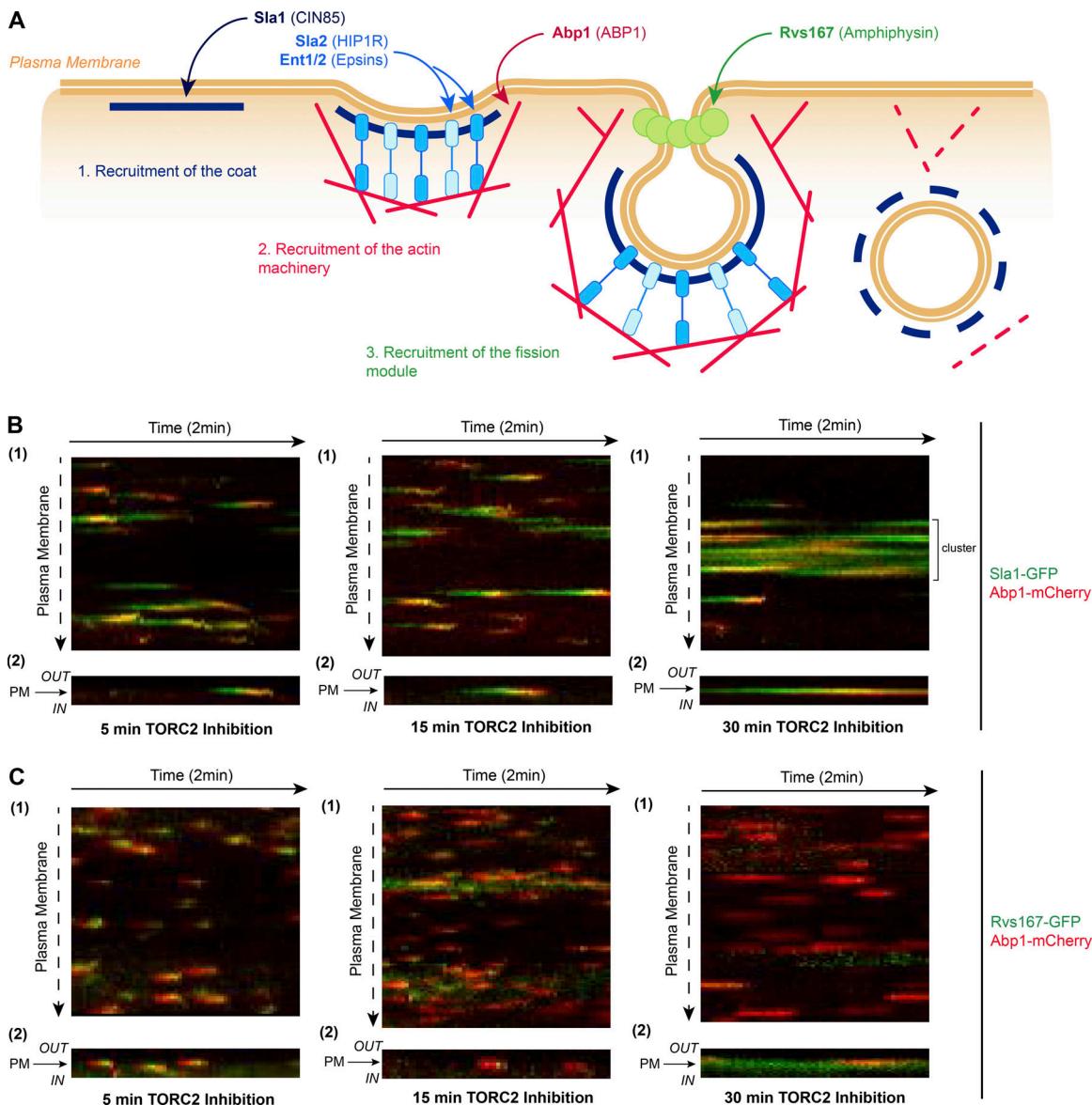
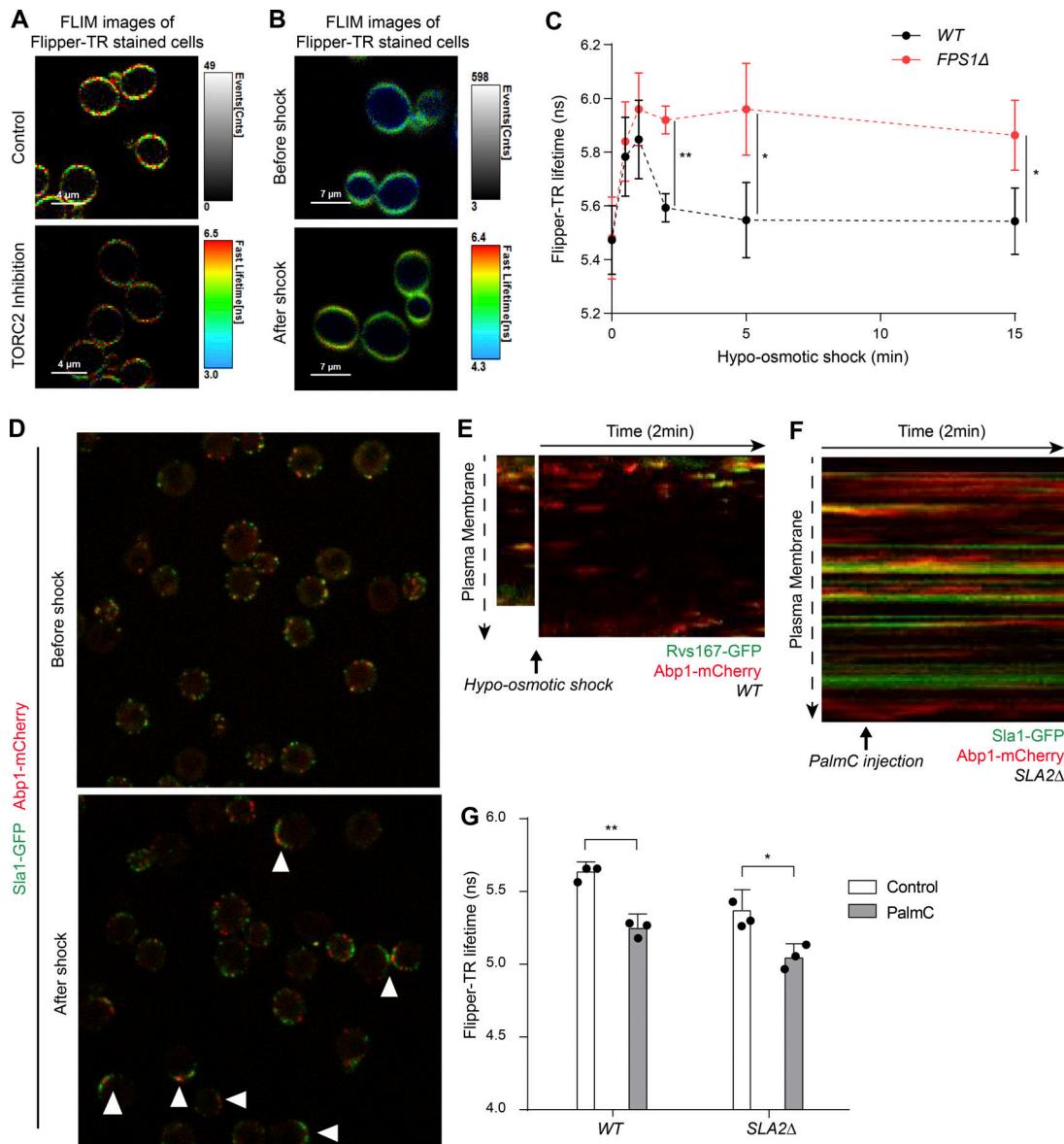


## Supplemental material

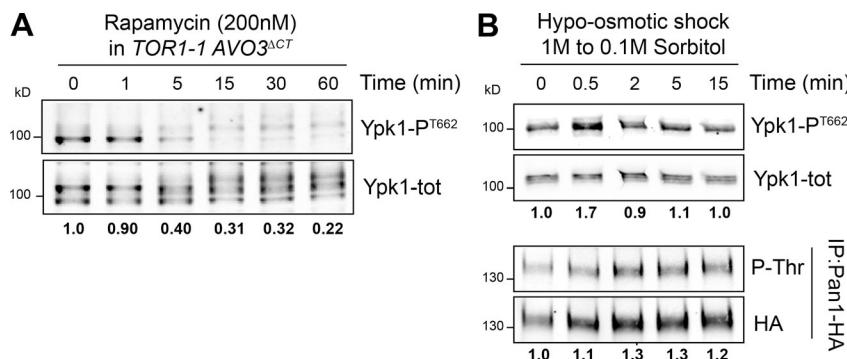
Riggi et al., <https://doi.org/10.1083/jcb.201901096>



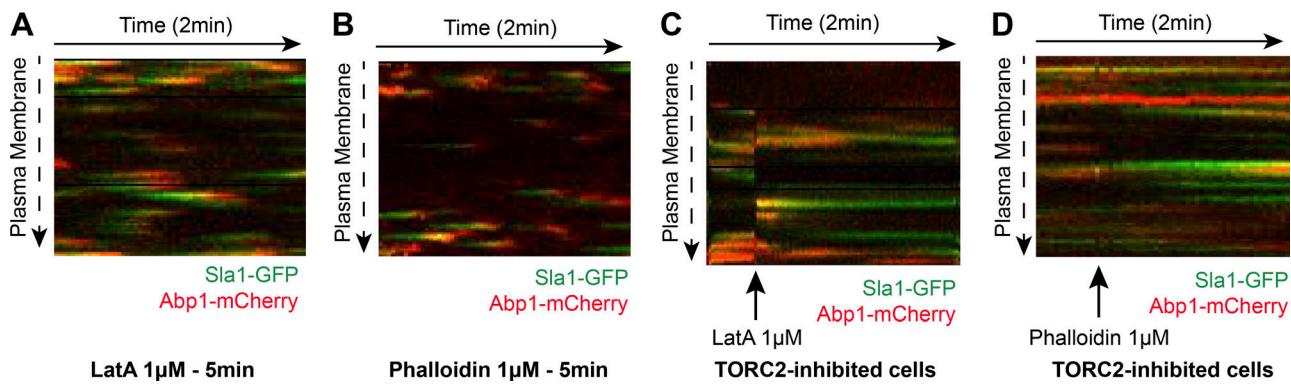
**Figure S1. TORC2 inhibition effects on endocytosis are progressive.** **(A)** Overview of endocytosis in yeast. The names of the proteins studied in this paper are indicated at the top of the figure. Mammalian orthologues are indicated between brackets. **(B and C)** Kymographs of *TOR1-1 AVO3Δ<sup>CT</sup>* cells tagged with Abp1-mCherry and either Sla1-GFP (B) or Rvs167-GFP (C), recorded after the indicated time of Rapamycin treatment. The kymographs were recorded either along (1) or across (2) the PM.



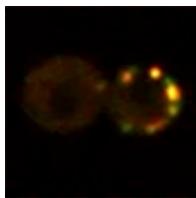
**Figure S2. Osmotic shocks and PalmC treatment affect PM tension in yeast.** **(A and B)** Color-coded FLIM images showing the Flipper-TR signal in *TOR1-1 AVO3 $\Delta^{CT}$*  cells before and after 1-h Rapamycin treatment (A) or in WT cells before and after hypo-osmotic shock (B). **(C)** Evolution of PM tension, monitored through the lifetime of the Flipper-TR probe measured by FLIM, upon hypo-osmotic shock in WT or *FPS1 $\Delta$*  cells. Error bars represent the propagated error of mean values calculated from three independent experiments (with  $n > 10$  cells; \*\*,  $P < 0.01$ ; \*,  $P < 0.05$ ). **(D)** Larger frames of *FPS1 $\Delta$*  cells expressing Sla1-GFP and Abp1-mCherry, from which the images in Fig. 2 C were cropped, acquired before and after hypo-osmotic shock. **(E)** Rvs167-GFP and Abp1-mCherry kymograph along the PM of a WT cell upon hypo-osmotic shock. **(F)** Sla1-GFP and Abp1-mCherry kymographs recorded as indicated along the PM of a *TOR1-1 AVO3 $\Delta^{CT}$  SLA2 $\Delta$*  cell, pretreated with Rapamycin for 1 h, upon PalmC injection. **(G)** PM tension, monitored through the lifetime of the Flipper-TR probe measured by FLIM, before and after PalmC treatment in WT or SLA2 $\Delta$  cells. Error bars represent the propagated error of mean values calculated from three independent experiments with  $n > 10$  cells (\*\*,  $P < 0.01$ ; \*,  $P < 0.05$ ). Source data are provided in Table S1.



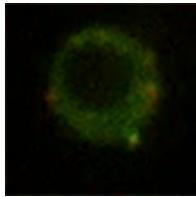
**Figure S3. Fpk-related phosphorylation of endocytic proteins are independent from PM tension.** **(A)** Rapamycin treatment achieves maximal TORC2 inhibition (monitored through Ypk1 T<sup>662</sup> phosphorylation signal compared with Ypk1-total signal) after only 5 min in *TOR1-1 AVO3<sup>ACT</sup>* cells. **(B)** The phosphorylation of immunoprecipitated (IP) Pan1-HA is not directly affected by PM tension manipulation through a hypo-osmotic shock. Source data are provided in Table S1.



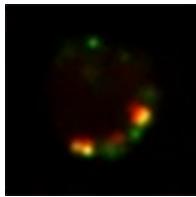
**Figure S4. F-actin manipulations through drug-stabilizing or destabilizing filaments do not mimic or rescue the uncoupling phenotype.** **(A and B)** Sla1-GFP and Abp1-mCherry kymographs recorded along the PM of a WT cell after 5 min of treatment with either 1 µM Latrunculin A (A) or Phalloidin (B). **(C and D)** Sla1-GFP and Abp1-mCherry kymographs recorded as indicated along the PM of a *TOR1-1 AVO3<sup>ACT</sup>* cell, pretreated with Rapamycin for 1 h, upon either 1 µM Latrunculin A (C) or Phalloidin (D) injection.



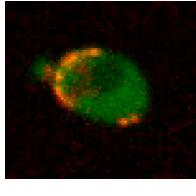
Video 1. ***TOR1-1 AVO3 $\Delta^{CT}$*  cell expressing **Abp1-mCherry and Sla1-GFP**.** Time between frames, 2 s.



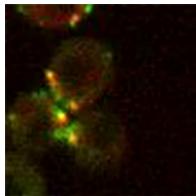
Video 2. ***TOR1-1 AVO3 $\Delta^{CT}$*  cell expressing **Abp1-mCherry and Rvs167-GFP**.** Time between frames, 2 s.



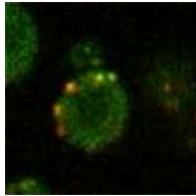
Video 3. **TORC2 inhibition induces a slow uncoupling and clustering endocytosis phenotype.** *TOR1-1 AVO3 $\Delta^{CT}$*  cell expressing Abp1-mCherry and Sla1-GFP after 1-h TORC2 inhibition with Rapamycin. Time between frames, 2 s.



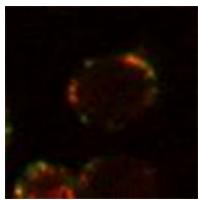
Video 4. **TORC2 inhibition prevents the recruitment of Rvs167 at the PM.** *TOR1-1 AVO3 $\Delta^{CT}$*  cell expressing Abp1-mCherry and Rvs167-GFP after 1-h TORC2 inhibition with Rapamycin. Time between frames, 2 s.



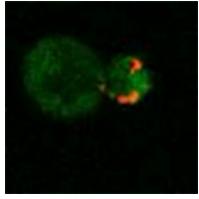
Video 5. **Hypo-osmotic shock induces a fast uncoupling and clustering endocytosis phenotype.** 1 M hypo-osmotic shock applied to a *TOR1-1 AVO3 $\Delta^{CT}$*  cell expressing Abp1-mCherry and Sla1-GFP. Time between frames, 2 s.



Video 6. **Hypo-osmotic shock induces a fast inhibition of Rvs167 recruitment.** 1 M hypo-osmotic shock applied to a *TOR1-1 AVO3 $\Delta^{CT}$*  cell expressing Abp1-mCherry and Rvs167-GFP. Time between frames, 2 s.



Video 7. **Decreasing PM tension partially restores the uncoupling and clustering endocytosis phenotypes.** PalmC treatment applied to a *TOR1-1 AVO3Δ<sup>CT</sup>* cell expressing Abp1-mCherry and Sla1-GFP after 1-h TORC2 inhibition with Rapamycin. Time between frames, 2 s.



Video 8. **Decreasing PM tension restores Rvs167 recruitment.** PalmC treatment applied to a *TOR1-1 AVO3Δ<sup>CT</sup>* cell expressing Abp1-mCherry and Rvs167-GFP after 1-h TORC2 inhibition with Rapamycin. Time between frames, 2 s.

Provided online is one table in Excel. Table S1 provides source data for Fig. 1, E–I; Fig. 2, A, C, E, and H; Fig. 3, A–D; Fig. 5, A–D; Fig. S2, C and F; and Fig. S3.