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Supplemental Information

TORC1 Determines Fab1 Lipid Kinase Function at Signaling Endosomes and Vacuoles

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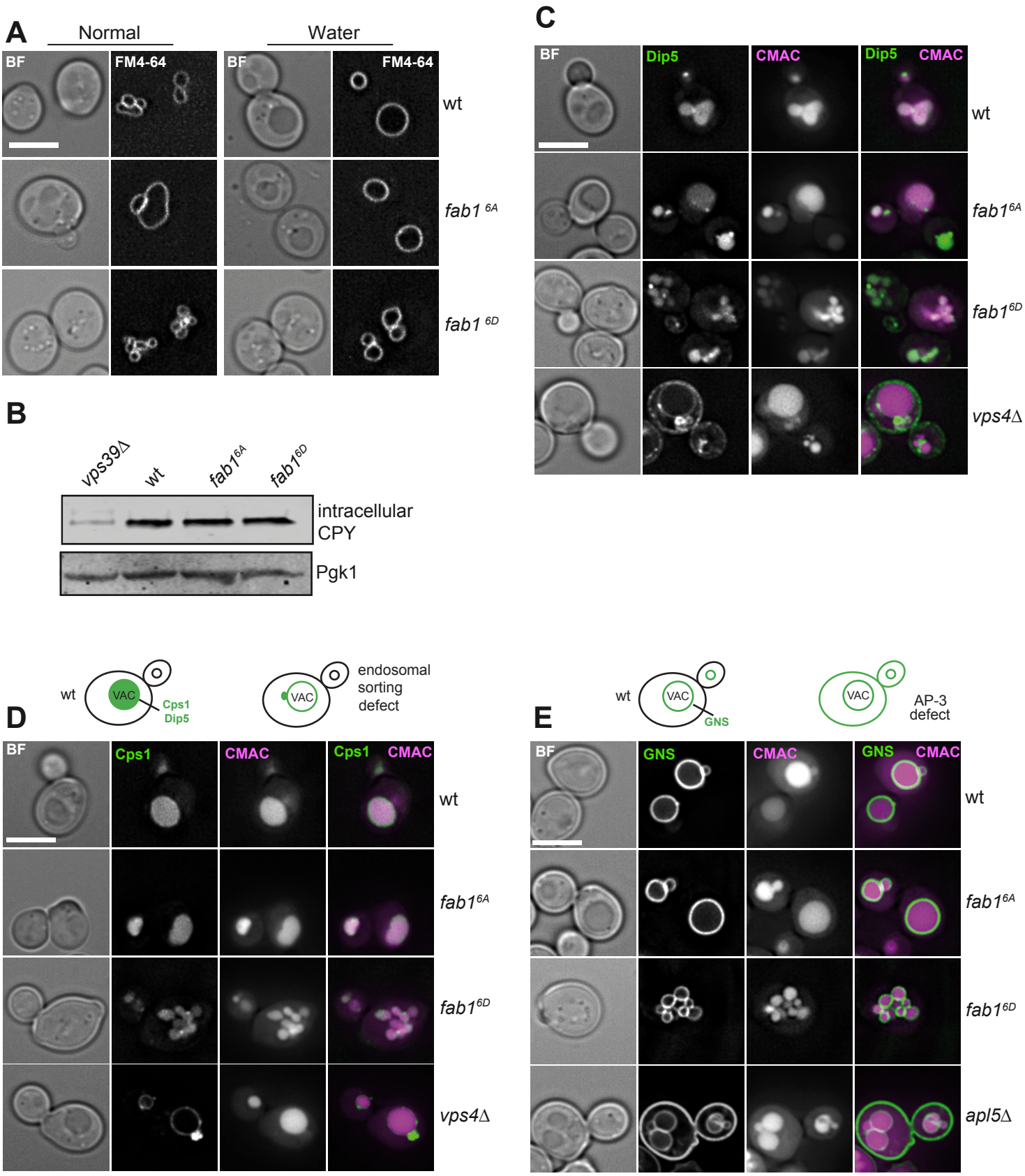

A
Normal
Water
BF
FM4-64
wt
fab1 6A
fab1 6D
B
vps39Δ
wt
fab1^6A
fab1^6D
intracellular CPY
Pgk1
C
BF
Dip5
CMAC
wt
fab1^6A
fab1^6D
vps4Δ
D
wt
VAC
Cps1
Dip5
endosomal sorting defect
Cps1
wt
fab1^6A
fab1^6D
vps4Δ
E
GNS
AP-3 defect
apl5Δ

Figure S1. Effect of Fab1 mutations on protein sorting to vacuole. Related to Figure 3. (A) Analysis of vacuolar morphology in *fab1* mutants after hypoosmotic shock. Cells were grown to exponential phase. After staining with FM4-64, cells were washed either with medium (normal), or twice with water, and vacuolar morphology was analyzed by fluorescence microscopy. Scale bar, 5 µm. (B-E) Analysis of vacuole sorting of selected fusion proteins along the endolysosomal pathway of *fab1* mutants. For the localization of CPY in cells whole cell lysates of the indicated strains were analyzed by SDS-PAGE and Western blotting (B). Sorting of GFP-tagged Dip5 (C), Cps1 (D), and GFP-Nyv1-Snc1 (GNS) (E) was analyzed in the indicated strains by fluorescence microscopy relative to CMAC stained vacuoles. Models in (D, E) indicate the expected outcome for wild-type and mutant cells. Scale bar, 5 µm.

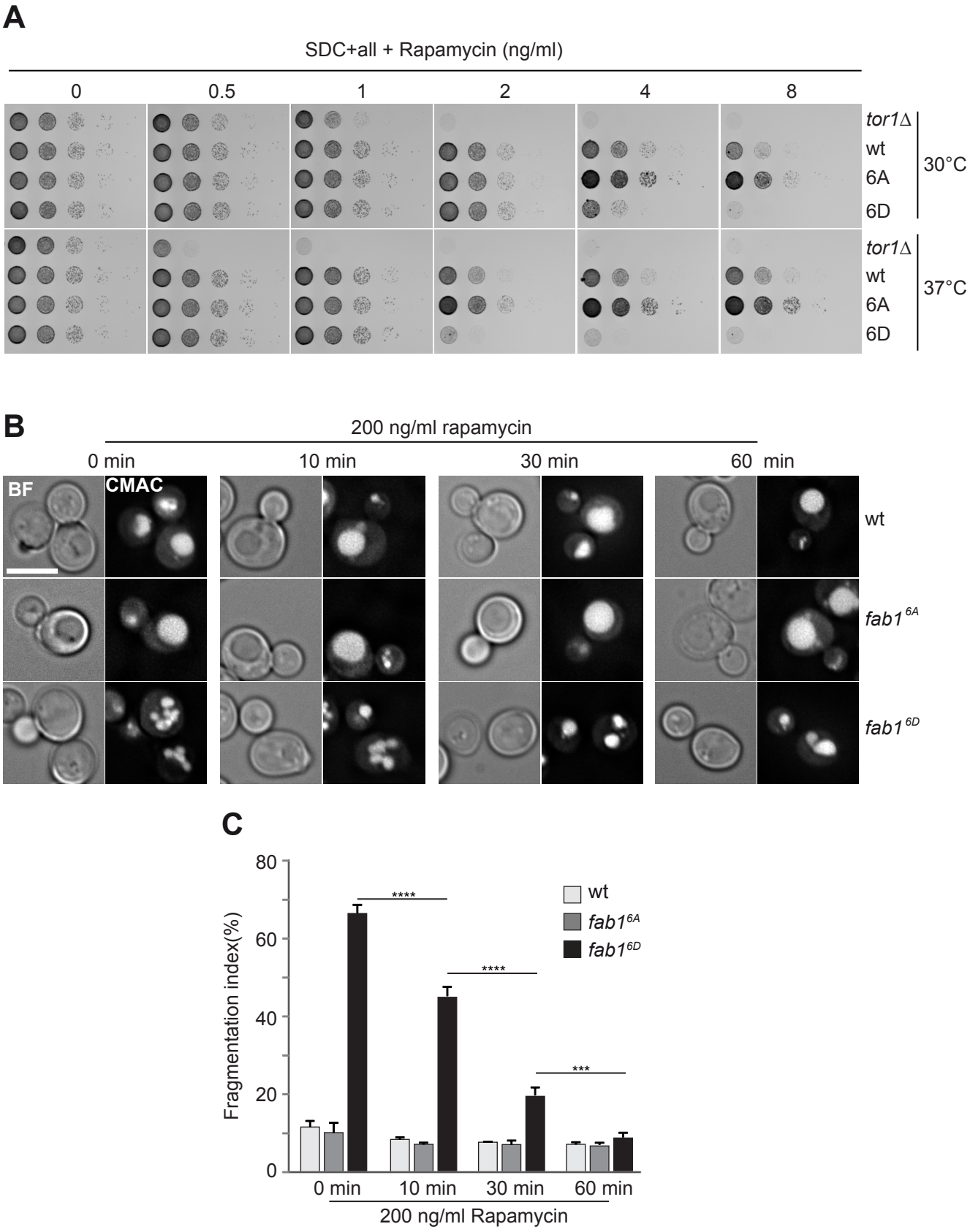
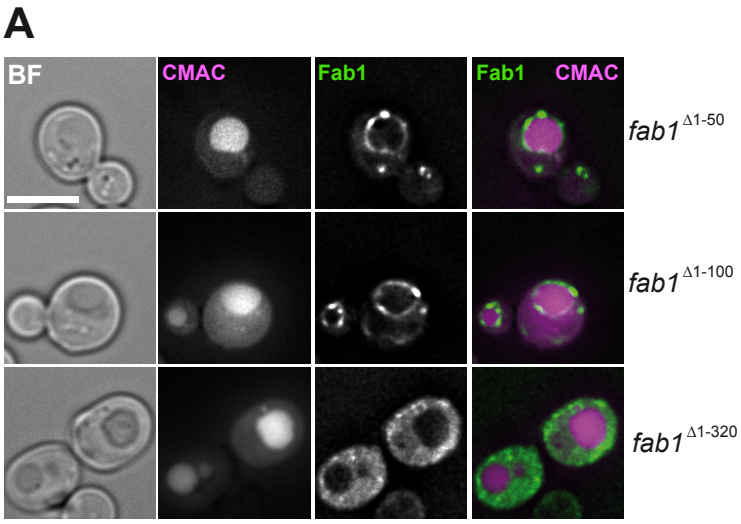
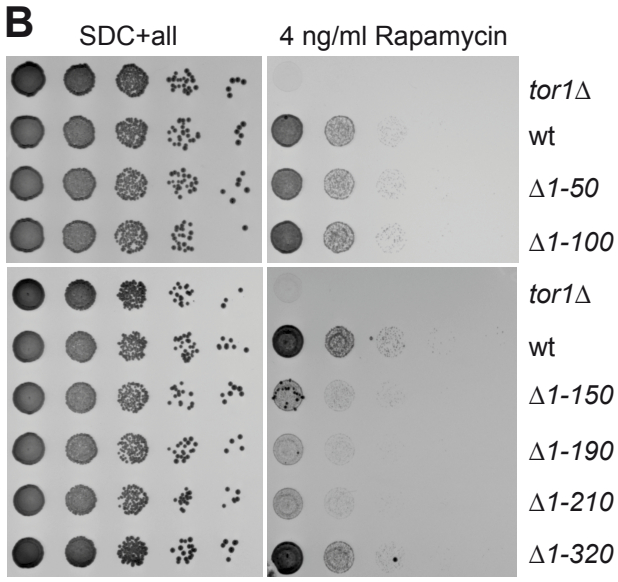

A
SDC+all + Rapamycin (ng/ml)
0
0.5
1
2
4
8
tor1Δ
wt
6A
6D
30°C
37°C
B
200 ng/ml rapamycin
0 min
10 min
30 min
60 min
BF
CMAC
wt
fab1 6A
fab1 6D
C
Fragmentation index(%)
80
60
40
20
0
****
****
***
wt
fab1 6A
fab1 6D
0 min
10 min
30 min
60 min
200 ng/ml Rapamycin

Figure S2. Growth of indicated strains on plates containing increasing amounts of rapamycin. Related to Figure 4. (A) Wild-type cells (wt), *tor1*Δ, and Fab1 mutant cells were grown as described in methods, and serial dilutions were spotted onto SDC plates containing the indicated concentrations of rapamycin. (B) Analysis of vacuolar morphology in wild-type and *fab1* mutant cells upon rapamycin addition. Cells were grown to log-phase and then stained with CMAC for 10 min. After washing cells twice with medium, 200 ng/ml rapamycin was added to cells and vacuolar morphology was analyzed by fluorescence microscopy at the indicated time points. Scale bar, 5 µm. (C) Quantification of vacuolar morphology shown in (B). Fragmentation index is shown as the percentage of cells containing more than 4 vacuoles. At least 100 cells were counted in three independent experiments. Results are means +/- SD. Significance was determined with a two-tailed Student's t-test (** * , p<0.0 0 1; **** , p<0.0001).


A
BF
CMAC
Fab1
Fab1 CMAC
fab1 Δ1-50
fab1 Δ1-100
fab1 Δ1-320


B
SDC+all
4 ng/ml Rapamycin
tor1Δ
wt
Δ1-50
Δ1-100
tor1Δ
wt
Δ1-150
Δ1-190
Δ1-210
Δ1-320

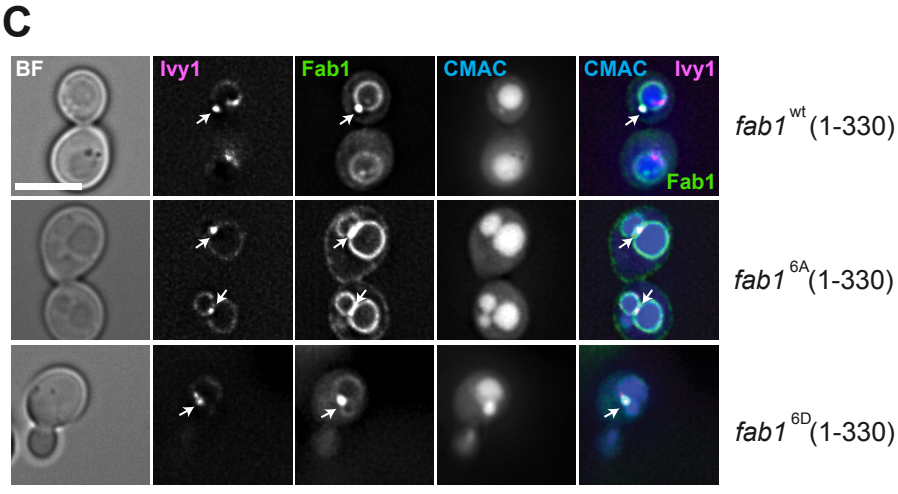

C
BF
Ivy1
Fab1
CMAC
CMAC Ivy1
Fab1
fab1 wt (1-330)
fab1 6A (1-330)
fab1 6D (1-330)

Figure S3. Regulation of Fab1 targeting to endosomes by its FYVE domain. Related to Figure 6. (A) Analysis of N-terminal truncations of Fab1. Fab1 was truncated at the indicated residues by insertion of the *PHO5* promoter together with N-terminal GFP and strains were analyzed as described in Figure 6A. (B) Growth of truncation strains on rapamycin-containing plates. Analysis was done as in Figure 6D. (C) Localization of the N-terminal part of Fab1 depends on phosphorylation. A plasmid encoding the GFP-tagged Fab1 fragments (aa 1-330) with the indicated mutations was inserted into the genome of wild-type cells carrying Ivy1-mKATE, and cells were analyzed as before.

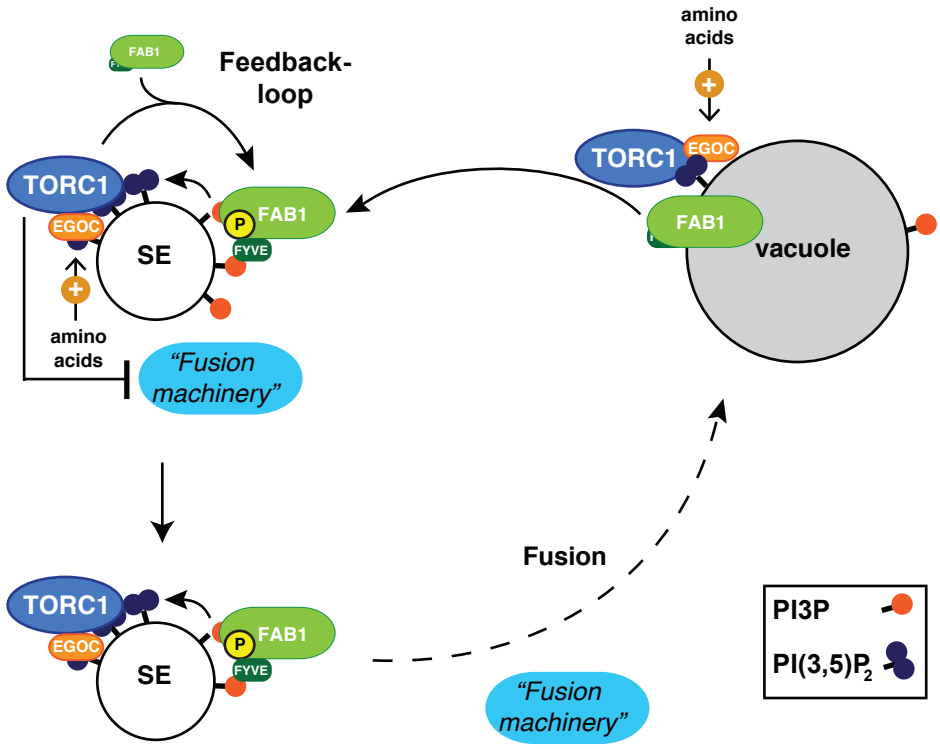

FAB1
Feedback-
loop
amino
acids
TORC1
EGOC
FAB1
P
FYVE
SE
vacuole
amino
acids
"Fusion
machinery"
Fusion
TORC1
EGOC
FAB1
P
FYVE
SE
"Fusion
machinery"
PI3P
PI(3,5)P2

Figure S4. Working model of Fab1 and TORC1 complex functions at endosomes and vacuoles. Related to Figure 6. The Fab1 complex (FAB1C) is shown in green, its FYVE domain in dark green. EGOC, EGO Complex. At the vacuole, Fab1 generates PI(3,5)P_2 and recruits TORC1. Phosphorylation of Fab1 makes the FYVE domain available to bind PI3P and causes its shift to signaling endosomes, where Fab1 generates PI(3,5)P_2 and recruits TORC1. As long as TORC1 is activated by available amino acids, it will keep Fab1 phosphorylated in a feedback-loop. Loss of input or Fab1 dephosphorylation may favor its shift to the vacuole. For details see text.