a     766 RH---------AVFATSESQTSQQTSSS--RGLTVEQLPPVAHFGSRRMRAEEFDKDDIT
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P22314|hUBA1       856 NFHMDFIVAASNLRAENYDIPSADRHKSKLIAGKIIPAIATTTAAVVGLVCLELYKVVQG
Q57XC5|TbUBA1a     833 NSHMDYITACSNLRATAYSIPPADVHYTKRIAGRIIPAMVTTTALVTGLVGIEALKYLLL
Q38DE8|TbUBA1b     975 NFHIDFVAATSNLRARNYDIPTQDRFKVKLVAGKIIPAIATTTAAVTGLALIEYFKALLS
Q4DYM1|TcUBA1a     818 NHHVEYITACSNMRAVAYNIPPADVHHTKRIAGKIIPAMVTTTALVTGLVGLEVLKRLLM
EKG03124.1TcUBA1b  975 NFHIDFVTAASNLRASNYDIPTQDRMKVKLVAGKIIPAIATTTSAVTGLALIEYFKALQG
Q9NF77|LmUBA1a     815 NHHVQFITYCSNLRARAYSIPVADFNQTKRIAGNIIPAMVTTTSLVTGLVGFEMLKYLLI
E9AFD0|LmUBA1b     915 NFQIDFVAAASNLRAENYGIPTQDRLKVKLVAGKIIPAIATTTSAVTGLGLIELFKVLQN

P22314|hUBA1       916 HRQ----------------------------LDSYKNGFLNLALPFFGFSEPLAA--PRH
Q57XC5|TbUBA1a     893 AHRENGAQGLAKANP-----IT--EKVQEEYLSLYRNAFVNVALPFMAFSDPIAA--PAK
Q38DE8|TbUBA1b      1035 NDVSCLRNGMLDIGT-----NN-YVLFERD-------------APLKHRTRVDKTYLPEQ
Q4DYM1|TcUBA1a     878 TQRQERSGMPANAVP-----TY--EEIQKQ-LSIYRNAFVNIALPFIAFSDPIIA--SGA
EKG03124.1TcUBA1b 1035 NDISCLRNGMIDVGT-----NN-YVLFERD-------------APIKNRTKIVSTYLPEQ
Q9NF77|LmUBA1a     875 QFHHARKPAVNGTGSSHGNFYLDADEEPEKLVTLFRSAFVNIALPFIAFSDPIIA--PSH
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Q57XC5|TbUBA1a     944 TVP-------MPDGSSVRWGIWDRIDIN----EGRDITVKELVSILEKR----------H
Q38DE8|TbUBA1b      1076 DYTYKKKVICLPEGY----TKYDMIEVP----ITKATTVQQFATELEKKLNTLLPTGMNA
Q4DYM1|TcUBA1a     928 TYP-------LPDGTSVRWGIWDRIDVN----EGRDLTVQELVTVLESR----------Y
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Q9NF77|LmUBA1a     933 SYA-------LPSGKKLRWGIWDRIDVS----EGRDMLVKELVQLLHDR----------Y
E9AFD0|LmUBA1b    1016 DYTYKKKIIRVPEGF----TKYDMIRIP----VTPATTVKAFAAALEAVLNKTLPEGADY

P22314|hUBA1       984 KLEITMLSQGV-SMLYSFFMPAAKLKERLDQPMTEIVS-RVS--KR-----KLGRHVRAL
Q57XC5|TbUBA1a     983 QLEIFIIALPCGKMVYSQFG-NVKDR---DKPVSVVVREKTK-GEE-----KDELSCICF
Q38DE8|TbUBA1b    1128 GCEVSAIGVGKG-SLWNGLPKHANTNCSLMDIIEKQKL-SEAGGKLPRPFWENRTHFHDL
Q4DYM1|TcUBA1a     967 QVELFIIALASGKIIYSQFG-NTKDR---GKPVSTVVLEKGE--QL-----QDGEDCCCL
EKG03124.1TcUBA1b 1128 SCEIVGIGVGHG-MLWNGSKKHANTNLSLMQLIEQQKM-TEAGGKLSQPFWQNRTQFCEL
Q9NF77|LmUBA1a     972 ELEVFMIALKNGKMIYTEFGGKAKDK---EKRVSEVAQDKGE--KV-----QDGIDYFDL
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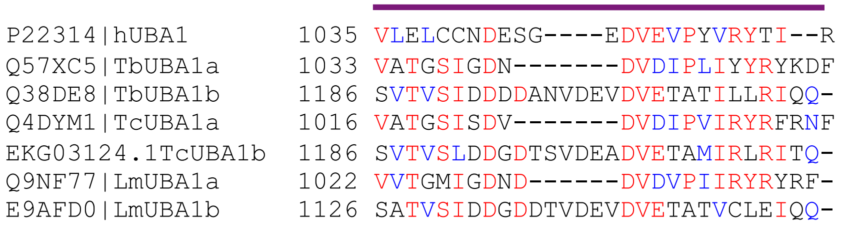

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Q4DYM1|TcUBA1a      1016 VATGSISDV-------DVDIPVIRYRFRNF
EKG03124.1TcUBA1b   1186 SVTVSLDDGDTSVDEADVETAMIRLRITQ-
Q9NF77|LmUBA1a      1022 VVTGMIGDND------DVDVPIIRYRYRF-
E9AFD0|LmUBA1b      1126 SATVSIDDGDDTVDEVDVETATVCLEIQQ-
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Figure S2. Amino acid alignments of hUBA1 and the trypanosomatid UBA1s used or mentioned in this study.

Alignments were made with ClustalOmega and coloured with Boxshade. Red residues are identical, blue residues at least 50% similar between sequences. Residues that interact with TAK-243 in ScUBA1 based on PDB 5L6J are highlighted (see Figure 4). Colouring is as follows: residues that do not differ between hUBA1 (and ScUBA1) and the UBA1s of T. brucei are in grey; the gatekeeper residue is in yellow; other residues that were studied and mutated in this study (Figure 5) are in blue; residues that were not mutated but were studied in the structural models (Figure 6) are in green. The lines above the sequences indicate the separate domains with colours as in Figure 1 and Figure S1: the IAD in orange, the FCCH and SCCH in pink, the AAD in red and the UFD in purple. The catalytic cysteine is indicated with an asterisk and the gatekeeper with an arrow.

Gene Locus	size (AA)	ThiF domains	UniProt ID	E1
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Tb927.8.2640	1055	2	Q57XC5	UBA1 (Ub)
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Tb927.9.4620	467	1	Q38F81	UBA3 (Nedd8)
Tb927.9.6040	387	1	Q38EW4	UBA5 (Ufm1)
Tb927.10.11180	754	1	Q389M1	ATG7 (ATG8)
Tb827.11.2330	505	1	Q386S6	MOCS3 (Urm1, S)
TB927.2.4020	603	1	Q586W2	APPBP1 (Nedd8)
Tb11.02.5410b	295	1	Q384R4	AOS1 (SUMO)

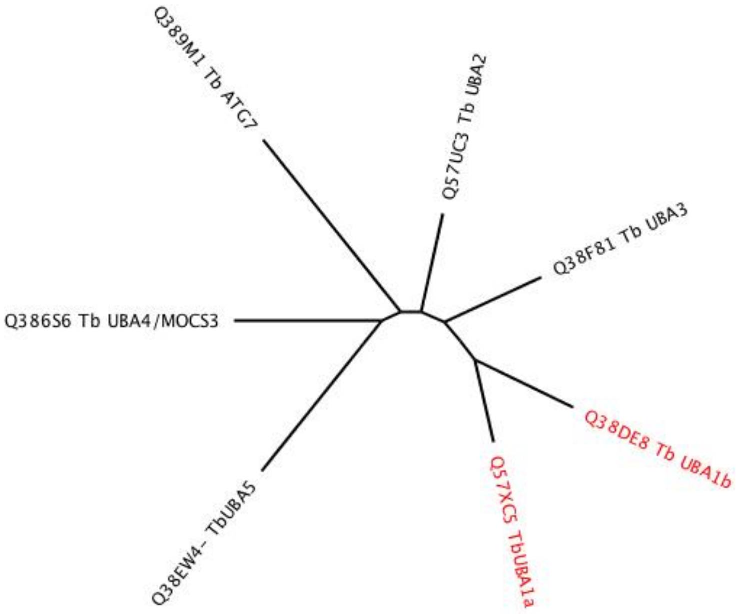

Figure S3. ThiF domain containing proteins in *T. brucei.*

Protein BLAST and ThiF domain (Pfam pf00899) searches in NCBI and TriTrypdp.org identified probable ortholgues of the indicated E1 proteins. AOS1 and UBA2 together form the E1 for SUMO, APPBP1 and UBA3 the E1 for Nedd8. These proteins, excluding AOS1 and APPBP1 without an active adenylation domain (AAD), where aligned in UniprotAlign, phylogenetic analysis was performed with PhyML3.0 (Dereeper A et al. Nucleic Acids Res. 2008 Jul 1;36) and the tree depicted with FigTree software.

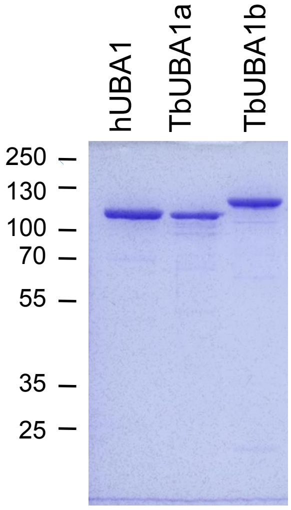

Figure S4. The purified human and T. brucei UBA1 proteins

The indicated UBA1s were purified from E. coli as described in Materials and Methods. A Coomassie stained acrylamide gel is shown.

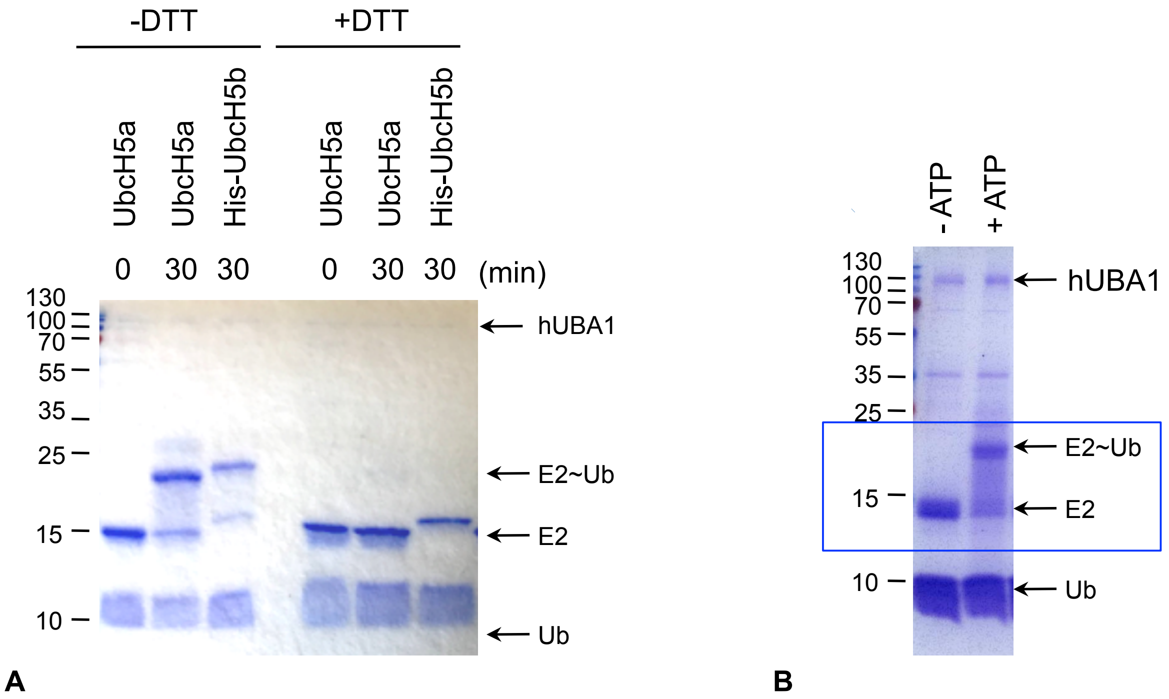

Figure S5. The E1-E2 transthioesterifcation reactions used in this study

E1-E2 transthioesterification reactions with hUBA1 were performed as described in Materials and Methods.

A. the transthioesterification reactions were performed with either UbcH5a purified in the laboratory or with commercial His6-UbcH5b (Enzo Life Sciences) for 0 or 30 min as indicated. The reactions were incubated with or without DTT before analysis by SDS-PAGE. The gel was Coomassie stained and dried. The disappearance of the E2~Ub conjugate in the presence of DTT illustrates that ubiquitin was bound to the E2 via a reducible thioester linkage.

B. the transthioesterification reactions were performed in the absence or presence of Mg~ATP (ATP) to show the dependence of the E2~Ub conjugate formation on ATP, as expected. The gel was captured following Coomassie staining using an ImageQuant LAS500 imager as for other gels in this study. The boxed area shows the part of the gels that are shown in Figures 1, 3, 5 and 7 of the main manuscript.

A

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hu Ub              1  MQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYN
T.brucei Ub        1  MQIFVKTLTGKTIALEVEASDTIENVKAKIQDKEGIPPDQQRLIFAGKQLEEGRTLADYN

hu Ub              61  IQKESTLHLVLRLRGG  76
T.brucei Ub        61  IQKESTLHLVLRLRGG  76
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B

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UbcH5a                 1  MALKRIQKELSDLQRDPPAHCSAGPVGD-DLFHWQATIMGPPDSAYQGGVFFLTVHFPTD
Q57W45 T. brucei E2    1  MALRRIQKELKDLERDPPANTSGGPVNESDLFNWKATIIGPEDSPYAGGLFFLNIHFPSD

UbcH5a                 60  YPFKPPKIAFTTKIYHPNINSNGSICLDILRSQWSPALTVSKVLLSICSLLCDPNPDDPL
Q57W45 T. brucei E2    61  YPFKPPKLQFTTKIYHPNINNNGGICLDILKDQWSPALTISKVLLSVCSLLTDPNPDDPL

UbcH5a                 120  VPDIAQIYKSDKEKYNRHAREWTQKYAM  147
Q57W45 T. brucei E2    121  VPDIARQYKTDRNAFNKTAMEWTRQYAM  148
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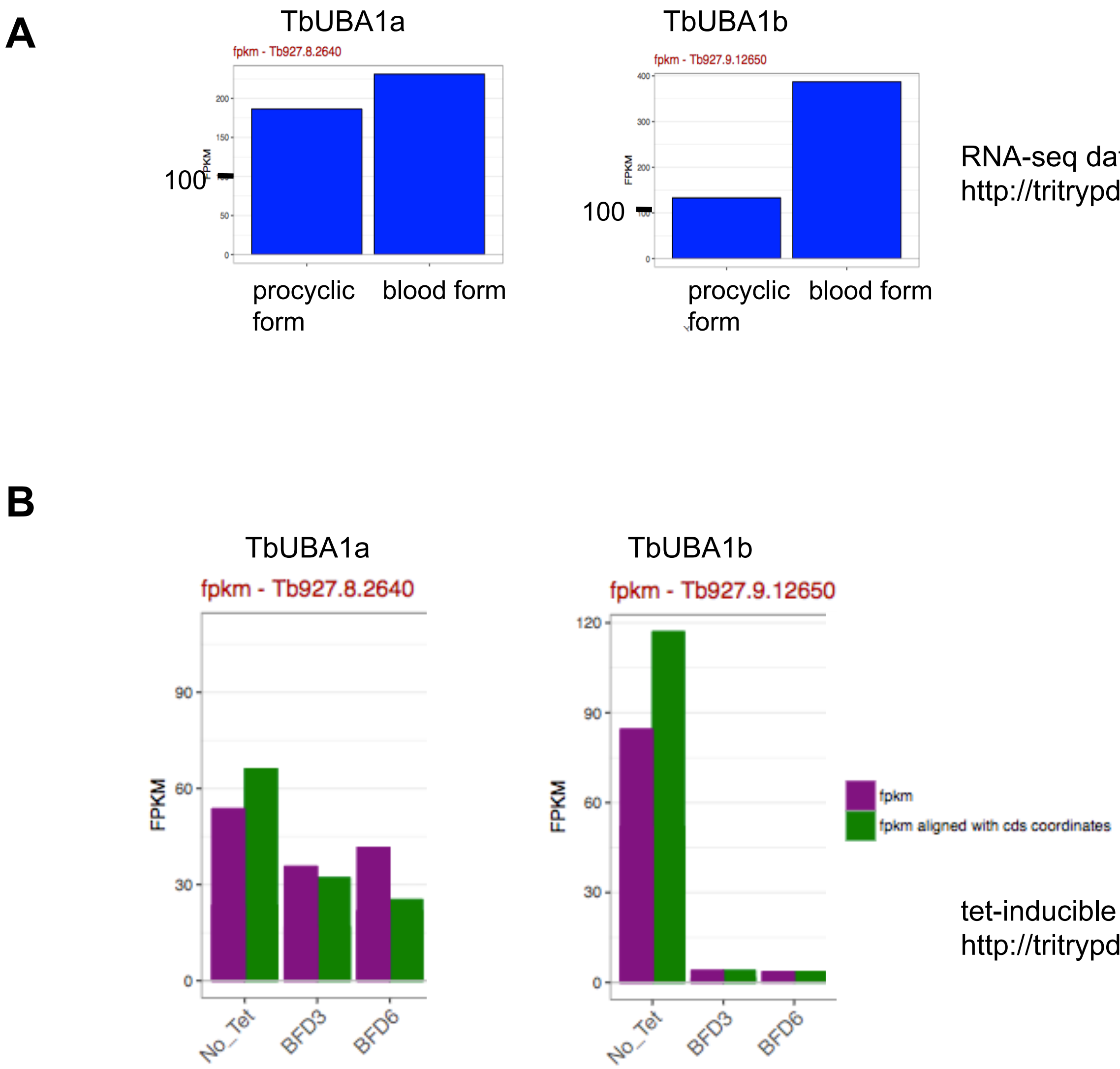
Figure S6. Alignments of human and T. brucei ubiquitin and E2.

A. Human and T. brucei ubiquitin (Q383T7) differ at 4 residues. Alignments were made with ClustalOmega and coloured with Expasy Boxshade. Identical residues are in red, similar residues in blue. Similarity groups are (G,A), (I,L,M,V), (D,E), (H,K,R) and (S,T).

B. T. brucei expresses an orthologue of the human E2 UbcH5a (E2D1, NP_003329.1) that is 73% identical (Q57W45). Alignment and colouring as in A.



RNA-seq data
http://tritrypdb.org

tet-inducible RNAi
http://tritrypdb.org

Figure S7. Expression of TbUBA1a and TbUBA1b; Effects of tet-inducible RNAi knockdown of TbUBA1a and TbUBA1b.

A. RNA-seq data from http://tritrypdb.org website showing that TbUBA1a and TbUBA1b are both expressed in the blood form as well as the procyclic form of T. brucei (Siegel T. N. et al. Nucleic Acid Res. 38 (15): 4946 (2010)).

B. Data from http://tritrypdb.org of a tetracycline-inducible RNAi knockdown study of 9891 genes in T. brucei (Alsford S. et al Genome Res 21(6):915 (2011)). Transcript levels before induction of RNAi (No Tet), after 3 days (BFD3) and 6 days (BFD6) are shown.

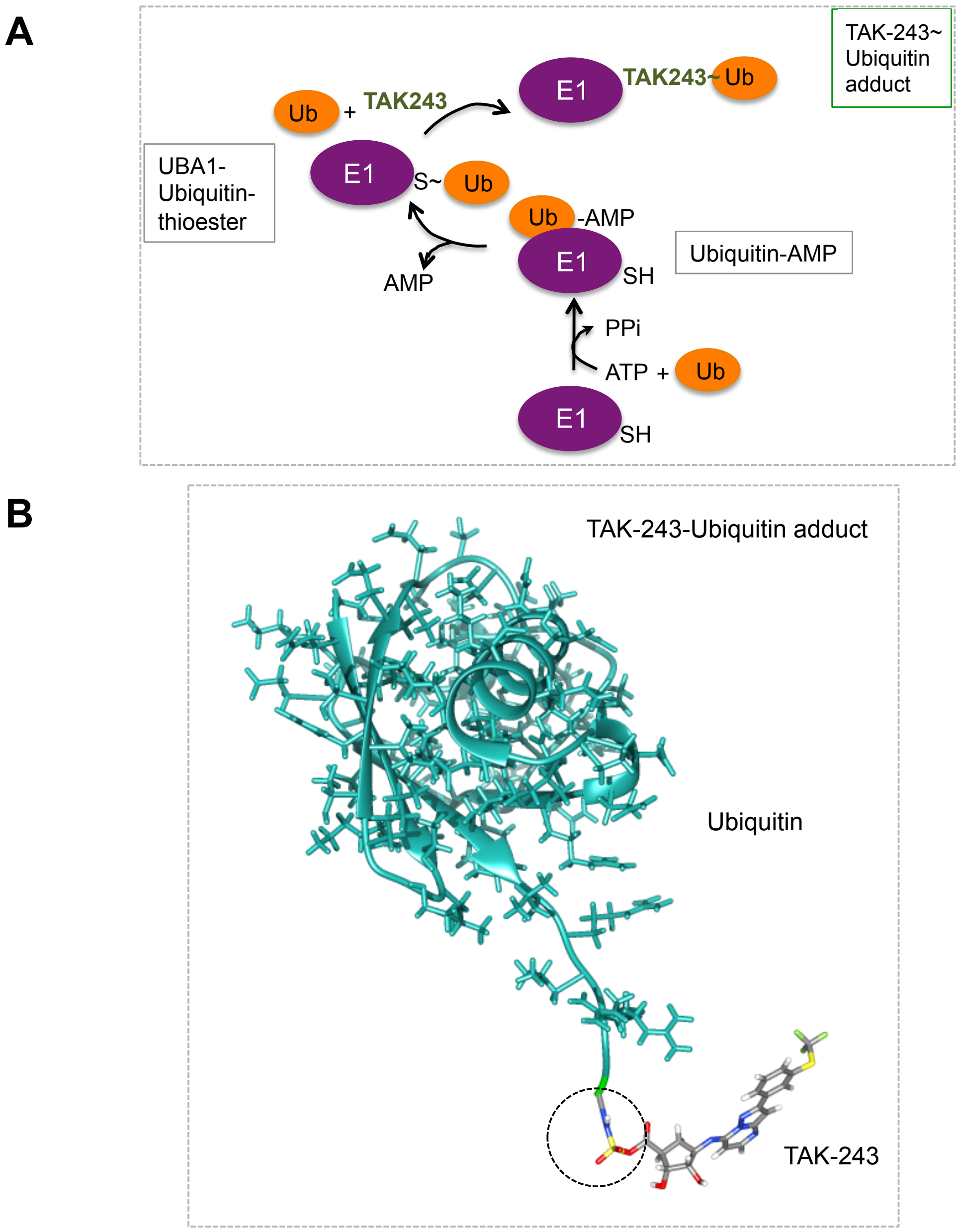


Figure S8. TAK-243 forms a covalent TAK-243~ubiquitin adduct that can not be released from UBA1.

A. In the presence of TAK-243, the thioester-bound ubiquitin on UBA1 is attacked so that a TAK-243~ubiquitin adduct is formed. This substrate-assisted inhibition was elucidated for MLN4924 and UBA3, the active subunit of Nedd8 E1 (Brownell et al. Molecular Cell 37:102, 2010).

B. The TAK-243~ubiquitin adduct bound to ScUBA1 (chain not shown) was taken from PDB 5L6J (Misra et al. Structure 25(7):1120, 2017). The circle indicates the covalent attachment of ubiquitin to the sulfamate nitrogen of TAK-243. The image was generated with UCSF Chimera (Pettersen et al. J Comput Chem 25: 1605-1612, 2004).

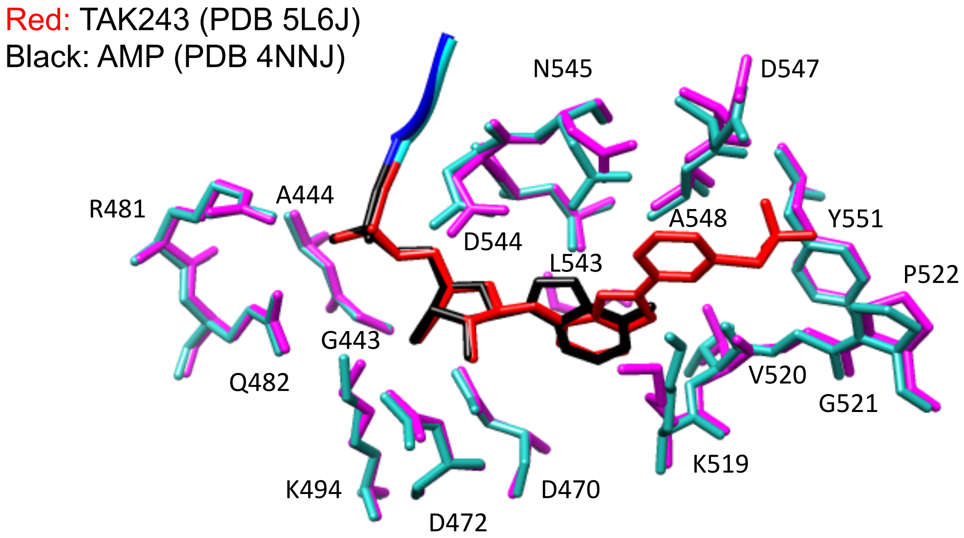


Figure S9. TAK-243 protrudes further into the adenylation site of UBA1 than AMP.

An overlay of residues from ScUBA1/TAK-243~ubiquitin (cyan, PDB 5L6J) with those from ScUBA1/AMP~ubiquitin (magenta, PDB 4NNJ). Only the residues that that interact with TAK-243 in the adenylation site are shown. AMP is in black, TAK-243 in red. Ubiquitin residues are in blue. The image was generated with UCSF Chimera (Pettersen et al. J Comput Chem 25: 1605-1612, 2004).

CLONING PRIMERS

TbUBA1a

For gateway cloning and to introduce an upstream HRV 3C protease cleavage site. This was done in two rounds of PCRs. The first round was with primers Tb26Fw1 and Tb26Rev; the second round with primers GwB1-3C using the product of the first PCR as input (nucleotides for the HRV 3C cleavage site are in italics, nucleotides complementary to TbUBA1a are underlined, recombination sites are in normal text):

Tb26Fw1: *CTGGAAGTGCTGTTTCAGGGCCCG*ACTAGTGAAGAGCAAAGACGG
Tb26Rev : GGGGACCACTTTGTACAAGAAAGCTGGGTGTTAAAAATCCTTGTACCTGTAATAAATTAGAGG
GwB1-3C GGGGACAAGTTTGTACAAAAAAGCAGGCTTCCTGGAAGTGCTGTTTCAGGGCCCG

TbUBA1b

For restriction enzyme cloning into EcoRI and NotI site of pGEX4T3 (nucleotides complementary to TbUBA1b are underlined):
Tb09EcoFw: TCCCCGAATTCCCCAAAACATCAACGGGATCGCTTTTCCAATTGTCC
Tb09NotRev: CCGCGGCCGCTTATTGTTGGATGCGCAAAAGTATTGTAGCGGTTTCAAC

LmUBA1a

For restriction enzyme cloning into EcoRI and NotI site of pGEX4T3 (nucleotides complementary to LmUBA1a are underlined):
LmEcoFw: TCCCCGAATTCCTTGTCCGAGGAGGAGCAAAAGAGG
LmNotRev: CCGCGGCCGCTTATTAGAAGCGATAACGGTAGCGGATGATGGGCAC

MUTAGENESIS PRIMERS

TbUBA1a

Q534P-Fw: GAGAAGGTGGCGCCAGAAACAGAAAATGTG
Q534P-Rev: CACATTTTCTGTTTCTGGCGCCACCTTCTC

E559D-Fw: CGCTTGACAATGTAGACAGCCGGAAATATGTTG
E559F-Rev: CAACATATTTCCGGCTGTCTACATTGTCAAGCG

S560A-Fw: CGCTTGACAATGTAGAAGCCCGGAAATATGTTG
S560A-Rev: CAACATATTTCCGGGCTTCTACATTGTCAAGCG

E559D,S560A-Fw: CGCTTGACAATGTAGACGCCCGGAAATATGTTG
E559D,S560A-Rev: CAACATATTTCCGGGCGTCTACATTGTCAAGCG

TbUBA1b

S631P-Fw: GATTACGTGGGACCCAACACGGAACAT
S631P-Rev: ATGTTCCGTGTTGGGTCCCACGTAATC

E656D,T657A-Fw: CTTGACAACATGGACGCTCGACTCTATGTTG
E656D,T657A-Rev: CAACATAGAGTCGAGCGTCCATGTTGTCAAG

Figure S10. Cloning an mutagenesis primers used in this study.

Full length gels for Figures 1 and 3

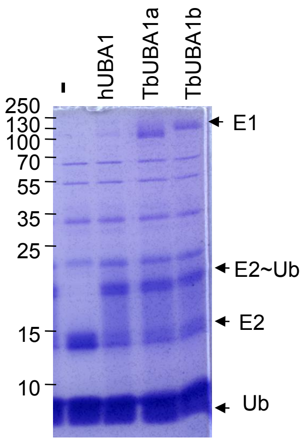


Figure 1D

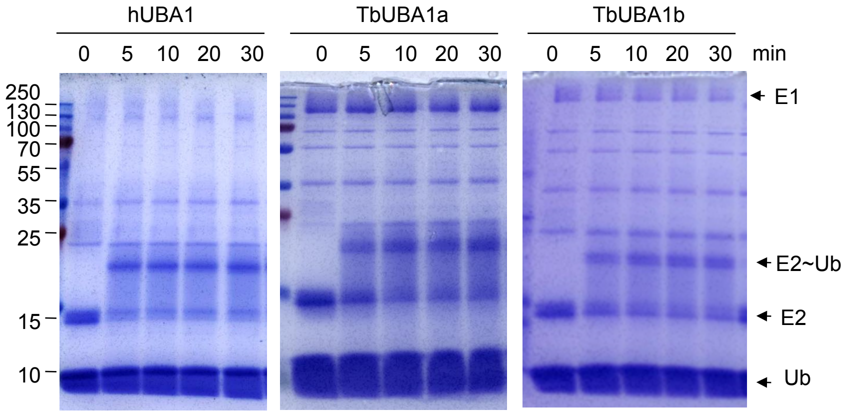


Figure 1E

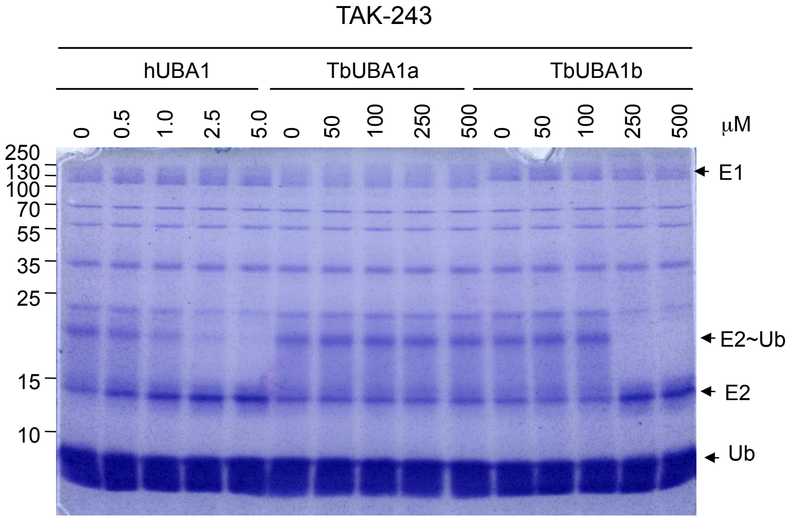


Figure 3A

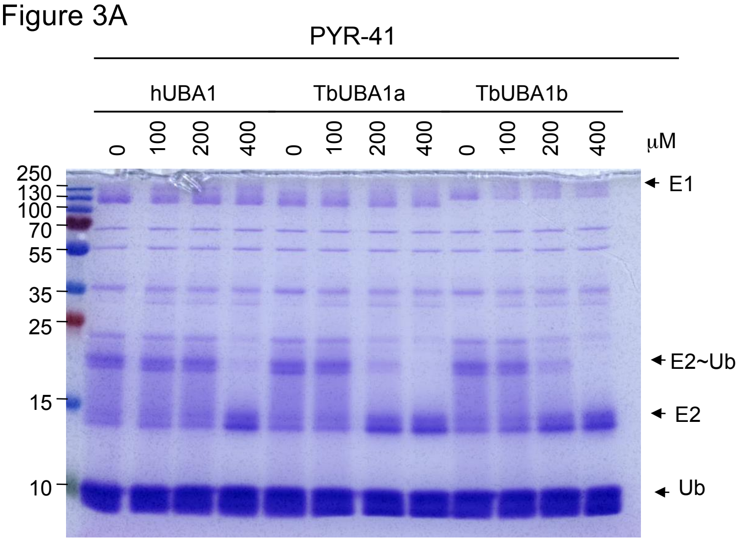
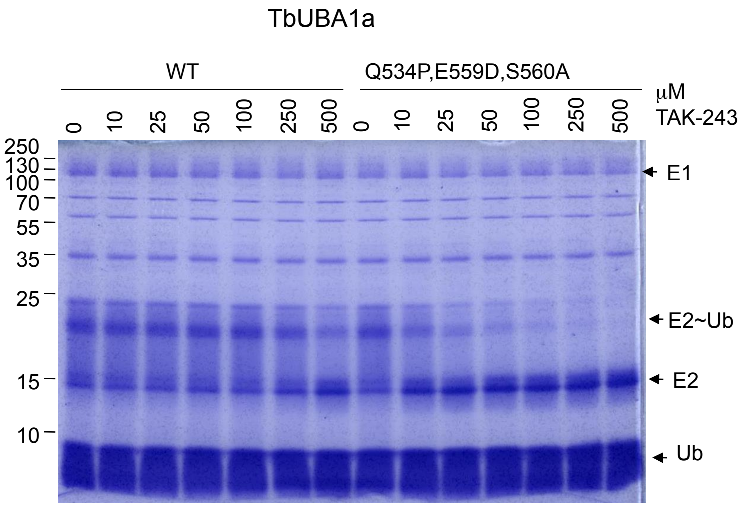
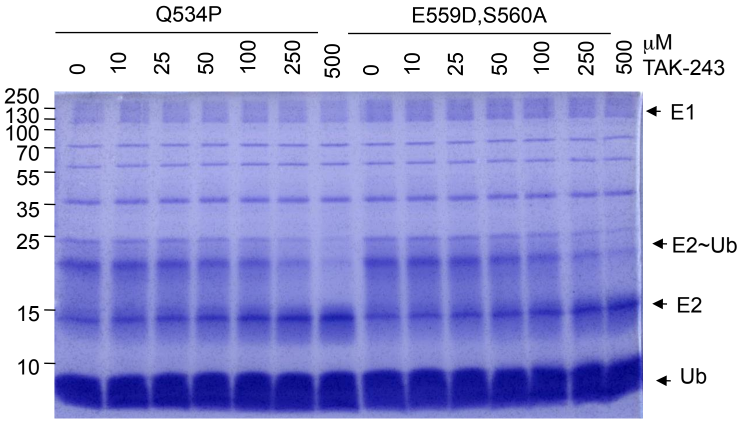
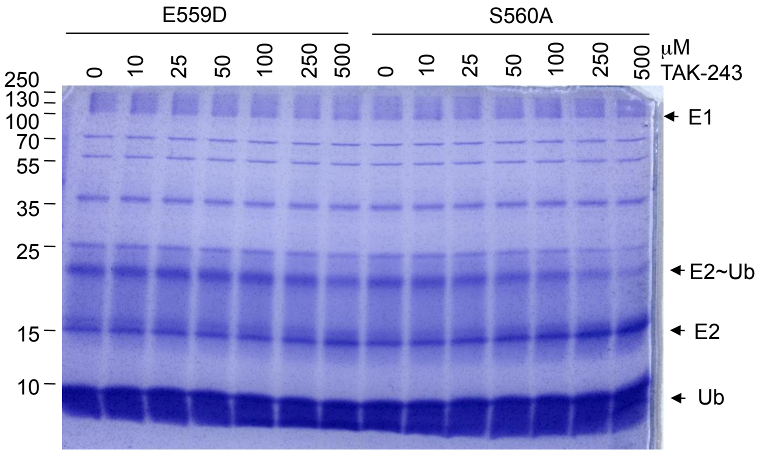


Figure 3C

Full length gels for Figure 5A*







* Note that the order of the mutants on these gels is different from the order in which they are displayed in Figure 5A.

Full length gels for Figure 5B* and C

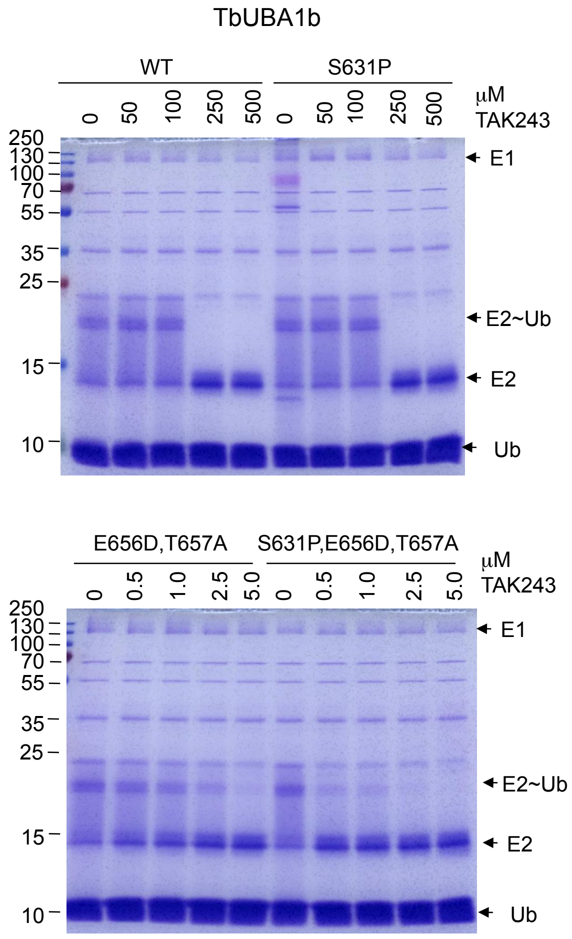


Figure 5B
* Note that the order of the mutants on these gels is different from the order in which they are displayed in Figure 5A.

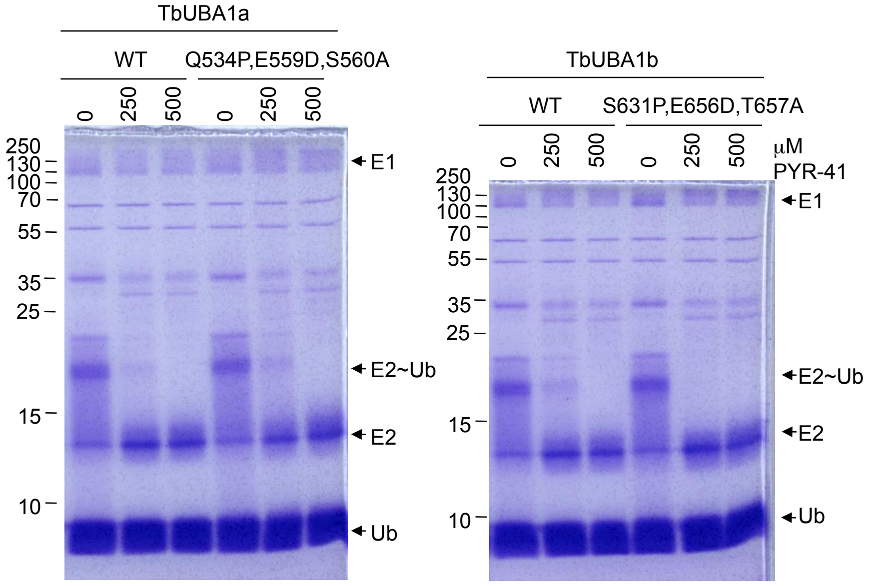


Figure 5C

Full length gel for Figure 7