

Supplemental Materials

Molecular Biology of the Cell

Bhattacharjee et al.

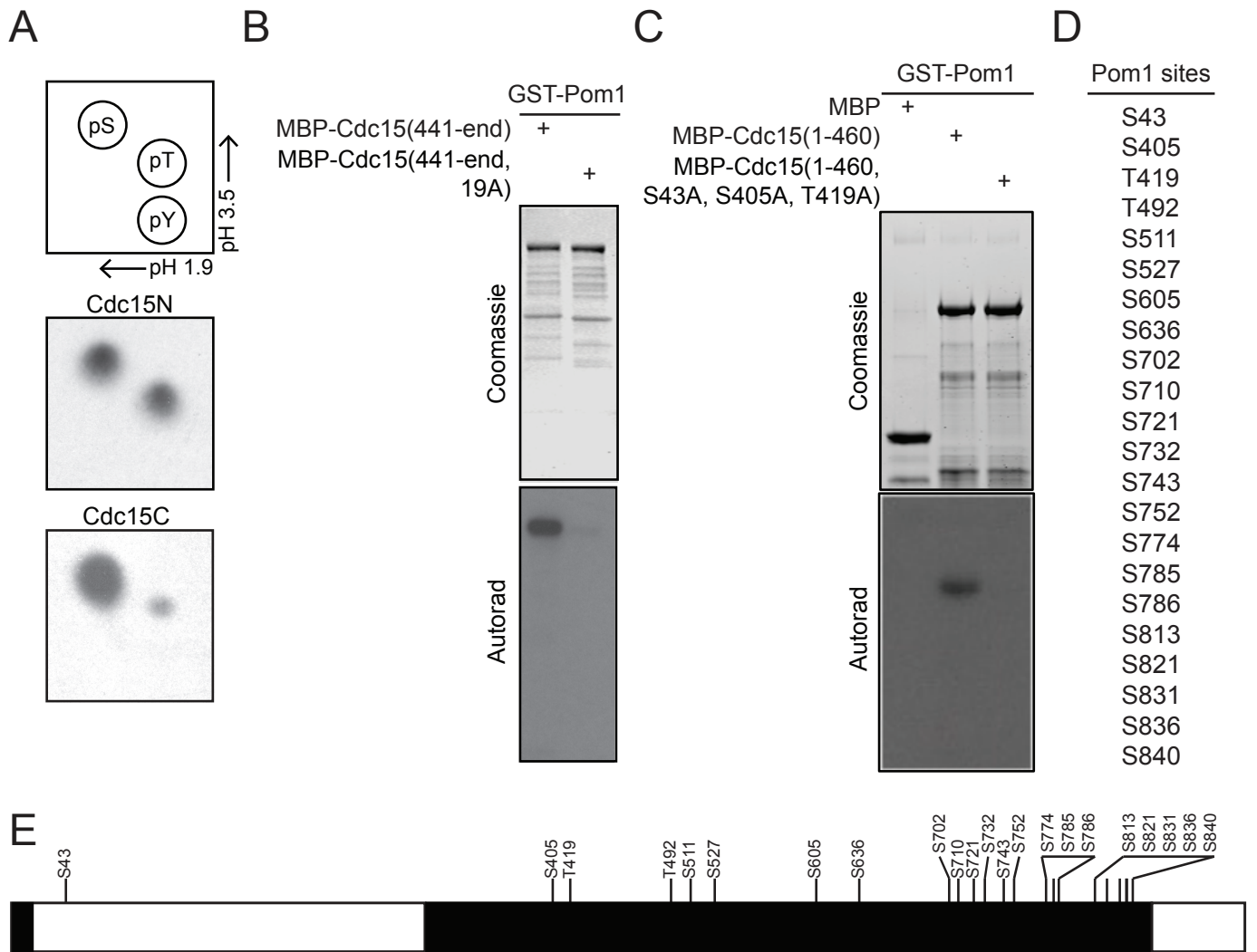


Figure S1

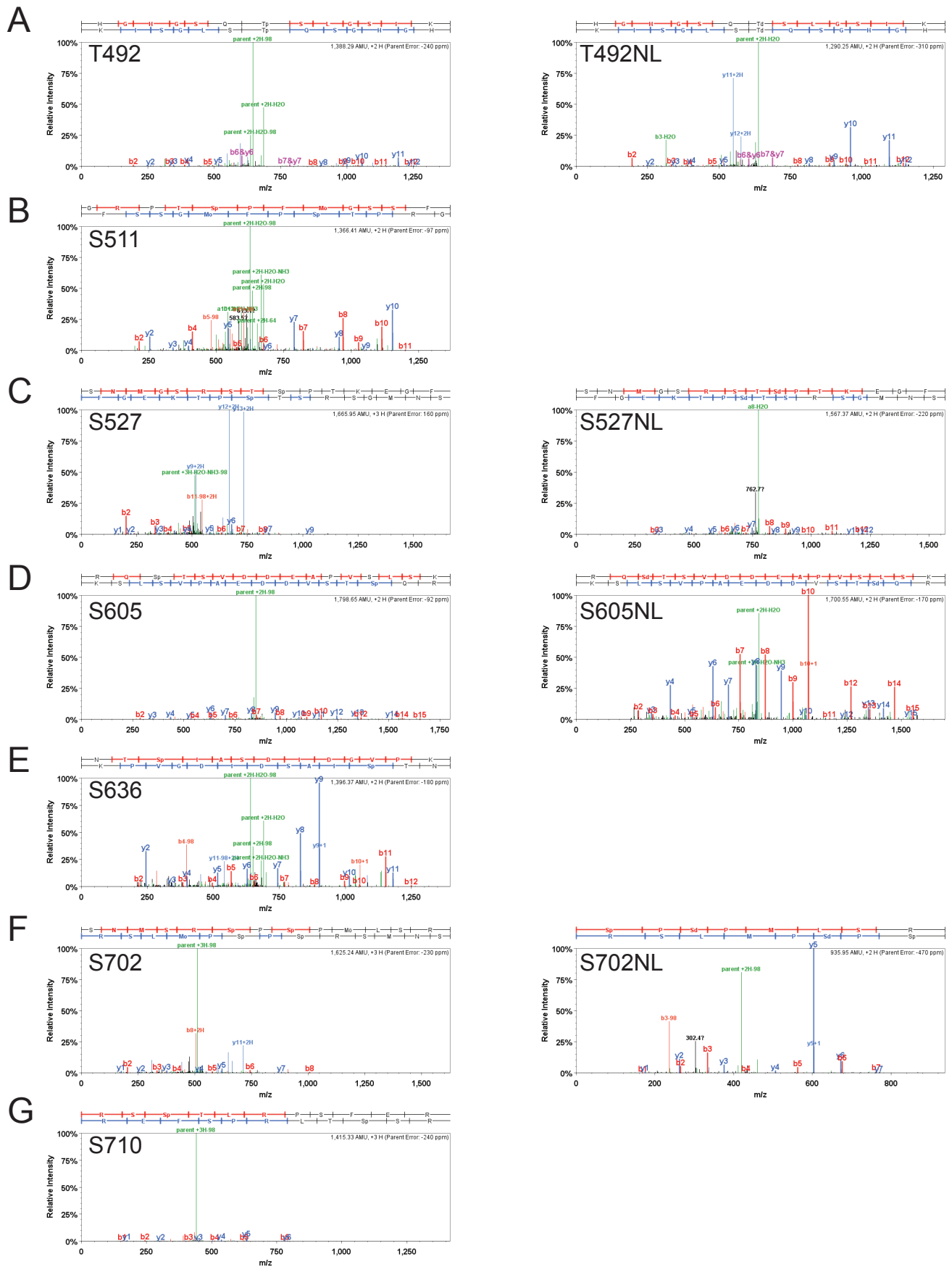


Figure S2

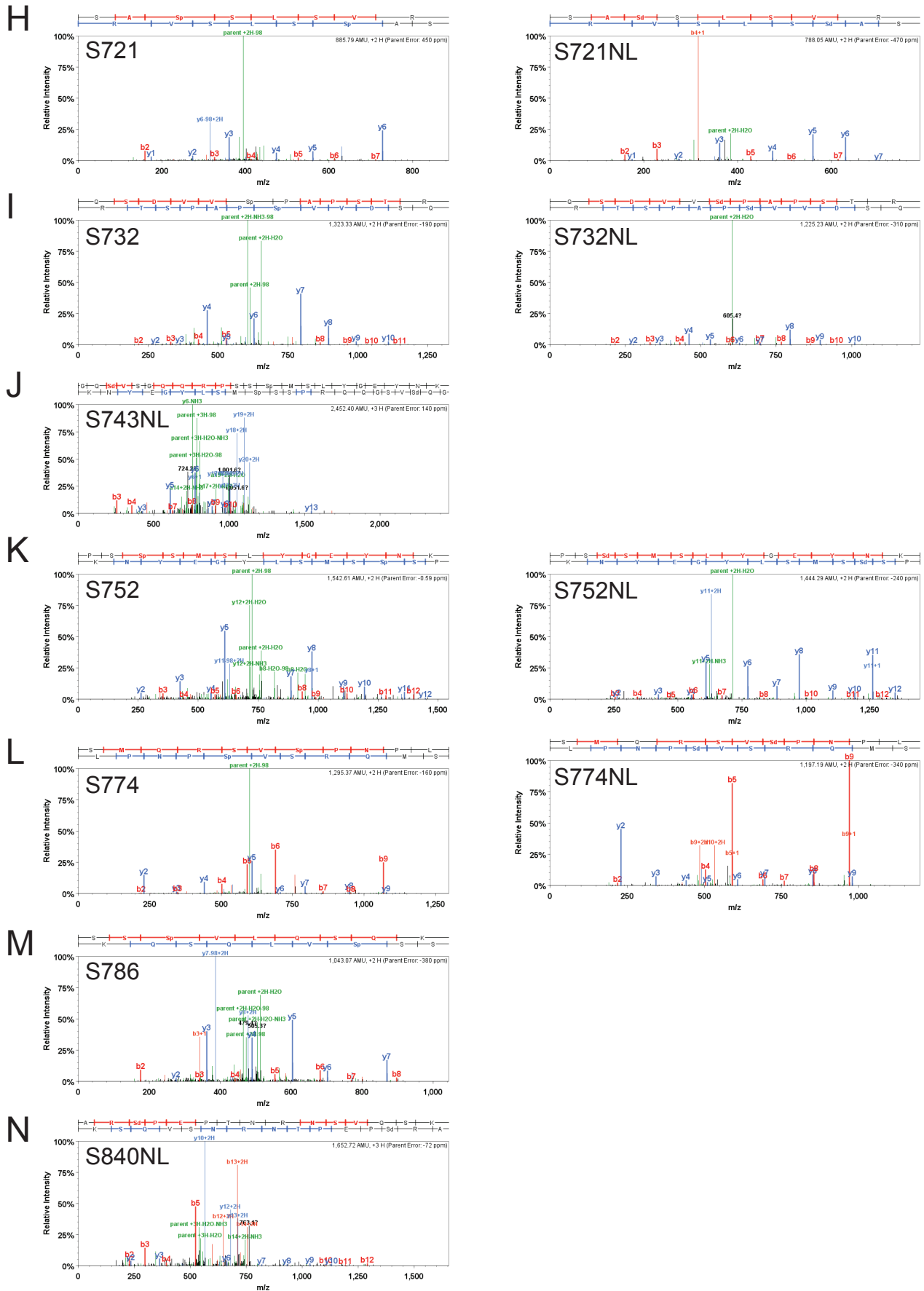


Figure S2-continued

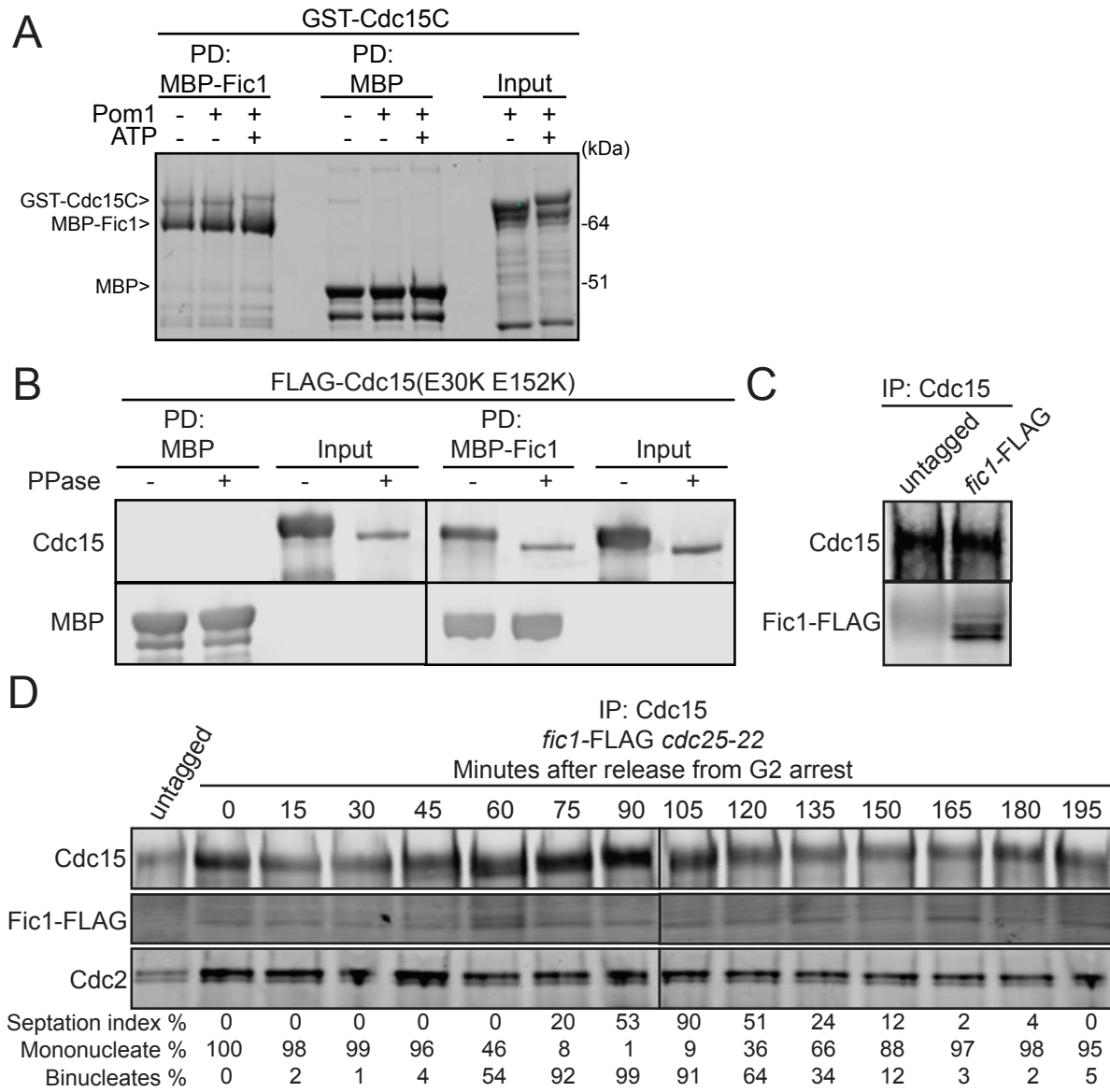


Figure S3

Supplemental Figure Legends

Figure S1. Identifying Cdc15 sites phosphorylated by Pom1. A) Phosphoamino acid analysis of indicated substrates phosphorylated in vitro by GST-Pom1. The positions of standard phosphorylated amino acids are indicated (top). Phosphoamino acids were detected by autoradiography (bottom). Cdc15N, MBP-Cdc15(1-460); Cdc15C, MBP-Cdc15(441-end). B-C) In vitro kinase assays using radiolabeled ATP, recombinant GST-Pom1 and indicated substrate proteins were separated by SDS-PAGE. Coomassie-stained gels of inputs (top) and autoradiography detection of ^{32}P incorporation (bottom) are shown. D) Table and E) scaled schematic of Cdc15 residues phosphorylated by Pom1 and mutated in the *cdc15-22A* and *cdc15-22D* mutants.

Figure S2. Identifying Cdc15 sites phosphorylated by Pom1. A-N) Spectra identifying phosphorylation of the indicated residue.

Figure S3. Cdc15 and Fic1 interact independently of Cdc15 phosphostatus. A) Coomassie-stained SDS-PAGE of binding assays between the indicated MBP-tagged and GST-tagged proteins. Amylose resin was used to pull down proteins. B) Full-length Cdc15-E30K E152K co-expressed with Pom1 in bacteria, purified, and treated with lambda phosphatase (PPase) before addition to MBP or MBP-Fic1. Coomassie-stained SDS-PAGE are shown. C) Immunoprecipitation with anti-Cdc15 from the indicated strains was separated by SDS-PAGE and analyzed by immunoblot. D) Cells were arrested in G2 by shifting to 36°C for 3.5 hours. Cells were synchronously released into the cell cycle by downshift to 25°C and samples were collected every 15 minutes. Anti-Cdc15 immunoprecipitates were resolved by SDS-PAGE and analyzed by immunoblotting to detect the indicated proteins. Cell cycle progress was monitored by determining the binucleate and septation indices from fixed cells stained with DAPI and methyl blue.

Table S1. *S. pombe* strains used in this study

Strain number	Genotype	Source
Figure 1		
KGY246	<i>ade6-M210 ura4-D18 leu1-32 h-</i>	Lab stock
KGY2711	<i>mid1::ura4+ ade6-M210 ura4-D18 leu1-32 h+</i>	Lab stock
KGY2001-2	<i>pom1-as1-tdTomato:nat_R ade6-M210 ura4-D18 leu1-32 h-</i>	This study
KGY4951-2	<i>mid1::ura4+ pom1-as1-tdTomato:nat_R ade6-M210 ura4-D18 leu1-32 h+</i>	This study
Figure 2		
YSM2629	<i>ura4-294::nmt82:myo52N-RFP-nup146::ura4+ pom1_{as1}-tomato::nat_R</i>	This study
YSM3695	<i>ura4-294::nmt82:myo52N-GFP-nup146::ura4+ leu1-32 h+</i>	This study
YSM3696	<i>pom1::kan_R ura4-294::nmt82-myo52N-GFP-nup146::ura4+ leu1-32 h-</i>	This study
Figure 3		
KGY4951-2	<i>mid1::ura4+ pom1-as1-tdTomato:nat_R ade6-M210 ura4-D18 leu1-32 h+</i>	This study
KGY5936-2	<i>mid1::ura4+ pom1-as1-tdTomato:nat_R cdc15-EGFP:kan_R ade6-M21X leu1-32 ura4-D18</i>	This study
KGY246	<i>ade6-M210 ura4-D18 leu1-32 h-</i>	Lab stock
KGY6924	<i>pom1::ura4+ leu1-32 ura4-D18 h-</i>	Lab stock
KGY11562-2	<i>cdc15-22A ade6-M210 ura4-D18 leu1-32 h-</i>	This study
KGY11563-2	<i>cdc15-22D ade6-M210 ura4-D18 leu1-32 h-</i>	This study
Figure 4		
KGY246	<i>ade6-M210 ura4-D18 leu1-32 h-</i>	Lab stock
KGY11562-2	<i>cdc15-22A ade6-M210 ura4-D18 leu1-32 h-</i>	This study
KGY2711	<i>mid1::ura4+ ade6-M210 ura4-D18 leu1-32 h+</i>	Lab stock
KGY14292	<i>cdc15-22A mid1::ura4+ ade6-M210, ura4-D18, leu1-32 h-</i>	This study
KGY1498-2	<i>mNG-cdc15 ade6-M21X ura4-D18 leu1-32 h-</i>	Lab stock
KGY19616	<i>mNG-cdc15-22A ade6-M21X ura4-D18 leu1-32 h-</i>	This study
KGY19617	<i>mNG-cdc15-22D ade6-M21X ura4-D18 leu1-32 h-</i>	This study
KGY14317	<i>mNG-cdc15 sid4-mNG:kan_R ade6-M21X ura 4-D18 leu 1-32</i>	This study
KGY14306	<i>mNG-cdc15-22A sid4-mNG:kan_R ade6-M21X ura 4-D18 leu 1-32</i>	This study
KGY14307	<i>mNG-cdc15-22D sid4-mNG:kan_R ade6-M21X ura 4-D18 leu 1-32</i>	This study
KGY19083	<i>rlc1-mNG:hyg_R sid4-mNG:kan_R ade6-M210 ura4-D18 leu1-32 h+</i>	Lab stock
KGY3982-2	<i>cdc15-22A rlc1-mNG:hyg_R sid4-mNG:kan_R ade6-M21X leu1-32 ura4-D18 h?</i>	This study
KGY3984-2	<i>cdc15-22D rlc1-mNG:hyg_R sid4-mNG:kan_R ade6-M21X leu1-32 ura4-D18 h</i>	This study
KGY4951-2	<i>mid1::ura4+ pom1-as1-tdTomato:nat_R ade6-M210 ura4-D18 leu1-32 h+</i>	This study

KGY8513-2	<i>cdc15-22D mid1::ura4+ pom1-as1-tdTomato:natR leu1-32 ade6-M210 ura4-D18 h+</i>	This study
Figure 6		
KGY246	<i>ade6-M210 ura4-D18 leu1-32 h-</i>	Lab stock
KGY19261	<i>pxl1::kan_R ade6-M210 leu1-32 ura4-D18 h-</i>	Lab stock
KGY2711	<i>mid1::ura4+ ade6-M210 ura4-D18 leu1-32 h+</i>	Lab stock
KGY4951-2	<i>mid1::ura4+ pom1-as1-tdTomato:nat_R ade6-M210 ura4-D18 leu1-32 h+</i>	This study
KGY8513-2	<i>cdc15-22D mid1::ura4+ pom1-as1-tdTomato:nat_R leu1-32 ade6-210 ura4-D18 h+</i>	This study
KGY8279-2	<i>mid1::ura4+ pom1-as1-tdTomato:nat_R pxl1::kan_R leu1-32 ade6-210 ura4-D18</i>	This study
KGY19718	<i>cdc15-22D mid1::ura4+ pom1-as1-tdTomato:nat_R pxl1::kan_R leu1-32 ade6-210 ura4-D18</i>	This study
KGY6008	<i>fic1::ura4+ leu1-32 ura4-D18 ade6-M210 h+</i>	Lab stock
KGY11374	<i>mid1::ura4+ pom1-as1-tdTomato:nat_R fic1::ura4+ leu1-32 ade6-210 ura4-D18 h-</i>	This study
KGY19717	<i>cdc15-22D mid1::ura4+ pom1-as1-tdTomato:nat_R fic1::ura4+ leu1-32 ade6-210 ura4-D18</i>	This study
Figure S3		
KGY247	<i>ade6-M210 ura4-D18 leu1-32 h+</i>	Lab stock
KGY6288	<i>fic1-FLAG:kan_R ade6-M216 leu1-32 ura4-D18 h-</i>	Lab stock
KGY6659	<i>cdc25-22 fic1-FLAG:kan_R ade6-M21X leu1-32 ura4-D18 h-</i>	Lab stock