Supplemental Materials

The dual role of fission yeast Tbc1/cofactor C orchestrates microtubule homeostasis in tubulin folding and as a GAP for GTPase Alp41/Arl2

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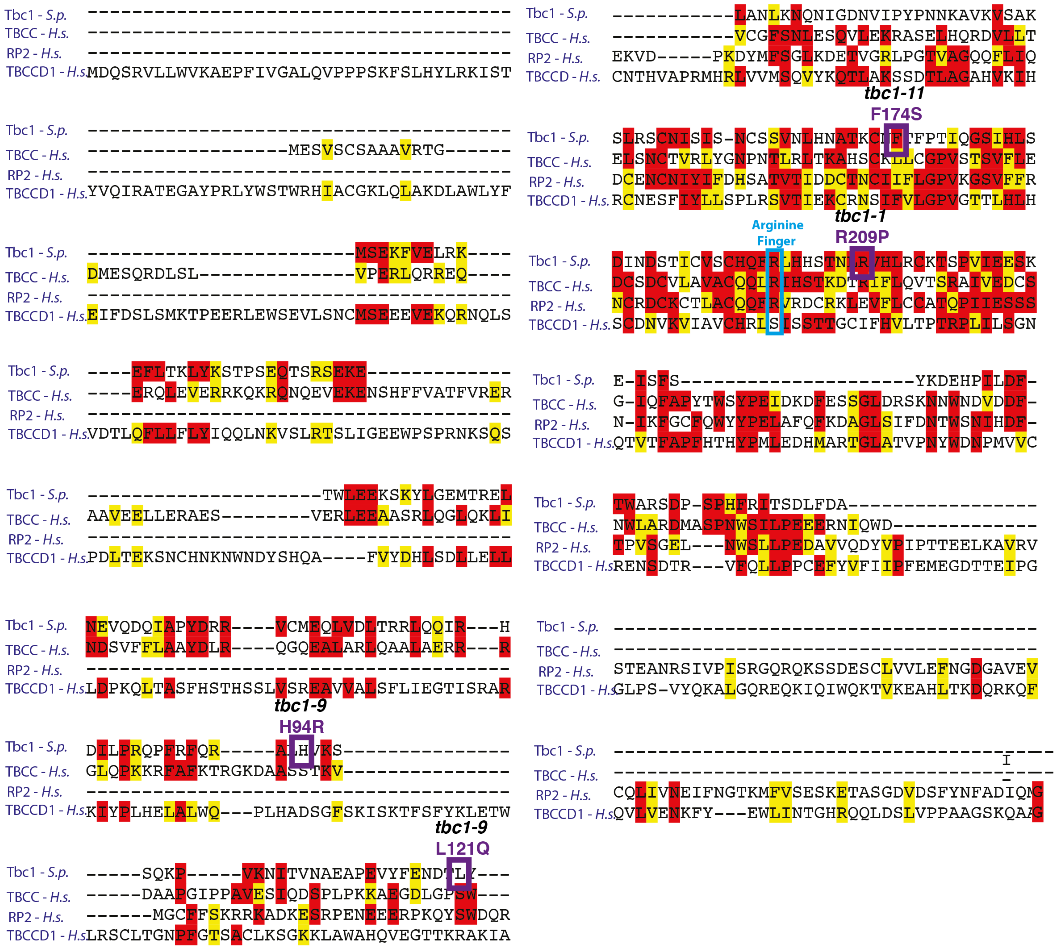
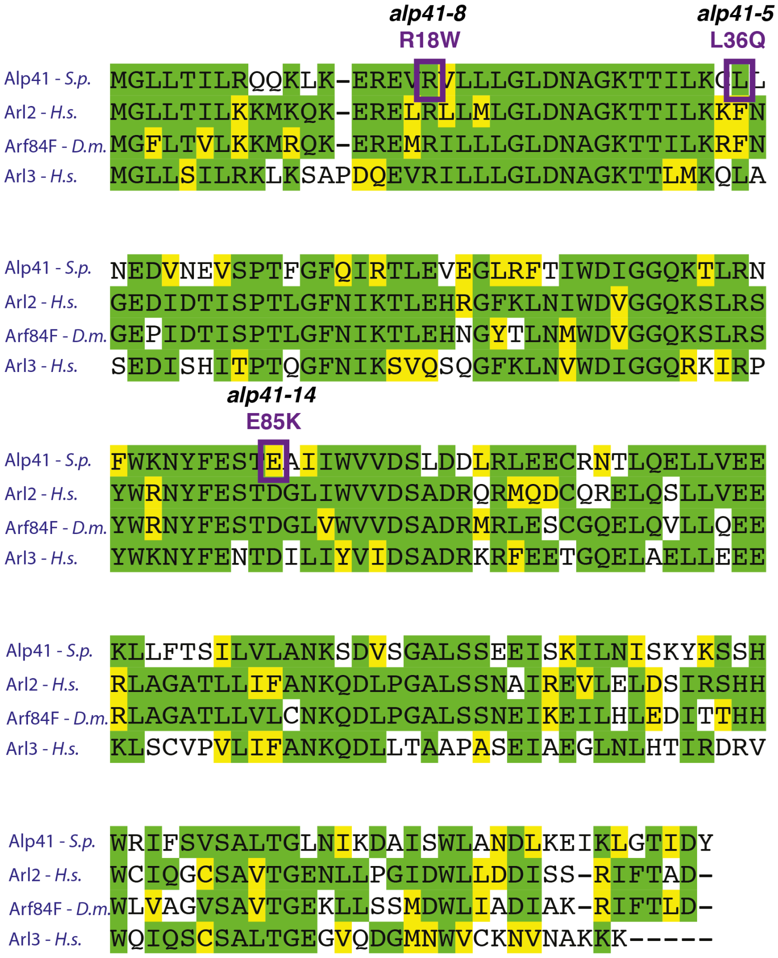
Supplemental Figure S1: Sequence alignment of Tbc1 and related molecules.
Supplemental Figure S2: Sequence alignment of Alp41 and related molecules.
Supplemental Figure S3: Prolonged overproduction of $\mathrm{Alp1^D}$ shows an eventual loss of microtubules.
Supplemental Figure S4: Overproduced Alp41 does not localize to microtubules.
Supplemental Figure S5: $\mathrm{Alp1^D}$ does not localize to microtubules in the absence of overproduction.
Supplemental Table S1: Fission yeast strains used in this study.



Supplemental Figure S1. Sequence alignment of Tbc1 and related molecules.
Amino acid sequences of *S. pombe* Tbc1 were aligned with human cofactor C, RP2 and TBCCD1 using Clustal W2 and hand adjustment. The blue label marks the conserved arginine finger. Residues marked with red show exact conservation, yellow marks residues with similar amino acid properties. Mutation sites of *tbc1* ts mutants are shown in violet.



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Alp41 - S.p.    MGLLTILRQQKLK-EREVRVLLLGLDNAGKTTILKCLL
Arl2 - H.s.     MGLLTILKKMKQK-ERELRLLMLGLDNAGKTTILKKFN
Arf84F - D.m.   MGFLTVLKKMRQK-EREMRILLLGLDNAGKTTILKRFN
Arl3 - H.s.     MGLLSILRKLKSAPDQEVRILLLGLDNAGKTTLMKQLA

Alp41 - S.p.    NEDVNEVSPTFGFQIRTLEVEGLRFTIWDIGGQKTLRN
Arl2 - H.s.     GEDIDTISPTLGFNIKTLEHRGFKLNIWDVGGQKSLRS
Arf84F - D.m.   GEPIDTISPTLGFNIKTLEHNGYTLNMWDVGGQKSLRS
Arl3 - H.s.     SEDISHITPTQGFNIKSVQSQGFKLNVWDIGGQRKIRP

Alp41 - S.p.    FWKNYFESTEAIIWVVDSLDDLRLEECRNTLQELLVEE
Arl2 - H.s.     YWRNYFESTDGLIWVVDSADRQRMQDCQRELQSLLVEE
Arf84F - D.m.   YWRNYFESTDGLVWVVDSADRMRLESCGQELQVLLQEE
Arl3 - H.s.     YWKNYFENTDILIYVIDSADRKRFEETGQELAELLEEE

Alp41 - S.p.    KLLFTSILVLANKSDVSGALSSEEISKILNISKYKSSH
Arl2 - H.s.     RLAGATLLIFANKQDLPGALSSNAIREVLELDSIRSHH
Arf84F - D.m.   RLAGATLLVLCNKQDLPGALSSNEIKEILHLEDITTHH
Arl3 - H.s.     KLSCVPVLIFANKQDLLTAAPASEIAEGLNLHTIRDRV

Alp41 - S.p.    WRIFSVSALTGLNIKDAISWLANDLKEIKLGTIDY
Arl2 - H.s.     WCIQGCSAVTGENLLPGIDWLLDDISS-RIFTAD-
Arf84F - D.m.   WLVAGVSAVTGEKLLSSMDWLIADIAK-RIFTLD-
Arl3 - H.s.     WQIQSCSALTGEGVQDGMNWVCKNVNAKKK-----
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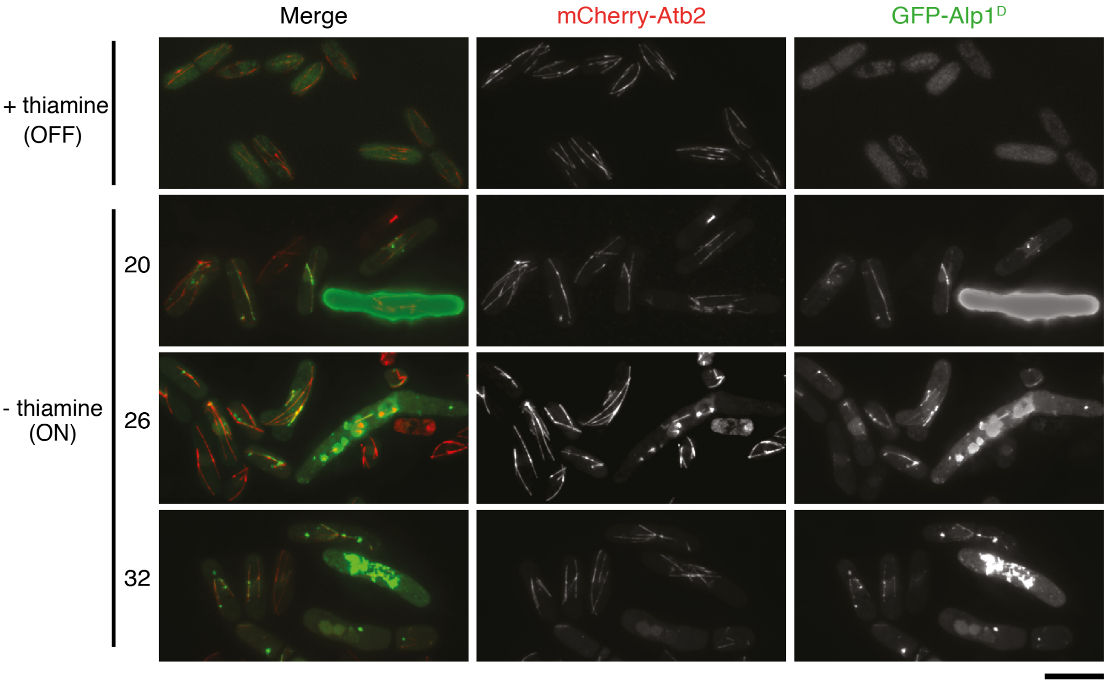
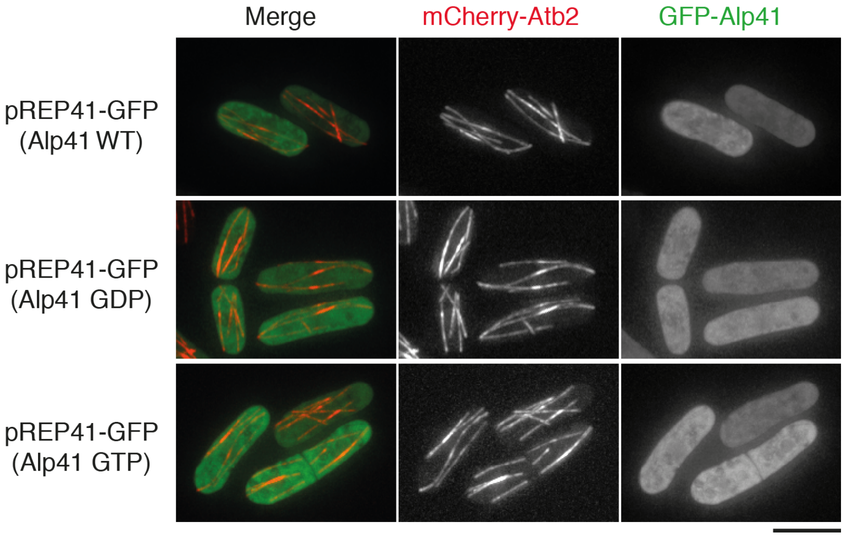
Supplemental Figure S2. Sequence alignment of Alp41 and related molecules. Amino acid sequences of *S. pombe* Alp41 were aligned with its human ortholog Arl2, related protein Arl3 and the *Drosophila melanogaster* ortholog Arf84F using Clustal W2 and hand adjustment. Residues marked with green show exact conservation, yellow marks residues with similar amino acid properties. Mutation sites of *alp41* ts mutants are shown in violet.

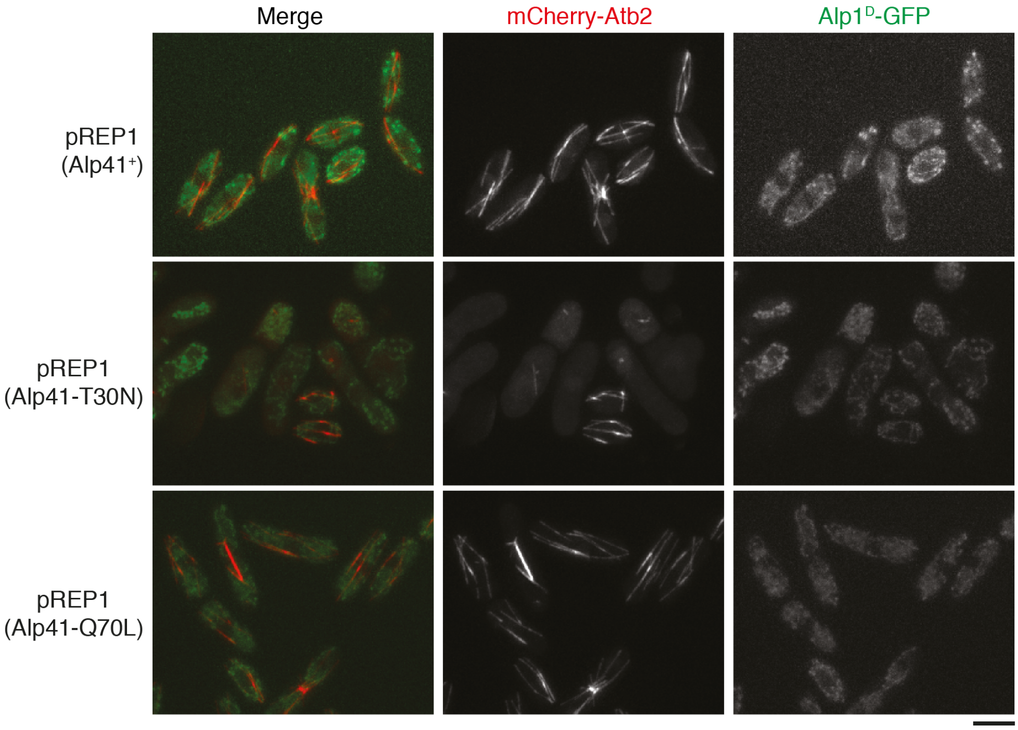


Supplemental Figure S3. Prolonged overproduction of Alp1^D shows an eventual loss of microtubules.

Cells overproducing Alp1^D over 20 to 32 h in minus thiamine conditions shows gradual accumulation of GFP-Alp1^D signal on the residual microtubules and eventual loss. Bar = 5 μm.



Supplemental Figure S4. Overproduced Alp41 does not localize to microtubules.
Cells containing mCherry-Atb2 were transformed with GFP-tagged Alp41 (wild-type, GDP-bound T30N and GTP-bound Q70L). Images were taken after 18 h of incubation in minus thiamine conditions. All forms of Alp41 show cytosolic localization and none on the microtubules. Bar = 5 µm.



Supplemental Figure S5. Alp1^D does not localize to microtubules in the absence of overproduction.

Alp1^D-GFP signals derived from the *alp1*$^+$ gene under its own promoter were observed under conditions in which various forms of Alp41 (wild-type, GDP-bound T30N and GTP-bound Q70L) were overproduced. Alp1^D does not localize to the microtubules in either form. Bar = 5 µm.

Supplemental Table S1. Fission yeast strains used in this study.

Strain	Genotype	Figure used
RM176	*h*$^{-}$ *alp1*$^{+}$*-3pk-kan*^{r} *leu1 ura4*	1C, 5A, 5B, 5C
RM473	*h*$^{-}$ *tbc1*$^{+}$*-3HA-hph*^{r} *alp21*$^{+}$*-FLAG-nat*^{r} *leu1 ura4*	1C
RM443	*h*$^{-}$ *tbc1*$^{+}$*-3HA-hph*^{r} *alp1*$^{+}$*-3pk-kan*^{r} *alp21*$^{+}$*-FLAG-nat*^{r} *leu1 ura4*	1C, 1D
RM385	*h*$^{-}$ *alp21*$^{+}$*-FLAG-nat*^{r} *leu1 ura4*	1D
RM475	*h*$^{-}$ *tbc1*$^{+}$*-3HA-hph*^{r} *alp1*$^{+}$*-3pk-kan*^{r} *leu1 ura4*	1D
RM036	*h*$^{-}$ *tbc1-1-hph*^{r} *leu1 ura4*	2A
RM052	*h*$^{-}$ *tbc1-9-hph*^{r} *leu1 ura4*	2A
RM057	*h*$^{-}$ *tbc1-11-hph*^{r} *leu1 ura4*	2A, 3F
RM376	*h*$^{-}$ *tbc1-11-hph*^{r} *SV40-GFP-atb2*$^{+}$ *leu1*$^{+}$ *ura4*	2B, 2C
RM733	*h*$^{-}$ *tbc1-11-hph*^{r} *SV40-GFP-atb2*$^{+}$ *cut11*$^{+}$*-RFP-nat*^{r} *leu1*$^{+}$ *ura4*	2B
DH2-8D	*h*$^{-}$ *alp1-1315 leu1*	2D, 5D
PR6	*h*$^{-}$ *alp11-924 leu1 ura4*	2D
RM064	*h*$^{-}$ *alp41-5-hph*^{r} *leu1 ura4*	3A
RM070	*h*$^{-}$ *alp41-8-hph*^{r} *leu1 ura4*	3A, 3F
RM082	*h*$^{-}$ *alp41-14-hph*^{r} *leu1 ura4*	3A, 3F
RM596	*h*$^{-}$ *alp41-5 aur*^{r}*-pnda3-GFP-atb2*$^{+}$ *leu1 ura4*	3B, 3C
RM128	*h*$^{-}$ *kan*^{r}*-nmt1-GFP-alp41*$^{+}$ *leu1 ura4*	3D
RM429	*h*$^{-}$ *tbc1*$^{+}$*-3HA-hph*^{r} *leu1 ura4*	3D
RM469	*h*$^{-}$ *tbc1*$^{+}$*-3HA-nat*^{r} *kan*^{r}*-nmt1-GFP-alp41*$^{+}$ *leu1 ura4*	3D
RM628	*h*$^{+}$ *alp41-8-hph*^{r} *tbc1-11-nat*^{r} *leu1 ura4 his2*	3F
RM356	*h*$^{-}$ *tbc1-11-hph*^{r} *alp41-14-nat*^{r} *leu1 ura4*	3F
RM521	*h*$^{-}$ *cut11*$^{+}$*-RFP-nat*^{r} *aur*^{r}*-pnda3-GFP-atb2*$^{+}$ *leu1 ura4 +pREP1*	4A
RM523	*h*$^{-}$ *cut11*$^{+}$*-RFP-nat*^{r} *aur*^{r}*-pnda3-GFP-atb2*$^{+}$ *leu1 ura4 +pREP1-alp41*$^{+}$	4A, 4B
RM525	*h*$^{-}$ *cut11*$^{+}$*-RFP-nat*^{r} *aur*^{r}*-pnda3-GFP-atb2*$^{+}$ *leu1 ura4 +pREP1-alp41-Q70L*	4A, 4B
RM527	*h*$^{-}$ *cut11*$^{+}$*-RFP-nat*^{r} *aur*^{r}*-pnda3-GFP-atb2*$^{+}$ *leu1 ura4 +pREP1-alp41-T30N*	4A, 4B
RM529	*h*$^{-}$ *cut11*$^{+}$*-RFP-nat*^{r} *aur*^{r}*-pnda3-GFP-atb2*$^{+}$ *leu1 ura4 +pREP1-alp41-T47A*	4A
RM637	*h*$^{-}$ *leu1 ura4 +pREP41-GFP-alp41*$^{+}$	5A
RM650	*h*$^{-}$ *tbc1*$^{+}$*-3HA-nat*^{r} *alp1*$^{+}$*-3pk-hph*^{r} *leu1 ura4 +pREP41-GFP-alp41*$^{+}$	5A
RM638	*h*$^{-}$ *leu1 ura4 +pREP41-GFP-alp41-Q70L*	5B
RM652	*h*$^{-}$ *tbc1*$^{+}$*-3HA-nat*^{r} *alp1*$^{+}$*-3pk-hph*^{r} *leu1 ura4+pREP41-GFP-alp41-Q70L*	5B
RM639	*h*$^{-}$ *leu1 ura4 +pREP41-GFP-alp41-T47A*	5C
RM653	*h*$^{-}$ *tbc1*$^{+}$*-3HA-nat*^{r} *alp1*$^{+}$*-3pk-hph*^{r} *leu1 ura4 +pREP41-GFP-alp41-T47A*	5C
RM520	*h*$^{-}$ *aur*^{r}*-pnda3-mCh-atb2*$^{+}$ *leu1 ura4 +pREP1-GFP-Alp1*$^{+}$	6A, 6B
RM738	*h*$^{-}$ *aur*^{r}*-pnda3-mCh-atb2*$^{+}$ *leu1 ura4 +pREP1-GFP-Alp1*$^{+}$ *+pREP2-alp41*$^{+}$	6C
RM739	*h*$^{-}$ *aur*^{r}*-pnda3-mCh-atb2*$^{+}$ *leu1 ura4 +pREP1-GFP-Alp1*$^{+}$ *+pREP2-alp41-Q70L*	6C
RM740	*h*$^{-}$ *aur*^{r}*-pnda3-mCh-atb2*$^{+}$ *leu1 ura4 +pREP1-GFP-Alp1*$^{+}$ *+pREP2-alp41-T30N*	6C

RM734	*h*$^{-}$ *aur*^{r}*-pnda3-mCh-atb2*$^{+}$ *leu1 ura4 +pREP41-GFP-Alp41*$^{+}$	S4
RM735	*h*$^{-}$ *aur*^{r}*-pnda3-mCh-atb2*$^{+}$ *leu1 ura4 +pREP41-GFP-Alp41-Q70L*	S4
RM736	*h*$^{-}$ *aur*^{r}*-pnda3-mCh-atb2*$^{+}$ *leu1 ura4 +pREP41-GFP-Alp41-T30N*	S4
RM672	*h*$^{-}$ *alp1*$^{+}$*-GFP-kan*^{r} *aur*^{r}*-pnda3-mCh-atb2*$^{+}$ *leu1 ura4 +pREP41-GFP-Alp41*$^{+}$	S5
RM673	*h*$^{-}$ *alp1*$^{+}$*-GFP-kan*^{r} *aur*^{r}*-pnda3-mCh-atb2*$^{+}$ *leu1 ura4 +pREP41-GFP-Alp41-Q70L*	S5
RM674	*h*$^{-}$ *alp1*$^{+}$*-GFP-kan*^{r} *aur*^{r}*-pnda3-mCh-atb2*$^{+}$ *leu1 ura4 +pREP41-GFP-Alp41-T30N*	S5