

Figure S1. *CDC15* is required for Sps1 post-translational modification. Wild type *CDC15* (LH875) and *cdc15-mn* (LH1069) strains containing *SPS1-13myc* were sporulated. Lysates were collected at the indicated times and prepared for immunoblotting. Pgk1 was used as a loading control.

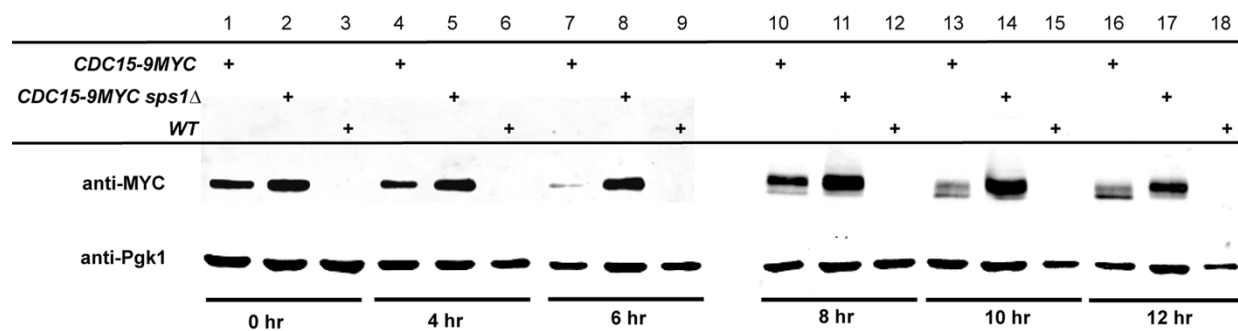


Figure S2. *SPS1* is not required for Cdc15 phosphorylation. Wild type (WT: LH177) and strains containing *CDC15-9myc* (LH1070) and *CDC15-9myc* in the *sps1Δ* strain (LH1083) were sporulated and lysates were prepared for immunoblotting. Lanes 1-9 were run on the same SDS-PAGE gel; Lanes 10-18 are on the same SDS-PAGE gel. Pgk1 was used as a loading control.

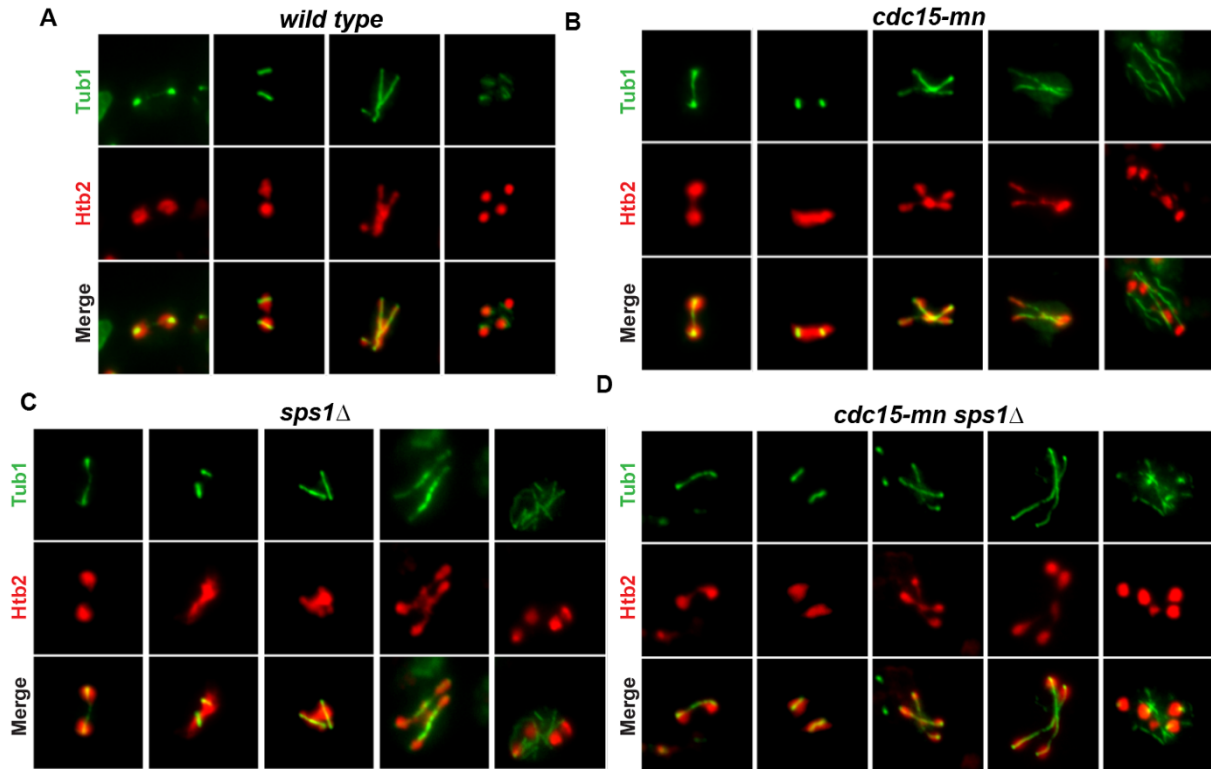


Figure S3. *SPS1* has a spindle disassembly defect. Microtubules were visualized in green using an anti-Tub1 antibody. Histones, in red, are visualized using *HTB2-mCherry*. Cells at different time points in meiosis, arrayed from early (left) to late (right), with varying terminal phenotypes shown for the mutant strains. Cells were fixed at appropriate times during sporulation using 3.7% methanol-free formaldehyde, spheroplasted, adhered to polylysine coated slides, stained with anti-Tub1 antibodies (monoclonal mouse 12G10 anti-Tub1 [Developmental Studies Hybridoma Bank]; 1:1000 concentration) followed by Cy2-conjugated donkey anti-mouse antibodies at 1:100 for one hour (JacksonImmno), and mounted in Vectashield mounting medium (Vector Labs). Images were captured using a wide-field microscope. Cells are of the following genotypes: (A) WT (LH902) (B) *cdc15-mn* (LH1072) (C) *sps1Δ* (LH976) (D) *cdc15-mn sps1Δ* (LH1067).

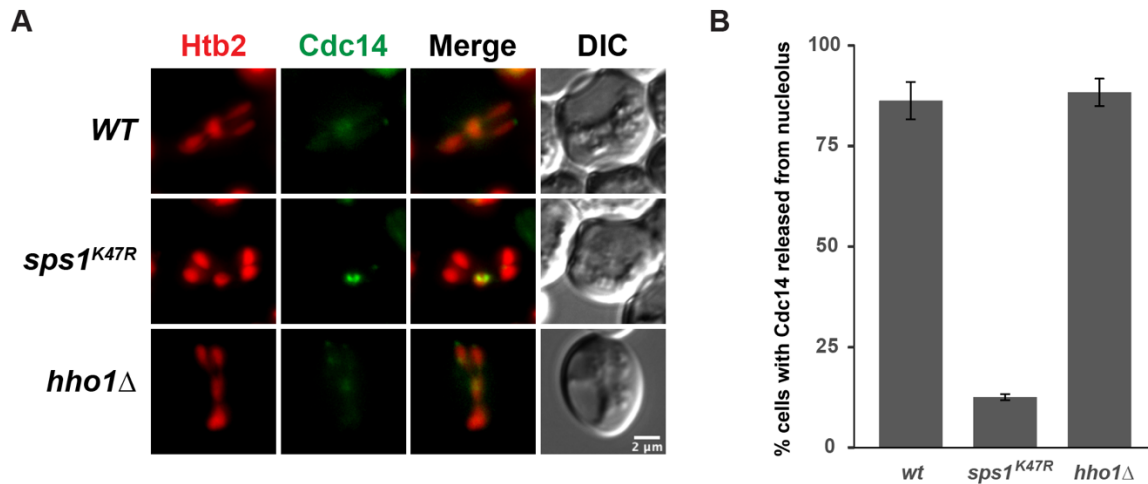


Figure S4. The sustained release of Cdc14 requires Sps1 kinase activity but not *HHO1*. (A) The Cdc14-GFP^{ENVY} fusion protein was visualized in WT (LH1077), *sps1^{K47R}* (LH1102), and *hho1Δ* (LH1103) cells. Representative images are shown from these strains. Histones were visualized using *Htb2-mCherry*. Images were captured using a widefield microscope. (B) Quantitation of cells in anaphase II with Cdc14 released from the nucleolus. Cells were sporulated in triplicate; at least 100 anaphase II cells were counted per strain. Error bars represent standard error of the mean. The *sps1^{K47R}* strain is significantly different from wild type, but the wild type and *hho1Δ* strains do not differ from each other (one-way ANOVA [F(2,8)=96.04, p<0.001], followed by Tukey HSD post hoc test (alpha = 0.01)).

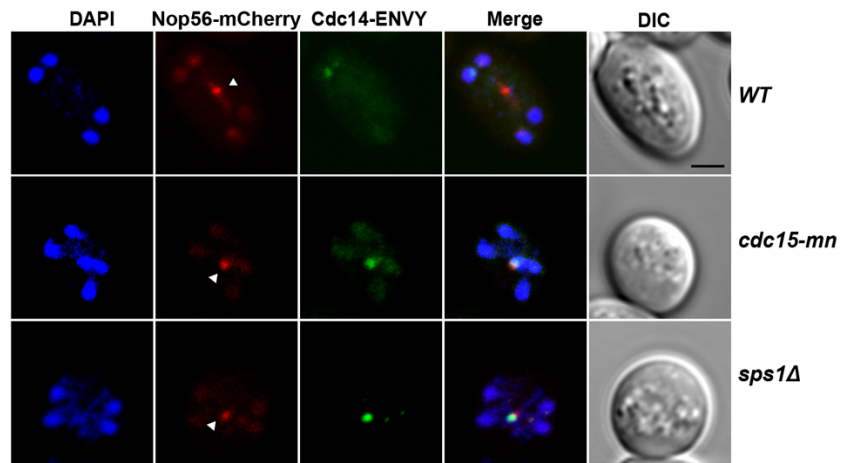


Figure S5. Cdc14 remains in the nucleolus in *cdc15* and *sps1* mutants. In anaphase II, Cdc14-ENVY co-localizes with the nucleolar marker Nop56 in *cdc15-mn* (LH1086) and *sps1Δ* (LH1085), but is released from the nucleolus in wild type cells (LH1084). White arrowhead points to nucleolus. Scale bar = 2 μ m.

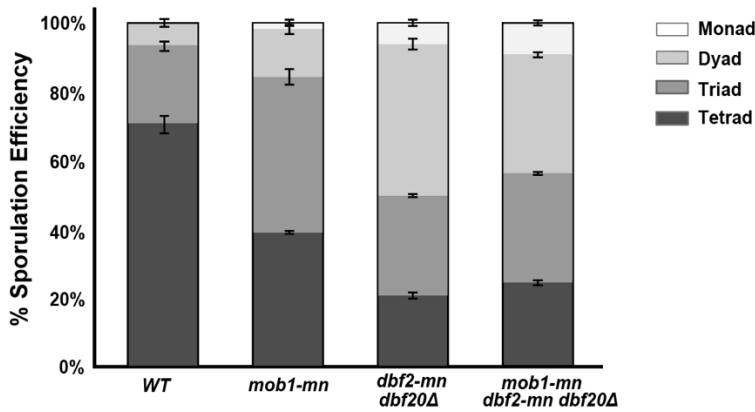


Figure S6. *MOB1*, *DBF2*, and *DBF20* affect spore number control. The number of spores packaged was assayed by counting refractile spores in wild type (LH902), *mob1-mn* (LH1087), *dbf2-mn dbf20Δ* (LH1088), *mob1-mn, dbf2-mn, dbf20Δ* (LH1068). Monads have 1 spore per ascus, dyads have 2, triads have 3 and tetrads have 4. Error bars depict the standard error of the mean. All three mutant strains are significantly different from wild type, but not from one another, in the fraction of mutant spores formed (one-way ANOVA [$F(3,8)=64$, $p<0.001$], followed by Tukey HSD post hoc test ($\alpha = 0.01$)).