SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1.

Western blot analysis showing protein levels of Rvs161 and Rvs167 harboring modifications in their BAR domain. (A) Strains carrying substitutions or truncations in the BAR domain of Rvs161 were used to assess the protein levels of Rvs161 and its partner, Rvs167. α-Swi6 was used as a loading control. (* Nonspecific band co-migrating with Rvs161. This cross-reacting band is clearly separated in *rvs161-ΔN* lane.) (B) Mutants carrying substitutions or truncations in the BAR domain of *RVS167* are compared to the wild-type to look for changes in their protein levels. (C) Double mutants harboring symmetrical changes in both *RVS161* and *RVS167* BAR domain were analyzed in the same manner.

Supplementary Figure 2.

Limited plate-mating analysis of *rvs161* BAR domain mutants. Haploid strains were mixed and allowed to mate on a YPD plate for 5hrs at 30ºC, which was then replica-plated onto a diploid selection plate, YPD+G418+NAT. The top panel shows an YPD mating plate and the bottom panel shows the diploid selection plate, both 24 h post-mating.

Supplementary Figure 3.

Serial spot dilution assay showing comparitive fitness of *rvs161* single BAR domain mutants and *rvs161 rvs167* double mutants on YPD and YPD+0.5M NaCl. Additional mutation in *RVS167* BAR domain enhances growth sensitivity to salt in all strains, except *AP* and *ΔN*.

Supplementary Figure 4.

Actin polarization defects in the BAR domain mutants. Actin structures in log-phase cells were stained with rhodamine-phalloidin and small-budded cells were assessed for actin patch polarization defects. Cells were defined as polarized when more than 75% of the total actin patches were localized in the daughter cell (n>100).

Supplementary Figure 5.

Lifetime of the early endocytic vesicle coat marker, Sla1-GFP, at the membrane surface in each BAR domain mutant background.

Supplementary Figure 6.

(A) Western blot analysis showing protein levels of C-terminally GFP-tagged Rvs167 in BAR domain mutants. α- Hexokinase was used as a loading control. (B) Functional complementation assay for C-terminally GFP-tagged Rvs167 strains used in this study. Serial spot dilution assays show growth sensitivity of untagged BAR domain double mutants compared to C-terminally GFP-tagged double mutants on different salt conditions (YPD+0.5M NaCl, YPD+0.7M NaCl).

Supplementary Figure 7.

(A) Growth sensitivity of BAR domain mutants on medium containing Aur1 inhibitor, AureobasidinA (0.1μg/ml). Serially diluted strains were spotted onto YPD, YPD+ AureobasidinA (0.1μg/ml) plates, which were grown for 2 days (YPD) or 4 days (YPD+AureobasidinA) at 30ºC. (B) Rvs167-GFP protein levels in the presence or absence of AureobasidinA (0.2μg/ml). Wild-type and *rvs161-RC rvs167-RC-GFP* strains were incubated with AureobasidinA (0.2μg/ml) for indicated times and were collected to compare GFP-tagged Rvs167 protein levels using western blot analysis.

Supplementary Movie 1.

Rvs167-GFP was visualized every second in a time course of two minutes total.

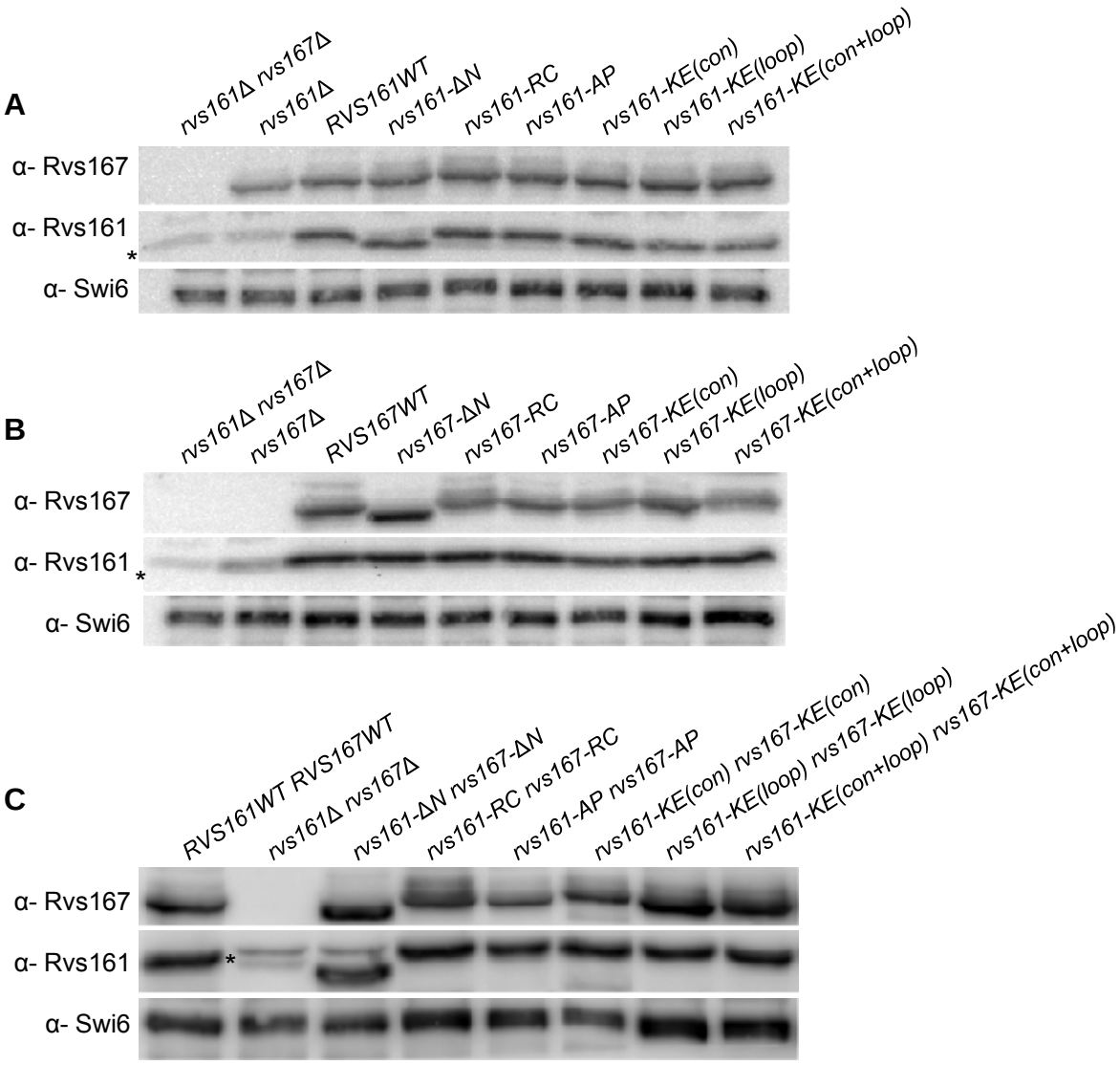
Supplementary Movie 2.

Rvs167-RC-GFP in *rvs161-RC* background was imaged every second in a time course of two minutes total.

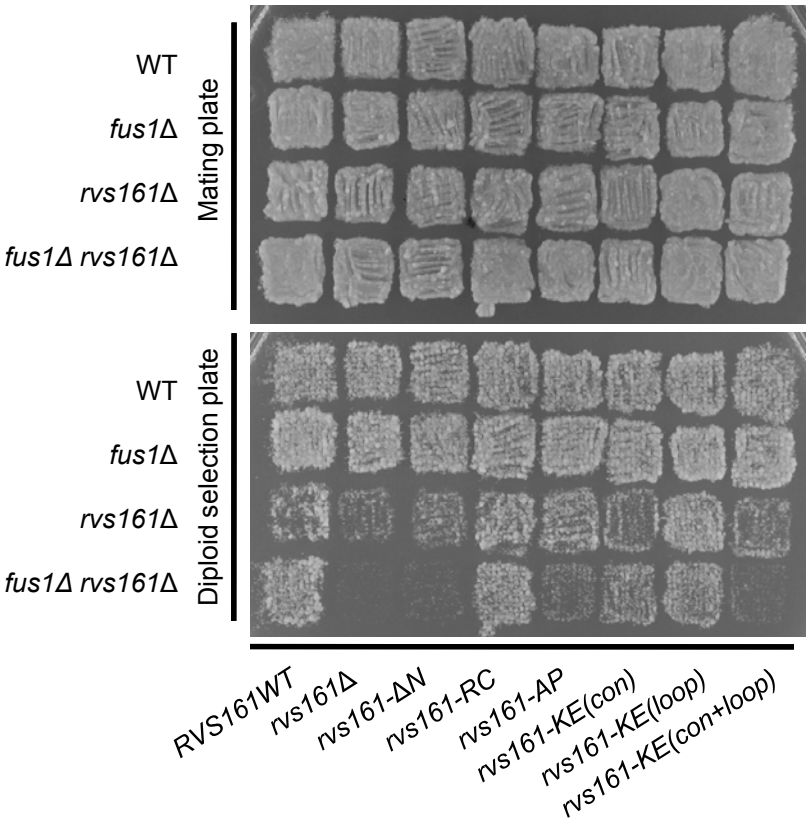
Supplementary Table 1.

Strains used in this study

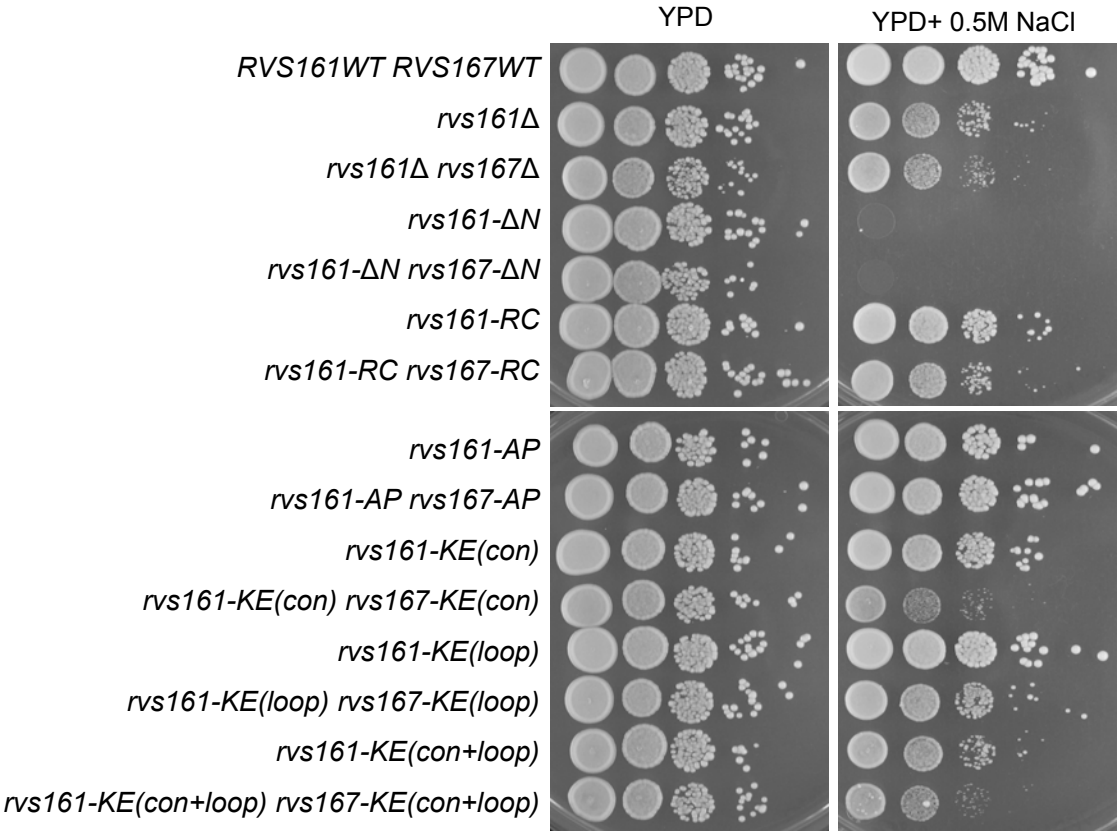
Supplementary Figure 1



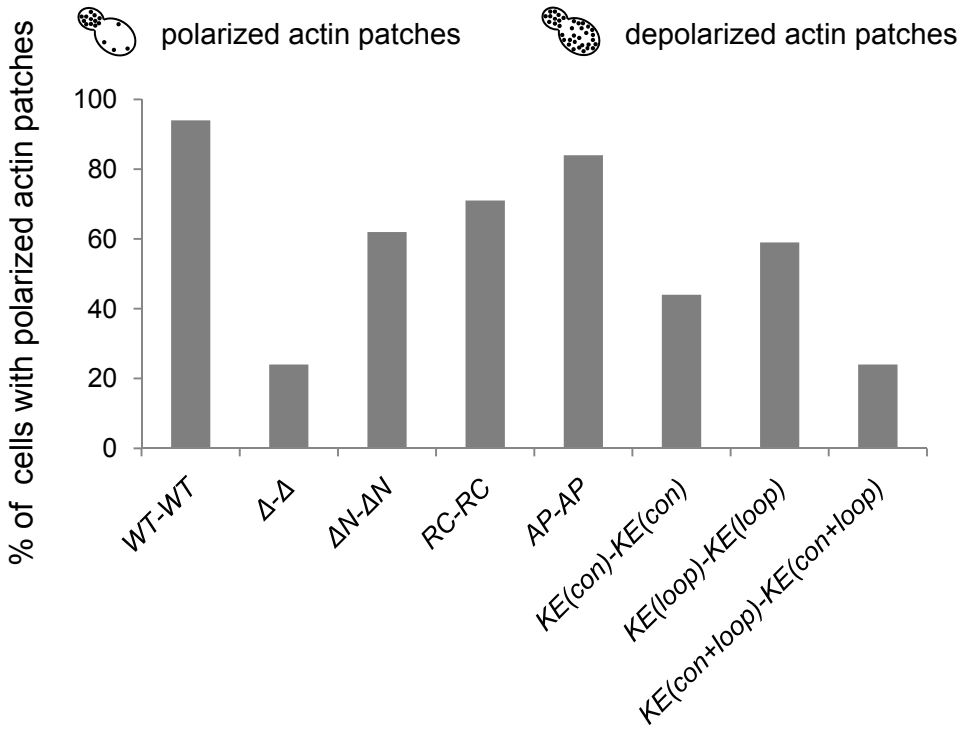
Supplementary Figure 2



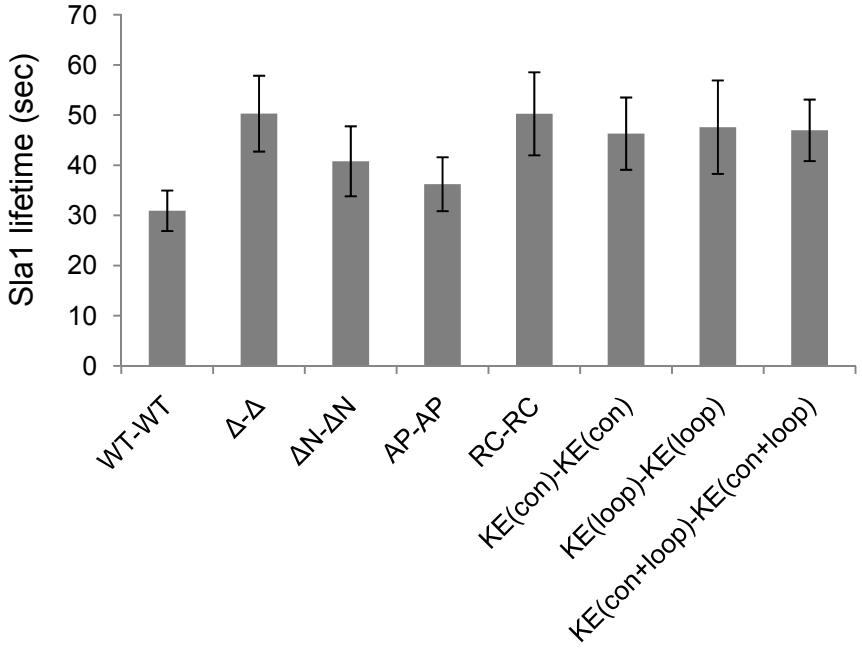
Supplementary Figure 3



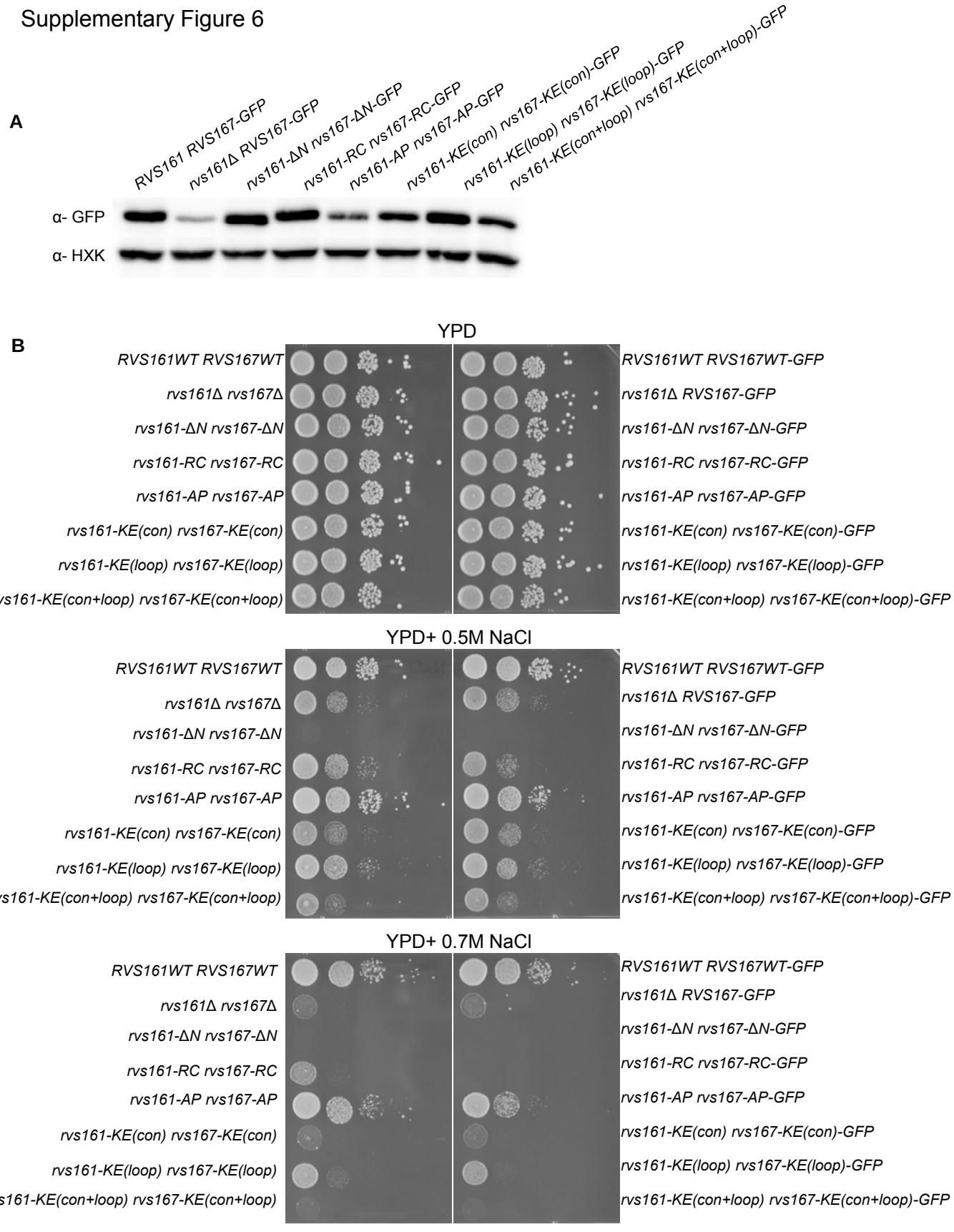
Supplementary Figure 4



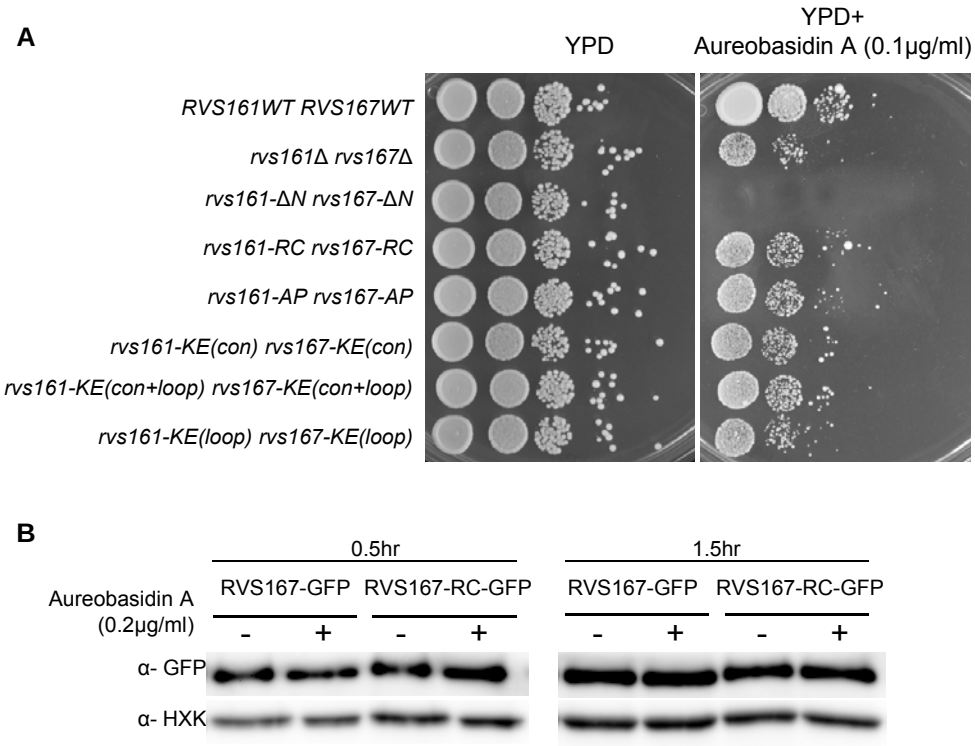
Supplementary Figure 5



Supplementary Figure 6



Supplementary Figure 7



Supplementary Table I. Yeast Strains Used in This Study

Yeast Strain	Strain Genotype	Source
rvs161 rvs167 strains used for BiFC analysis		
BY4646	BY4422 *MATα RVS167-VC::kan::Hph*	This study
BY4647	BY4741**a** *Nat::RVS161-VN::His3*	This study
BY4648	diploid of BY4646 x BY4647	This study
BY4674	BY4422 *MATα EDE1-VC::kan*	This study
BY4675	BY4741**a** *EDE1-VN::His3*	This study
BY4649	BY4741**a** *RVS167-VN::His3::Hph*	This study
BY4678	diploid of BY4674 x BY4649	This study
BY4677	diploid of BY4675 x BY4646	This study
BY4650	diploid of BY4646 x BY4649	This study
BY4651	BY4650 *ABP1-mCherry::URA3*	This study
BY4652	BY4049 *MATα rvs161Δ::URA3 RVS167-VC::kan::Hph*	This study
BY4653	BY4741a *rvs161Δ::URA3 RVS167-VN::His3*	This study
BY4654	diploid of BY4652 x BY4653	This study
BY4655	BY4654 with pRS315-RVS161	This study
Strains used in limited-mating assays		
BY4741a	*MAT***a** *his3Δ1 leu2Δ0 ura3Δ0 met15Δ0*	Brachmann *et al.* (1998)
	BY4741**a** *fus1Δ::kan*	Deletion consortium
BY4406	BY4741**a** *rvs161Δ::URA3*[1,2,3]	This study
BY4405	BY4741**a** *rvs161Δ::URA3 fus1Δ::kan*[1,2,3]	This study
rvs161 alone		
BY4360	*MATα his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0 can1Δ0 mfa1Δ::MFA1pr-HIS3 Nat::RVS161+*	This study
BY4049	BY4360 *Nat::rvs161Δ::URA3*	This study
BY4366	BY4360 *Nat::rvs161-ΔN*	This study
BY4061	BY4360 *Nat::rvs161-R35C*	This study
BY4057	BY4360 *Nat::rvs161-A175P*	This study
BY4362	BY4360 *Nat::rvs161-K136E, K140E*	This study
BY4613	BY4360 *Nat::rvs161-K157E, K160E*	This study
BY4364	BY4360 *Nat::rvs161-K136E, K140E, K157E, K160E*	This study
rvs167 alone		
BY4051	*MAT***a** *RVS167*$^{+}$*::Hph his3Δ1 leu2Δ0 ura3Δ0 met15Δ0*	This study
BY4063	BY4051 *rvs167Δ::URA3 (1-890nt)::Hph*	This study
BY4369	BY4051 *rvs167-ΔN::Hph*	This study
BY4371	BY4051 *rvs167-R37C::Hph*	This study
BY4373	BY4051 *rvs167-A190P::Hph*	This study
BY4375	BY4051 *rvs167-K148E, K152E::Hph*	This study
BY4377	BY4051 *rvs167-K170E, K175E ::Hph*	This study

BY4379	BY4051 *rvs167-K148E K152E, K170E, K175E ::Hph*	This study

rvs161 rvs167 strains assayed for salt sensitivitity and Lucifer Yellow uptake[2]		
BY4422	*MATα Nat::RVS161+ RVS167+::Hph his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0 can1Δ0 mfa1Δ::MFA1pr-HIS3*	This study
BY4395	BY4422 *Nat::rvs161Δ::URA3 rvs167Δ::URA3::Hph*	This study
BY4434	BY4422 *Nat::rvs161-ΔN rvs167-ΔN::Hph*	This study
BY4399	BY4422 *Nat::rvs161-R35C rvs167-R37C::Hph*	This study
BY4397	BY4422 *Nat::rvs161-A175P rvs167-A190P::Hph*	This study
BY4400	BY4422 *Nat::rvs161-K136E, K140E rvs167-K148E, K152E::Hph*	This study
BY4622	BY4422 *Nat::rvs161-K157E, K160E rvs167-K170E, K175E ::Hph*	This study
BY4412	BY4422 *Nat::rvs161-K136E, K140E, K157E, K160E rvs167-K148E, K152E, K170E, K175E ::Hph*	This study

rvs161 rvs167 assayed for Sla1-GFP retraction		
BY4527	*MATα his3-Δ200 ura3-52 leu2-3,112 SLA1-GFP::kan ABP1-RFP::His3*	DCT214 (Drubin Laboratory)
BY4572	BY4527 *MAT**a** Nat::RVS161+ RVS167+::Hph*	This study
BY4571	BY4527 *MATα Nat::rvs161Δ::URA3 rvs167Δ::URA3::Hph*	This study
BY4573	BY4527 *MATα Nat::rvs161-ΔN rvs167-ΔN::Hph*	This study
BY4577	BY4527 *MATα Nat::rvs161-R35C rvs167-R37C::Hph*	This study
BY4574	BY4527 MAT**a** *Nat::rvs161-A175P rvs167-A190P::Hph*	This study
BY4579	BY4527 MAT**a** *Nat::rvs161-K136E, K140E rvs167-K148E, K152E::Hph*	This study
BY4578	BY4527 MAT**a** *Nat::rvs161-K157E, K160E rvs167-K170E, K175E ::Hph*	This study
BY4575	BY4527 MAT**a** *Nat::rvs161-K136E, K140E, K157E, K160E rvs167-K148E, K152E, K170E, K175E ::Hph*	This study

rvs161 rvs167 strains assayed for Rvs167 localization		
BY4580	BY4422 *Nat::RVS161+ RVS167+-GFP::kan::Hph*	This study
BY4599	BY4422 *Nat::rvs161Δ::URA3 RVS167+-GFP::kan*	This study
BY4581	BY4434 *Nat::rvs161-ΔN rvs167-ΔN-GFP::kan::Hph*	This study
BY4585	BY4399 *Nat::rvs161-R35C rvs167-R37C-GFP::kan::Hph*	This study
BY4582	BY4397 *Nat::rvs161-A175P rvs167-A190P-GFP::kan::Hph*	This study
BY4588	BY4400 *Nat::rvs161-K136E, K140E rvs167-K148E, K152E-GFP::kan::Hph*	This study
BY4627	BY4622 *Nat::rvs161-K157E, K160E rvs167-K170E, K175E-GFP::kan::Hph*	This study
BY4587	BY4412 *Nat::rvs161-K136E, K140E, K157E, K160E rvs167-K148E, K152E, K170E, K175E-GFP::kan::Hph*	This study
BY4600	BY4580 *Nat::RVS161+ RVS167+-GFP::kan::Hph ABP1-mCherry::URA3*	This study
BY4605	BY4585 *Nat::rvs161-R35C rvs167-R37C-GFP::kan::Hph ABP1-mCherry::URA3*	This study
BY4603	BY4582 *Nat::rvs161-A175P rvs167-A190P-GFP::kan::Hph ABP1-mCherry::URA3*	This study

rvs161 rvs167 strains used for genetic interaction with *INP52*		
BY4702	*MAT***a** *Nat::RVS161+ RVS167+::Hph hoΔ::kan his3Δ1 leu2Δ0 ura3Δ0 lyp1ΔSTE3pr-LEU2 can1Δ::STE2pr-his5*	This study
BY4703	BY4702 *rvs161Δ::Nat rvs167Δ::Hph hoΔ::kan*	This study
BY4704	BY4702 *Nat::rvs161-ΔN rvs167-ΔN::Hph hoΔ::kan*	This study
BY4705	BY4702 *Nat::rvs161-R35C rvs167-R37C::Hph hoΔ::kan*	This study
BY4706	BY4702 *Nat::rvs161-A175P rvs167-A190P::Hph hoΔ::kan*	This study
BY4707	BY4702 *Nat::rvs161-K137E, K141E rvs167-K148E, K152E::Hph hoΔ::kan*	This study
BY4708	BY4702 *Nat::rvs161-K157E, K160E rvs167-K170E, K175E ::Hph hoΔ::kan*	This study
BY4709	BY4702 *Nat::rvs161-K137E, K141E, K157E, K160E rvs167-K148E, K152E, K170E, K175E ::Hph hoΔ::kan*	This study
BY4710	*MAT***a** *Nat::RVS161+ RVS167+::Hph inp52Δ::kan his3Δ1 leu2Δ0 ura3Δ0 lyp1ΔSTE3pr-LEU2 can1Δ::STE2pr-his5*	This study
BY4711	BY4702 *rvs161Δ::Nat rvs167Δ::Hph inp52Δ::kan*	This study
BY4712	BY4702 *Nat::rvs161-ΔN rvs167-ΔN::Hph inp52Δ::kan*	This study
BY4713	BY4702 *Nat::rvs161-R35C rvs167-R37C::Hph inp52Δ::kan*	This study
BY4714	BY4702 *Nat::rvs161-A175P rvs167-A190P::Hph inp52Δ::kan*	This study
BY4715	BY4702 *Nat::rvs161-K137E, K141E rvs167-K148E, K152E::Hph inp52Δ::kan*	This study
BY4716	BY4702 *Nat::rvs161-K157E, K160E rvs167-K170E, K175E ::Hph inp52Δ::kan*	This study
BY4717	BY4702 *Nat::rvs161-K137E, K141E, K157E, K160E rvs167-K148E, K152E, K170E, K175E ::Hph inp52Δ::kan*	This study

[1] This strain may be can1Δ0 and/ or *mfa1Δ::MFA1pr-HIS3*

[2] Some of the strains in this group are *met15Δ0* and/or *lys2Δ0*

[3] Some of these strains may be *met15Δ0*