Expanded View Figures

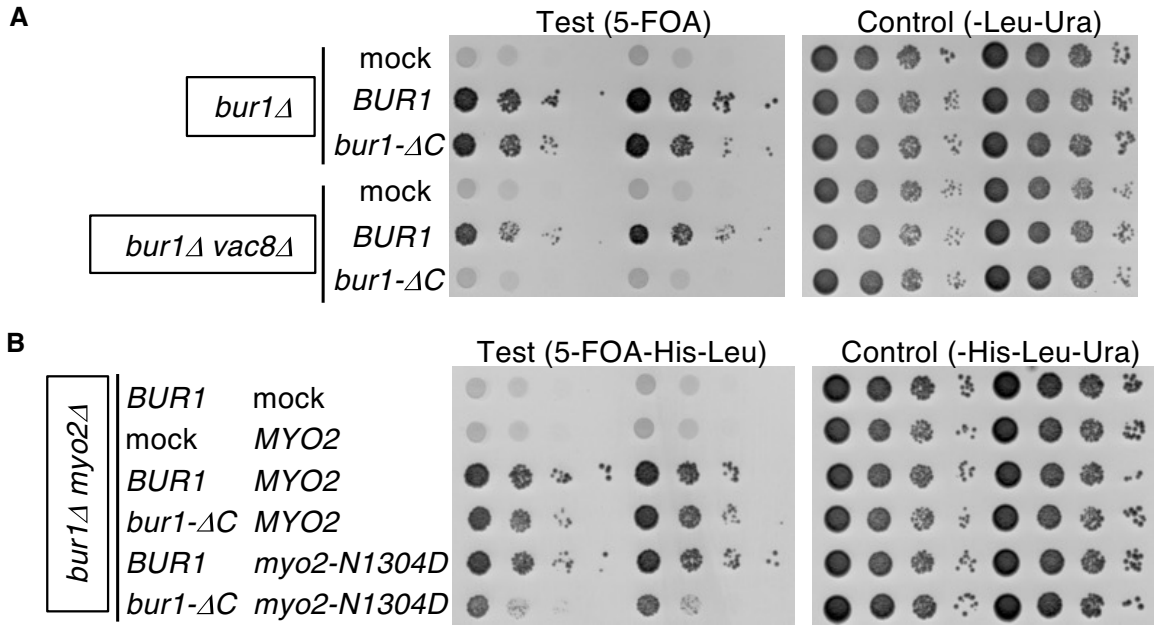

Figure EV1. Mutation of *BUR1* combined with mutations in vacuole inheritance exhibit synthetic growth defects.

A The *bur1-ΔC* mutant exhibits a synthetic growth defect with a *vac8Δ* mutant. Plasmids were transformed into a *bur1Δ*, or a *bur1Δ vac8Δ* mutant containing YCp50 [*URA3*] *BUR1*. Plasmids tested were pRS415 [*LEU2*] (mock), pRS415 *BUR1*, or pRS415 *bur1-ΔC*. Transformed colonies were cultured in liquid medium and serial dilutions spotted onto SC+5-FOA or SC-Leu-Ura plates. Plates were incubated at 24°C for 3 days.

B The *bur1-ΔC* mutant also exhibits a synthetic growth defect with *myo2-N1304D* mutant. Plasmids were transformed into a *bur1Δ myo2Δ* mutant containing YCp50 [*URA3*] *BUR1/MYO2*. Plasmids tested were pRS415 [*LEU2*] (mock), pRS415 *BUR1*, or pRS415 *bur1-ΔC*, and pRS413[*HIS3*] (mock), pRS413 *MYO2*, or pRS413 *myo2-N1304D*. Transformed colonies were cultured in liquid medium and serial dilutions spotted onto SC-His-Leu+5-FOA or SC-His-Leu-Ura plates. Plates were incubated at 24°C for 3 days.

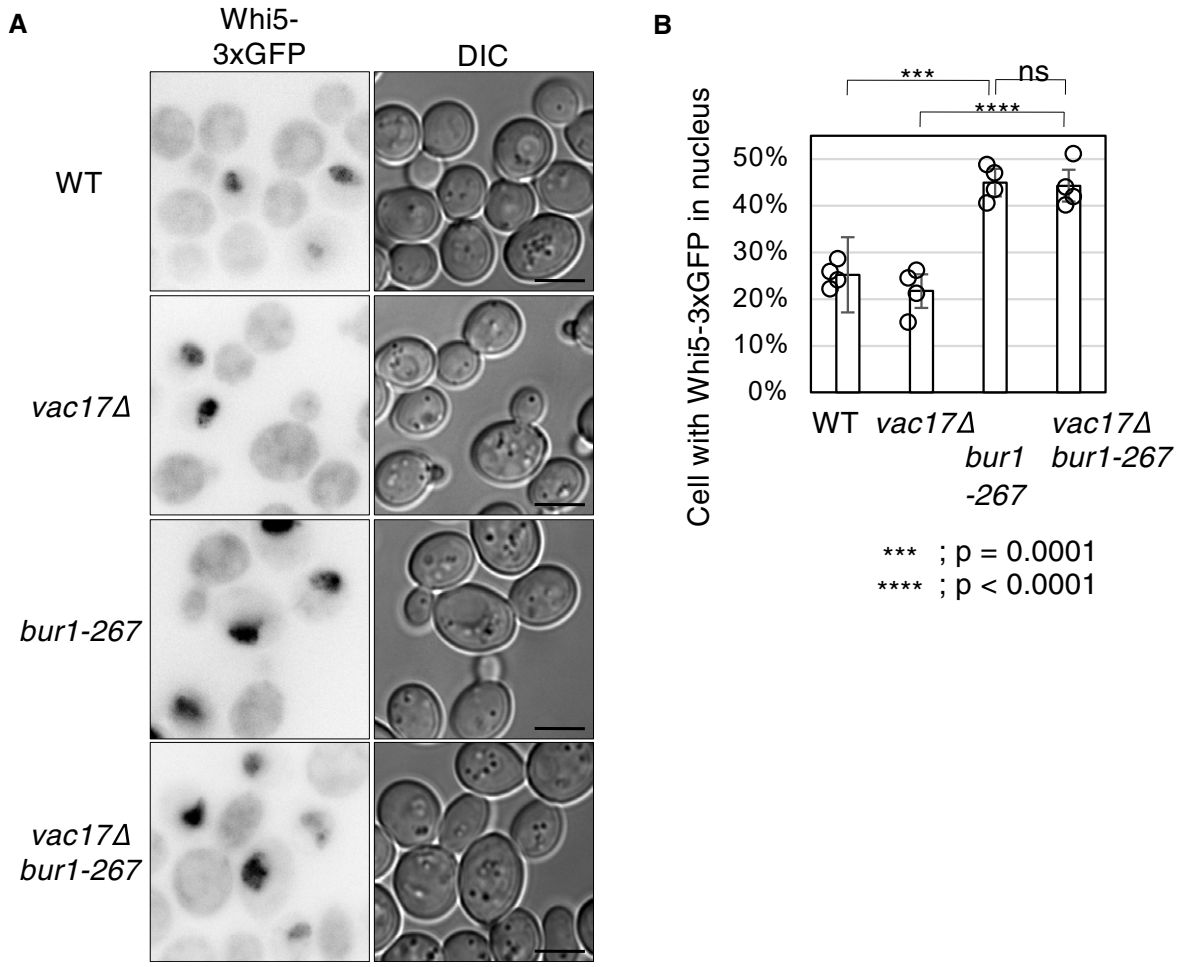

Figure EV2. A truncation mutation of *bur1-267* exhibits a cell cycle delay.

A, B Both the *bur1-267* single and *bur1-267 vac17Δ* double mutants show accumulation of Whi5 in the nucleus. WT, *vac17Δ*, *bur1-267*, and *bur1-267 vac17Δ* cells, which express Whi5-3xGFP from its endogenous locus, were incubated at 24°C. Cells were scored for the presence of Whi5-3xGFP in the nucleus. Error bars; SD calculated from four independent experiments with at least 100 cells counted in each strain/experiment. *P*-value determined with a one-way ANOVA and Tukey *post hoc* test. ***(*P*-value $< 1 \times 10^{-3}$), ****(*P*-value $< 1 \times 10^{-4}$). Scale bar, 2 μm.

Figure EV3. *BUR1* functions in parallel with the TORC1 pathway.

A *In vivo* phosphorylation of Sch9 is reduced in *bur1-*ΔC, *bur1-*ΔC *vac17*Δ, *bur1-267*, or *bur1-267 vac17*Δ cells. WT, *vac17*Δ, *bur1-*ΔC, *bur1-*ΔC *vac17*Δ, *bur1-267*, or *bur1-267 vac17*Δ cells expressing *SCH9-HA* from the *URA3* locus were grown in YPD. (Top panel) Cell lysates were separated in phos-tag containing SDS–PAGE gels and analyzed by immunoblot with antibodies directed against HA. (Bottom panel) Quantification of the ratio of upper-Sch9 to lower Sch9 on phos-tag gel Western blots. Red bar; average in each strain. Error bars; SD calculated from three independent experiments. *P*-value determined with a one-way ANOVA and Tukey *post hoc* test. ***(*P*-value $< 1 \times 10^{-3}$), ****(*P*-value $< 1 \times 10^{-4}$).

B Plasmids were transformed into a *bur1Δ vac17Δ* mutant containing YCp50 [*URA3*] *BUR1*. Plasmids tested were pRS415 [*LEU2*] (mock), pRS415 *BUR1*, or pRS415 *bur1-*ΔC, with pRS413 [*HIS3*] (mock), pRS413 *VAC17*, pRS423 [*HIS3*] (2 μ), or pRS423 *TOR1*. Transformed colonies were cultured in liquid medium and serial dilutions spotted onto 5-FOA-His-Leu or SC-His-Leu-Ura plates. Plates were incubated at 24°C for 3 days.

C Over-expression of *TOR1* rescued the rapamycin sensitivity of the *bur1-*ΔC mutant. Plasmids were transformed into WT, *tor1Δ*, *bur1-*ΔC, or *tor1*Δ *bur1-*ΔC mutant. Plasmids tested were pRS426 [*URA3*] (2 μ), pRS426 *TOR1*, pRS426 *BUR1*, or pRS426 *bur1-ΔC*. Transformed colonies were cultured in liquid SC-Ura medium and serial dilutions spotted onto SC-Ura plate with 0, 4, or 8 ng/ml of rapamycin added. Plates were incubated at 24°C for 2 days.

D Low copy or over-expression of *BUR1* did not rescue the growth defect of the *tor1*Δ *vac17*Δ mutant. Plasmids were transformed into a *vac17Δ tor1Δ* mutant containing pRS416 [*URA3*] *VAC17*. Plasmids tested were pRS415 [*LEU2*] (mock), pRS415 *VAC17*, pRS415 *TOR1*, pRS415 *TOR2*, pRS415 *BUR1*, pRS425 [*LEU2*] (2 μ), pRS425 *BUR1*, or pRS425 *bur1-ΔC*. Transformed colonies were streaked onto 5-FOA or -Leu-Ura plates. Plates were incubated at 24°C for 3 days.

E Over-expression of *BUR1* and *BUR2* did not rescue the growth defect of the *tor1*Δ *vac17*Δ mutant. Plasmids tested were pRS425 [*LEU2*] (2 μ), or pRS425 *BUR1*, with pVT102-His (2 μ), or pVT102-His *BUR2*, which encodes the *BUR1* cyclin. pRS315 *TOR1* and pRS413 *VAC17* were used as positive controls.

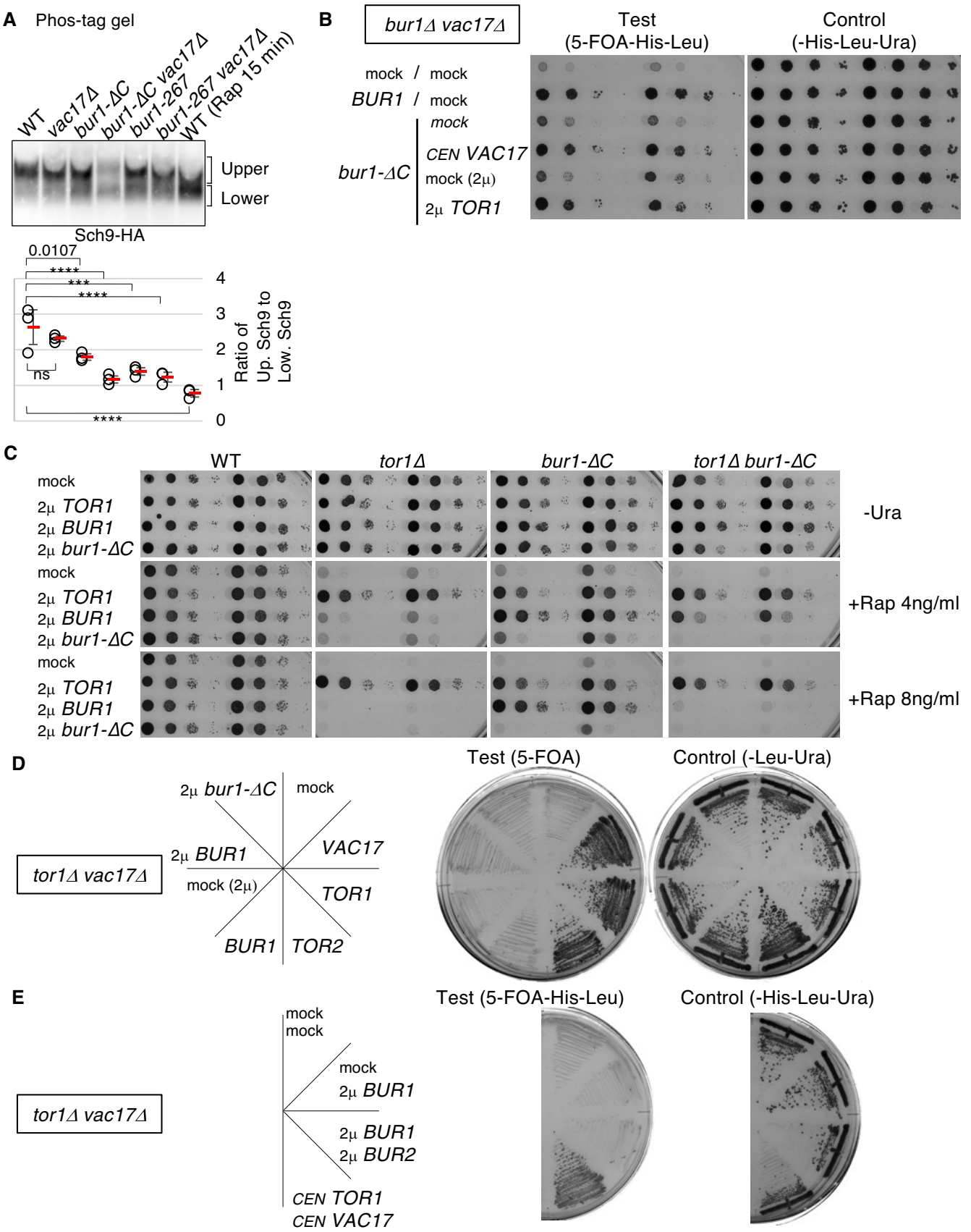
A
Phos-tag gel
WT
vac17Δ
bur1-ΔC
bur1-ΔC vac17Δ
bur1-267
bur1-267 vac17Δ
WT (Rap 15 min)
Upper
Lower
Sch9-HA
0.0107
****
***
****
ns
****
Ratio of Up. Sch9 to Low. Sch9
B
bur1Δ vac17Δ
Test (5-FOA-His-Leu)
Control (-His-Leu-Ura)
mock / mock
BUR1 / mock
bur1-ΔC
mock
CEN VAC17
mock (2μ)
2μ TOR1
C
WT
tor1Δ
bur1-ΔC
tor1Δ bur1-ΔC
mock
2μ TOR1
2μ BUR1
2μ bur1-ΔC
-Ura
+Rap 4ng/ml
+Rap 8ng/ml
D
tor1Δ vac17Δ
2μ bur1-ΔC
mock
2μ BUR1
VAC17
mock (2μ)
TOR1
BUR1
TOR2
Test (5-FOA)
Control (-Leu-Ura)
E
tor1Δ vac17Δ
mock
mock
mock
2μ BUR1
2μ BUR1
2μ BUR2
CEN TOR1
CEN VAC17
Test (5-FOA-His-Leu)
Control (-His-Leu-Ura)

Figure EV3.

Figure EV4. The Bur1 phospho-sites on Sch9 are important for normal cell growth.

A Bur1 is associated with Sch9. Purified bacterially expressed GST-Sch9 protein pulled down Bur1 from yeast lysates. Proteins pulled down by GST, GST-Sch9 (1–390), GST-Sch9 (391–824), and 8% of input yeast lysate analyzed by immunoblot with anti-HA antibodies (top), or by Coomassie brilliant blue (CBB) (bottom).

B Over-expression of Bur1 C-terminal region in wild-type yeast does not affect their sensitivity to rapamycin or their growth on normal synthetic media. Plasmids tested were pRS426 [*URA3*] (mock), pRS426 *BUR1*, pRS426 *BUR1-Venus*, or pRS426 *bur1(358-end)-Venus*. Transformed colonies were cultured in liquid medium and serial dilutions were spotted onto SC-Ura plates with 0, 4, 8, or 16 ng/ml of rapamycin added. Plates were incubated at 24°C for 2 days.

C Over-expression of the Bur1 C-terminal region does not rescue the rapamycin sensitivity of *bur1*-Δ*C* cells.

D Western blot analysis of cells expressing Bur1-Venus or Bur1(358-657)-Venus from CEN or 2 μ plasmids. Cell lysates were analyzed by immunoblot with antibodies directed against GFP (top) and Pgk1 (loading control).

E An Sch9 mutant that is missing the nine novel Bur1 phosphorylation sites identified in this study is not functional, and an Sch9 mutant which is mutated at only three novel adjacent Bur1 sites identified in this study exhibits a growth defect. Plasmids were transformed into a *sch9*Δ *vac17Δ* mutant containing pRS416 [*URA3*] *SCH9*. Plasmids tested were pRS413 [*HIS3*] (mock), pRS413 *SCH9*, pRS413 *sch9-9A*, pRS413 *sch9-3A*, or pRS413 *sch9-3D/E*. Transformed colonies were cultured in liquid medium and serial dilutions were spotted onto 5-FOA or SC-His-Ura plates. Plates were incubated at 24°C for 2 days.

F The phospho-mimetic sch9-2D3E mutant as well as sch9-3D/E does not rescue the growth defect of the *bur1*-Δ*C vac17Δ* mutant. Plasmids tested were pRS415 *bur1*-Δ*C* with pRS413 (mock), pRS413 *VAC17*, pVT102-H (2 μ), pVT102-H *SCH9*, pVT102-H *sch9-2D3E*, or pVT102-H *sch9-3D/E*. Transformed colonies were cultured in liquid medium, and serial dilutions were spotted onto 5-FOA-His-Leu, 5-FOA-His, or SC-His-Leu-Ura plates, and incubated at 24°C for 3 days.

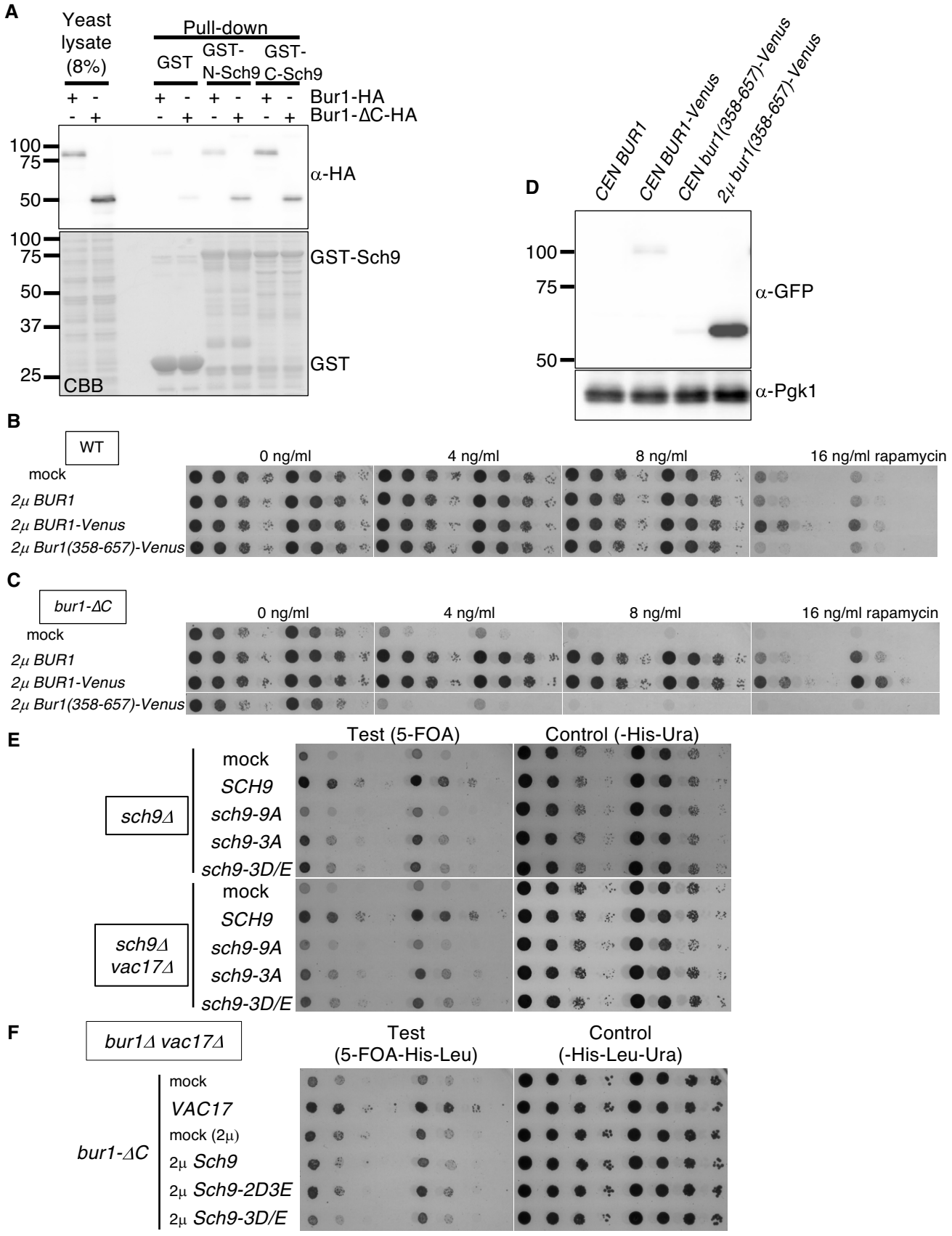
A
Yeast lysate (8%)
Pull-down
GST
GST-N-Sch9
GST-C-Sch9
Bur1-HA
Bur1-ΔC-HA
100
75
50
α-HA
100
75
50
37
25
GST-Sch9
GST
CBB
D
CEN BUR1
CEN BUR1-Venus
CEN bur1(358-657)-Venus
2μ bur1(358-657)-Venus
100
75
50
α-GFP
α-Pgk1
B
WT
mock
2μ BUR1
2μ BUR1-Venus
2μ Bur1(358-657)-Venus
0 ng/ml
4 ng/ml
8 ng/ml
16 ng/ml rapamycin
C
bur1-ΔC
mock
2μ BUR1
2μ BUR1-Venus
2μ Bur1(358-657)-Venus
0 ng/ml
4 ng/ml
8 ng/ml
16 ng/ml rapamycin
E
Test (5-FOA)
Control (-His-Ura)
sch9Δ
mock
SCH9
sch9-9A
sch9-3A
sch9-3D/E
sch9Δ vac17Δ
mock
SCH9
sch9-9A
sch9-3A
sch9-3D/E
F
bur1Δ vac17Δ
Test (5-FOA-His-Leu)
Control (-His-Leu-Ura)
bur1-ΔC
mock
VAC17
mock (2μ)
2μ Sch9
2μ Sch9-2D3E
2μ Sch9-3D/E

Figure EV4.

Figure EV5. Bur1 is distributed between the nucleus and cytoplasm.

A The C-terminal truncation of Bur1 exhibited a reduced cytoplasmic localization. Bur1-mNG or Bur1-ΔC-mNG with histone H2B (Htb2)-mCherry were expressed from their endogenous loci. Scale bar, 2 μm.

B Quantification of Bur1-mNG intensity in the cytoplasmic region. Ratios of cytoplasmic/total cellular mNG intensity were calculated for both Bur1 and Bur1-ΔC. Statistical significance (*P*-value) was determined with student *t*-test. ****(*P*-value $< 1 \times 10^{-4}$). Red bar; average in each strain. Error bar; SD calculated from at least 300 cells were measured in each strain.

C Western blot analysis of cells expressing Bur1-mNG or Bur1-ΔC-mNG. Cell lysates were analyzed by immunoblot with antibodies directed against mNG (top) and Pgk1 (loading control).

D The cytosolic pool of Bur1 exhibits some change during cell cycle. Wild-type yeast, which express Bur1-mNG and histone Htb2-mCherry from endogenous loci, were synchronized using α-mating factor arrest and release. Released cells from the α-factor were imaged every 15 min by microscope. For fluorescent intensity analysis, images were analyzed using CellProfiler software (Top panel). Quantification of Bur1-mNG intensity in the cytoplasmic region. Ratios of nuclear/cytoplasm and cytosolic/cell mNG intensity were calculated for Bur1-mNG. (Bottom panel) Cell cycle synchronicity was monitored using cell morphology combined with histone Htb2-mCherry fluorescence to assess the location of the nucleus: unbudded cell, bud without nucleus, or cell during nuclear segregation.

E Full-length Bur1 localizes in the nucleus, while Bur1-ΔN (50–657) mainly localizes in the cytoplasmic region in cells. Scale bar, 2 μm.

F Bur1-ΔN supports the viability of the *bur1*Δ and *bur1*Δ *vac17*Δ mutants. Plasmids were transformed into a *bur1Δ* or *bur1Δ vac17Δ* double mutant containing YCp50 [*URA3*] *BUR1*. Plasmids tested were pRS415 [*LEU2*] (mock), pRS415 *BUR1*, pRS415 *bur1-ΔN*, or pRS415 *bur1-ΔC*. Transformed colonies were cultured in liquid medium and serial dilutions spotted onto 5-FOA or SC-Leu-Ura plates. Plates were incubated at 24°C for 3 days.

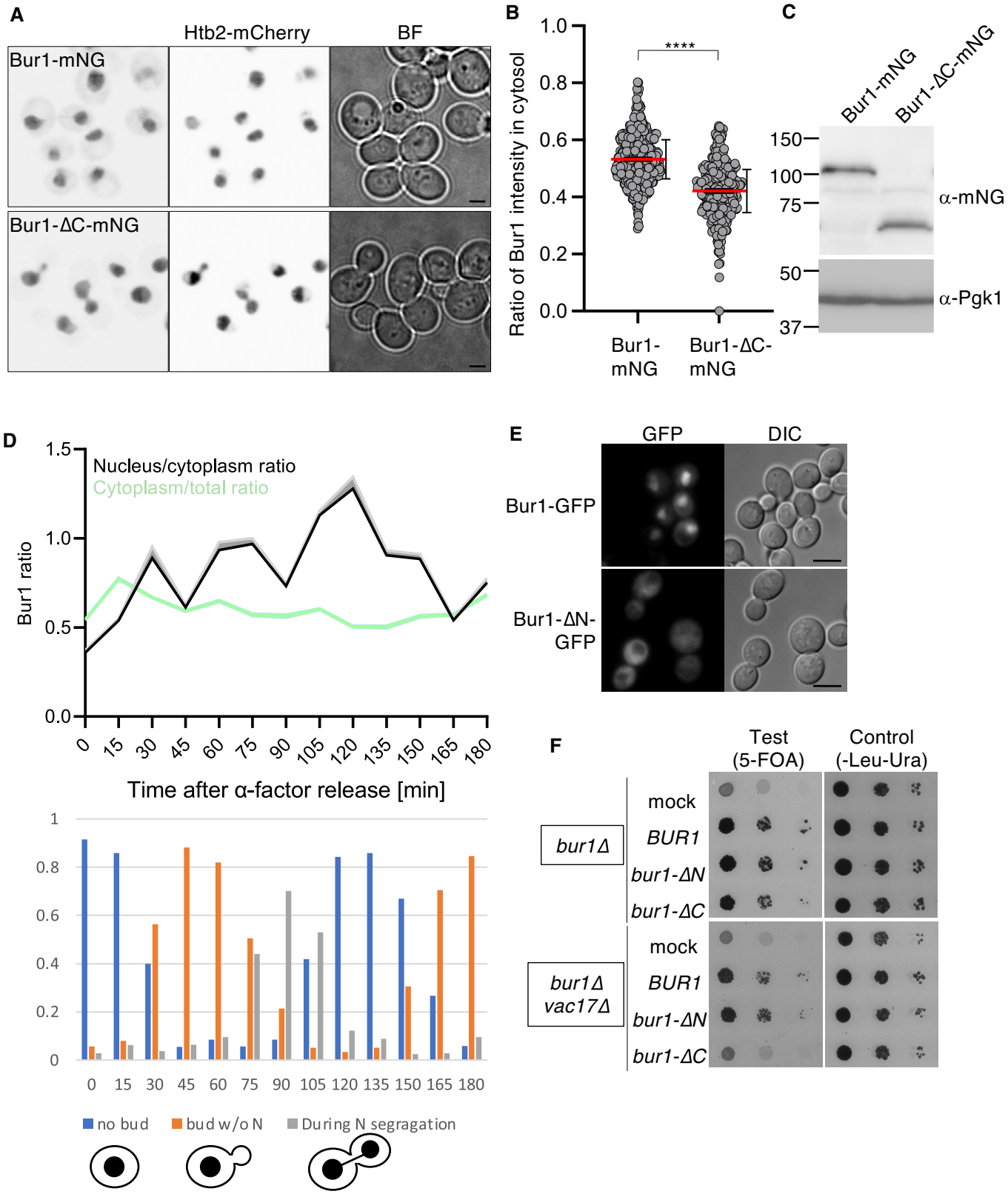
A
Htb2-mCherry
BF
Bur1-mNG
Bur1-ΔC-mNG
B
Ratio of Bur1 intensity in cytosol
****
Bur1-mNG
Bur1-ΔC-mNG
C
Bur1-mNG
Bur1-ΔC-mNG
α-mNG
α-Pgk1
D
Nucleus/cytoplasm ratio
Cytoplasm/total ratio
Bur1 ratio
Time after α-factor release [min]
no bud
bud w/o N
During N segragation
E
GFP
DIC
Bur1-GFP
Bur1-ΔN-GFP
F
Test (5-FOA)
Control (-Leu-Ura)
bur1Δ
bur1Δ vac17Δ
mock
BUR1
bur1-ΔN
bur1-ΔC

Figure EV5.