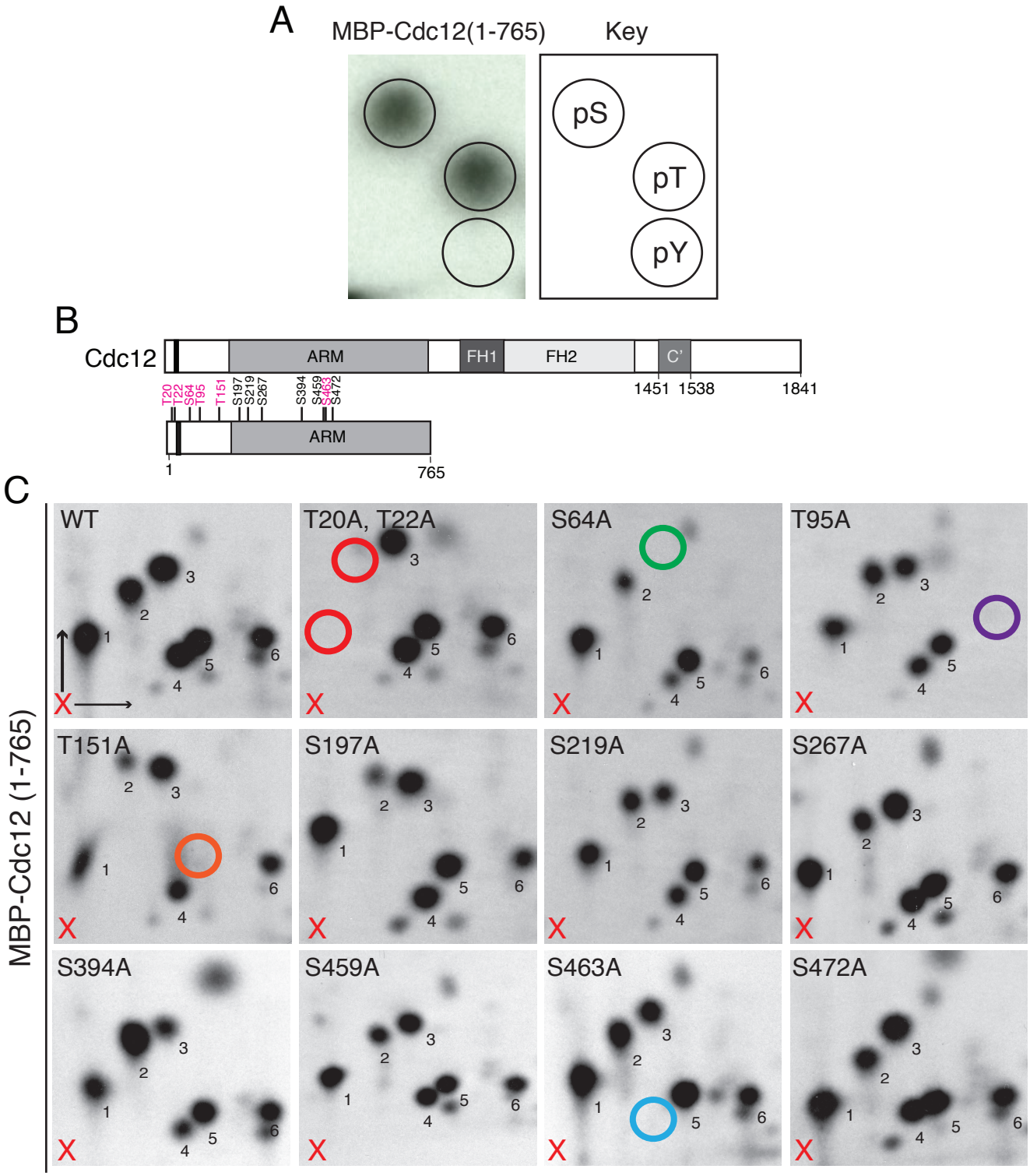
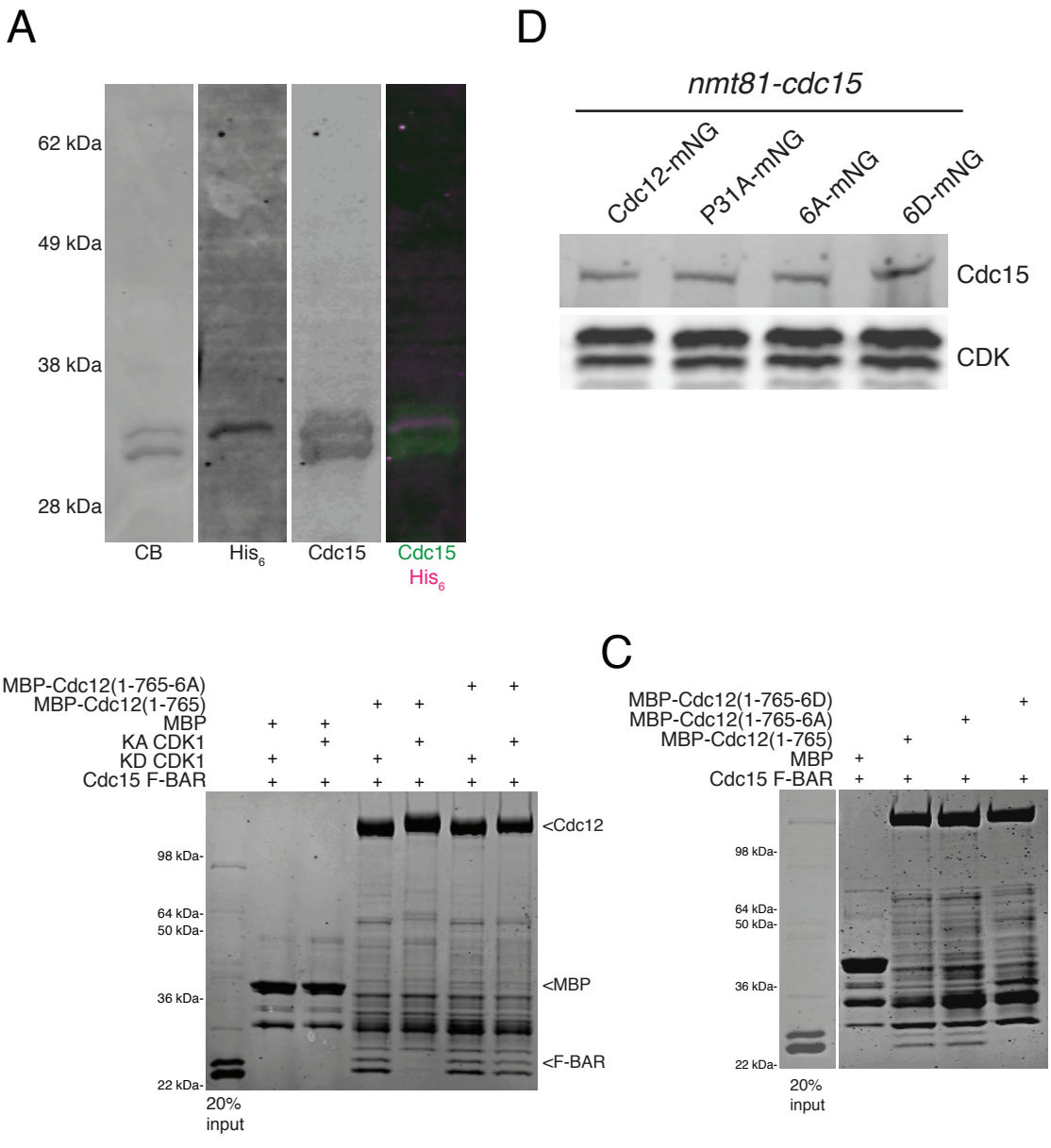
Supplemental Materials

Molecular Biology of the Cell

Willet et al.



Willet et al Figure S1



Willet et al Figure S2

Supplemental figure 1. Identification of Cdk1-phosphorylated sites on Cdc12's N-terminus. (A) Phosphoamino acid analysis of the MBP-Cdc12(1-765) fragment phosphorylated by Cdk1 complex. The positions of phosho-serine, phospho-theonine and phospho-tyrosine standards are indicated in the key. (B) A schematic, drawn to scale, of full-length Cdc12 and the N-terminal fragment (1-765) with the domains indicated. All serines and threonines within S/T-P motifs are labeled and were tested in C. Residues in magenta were confirmed as the *in vitro* phosphorylated residues. (C) Phospho-tryptic peptide analysis of MBP-Cdc12(1-765) wt and mutant fragments phosphorylated by Cdk1. The positions of the origin are indicated by the red "X", and major spots are numbered. The anode is on the left. Loss of phosphorylated species for the T20A T22A, S64A, T95A, T151A and S463A mutants are indicated by red, green, purple, orange and light blue circles, respectively.

Supplemental figure 2. *cdc15* expression *in vivo* and *in vitro.* (A) Cdc15(19-312) resolved by SDS-PAGE and either CB stained or labeled with anti-His_6 (magenta) and anti-Cdc15 (green) antibodies. (B) The full CB gel of the experiment from Figure 1E. (C) The Full CB gel of the experiment from Figure 1F. (D) Expression of Cdc15 from the indicated genotypes in Figure 3D. CDK was used as a loading control.

Supplemental Table 1. *S. pombe* strains used in this study.

	Genotype	Source
	Figure 1	
KGY246	*ade6-M210 leu1-32 ura4-D18*h-	Lab stock
KGY3733	*mts3-1 ade6-M210 leu1-32 ura4-D18* h+	Lab stock
KGY14891	*mts3-1 cdc12-HA$_3$:kan^R ade6-M21X leu1-32 ura4-D18*h-	This study
KGY18392	*mts3-1 cdc12-6A-HA$_3$:kan^R ade6-M210 leu1-32 ura4-D18* h-	This study
KGY3067	*cdc12-HA$_3$:kan^R ade6-M210 leu1-32 ura4-D18* h-	Lab stock
	Figure 2	
KGY12968	*cdc12-6D:kan^R ade6-M210 leu1-32 ura4-D18*h-	This study
KGY6614	*myo2-E1 ade6-M21X leu1-32 ura4-D18*h+	M.Balasubramanian
KGY12700	*cdc12-6A:kan^R ade6-M210 leu1-32 ura4-D18* h-	This study
KGY18306	*myo2-E1 cdc12-6A:kan^R ade6-M21X leu1-32 ura4-D18*h+	This study
KGY6614	*rng2-D5 ade6-M21X leu1-32 ura4-D18*h+	Lab stock
KGY14978	*rng2-D5 cdc12-6A:kan^R ade6-M21X leu1-32 ura4-D18*h-	This study
KGY18304	*rng2-D5 cdc12-6D:kan^R ade6-M21X leu1-32 ura4-D18*h+	This study
KGY2711	*mid1::ura4$^+$ade6-M210 leu1-32 ura4-D18* h+	Lab stock
KGY18303	*mid1::ura4$^+$cdc12-6A:kan^R ade6-M21X leu1-32 ura4-D18*h+	This study
	*mid1::ura4$^+$cdc12-6D:kan^R ade6-M21X leu1-32 ura4-D18*h+	
KGY18305	*cdc15-140 ade6-M210 leu1-32 ura4-D18 h*+	This study
	*cdc15-140 cdc12-6A:kan^R ade6-M210 leu1-32 ura4-D18*h-	
KGY1008	*cdc15-140 cdc12-6D:kan^R ade6-M210 leu1-32 ura4-D18*h+	Lab stock
KGY18177		This study

KGY18238		This study

Figure 3

KGY16917	*cdc12-mNG:kan^R sid4-GFP:kan^R ade6-M210 leu1-32 ura4-D18*h-	This study
KGY2341-2	*cdc12-P31A-mNG:kan^R sid4-GFP:kan^R ade6-M210 leu1-32 ura4-D18* h-	This study
KGY2373-2	*cdc12-6D-mNG:kan^R sid4-GFP:kan^R ade6-M210 leu1-32 ura4-D18*h+	This study
KGY2405-2	*cdc12-6A-mNG:kan^R sid4-GFP:kan^R ade6-M210 leu1-32 ura4-D18*h+	This study
KGY17015	*cdc25-22 cdc15-11A cdc12-mNG:kan^R ade6-M210 leu1-32 ura4-D18* h-	Lab stock
KGY2402-2	*cdc25-22 cdc15-11A cdc12-P31A-mNG:kan^R ade6-M210 leu1-32 ura4-D18* h+	This study
KGY2410-2	*cdc25-22 cdc15-11A cdc12-6D-mNG:kan^R ade6-M210 leu1-32 ura4-D18* h+	This study
KGY2412-2	*cdc25-22 cdc15-11A cdc12-6A-mNG:kan^R ade6-M210 leu1-32 ura4-D18*h-	This study

Figure 4

KGY15739	*pAct1 LifeAct-mCherry:leu1$^+$ sid4-GFP:kan^R ade6-M216 leu1-32 ura4-D18*h-	Lab stock
KGY16615	*cdc12-P31A:kan^R pAct1 LifeAct-mCherry:leu1$^+$ sid4-GFP:kan^R ade6-M216 leu1-32 ura4-D18* h+	Lab stock

KGY18368	*cdc12-6D:kan^R pAct1 LifeAct-mCherry:leu1$^+$ sid4-GFP:kan^R ade6-M216 leu1-32 ura4-D18*h+	This study
KGY18240	*cdc12-6A:kan^R pAct1 LifeAct-mCherry:leu1$^+$ sid4-GFP:kan^R ade6-M216 leu1-32 ura4-D18* h+	This study
KGY16700	*mCherry-cdc15 sid4-RFP:kan^R ade6-M21X leu1-32 ura4-D18*h+	Lab stock
KGY16718	*cdc12-P31A:kan^{R}mCherry-cdc15 sid4-RFP:kan^R ade6-M21X leu1-32 ura4-D18* h+	Lab stock
KGY18626	*cdc12-6D:kan^R mCherry-cdc15 sid4-RFP:kan^R ade6-M21X leu1-32 ura4-D18* h-	This study
KGY18625	*cdc12-6A:kan^R mCherry-cdc15 sid4-RFP:kan^R ade6-M21X leu1-32 ura4-D18* h+	This study
KGY16924	*ain1-GFP:kan^R sid4-GFP:kan^R ade6-M21X leu1-32 ura4-D18*h-	Lab stock
KGY16903	*cdc12-P31A:kan^R ain1-GFP:kan^R sid4-GFP:kan^R ade6-M21X leu1-32 ura4-D18*h-	Lab stock
KGY18621	*cdc12-6D:kan^R ain1-GFP:kan^R sid4-GFP:kan^R ade6-M21X leu1-32 ura4-D18*h+	This study
KGY18668	*cdc12-6A:kan^R ain1-GFP:kan^R sid4-GFP:kan^R ade6-M21X leu1-32 ura4-D18*h+	This study

Figure 5

KGY7450	*rlc1-GFP:ura$^+$ sid4-GFP:kan^R ade6-M21X leu1-32 ura4-D18*h-	Lab stock

KGY15636	*cdc12-P31A:kan^R rlc1-GFP:ura$^+$ sid4-GFP:kan^R ade6-M21X leu1-32 ura4-D18*h-	Lab stock
KGY18132	*cdc12-6D:kan^R rlc1-GFP:ura4$^+$ sid4-GFP:kan^R ade6-M21X leu1-32 ura4-D18*h-	This study
KGY18217	*cdc12-6A:kan^R rlc1-GFP:ura4$^+$ sid4-GFP:kan^R ade6-M21X leu1-32 ura4-D18*h+	This study