**E08-05-0455 Luca**

**Supplemental figures**

**Figure S1** Cbk1 inhibition delays bud emergence in G0 synchronized cells. *cbk1-as* (FLY2084) cells were synchronized in G0 and released into medium +/- 5uM 1NA-PP1. The number of buds was scored over 2 hours.

**Figure S2** Viability and morphologies of conditional *cbk1* mutants. The viabilities of *cbk1-as* cells +/- 1NA-PP1 (top left) and *cbk1-8* cells at 22°C and 37°C (bottom left) were determined by counting the number of colony forming units over 8 hours. Representative DIC images of the cells after 8 hours in 1NA-PP1 or at 37°C are shown on the right. Note that most of the *cbk1-8* cells have lysed by 8 hours at 37°C.

**Figure S3** Cbk1 inhibition in synchronized *cbk1-as SSD1* and *cbk1-as ssd1-d* cells causes a modest S phase delay. (S3-A) This is a duplicate experiment for Fig1 B.

**Figure S4** Cbk1 inhibition delays growth. *cbk1-as* cells (FLY2084) were synchronized in G0 and released to fresh medium until ~40% of cells formed small buds (time 0). The culture was split in two and treated with either DMSO (untreated) or 1NA-PP1. Samples were fixed at designated intervals. The percent of unbudded, small budded and large budded cells were plotted over time. Note that by 60' the DMSO cells already lost synchrony.

**Figure S5** Uncropped immunoblot and corresponding Coomassie Blue stained gel for Figure 4A, which demonstrate that Cbk1 phosphorylates Sec2 in vitro.

**Figure S6** Media secretion assays with wild type, *dse4*Δ, Dse4-TAP, *cts1*Δ and Cts1-TAP cells. The ~125 kD Cbk1 and Ace2-dependent protein (arrowhead) is present in *dse4*Δ and *cts1*Δ cells and does not appear larger in molecular weight in Dse4-TAP or Cts1-TAP cells. Thus, the 125 kD Cbk1 and Ace2-dependent protein cannot be Dse4 or Cts1. The yeast strains used in this experiment are BY4741, FLY2704, FLY2866, FLY2867, FLY2885 and FLY2886.

**Figure S7**. *cbk1-8* is lethal or causes severe growth defects when combined with trafficking mutants *sec2-41, sec16-2, cog1*Δ and *ypt6*Δ. Top panel: tetrads from a cross between wild type cells (BY4741) and a *cbk1-8 ypt6*Δ double mutant, which was obtained from the cross between *cbk1-8* and *ypt6*Δ cells (FLY2661, FLY2766). Some *cbk1-8 ypt6*Δ clones were viable but exhibit severe slow growth phenotypes.

Table: Synthetic interactions between *cbk1-8* and the trafficking mutants *sec2-41, sec16-2*, *cog1*Δ and *ypt6*Δ. The significance of missing or under-represented double mutants was determined by the Chi-square test. Clones of *cbk1-8 cog1*Δ and *cbk1-8 ypt6*Δ double mutants that were alive were backcrossed to wild type parental strains (BY4741or BY4742) and the segregation patterns strongly support synthetic lethality or synthetic growth interactions. Strains used for the crosses were FLY2661, NY770, NY416, FLY2764, FLY2765, FLY2766.

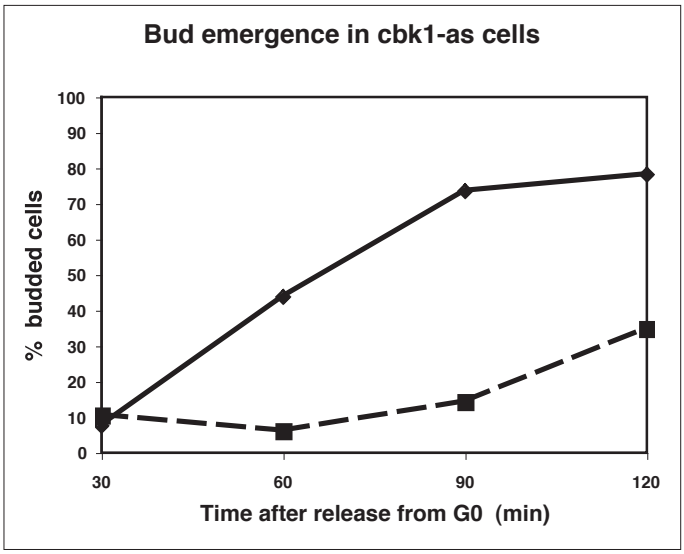
**Figure S8. Cbk1 inhibition diminishes secretory vesicle accumulation in *sec6-2* cells.** *cbk1-as sec6-2* cells (FLY2789*)* were synchronized in early S phase by G0 block and release. When ~50% of the cells developed small buds, the cells were incubated at 22°C for 30' +/- 1NA-PP1. The cells were then shifted to 37°C for 15' (to inactivate Sec6) and samples were collected for EM. (A-D) EM images of representative *cbk1-as sec6-2* cells. (n=nucleus; v=vacuole; arrows indicate vesicles). (E) The average number of secretory vesicles per EM section of *cbk1-as sec6-2* cells is plotted.

**Table S1. Dosage suppressors of *cbk1-8***

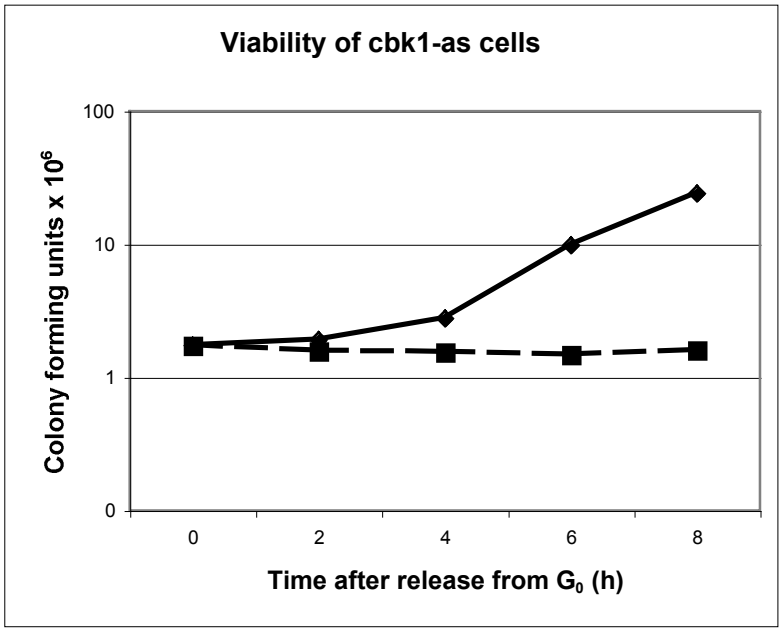
| Clone # | ORFs in plasmid | Putative or confirmed suppressors | Growth at 34C | Growth at 37C | Growth in Hyg B |
|---|---|---|---|---|---|
| YGPM-18j03 | [WBP1], YEL001C, **MNN1**,NOP16, PMI40, FMP52, YND1, [NUG1] | Mnn1 mannosytranferase | ++++ | + | ++++ |
| YGPM-14a05 | [EAP1], TOR2, YKL202W, [**MNN4**] | Mnn4 (*confirmed*) positive regulator of mannosylphosphate transferase | ++++ | ++++ | ++++ |
| YGPM-10a12 | [KTR6],OAZ1, ARL3, **MNN9**, DIG1, CAM1, SGF1, ELC1, [VPS16]* | Mnn9 subunit of Golgi manno-syltransferase complex | +++ | ++ | ++ |
| YGPM-28f19 | [RPN6], YDL096C, **PMT1**, YDL094C, **PMT5**, SRP14, UBX3, RAM1, YDL089W | Pmt1 (*confirmed*) O-mannosyltransferase | ++++ | ++++ | ++++ |
| YGPM-20h07 | YOR318C, HSH49, GNT1, **PMT3**, [YOR322C] | Pmt3 (*confirmed*) O-mannosyltransferase | ++++ | +++ | +++ |
| YGPM-10e17 | RPS7A, YOR097C, NUP1, **KTR1** CRC1, RAS1, YOR102W, [OST2] | Ktr1 Alpha-1,2-mannosyltransferase | ++++ | +++ | +++ |
| YGPM-15f24 | [GLG1], TIF1, UTP30, **KTR2,** SNR42, TFA2, LAS1, YKR064W, FMP18, [CCP1] | Ktr2 Alpha-1,2-mannosyltransferase | ++++ | ++ | ++++ |

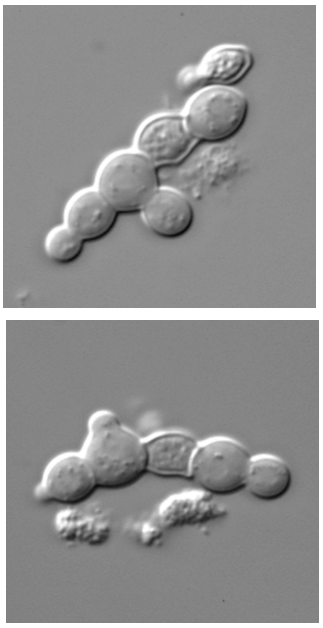
Plasmids encoding single yeast genes (from Dr. Aaron Gitler, UPenn) were used to confirm that *MNN4*, *PMT1* and *PMT3* are dosage suppressors of *cbk1-8*. The genes in brackets denote incomplete yeast ORFs on the plasmid.

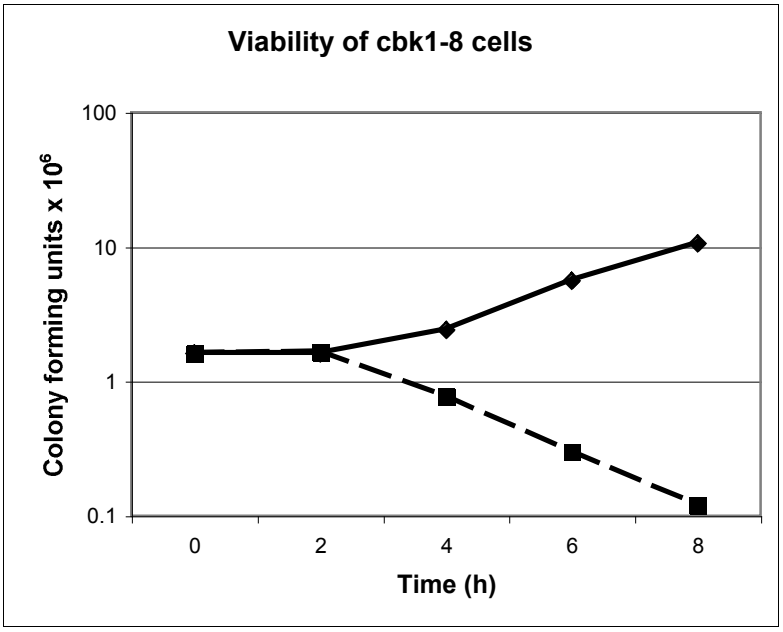
# SUPPLEMENT FIG. S1



# SUPPLEMENT FIG. S2

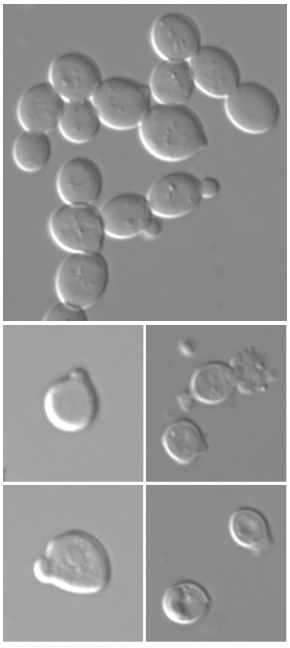


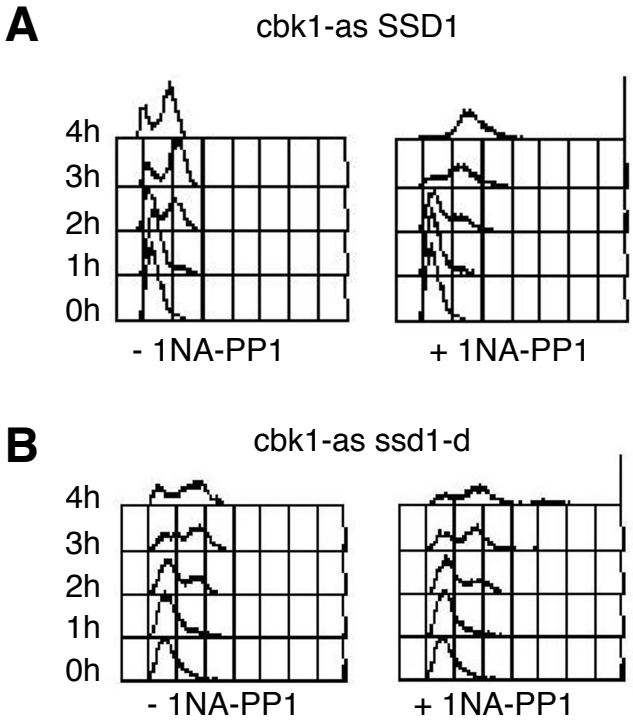



22C

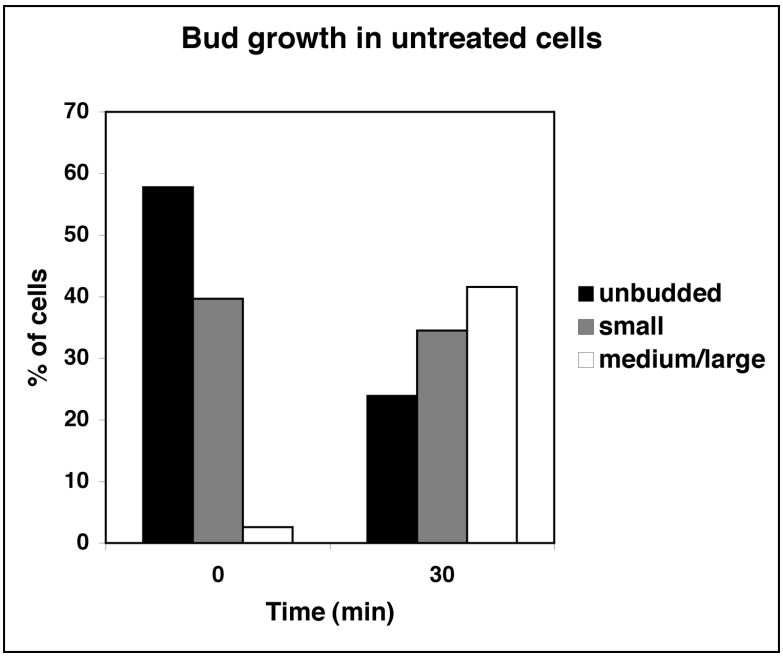
37C

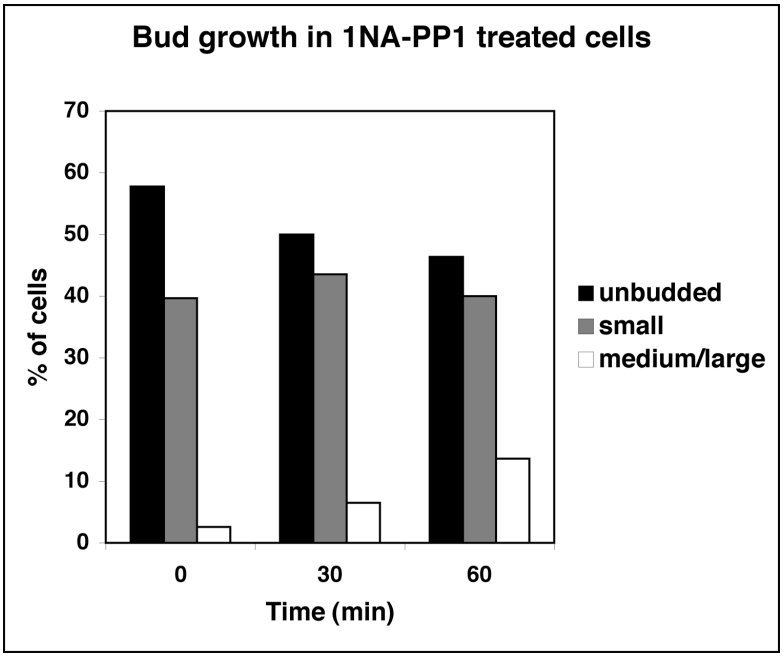


# SUPPLEMENT FIG. S3

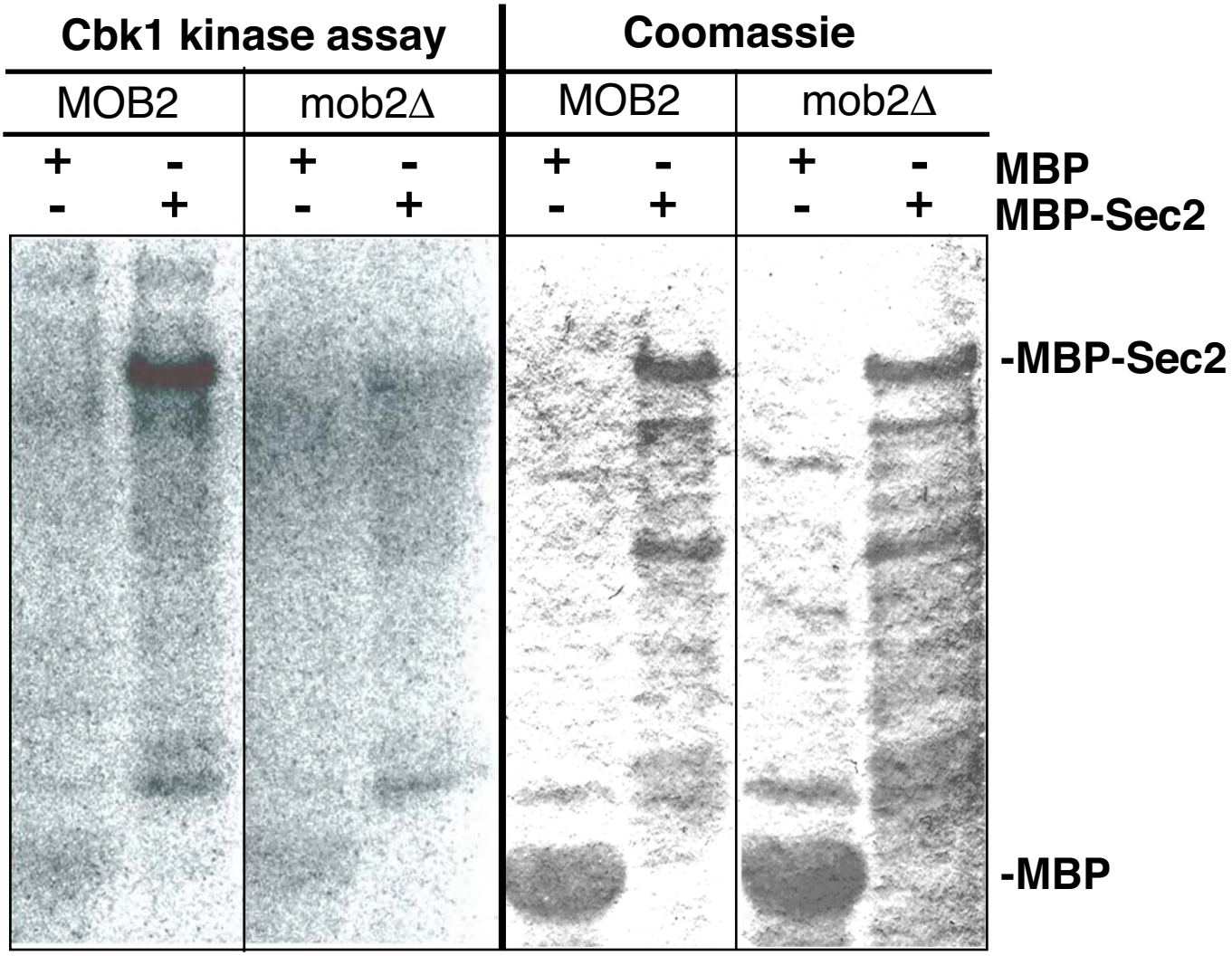


## SUPPLEMENT FIG. S4

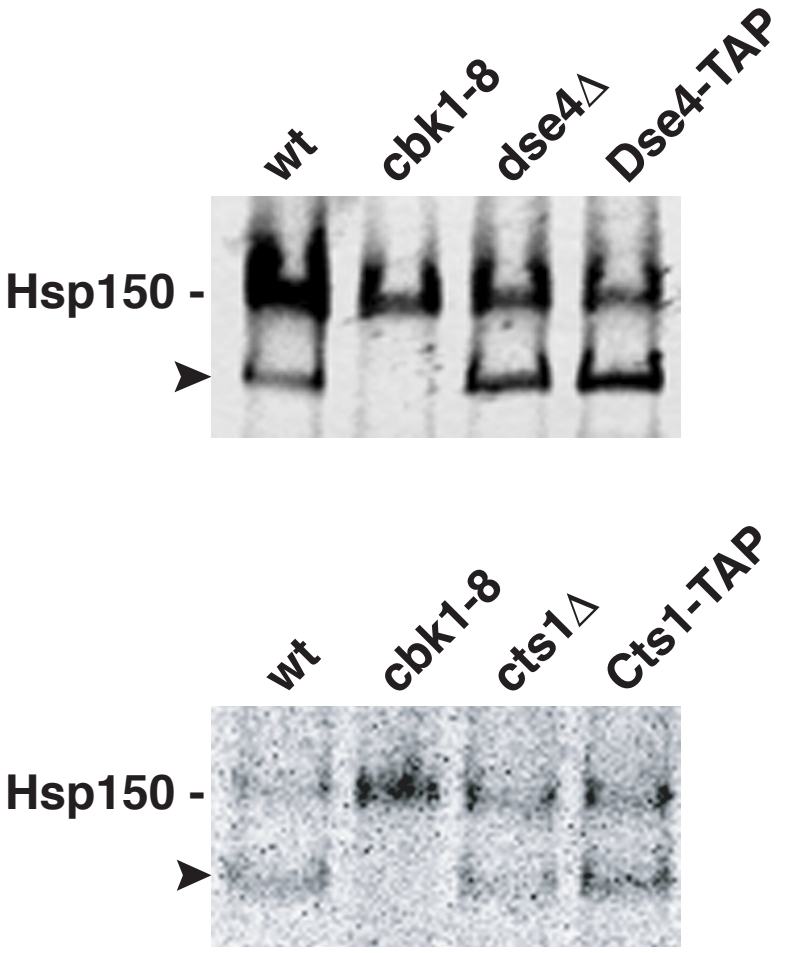


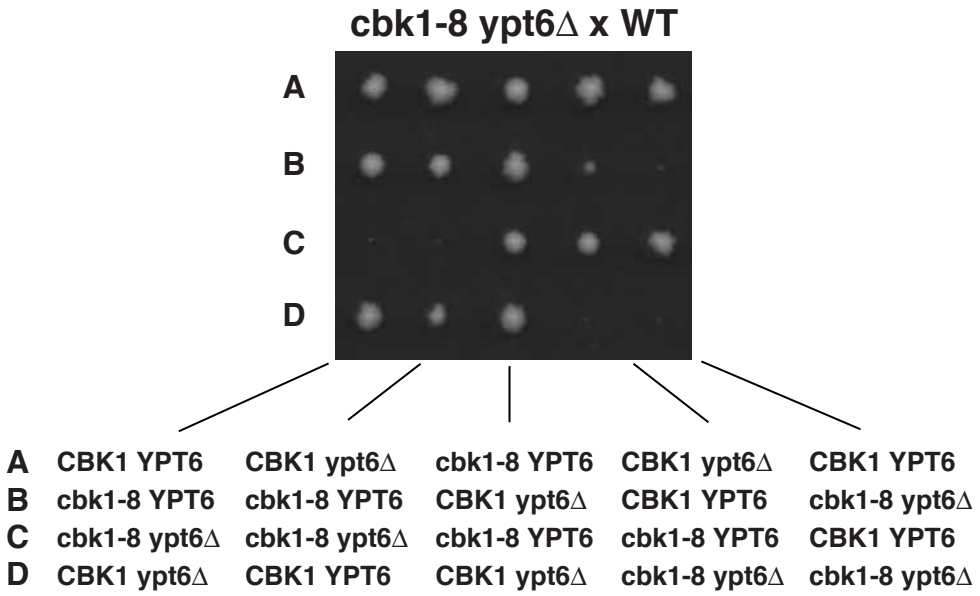
# Figure S5


Cbk1 kinase assay
Coomassie
MOB2
mob2Δ
MOB2
mob2Δ
+ - + - + - + -
- + - + - + - +
MBP
MBP-Sec2
-MBP-Sec2
-MBP

**SUPPLEMENT FIG. S6**



## SUPPLEMENT FIG. S7




| Synthetic interaction between cbk1-8 and trafficking mutants | | | | | |
|---|---|---|---|---|---|
| | Actual | | Expected | | |
| | Double mutants | Number of spores | Double mutants | Number of spores | chi $^2$ |
| sec2-41 cbk1-8 | 0 | 48 | 12 | 48 | 0.000532006 |
| sec16-2 cbk1-8 | 0 | 40 | 10 | 40 | 0.001565402 |
| cog1Δ cbk1-8 | 3 | 27 | 6.75 | 27 | 0.148914738 |
| ypt6Δ cbk1-8 | 6 | 40 | 10 | 40 | 0.205903321 |
| | | | | | |
| cog1Δ cbk1-8* | 3 | 75 | 18.75 | 75 | 0.000275504 |
| ypt6Δ cbk1-8* | 5 | 92 | 23 | 92 | 0.00017455 |

*** back-crosses of double mutants to wild type parental strain**

# SUPPLEMENT FIG. S8