Supplementary Figures

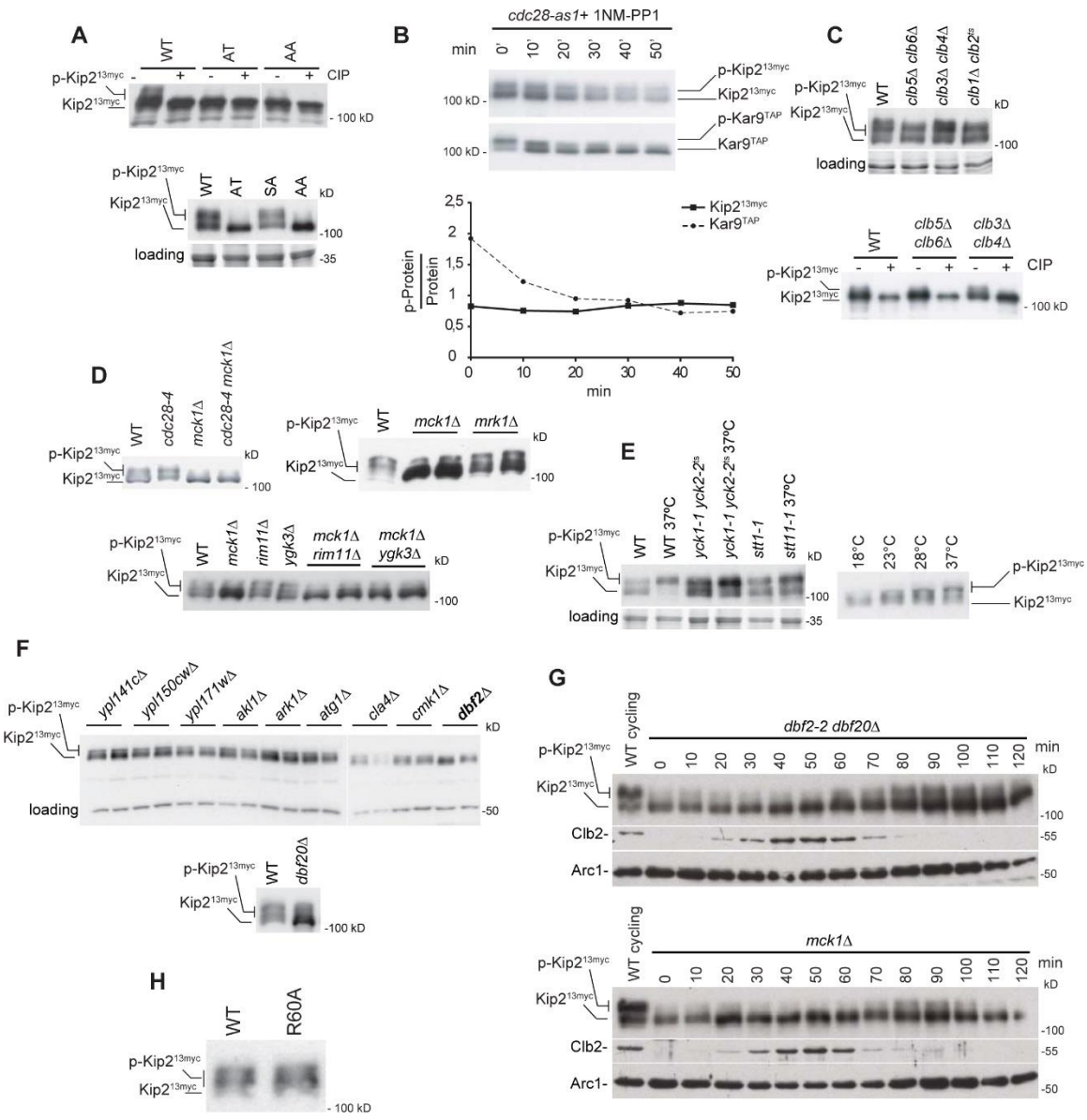

Fig. S1

(A) Phosphorylation and expression levels of Kip2^{13myc} variants. Upper panel: Cell lysates expressing depicted variants were treated at 25ºC with (+) or without (-) calf intestine phosphatase (CIP), followed by western blot against the myc tag. WT: Kip2^{13myc}, AA corresponds to Kip21^{3myc}-S63A T275A. Lower panel: cell lysates of indicated Kip2^{13myc} variants, followed by western blot against the myc tag.

(**B**) Upper panel: Phosphorylation of Kip2^{13myc} does not decrease upon inhibition of Cdc28-as1 with 1NM-PP1. As an internal control, Kar9^{TAP} is expressed in the same cells. Lower panel: quantification of the western blot in the upper panel.

(**C**) Kip2^{13myc} phosphorylation in depicted cyclin mutant cells. Upper panel: cell extracts, lower panel: immunoprecipitated Kip2^{13myc}, with and without phosphatase treatment.

(**D**) Kip2^{13myc} phosphorylation in cells with the hypomorphic *cdc28-4* mutation, in *cdc28-4 mck1*Δ cells and in the depicted mutants of yeast GSK-3 homologues.

(**E**) Kip2^{13myc} phosphorylation in cells with the mutations in the *PKC* gene (*stt1-1*) and in the genes for CKI *(yck1*Δ *yck2^{ts})*. Note that Kip2^{13myc} phosphorylation increases with increasing temperature.

(**F**) Kip2^{13myc} phosphorylation *dbf2*Δ and in *dbf20*Δ cells (western blot against Kip2^{13myc} of cell extracts). For comparison, the phosphopattern of Kip2^{13myc} in other kinase mutants is shown.

(**G**) Increase Kip2 phosphorylation in anaphase is reduced in *dbf2-2 dbf20*Δ and *mck1*Δ cells. Note that the *dbf2-2 dbf20*Δ time course was performed at the permissive temperature to avoid arrest, therefore some residual Dbf2 activity is still present in these cells.

(**H**) Kip2^{13myc} phosphorylation is not reduced upon the R60A mutation.

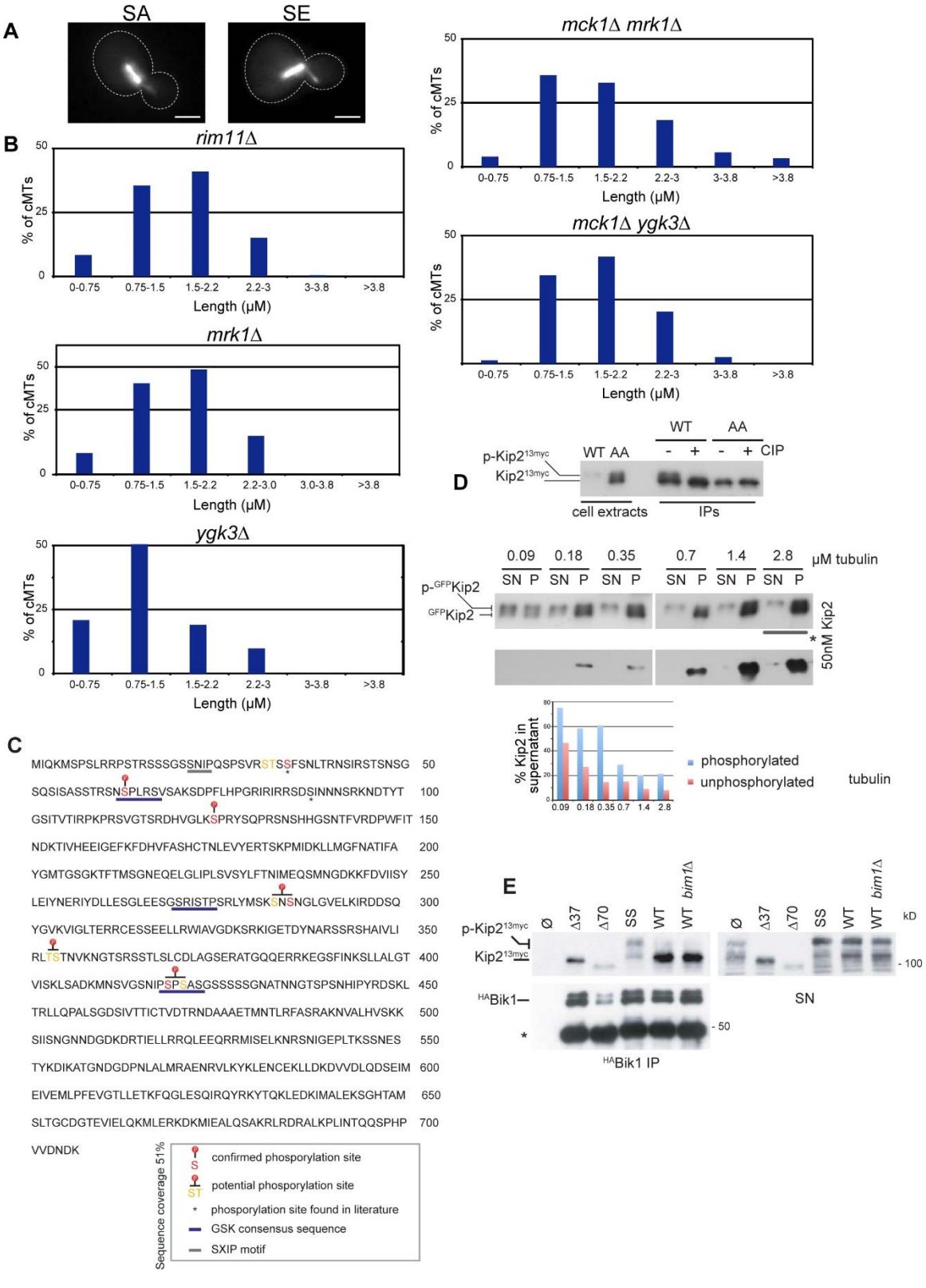

Fig. S2

(**A**) Representative images from cells showing the microtubule cytoskeleton of cells expressing Kip2-SA or Kip2-SE and GFP-Tub1. Bar: 2 µm

(**B**) Length distribution of G2/M aMTs in strains with single and double deletions for GSK-3 homologues. 450 aMTs were counted for each strain.

(**C**) Sequence of Kip2 showing the phosphorylation sites identified by mass spectrometry of His_6-^{GFP}Kip2 purified from SF9 cells (see also western blot in S2D, upper panel). Note that not all possible GSK-3 consensus sites in the N-terminus (aa1-80) are shown. Red residues: phosphorylation sites identified by mass spectrometry, Yellow residues: most likely a phosphorylated residue (fragment was phosphorylated, however assignment of phosphorylation to one exact residue is uncertain)

(**D**) Upper panel: Western blot of His_6-^{GFP}Kip2 expressed in SF9 cells showing a shift due to phosphorylation, which is absent in His_6-^{GFP}Kip2-AA (extracts) and alkaline phosphatase treatment (CIP) of the proteins after immunoprecipitation (IPs).

Middle panel: Hyper-phosphorylated His_6-^{GFP}Kip2 from insect cells has a lower affinity to taxol-stabilised microtubules than unphosphorylated Kip2. Anti-GFP and anti-tubulin western blot of microtubule sedimentation assay, with *in vitro* polymerised MTs (taxol) (increasing concentration) and recombinant His_6-^{GFP}Kip2 (constant concentration 0.05µM) purified from SF9 cells. SN: supernatant, P: pellet.

Lower panel: Quantification of the partitioning of Kip2 phosphoisoforms in the supernatant of the western blot shown above. The upper half of the His_6-^{GFP}Kip2 band was quantified as "phosphorylated" while the lower half as "unphosphorylated" and depicted as percentage of the total amount (adding the amounts in supernatant and pellet).

(**E**) Bik1 interacts with hypo-phosphorylated Kip2 independently of Bim1 and the Kip2 N-terminal extension. Immunoprecipitations of ^{HA}Bik1 from *kip2*Δ *or kip2*Δ *bim1*Δ cells expressing plasmid-borne wild-type Kip2^{13myc} (WT) or indicated variants. Note that Bik1 co-precipitates mainly un- (or hypo)-phosphorylated Kip2 isoforms and that this interaction does not depend on the N-terminus of Kip2 (Δ37/Δ70) or the

Kip2-Bim1 interaction (it occurs in *bim1*Δ, and the variants SS). P_{GAL}-^{3HA}*BIK1* expression was induced for 3h prior to immunoprecipitation. Immunoprecipitated proteins (IP) and extracts after the immunoprecipitation (SN) were probed in western blots with anti-HA and anti-myc antibodies. Asterisk indicates antibody heavy chain.

Supplementary Tables

Click here to Download Supplementary Tables