Supporting Information

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SI Methods

Plasmids and Antibodies. The coding sequence for human Ube2S was cloned into pMAL-TEV using *Fse*I/*Asc*I restriction sites introduced by PCR. When mentioned, a C-terminal His_6 tag was added before the stop codon by PCR. Ube2S was also cloned into pET28 for expression in bacteria, pCS2-ZZ/TEV for expression by in vitro transcription/translation (IVT/T), and pCS2, pCS2-HA, pCS2-myc, and pcDNA5 for expression in human cells. Mutations were introduced into these vectors by site-directed mutagenesis and *Dpn*I-digestion to yield $Ube2S^{C95S}$. The deletion of C-terminal amino acids ($Ube2S^{\Delta C}$) were constructed by PCR and *Fse*I/*Asc*I cloning. Similarly, the coding sequence for *Drosophila melanogaster* Ube2S and UbcH10/Vihar was amplified by PCR and cloned into pMAL-TEV using *Fse*I/*Asc*I restriction sites. The coding sequences of cyclin A2, Tpx2, Plk1, Cdh1, Cdh1-mutants (amino acids 1–125; 1–181; WD-domains only), Brca1 (1–600), Apc11, and Efp were cloned into pCS2 for IVT/T; UbcH10 was cloned into pCS2-ZZ/TEV for IVT. Human Cdh1 was cloned into pFBAC-His_6 for purification in insect cells. Two polyclonal antibodies against human Ube2S were kindly provided by Deborah Zajchowski and Rick Feldman (Bayer). The following commercial antibodies were used: αUbcH10, αUbcH5 (Boston Biochem; A-650, A-615); αCdc27, αCdc20, αcyclin A, αcyclin B1, αMad2, αHA (Santa Cruz; sc-9972, sc-13162, sc-751, sc-594, sc-245, sc-6329, sc-805); αCdh1, α-γtubulin, α-βtubulin, αFLAG (Sigma; C7855, T3320, C4585, F3165); αPlk1 (Upstate; 06–813); α-βactin, αAurora A (Abcam; ab8226, ab12875); αsecurin (MBL; K0090–3); αEmi1 (Invitrogen; 38–5000); αUbe2S (Strategic Diagnostics; 2257.00.02); and αTpx2 (Novus; NB500–179).

Purification of Recombinant Ube2S. BL21/DE3 (RIL) bacteria were transformed with the pMAL-TEV-$Ube2S^{His\text{-}6}$ construct and grown in LB. Protein expression was induced by 1 mM IPTG, and bacteria were grown for 14 h at room temperature. Lysates were prepared in lysis buffer [LB, 50 mM sodium phosphate (pH 8), 500 mM NaCl, 10 mM imidazole, and 0.1% Tween 20] by treatment with lysozyme and sonication on ice. Lysates were cleared and the recombinant protein was purified on NiNTA-agarose. Beads were washed in wash buffer [WB, 50 mM sodium phosphate (pH 8), 500 mM NaCl, 20 mM imidazole, and 0.1% Tween 20]. Recombinant ^{M}BPTEV protease was added in cleavage buffer [20 mM Tris (pH 7.5), 500 mM KCl, 10% glycerol, and 0.5 mM DTT], and beads were incubated at 4 °C on a roller drum over night. The beads were washed, and the supernatant was discarded. Ube2SHis-6 was eluted in elution buffer [50 mM sodium phosphate (pH 8), 500 mM NaCl, and 200 mM imidazole], and dialyzed into storage buffer [20 mM Tris (pH 7.5, 500 mM KCl, 10% glycerol, and 2 mM DTT] by changing the buffer three times in 1-h intervals. The *D. melanogaster* Ube2S and UbcH10/Vihar were purified on amylose resin and eluted by TEV cleavage overnight as described above.

Proteins. UbcH10, $UbcH10^{C114S}$, UbcH5c, $UbcH5c^{C85S}$, E2–25K, Ubc13, Uev1A, E1, securin, securin mutants, and Emi1 were purified from BL21/DE3 (RIL) or SF9 cells (E1) using His_6 tags as described before (1, 2). Purified ubiquitin, ubiquitin mutants, and 26S proteasomes were obtained from Boston Biochem. $^{His\text{-}6}$Cdh1 was expressed in insect cells. Cells were lysed in 50 mM sodium phosphate, 500 mM NaCl, 25 mM imidazole, and 1% Triton X-100 and repeated douncing on ice. The protein was purified to homogeneity using NiNTA agarose (Qiagen).

In Vitro Ubiquitination Reactions. For approximately 10 ubiquitination reactions, human APC/C was purified from 1.5 mL concentrated extracts of cells synchronized in G1 by immunoprecipitation using 75 μL αCdc27-antibodies and 100 μL Protein G-agarose (Roche) as described before (1, 2). When indicated, APC/C was precipitated from a 1.5-mL extract prepared from quiescent T24 cells arrest by serum starvation for 72 h. The washed beads were incubated with 50 nM E1, E2 (concentrations and E2 as indicated; usually 50 nM UbcH10 and 100 nM Ube2S), 1 mg/mL ubiquitin or ubiquitin mutants, energy mix (20 mM ATP, 15 mM creatine phosphate, and creatine phosphokinase), and 1 mM DTT at room temperature for the indicated times. ^{35}S-labeled substrates were synthesized by IVT/T and added to the reactions, which were analyzed by SDS gel electrophoresis and autoradiography.

Immunoprecipitation Reactions. HeLa or 293T cells were either grown without synchronization or synchronized in S phase by thymidine treatment, mitosis by a thymidine/nocodazole protocol (1), and in G1 by release from a thymidine/nocodazole arrest for 2, 4, and 6 h respectively. Cells were collected by scraping and lysed in IP buffer [50 mM HEPES (pH 7.5), 150 mM NaCl, 1.5 mM $MgCl_2$, 5 mM KCl, 0.1% Tween 20, and 2 mM DTT]. The precleared lysates were incubated with rabbit IgG, αUbe2S-antibody (kindly provided by Deborah Zajchowski and Rick Feldman), αCdc27 (monoclonal, Santa Cruz), FLAG-agarose (Sigma), or HA-matrix (Roche) for 4 h at 4 °C. When required, protein G-agarose was added for additional 60 min. Beads were extensively washed and eluted in SDS-gel buffer. Samples were analyzed by 10% SDS/PAGE and Western blotting.

Purification of Ube2S Complexes for Mass Spectrometry. We established a 293T cell line, which expresses ^{FLAG}Ube2S under the control of an inducible Tet promoter, according to the manufacturer's instructions (Invitrogen). Thirty-five 15-cm dishes of 293T::^{FLAG}Ube2S cells were grown in DMEM, and expression of ^{FLAG}Ube2S was induced by addition of 1 μg/mL doxycyclin for 36 h. Cells were harvested and incubated in SB buffer [50 mM HEPES (pH 7.5), 150 mM NaCl, 1.5 mM $MgCl_2$, and 5 mM KCl] for 30 min on ice. Subsequently, the cells were lysed with a Dounce homogenizer and passaged through a 201/2G needle several times. NaCl was added to a final concentration of 150 mM before the lysate was centrifuged at 4 °C for 30 min. The clarified extracts were then incubated with 400 μL FLAG-agarose (Sigma) on a rotator for 4 h at 4 °C. The beads were extensively washed with SB/150 mM NaCl, and eluted three times with 100 μg/mL FLAG-peptide. The elutions were pooled, and the FLAG-peptide was removed and samples were concentrated using 10 MWCO spin columns (Millipore). The samples were subjected to MudPIT mass spectrometry at the UCB Proteomics/Mass Spectrometry Laboratory (P/MSL). Peptides present only in ^{FLAG}Ube2S-IPs were selected for further analysis.

Sucrose Gradient Centrifugations. Extract preparation was described before (2). Three-hundred-microliter extracts were loaded on 5–40% sucrose gradient and centrifuged for 20 h, 30,000 rpm, at 4 °C (21). Fractions were supplemented with BSA, precipitated with TCA, and analyzed by SDS/PAGE and Western blot using specific antibodies against Ube2S, UbcH10, Cdc20, Cdh1, and Cdc27. Western signals were quantified using Quantity One (Bio-Rad) and visualized by SigmaPlot.

Pulldown Assays. Recombinant MBP or ^{MBP}Ube2S were incubated with amylose resin (Stratagene) in IP buffer [50 mM HEPES (pH 7.5), 150 mM NaCl, 1.5 mM $MgCl_2$, 5 mM KCl, 0.1% Tween 20, and 2 mM DTT] for 2 h at 4 °C. Beads were washed and incubated with either 300 μL G1-extract, 15 μL IVT/T in IP-buffer, or 5 μg His-6Cdh1 in IP-buffer. Reactions were incubated for 4 h at 4 °C on a roller drum. Beads were extensively washed and eluted either in SDS gel buffer or PBS/10 mM maltose. Reactions were analyzed after SDS/PAGE by Coomassie staining or Western blotting as indicated.

RNAi, Immunofluorescence, and Quantification. *Drosophila* Kc167 cells were transfected with 10 μg/mL dsRNA directed against Ube2S and/or Vihar using DOTAP (Roche) as a transfecting agent following the manufacturer's instructions. Control cells were treated with DOTAP but no dsRNA. After 4 days, cells were settled onto positively-charged glass slides and fixed with 4% formaldehyde in PBST (PBS; 0.1% Triton X-100) for 10 min. After three washes with PBST, cells were blocked in 5% milk in PBST (MPBST) for 10 min before primary antibody incubation overnight at 4 °C, followed by three washes of 10 min in PBST. The secondary antibodies used were Alexa 488, Alexa 546, or Alexa 647 (Molecular Probes) conjugates, and were incubated at 1:500 dilutions for 45 min at room temperature in MPBST. After three 10-min washes in PBST, cells were mounted in 2.5% DABCO and 1 μg/mL DAPI in 50% glycerol. Primary antibodies used were: mouse anti-tubulin (Sigma) 1:500, rabbit anti-Phospho-H3 (Upstate) 1:500, and rabbit anti-Cnn (gift of T. Kaufman) 1:200. All images were taken on a Deltavision Spectris Microscope and deconvolved using softWoRx (Applied Precision). Images were taken as z-stacks of 0.3-μm increments, using oil-immersion objectives.

Quantification of mitotic index: large fields of cells were captured with a 40× objective, cells positive for the mitotic marker anti-Phospho-H3 were counted over the total number of cells.

Quantification of spindle and centrosome defects: 40–60 mitotic cells per RNAi experiment were imaged and mitotic cells were scored as defective or normal based on chromosome, centrosome and spindle morphology compared to control cells. The significance of differences was determined using the χ^2 test and P values.

Human UbcH10 and Ube2S were depleted by specific siRNA in HeLa and U2OS cells. Both cell lines behaved identical. Three different siRNAs were tested for UbcH10 (Dharmacon smartpool; two custom-made siRNA against the 3′-UTR of UbcH10 made by Dharmacon), and six different siRNA were tested for Ube2S (Dharmacon smartpool; siRNA against 3′-UTR: GGCACTGGGACCTGGATTT {used in most experiments}; CCTCCAACTCTGTCTCTAA; predesigned siRNA from Ambion: GCCTGCTGATCCACCCTAA; TGACGACACTGACCGCAGA; GGTCTGTTCCGCATGAAAC). siRNA against Emi1, Mad2, $p31^{comet}$, and Usp44 were described before (3, 4). siRNA against Cdh1 was from Dharmacon (smartpool). Cells were transfected by oligofectamine directly on coverslips for immunofluorescence or in 12-well plates for Western analysis. Cells were stained for endogenous human cyclin A2, cyclin B1, and Tpx2, as described before (5).

Live Imaging. Time-lapse imaging was carried out using a Deltavision Spectris Microscope (Applied Precision). S2 cells expressing mCherry-tubulin and H2B-GFP (gift of G. Goshima and R. Vale, UCSF), and S2 cells expressing cyclin B-GFP (gift of Eric Griffis and Ron Vale, UCSF) were transfected with dsRNA as above and mounted using the 'hanging drop' method in Schneider medium (Gibco) supplemented with 10% fetal calf serum and antibiotics/antimycotics (Gibco). For the mitotic arrest time-lapse videos, randomly selected mitotic cells were imaged every 15 min for a total of 3 h ($n > 5$ per RNAi experiment). For the centrosome defects, cells were imaged every 2 min for 1–2 h ($n > 5$ per RNAi experiment). For monitoring cyclin B-GFP degradation, cells were imaged every 3 min for the control RNAi and every 10 min for the Ube2s RNAi. Videos were deconvolved, equalized and quick-projected using Softworks (Applied Precision).

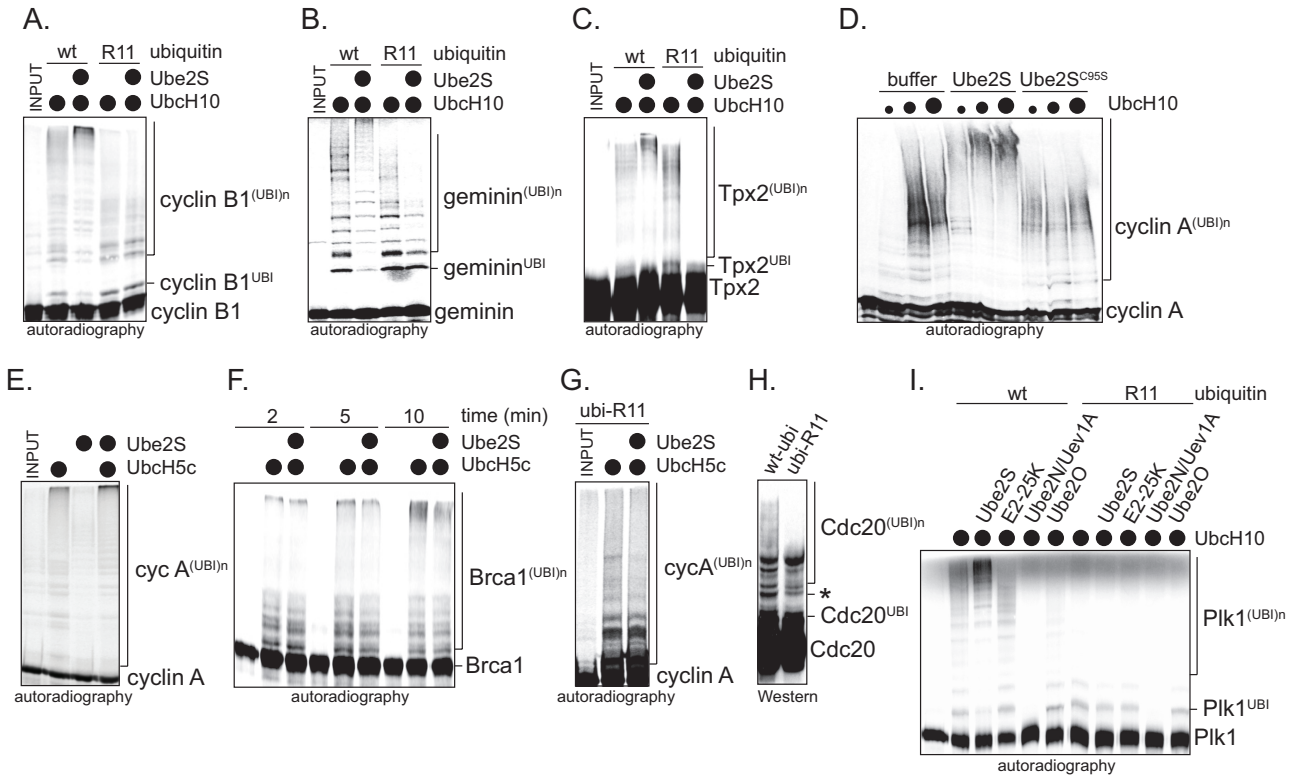

Fig. S1. Ube2S extends K11-linked ubiquitin chains on APC/C substrates. (*A*) Ube2S extends K11-linked ubiquitin chains on cyclin B1. APC/C^{Cdh1} and UbcH10/Ube2S were used to ubiquitinate ^{35}S-cyclin B1. The reactions were performed in the presence of ubiquitin or ubi-R11, and reaction products were detected by autoradiography. (*B*) Ube2S extends K11-linked ubiquitin chains on geminin. The ubiquitination of ^{35}S-geminin by APC/C^{Cdh1} in the presence of ubiquitin and ubi-R11 was performed as described above. (*C*) Ube2S extends K11-linked ubiquitin chains on Tpx2. The ubiquitination of ^{35}S-Tpx2 by APC/C^{Cdh1} in the presence of ubiquitin and ubi-R11 was performed as described above. (*D*) Ube2S extends ubiquitin chains beyond the length achieved by UbcH10 and APC/C alone. APC/C^{Cdh1} and increasing concentrations of UbcH10 ([UbcH10]≈10–50 nM) were used to promote ubiquitination of ^{35}S-cyclin A. Ube2S or Ube2S^{C95S} were added where indicated. The reaction products were separated on long 5–15% SDS gradient gels, and visualized by autoradiography. (*E*) Ube2S can extend ubiquitin chains formed by APC/C and UbcH5c. APC/C^{Cdh1} was used to promote the ubiquitination of ^{35}S-cyclin A. Ube2S and UbcH5c were added when indicated. This reaction contained an increased concentration of ^{35}S-cyclin A, including more reticulocyte lysate. E2 enzymes present in reticulocyte lysate are responsible for weak chain initiation observed in the reaction containing only Ube2S. Reaction products were separated on long 5–15% SDS gradient gels and analyzed by autoradiography. (*F*) Ube2S does not extend ubiquitin chains formed by Brca1. ^{35}S-Brca1 was incubated with E1, UbcH5c, and ubiquitin in the presence of buffer or Ube2S, and the time course of ubiquitination at 16 °C was analyzed after autoradiography. Addition of Ube2S has no effect on chain length. (*G*) Ube2S is unable to extend ubiquitin chains nucleated by UbcH5c, if ubi-R11 is present. The ubiquitination of ^{35}S-cyclin A by APC/C^{Cdh1} was analyzed in the presence of ubi-R11. (*H*) Ube2S extends K11-linked ubiquitin chains with APC/C^{Cdc20}. The ubiquitination of Cdc20 was analyzed after APC/C^{Cdc20} was precipitated from mitotic extracts and incubated with E1, p31^{comet}, and UbcH10/Ube2S. The reaction was performed in the presence of wt-ubiquitin or ubi-R11. Ubiquitinated Cdc20 was detected by Western blotting. I. The chain elongating E2 enzymes E2–25K and Ube2N/Uev1A do not cooperate with UbcH10 and APC/C^{Cdh1} in promoting chain assembly. The ubiquitination of ^{35}S-Plk1 by APC/C^{Cdh1} was initiated by incubation with UbcH10, and chain elongation was catalyzed by addition of Ube2S, E2–25K, or Ube2N/Uev1A. The reactions were performed in the presence of wt-ubiquitin and ubi-R11, and products were detected by autoradiography.

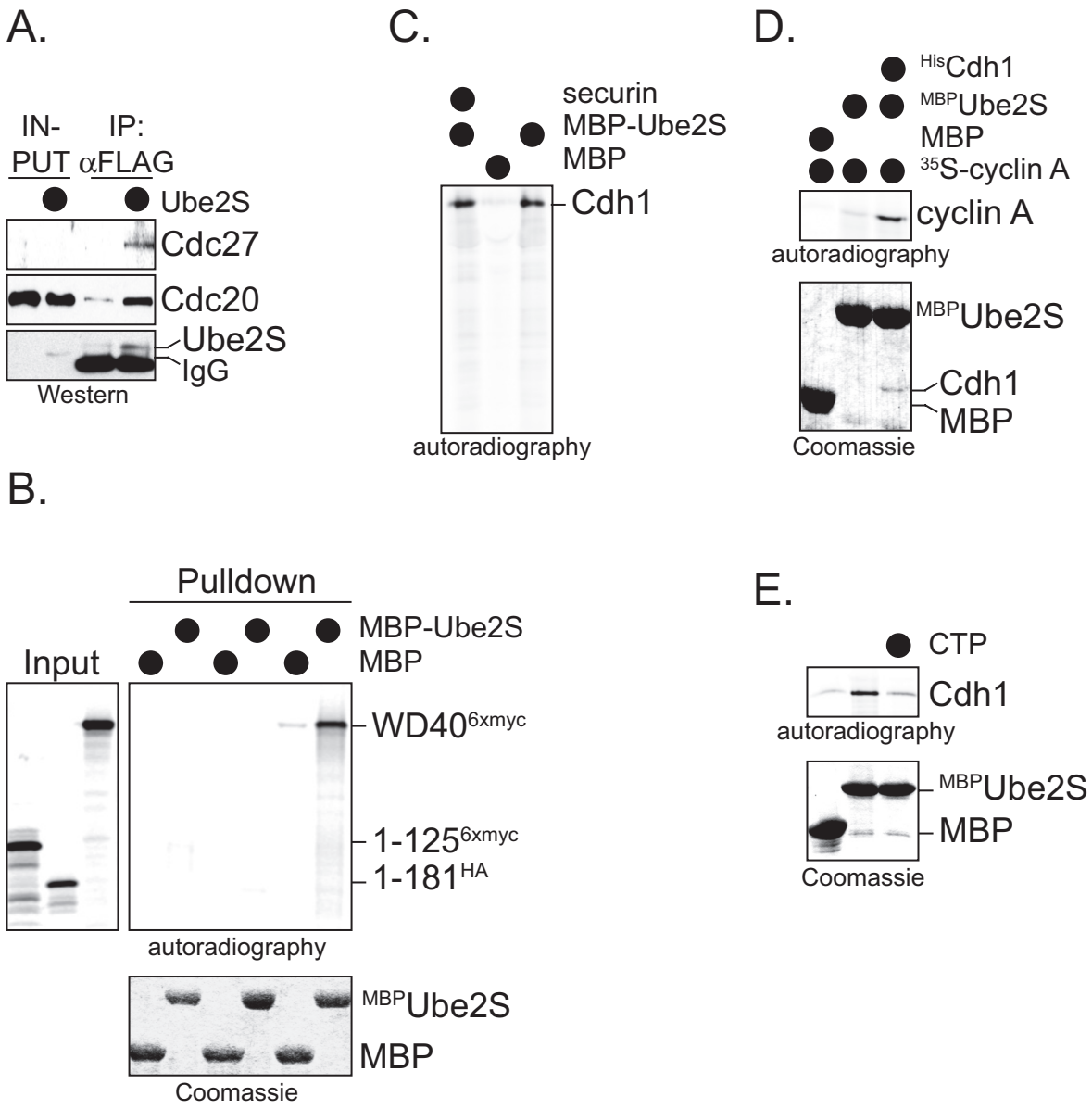

Fig. S2. Ube2S interacts with APC/C-activators. (*A*) ^{FLAG}Ube2S binds Cdc20 and core APC/C in mitosis. 293T cells were transfected with ^{FLAG}Ube2S and synchronized in mitosis. ^{FLAG}Ube2S was precipitated on FLAG-agarose, and co-purifying proteins were detected by Western blotting. (*B*) The interaction between Ube2S and ^{35}S-Cdh1 is mediated by the WD40-repeat domain of Cdh1. MBP or ^{MBP}Ube2S were coupled to amylose resin and incubated with the indicated Cdh1-truncation mutants. Bound proteins were detected by autoradiography. (*C*) APC/C substrates do not compete for binding of Ube2S to Cdh1. MBP or ^{MBP}Ube2S were immobilized on amylose resin and incubated with ^{35}S-Cdh1 either alone or in presence of an approximate 100-fold excess of the unlabeled APC/C substrate securin. Beads were extensively washed, eluted, and bound proteins were analyzed by autoradiography. (*D*) Cdh1 can bind substrates and Ube2S at the same time. MBP or ^{MBP}Ube2S were incubated with the APC/C substrate ^{35}S-cyclin A alone, or with ^{35}S-cyclin A and recombinant ^{His}Cdh1. The reaction products were analyzed by Coomassie staining and autoradiography. The substrate cyclin A is found in Ube2S complexes only in the presence of ^{His}Cdh1, indicating that Cdh1 can bind to Ube2S and substrate at the same time. (*E*) The C terminus of Ube2S is a binding element for Cdh1. ^{35}S-Cdh1 was incubated with the C-terminal Ube2S peptide and then added to MBP or ^{MBP}Ube2S-beads. Bound proteins were detected by autoradiography.

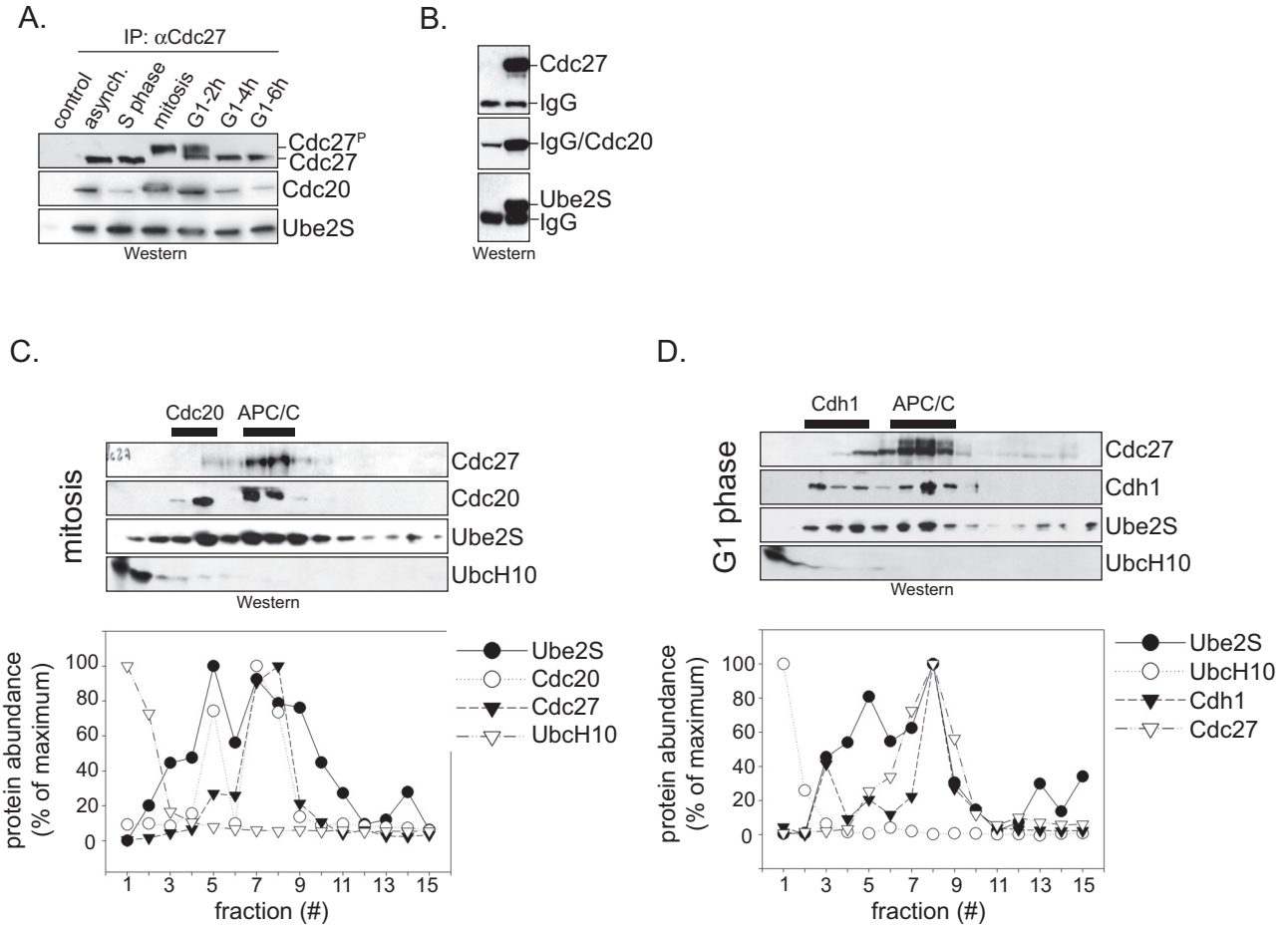

Fig. S3. Ube2S associates with APC/C during mitosis and G1. (*A*) Ube2S binds APC/C. The core APC/C-subunit Cdc27 was precipitated from extracts of synchronized HeLa cells using monoclonal αCdc27-antibodies, and bound Ube2S and Cdc20 were detected by Western blotting. (*B*) Ube2S binds core APC/C during mitosis. 293T cells stably expressing ^{FLAG}Ube2S were arrested in mitosis. ^{FLAG}Ube2S was precipitated, and co-purifying Cdc27 and Cdc20 were detected by Western blotting. Cdc20 runs very closely to IgG, explaining the background in the control reaction. (*C* and *D*) Ube2S co-fractionates with APC/C in extracts of HeLa S3 cells in mitosis (*C*) and G1 (*D*), as detected by sucrose gradient centrifugation. Sucrose gradient centrifugations of mitotic or G1 extracts were fractionated, proteins were precipitated with TCA, and the indicated proteins were detected by Western blotting using specific antibodies. The signal intensity of the Western blots was quantified using Quantity One; it shows that the peaks of the Ube2S, Cdc20/Cdh1, and APC/C blots overlap.

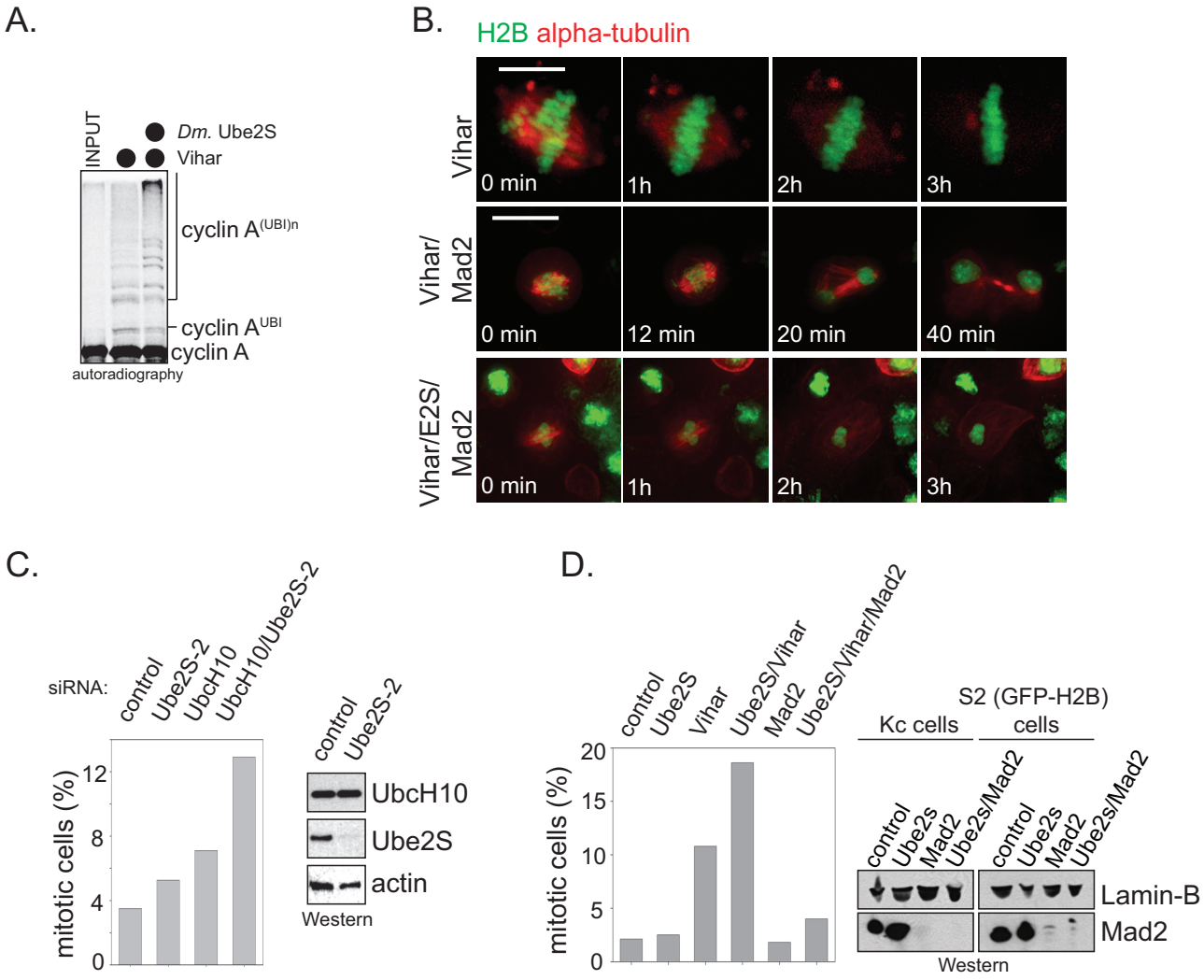

Fig. S4. Effects of Ube2S-depletion. (*A*) *Drosophila* Vihar/UbcH10 and Ube2S catalyze ubiquitin chain formation by APC/C. ^{35}S-cyclin A was incubated with human APC/C^{Cdh1} and recombinant *Drosophila* Vihar/UbcH10 and *Drosophila* Ube2S. The reaction products were detected by autoradiography. (*B*) The depletion of UbcH10/Vihar by RNAi in S2 cells stably expressing histone H2B-GFP and α-tubulin-mCherry causes a mitotic arrest, which is rescued by co-depletion of Mad2. If Ube2S is depleted in addition to Vihar, Mad2 does not rescue the mitotic arrest, and cells slip into G1 without chromosome segregation. (*C*) The co-depletion of Ube2S and UbcH10 causes mitotic arrest. HeLa cells were treated with the indicated siRNAs. The number of mitotic cells in the bulk population was determined 48 h later. The siRNA against Ube2S is different from the siRNA used for Fig. 5. The right panel shows the efficiency of depletion, as determined by Western analysis. (*D*) The co-depletion of Ube2S and UbcH10/Vihar by RNAi causes a pronounced mitotic arrest in S2 cells, as scored by pH3 staining. The right panel shows depletion of Mad2 in both Kc and S2 cells, as detected by Western blotting.

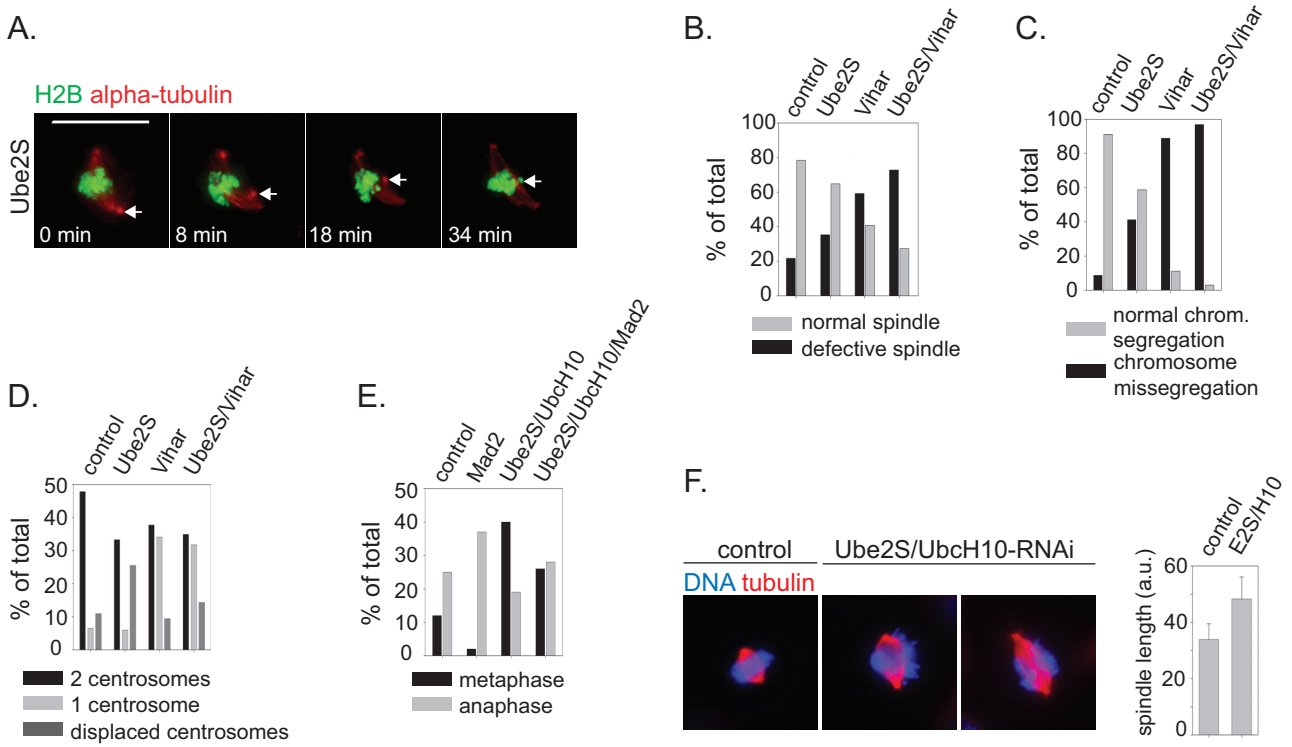

Fig. S5. Depletion of Ube2S and UbcH10 causes widespread spindle defects. (*A*) Ube2S-depletion leads to spindle pole detachment. *Drosophila* S2 cells stably expressing H2B-GFP and α-tubulin-mCherry were treated with RNAi against Ube2S, and imaged by time-lapse microscopy. The arrow indicates a spindle pole, which detaches from the spindle in the RNAi-treated cells. (*B*) Quantification of spindle defects in *Drosophila* Kc cells treated with the indicated siRNA and analyzed by fluorescence microscopy against tubulin. (*C*) Quantification of chromosome missegregation events in postmetaphase Kc cells treated with RNAi as described above. (*D*) Quantification of centrosome defects in RNAi-treated Kc cells measured by immunofluorescence with anti-centrosomin (Cnn) antibodies. (*E*) Depletion of Ube2S and UbcH10 delays mitosis in HeLa cells even in the absence of Mad2. Mitotic cells in metaphase or anaphase were counted after depletion of Mad2, Ube2S/UbcH10, or Mad/Ube2S/UbcH10 by specific siRNA. (*F*) Ube2S and UbcH10 are required for proper spindle formation. Depletion of Ube2S and UbcH10 from HeLa cells results in spindle elongation, chromosome congression defects, and spindle pole abnormalities. Representative figures are shown, in which DNA is detected by DAPI (blue), and the spindle is visualized by immunofluorescence against α-tubulin (red). The length of multiple spindles was measured in control and depleted cells, which is shown in the right panel.

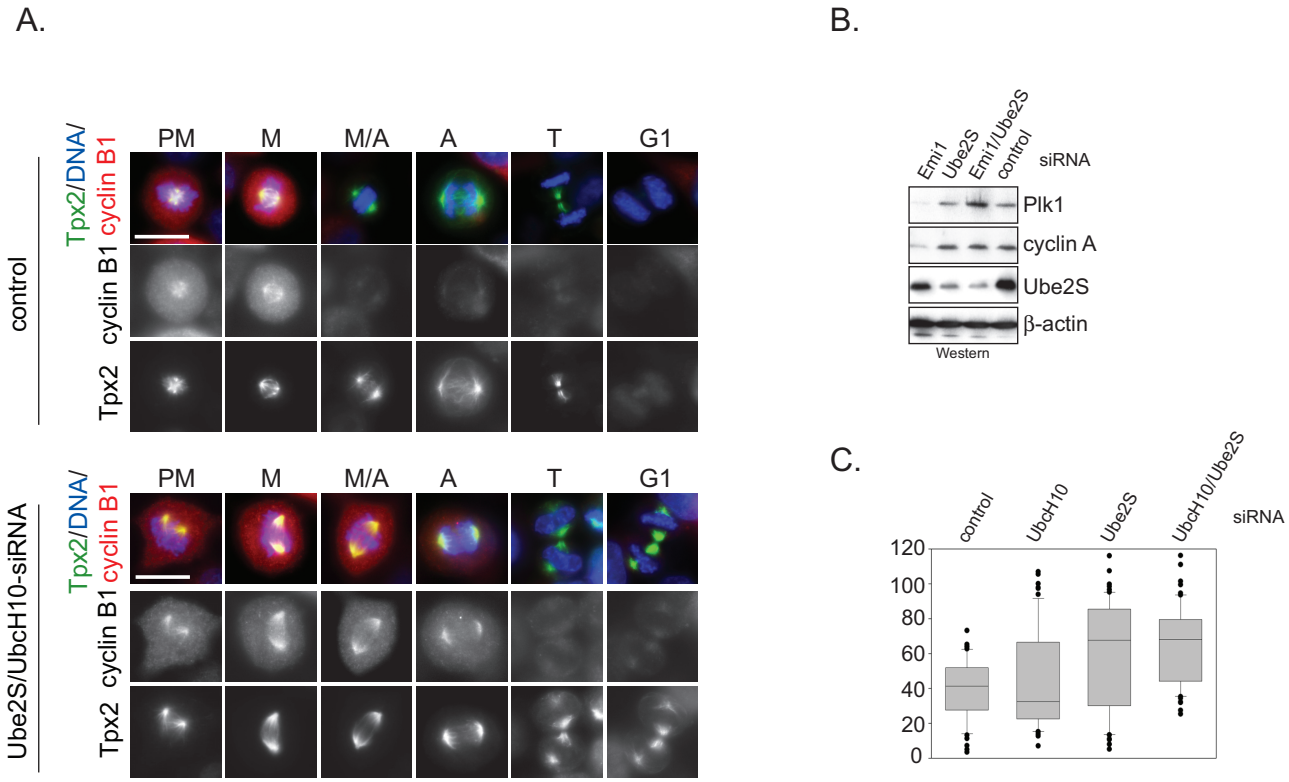

Fig. S6. Ube2S is crucial for APC/C-activity in vivo. (*A*) Loss of Ube2S and UbcH10 stabilizes cyclin B1 and Tpx2 on the spindle. HeLa cells were transfected with the indicated siRNA, and stained with antibodies against cyclin B1 (red) and Tpx2 (green). DNA was detected by DAPI-staining (blue). Representative images of all mitotic stages are shown. (Scale bar, 10 μm.) Co-depletion of Ube2S and UbcH10 results in strong stabilization of cyclin B1 and Tpx2. (*B*) Ube2S is required for APC/C^{Cdh1} activity in interphase. HeLa cells were treated with control siRNA or specific siRNAs against Emi1 (to activate APC/C and degrade APC/C substrates), Ube2S, or both. The indicated proteins were detected by Western blots. (*C*) Ube2S is required for Tpx2-degradation in cells. HeLa cells were stained for endogenous Tpx2 by immunofluorescence. The fluorescence intensity in G1 cells was measured and plotted for approximately 100 cells per experiment.

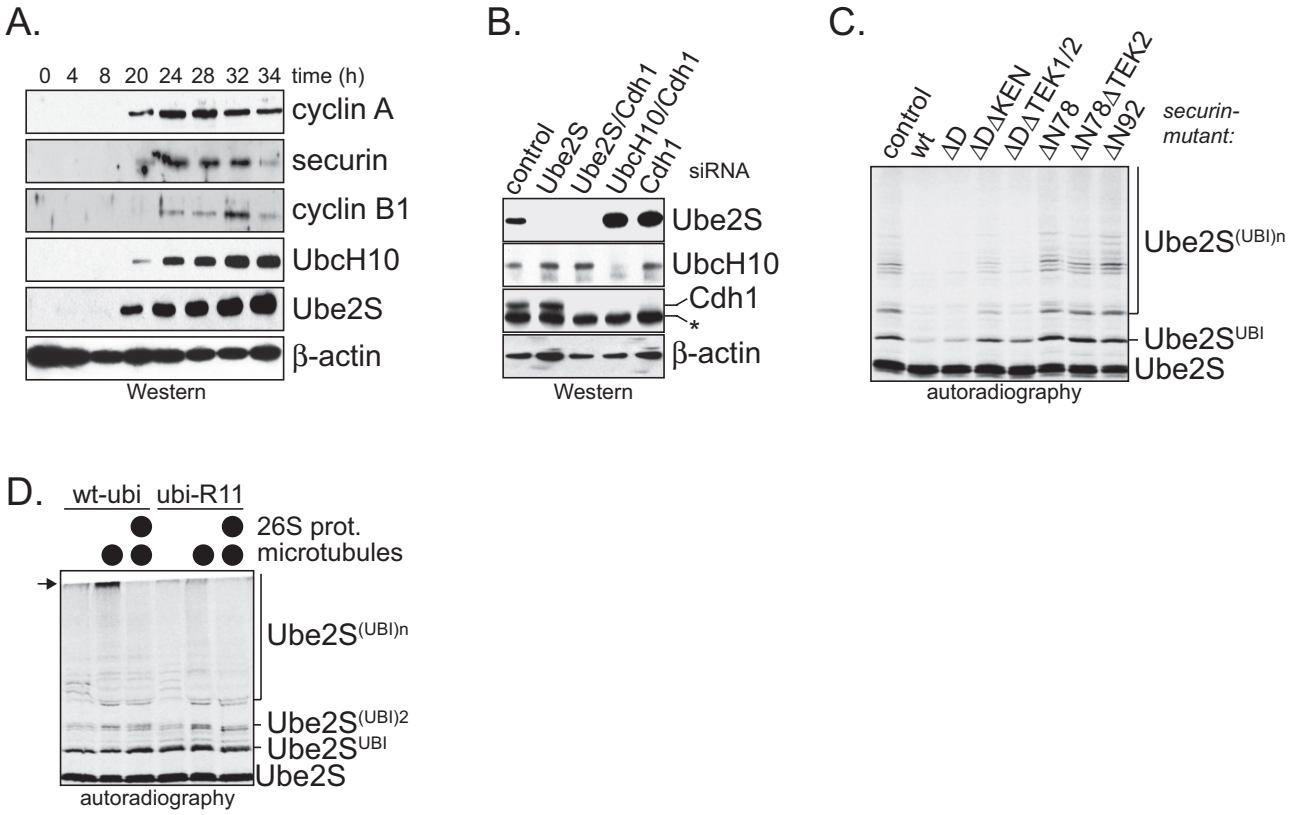

Fig. S7. APC/C-dependent ubiquitination and degradation of Ube2S. (*A*) Ube2S is co-regulated with UbcH10 during quiescence. T24 cells were synchronized in quiescence by serum starvation, and allowed to reenter the cell cycle by serum stimulation. The levels of the indicated proteins, including Ube2S, were measured by Western blotting. (*B*) Ube2S levels increase after APC/C^{Cdh1}-inhibition in vivo. HeLa cells were treated with the indicated siRNAs, and the levels of Ube2S, Cdh1, UbcH10, and β-actin were determined by Western blotting. The asterisk marks a cross-reactive band of the Cdh1-antibody. (*C*) APC/C substrates inhibit ubiquitination of Ube2S by APC/C^{Cdh1}. APC/C^{Cdh1} was incubated with the recombinant APC/C substrate securin or the indicated securin mutants and tested for its capability to ubiquitinate ^{35}S-Ube2S. UbcH5c was added as E2. Reaction products were detected by autoradiography. (*D*) The ubiquitination of Ube2S by APC/C^{Cdh1} is stimulated by microtubules, proceeds by formation of K11-linked ubiquitin chains, and results in Ube2S degradation. ^{35}S-Ube2S was incubated with APC/C^{Cdh1} and UbcH5c in the presence taxol-stabilized microtubules and ubiquitin or ubi-R11. Purified 26S proteasomes were added when indicated. The reaction products were detected by autoradiography.

Table S1. Ube2S interaction partners in asynchronously grown 293T cells

APC/C	APC1 (6); APC2 (3); APC4 (5); APC5 (4); APC6 (3); APC7 (7); APC10 (2); Cdc23 (4); Cdc27 (2)
Ribosome	RPS3 (5); RPS3a (3); RPS5 (5); RPS6 (3); RPS7 (4); RPS8 (4); RPS8c (2); RPS9 (3); RPS13 (3); RPS14 (2); RPS15a (2); RPS16 (3); RPS19 (3); RPS20 (2); RPS21 (2); RPS25 (3); RPS27a (9); RPS28 (3); RPSA (4); RPL5 (6); RPL6 (5); RPL7 (4); RPL7a (6); RPL8 (4); RPL9 (3); RPL9 (3); RPL10a (3); RPL11 (5); RPL12 (2); RPL13 (5); RPL13a (2); RPL17-like (2); RPL18 (3); RPL18a (2); RPL19 (2); RPL24 (5); RPL26 (2); RPL27a (2); RPL28 (3); RPL30 (2); RPL38 (2); RPLP0 (3); RPLP2 (3); LOC388339 (3); LOC389342 (5); LOC439992 (3); LOC100129902 (2)
Ribosome-associated	EIF6 (2); SRP14 (2); PA2G4 (3); EF2 (11); PABPC1 (2); EIF3; ILF2 (2); RACK1 (2)
Other	α-tubulin (2); β-tubulin (4); nucleolin (3); ASPM (2); Nek2; Aurora A; MAST1

Interaction partners of Ube2S in asynchronously grown 293T cells. ^{FLAG}Ube2S was immunopurified from a stable 293T cell line by FLAG-agarose and eluted by FLAG-peptide. Elutions were concentrated, and proteins were identified by MudPIT-mass spectrometry (Berkeley Proteomic Center). Proteins specifically identified in Ube2S-IPs are listed, and the number of unique peptides is shown in the brackets.