Supplementary Information

CAND1 controls *in vivo* dynamics of the Cullin 1-RING ubiquitin ligase repertoire

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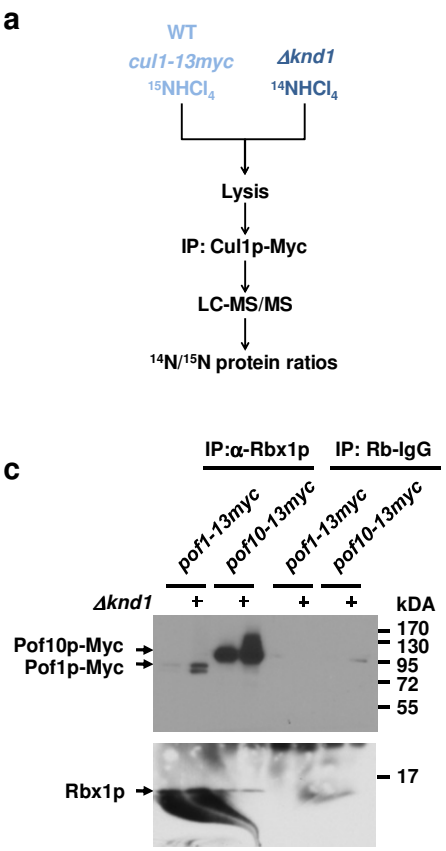


b

Spectral counts of Cul1p-associated proteins isolated from ^{15}N labeled wildtype cell lysate in the presence of ^{14}N lysate

	Spectral Counts	
Protein	^{15}N	^{14}N
Cul1p	136	1
Ned8p	3	0
Skp1p	54	1
Rbx1p	5	0
Pof1p	13	0
Pof7p	2	0
Pof10p	15	2
Pof15p	8	0
Pop1p	2	0
Ssa2p	44	34
Tdh1p	38	45
Ef1a-b	31	31
Rpl8-1p	11	10
Rpl26p	10	12
Tef3p	8	8

Supplementary Figure S1 Increased levels of CRL1 complexes in Δ*knd1* cells

(a) Outline of the control experiment to confirm that CRL1 do not rearrange after cell lysis and during immunopurification. Note that only the wildtype cell lysate labeled with ^{15}N contained a tagged allele for affinity purification of Cul1p (*cul1-13myc*).

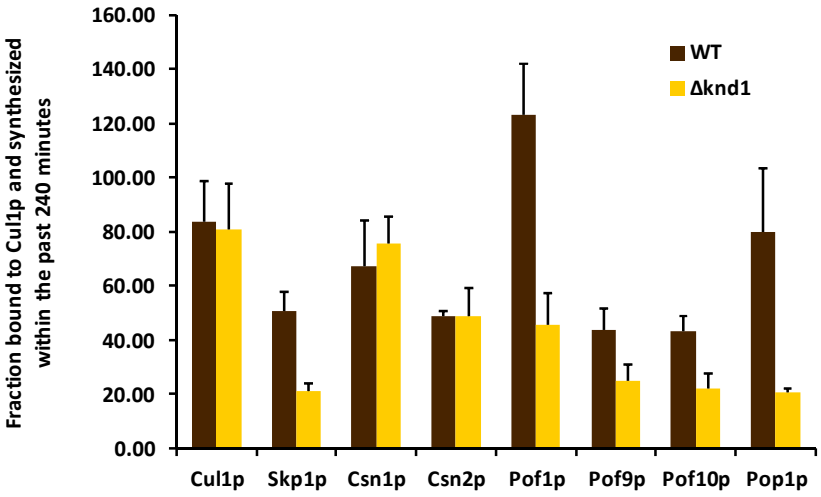
(b) Spectral counts of Cul1p-associated proteins (green) and unspecifically retrieved proteins (yellow) separated by isotope.

(c) The same cell lysates as those used for the FBP immunoprecipitation experiment shown in Fig. 1d were used for immunoprecipitation with anti-Rbx1p antibody or with rabbit IgG as a control. Co-precipitation of Pof1p-Myc and Pof10-Myc was assessed by immunoblotting with Myc antibodies. The corresponding total cell lysates are shown in Fig. 1d.

a **Fraction of newly synthesized CRL1 components purified via anti-Myc antibodies from Cul1p-13myc-tagged wildtype or Δ*knd1* cells after ^{15}N pulse-labeling for 240 minutes**

	$^{15}N/^{14}N*100$, WT			$^{15}N/^{14}N*100$, Δ*knd1*		
Protein	WT	**SD**	**RSD**	Δ*knd1*	**SD**	**RSD**
Cul1p	83.59	15.15	0.18	81.07	16.73	0.21
Skp1p	50.80	7.19	0.14	21.01	2.75	0.13
Csn1p	67.31	16.67	0.25	75.70	9.86	0.13
Csn2p	48.94	1.86	0.04	48.76	10.62	0.22
Pof1p	123.41	18.63	0.15	45.61	11.85	0.26
Pof9p	43.89	7.92	0.18	24.78	6.28	0.25
Pof10p	43.25	5.59	0.13	22.07	5.57	0.25
Pop1p	79.98	23.67	0.30	20.78	1.04	0.05

b

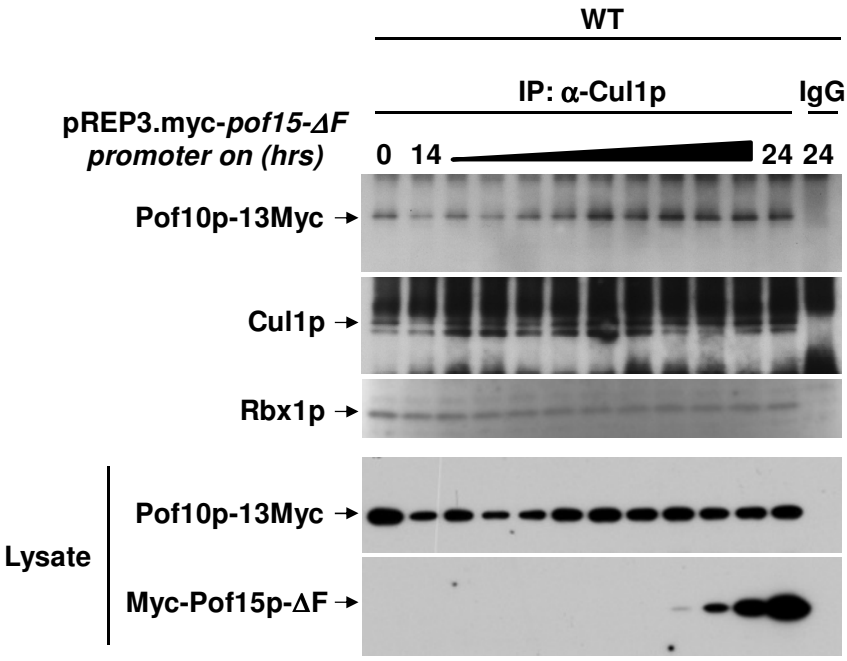


Supplementary Figure S2. Effect of CAND1/Knd1p on CRL1 dynamics

^{15}N pulse-labeling of wildtype and Δ*knd1* cells was carried out for 240 minutes, followed by lysate preparation, Cul1p IP, and quantitative analysis by LC-MS/MS.

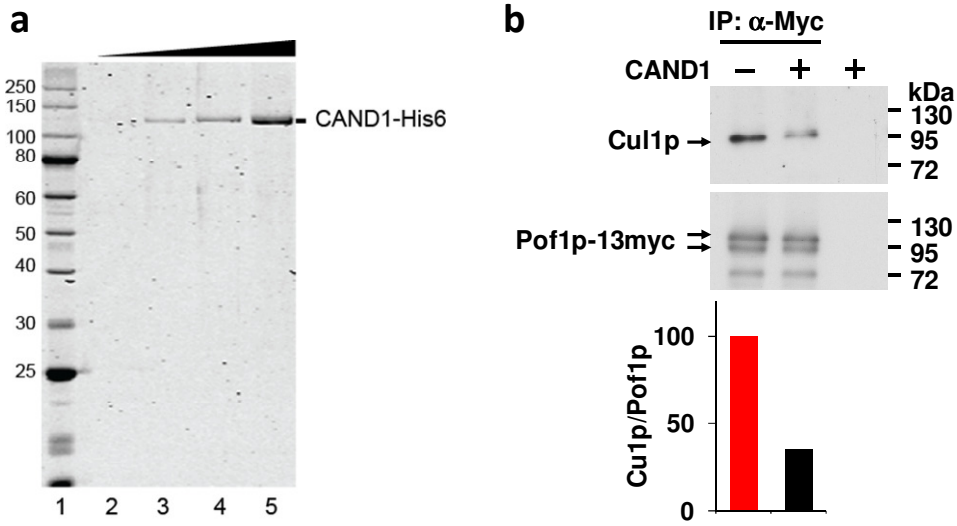
(a) The table lists the fractions of Cul1p-associated proteins synthesized during the 240 minute labeling period. SD = standard deviation, RSD = Relative standard deviation.

(b) A bar graph of the data in (a).



Supplementary Figure S3. FBP competition depends on the integrity of the F-box motif

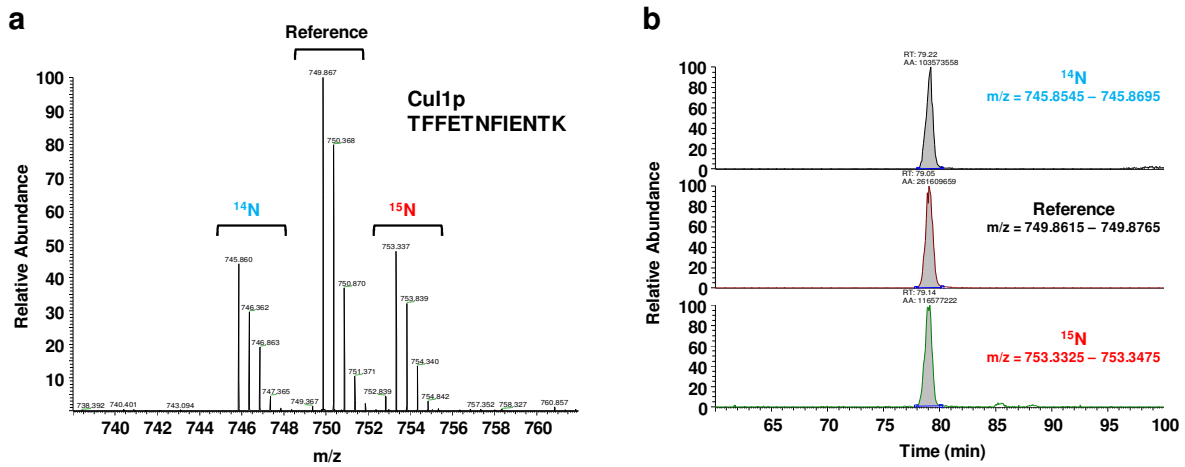
The same experiment as described in Fig. 3 except that a Myc epitope-tagged Pof15p lacking 7 residues of the F-box motif (=Pof15p-ΔF) was expressed from a pREP3 plasmid containing an inducible promoter in strains that harbor endogenously tagged Pof10p-Myc. Myc-Pof15p-ΔF expression was switched on by the removal of thiamine for 14 – 24 h as indicated. CRL1 complexes were immunoprecipitated with anti-Cul1p antibodies and monitored for the levels of co-precipitated Pof10p-Myc and Myc-Pof15p-ΔF. As a specificity control, the 24 h lysate was used for immunoprecipitation with rabbit IgG. The bottom panel shows total lysates. Unlike wildtype Pof15p (Fig. 3), mutant Pof15p-ΔF is unable to displace Pof10p-Myc from Cul1p.



Supplementary Figure S4. Recombinant human CAND1 used for FBP replacement experiments

(a) His-tagged human CAND1 was expressed from a baculovirus in insect cells and purified. Increasing amounts of a 9.3 uM solution of purified protein were loaded on a gel and stained with Coomassie brilliant Blue. Lane 1: 0.02 ul; 2: .05 ul; 3: .2 ul; 4: .5 ul.

(b) Pof1p-Myc-associated protein complexes were immunopurified from Δ*knd1* cells and incubated with 1 µg of recombinant His-tagged human CAND1 for 30 minutes. 1 µg of bovine serum albumin was used as control instead of CAND1. The complexes were analyzed for the levels of Cul1p and Pof1p-Myc by immunoblotting with Cul1p and Myc antibodies, and signals were quantified.



Supplementary Figure S5. AQUA analysis of Cul1p

(a) MS chromatogram of the spiked Cul1p reference peptide TFFETNFIENTK (100 fmol) and the corresponding ^{14}N- and ^{15}N-labeled peptides retrieved from a 1:1 mixture of wildtype and Δ*knd1* cell lysate.

(b) Peak integrations were obtained through application of extracted ion chromatograms over 10-ppm mass intervals on Qual Browser (Thermo Fisher).

Supplementary Table S1: Relative abundance ratios of CRL1 components purified from wildtype (^{15}N) and Δ*knd1* (^{14}N) cells

Protein	Experiment 4974 (EXP 1) Δ*knd1* /WT	SD	Experiment 5025 (EXP 2) Δ*knd1* /WT	SD	Experiment 5279 (EXP 3) Δ*knd1* /WT	SD	All Experiments Δ*knd1* /WT	SD	p-value
Cul1p	**1.06**	0.05	**0.87**	0.05	**0.92**	0.05	**0.95**	0.10	0.47
Rbx1p	**0.98**	0.40	**0.88**	0.10	**0.87**	0.07	**0.91**	0.06	0.12
Skp1p	**0.80**	0.07	**0.66**	0.07	**0.69**	0.03	**0.72**	0.07	0.02
CSN1p	**1.01**	0.21	**1.06**	0.19	**1.21**	0.12	**1.09**	0.10	0.26
CSN2p	**0.92**	0.22	**0.99**	0.12	**1.11**	0.27	**1.01**	0.10	0.92
CSN4p			**0.77**	0.01			**0.77**	0.01	na
CSN5p	**1.18**	0.17	**1.15**	0.29	**1.26**	0.09	**1.20**	0.06	0.03
Pof1p	**1.47**	0.19	**1.20**	0.11	**1.33**	0.27	**1.33**	0.14	0.05
Pof5p	**0.37**	na	**0.37**	0.07	**0.58**	na	**0.44**	0.12	0.02
Pof7p	**1.91**	0.17	**2.52**	na	**2.09**	0.31	**2.17**	0.31	0.02
Pof9p	**1.66**	0.19			**2.52**	0.21	**2.09**	0.61	0.24
Pof10p	**2.08**	0.18	**1.82**	0.21	**1.93**	0.12	**1.94**	0.13	0.01
Pof11p	**0.75**	0.07	**0.65**	na	**0.66**	0.09	**0.69**	0.06	0.01
Pof14p	**1.95**	na	**1.49**	0.11	**1.98**	0.41	**1.81**	0.27	0.04
Pof15p	**0.26**	0.02	**0.19**	0.02	**0.13**	0.02	**0.19**	0.07	0.00
Pop1p	**0.60**	0.07	**0.49**	0.05	**0.38**	0.04	**0.49**	0.11	0.02
Pop2p	**0.99**	na			**0.91**	0.23	**0.95**	0.06	0.27
Average ratio of all proteins identified	0.95		1.10		1.01				

na = not applicable
SD = Standard deviation