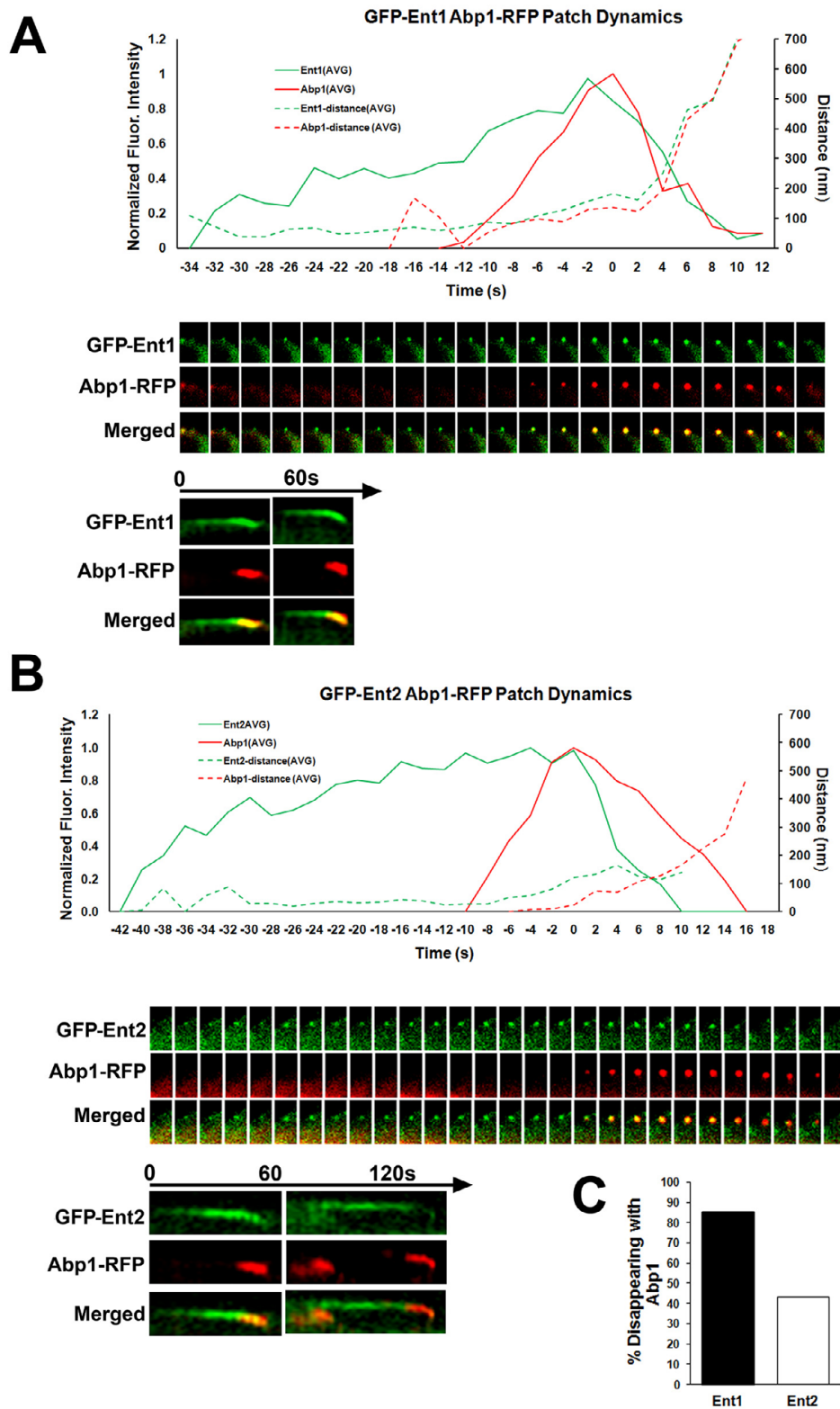
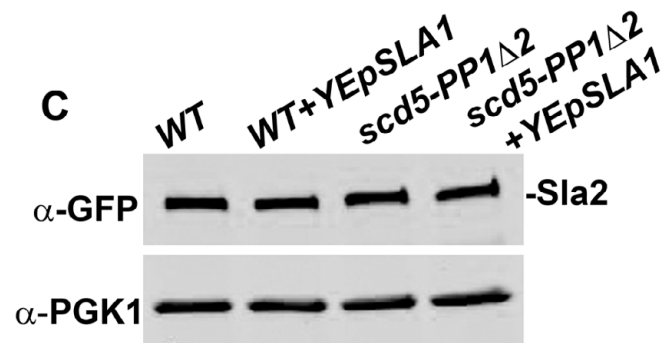
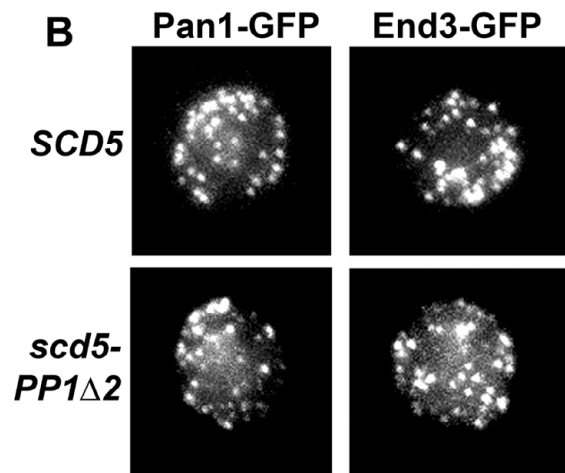
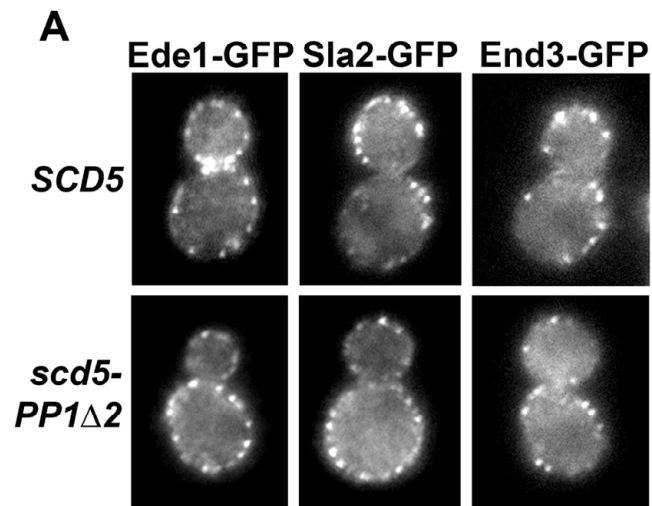


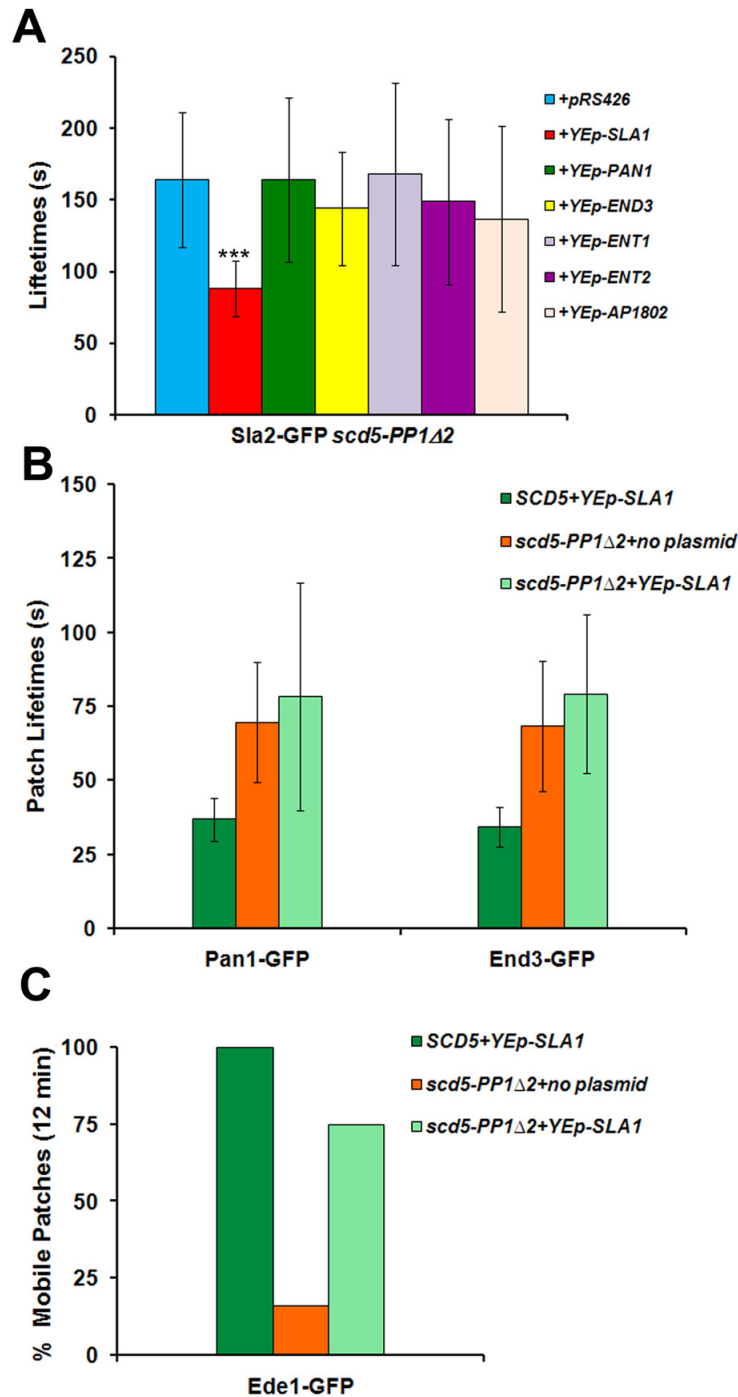
**Fig S1. Yap1801 and Yap1802 endocytic patch dynamics in wild-type cells.** (A) GFP-YAP1801 (SL5482) and (B) GFP-YAP1802 (SL6365), paired with Abp1-RFP, were generated for endocytic patch analysis in wild-type cells. A, B top panels: Graphs show normalized fluorescence intensity profiles averaged from three patches. Profiles from individual patches were aligned relative to their peak Abp1-RFP intensity (Time 0) and the average intensity at each time point was then plotted (solid lines). The inward movement of GFP or RFP (distance in nm) is also the average of three patches plotted versus time (dotted lines). A, B middle panels are tile views of patches from movies of GFP-Yap1801 or GFP-Yap1802 paired with Abp1-RFP. A, B bottom panels show kymographs of patches from movies of GFP-Yap1801 or GFP-Yap1802 paired with Abp1-RFP. Cortical patches shown were obtained from 4 min two-color movies (1 frame/2s).



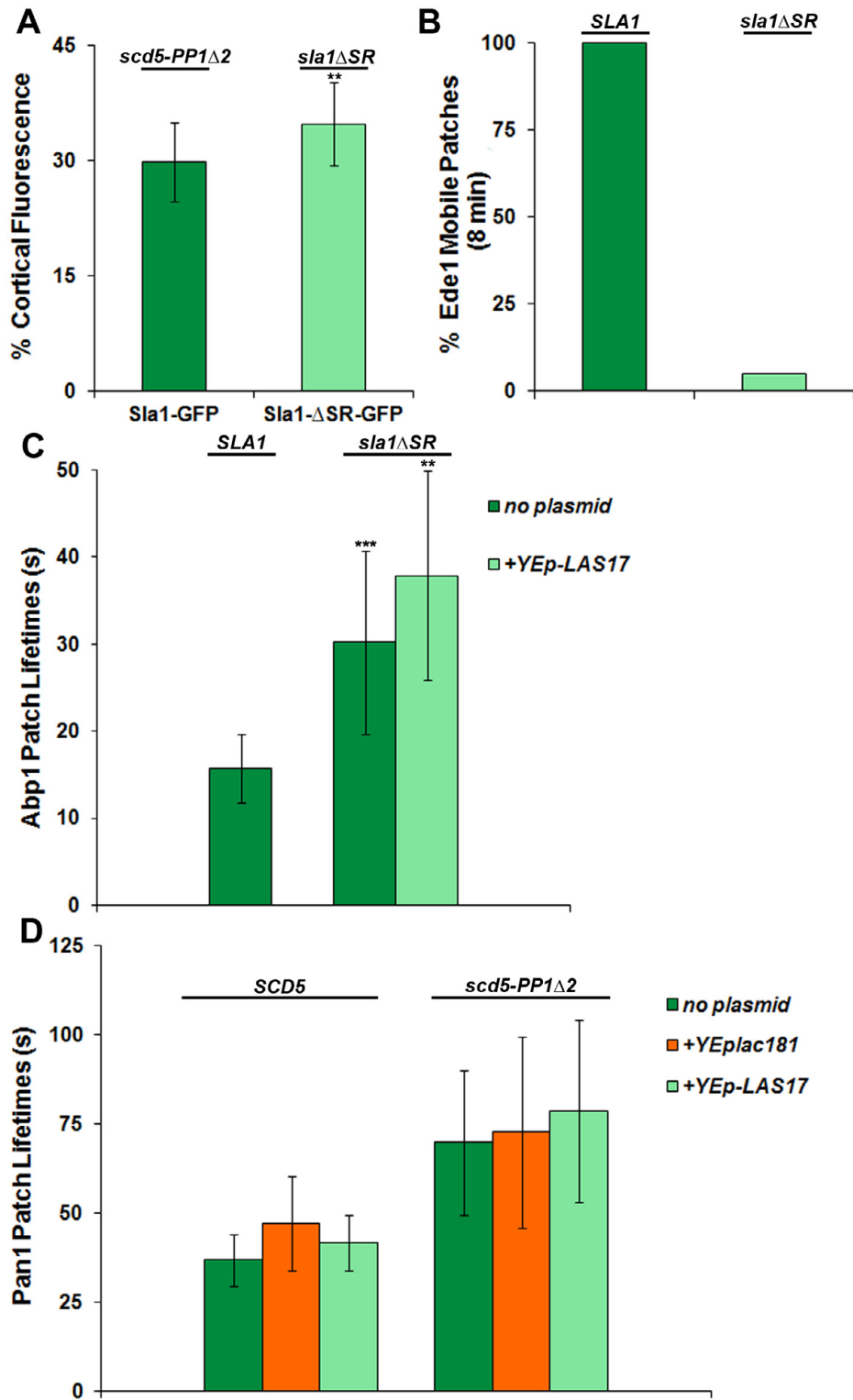
**Fig S2. Ent1 and Ent2 endocytic patch dynamics in wild-type cells.** Movies of (A) GFP-Ent1 (SL5480) and (B) GFP-Ent2 (SL5481), paired with Abp1-RFP, were generated for patch analysis in wild-type cells. (A, B) Graphs, patch tile views and kymographs were generated as described in Supplemental Figure 1. Cortical patches were obtained from 4 min two-color movies (1 frame/2s). (C) Quantification of percent of GFP-Ent1 or GFP-Ent2 patches disappearing with Abp1-RFP (n=30 patches for each).



**Fig S3. Cortical localization and patch densities of coat factors, and Sla2-GFP expression levels.** (A) Epifluorescence images at the medial focal plane of wild-type and *scd5-PP1Δ2* cells, respectively, expressing Ede1-GFP (SL6032, SL6029), Sla2-GFP (SL6026, SL6023), or End3-GFP (SL6039, SL6041). (B) Representative projection images of deconvolved Z stacks from unbudded wild-type and *scd5-PP1Δ2* cells, respectively, expressing Pan1-GFP (SL5425, SL5429) and End3-GFP (SL6039, SL6041). (C) Sla2-GFP protein levels were determined by immunoblot analysis in wild-type (SL6026) and *scd5-PP1Δ2* (SL6023) cells, with and without the *SLA1* overexpression plasmid, pJSC66 (YEp-*SLA1*).



**Fig S4. Suppression of patch dynamics in *scd5-PP1Δ2* by *SLA1* overexpression.** (A). Only *SLA1* overexpression suppresses Sla2-GFP dynamics in the *scd5* mutant: *SLA2-GFP ABP1-RFP scd5-PP1Δ2* strains (SL6023) were transformed with 2 $\mu$  overexpression plasmids: pRS426, pJSC66 (YEp-*SLA1*), pJSC65 (YEp-*PAN1*), pSR1 (YEp-*END3*), pENT1 (YEp-*ENT1*), pENT2 (YEp-*ENT2*), pYAP1802 (YEp-*YAP1802*) and lifetimes of Sla2-GFP were analyzed. n=15-30 patches for each transformant; (\*\*\*) denotes p<0.0001 compared to empty vector in a student's t-test. (B) Overexpression of *SLA1* (YEp-*SLA1*) does not suppress the delay of Pan1-GFP (SL5429) or End3-GFP (SL6041) caused by the *scd5-PP1Δ2* mutation. Also *SLA1* overexpression had no effect on *SCD5* cells expressing Pan1-GFP (SL5425) or End3-GFP (SL6039). (C) Mobility of Ede1-GFP (SL6029) patches was partially restored in the *scd5* mutant cells when overexpressing *SLA1* (YEp-*SLA1*). "Percent Mobile Patches" indicates % Ede1 patches (n=100) found at the start of the movie that complete internalization in the first 12 min of the movie. *SLA1* overexpression had no effect on Ede1-GFP (SL6029) mobility in wild-type cells.



**Fig S5. Sla1 SR domain is important for cortical patch localization/dynamics and overexpression of *LAS17* has no effect on Pan1-GFP in *scd5* mutant cells.** (A) % Cortical fluorescence of Sla1-GFP in the *scd5* mutant (SL5411) or Sla1ΔSR-GFP as the sole source of Sla1 (SL6310). n=20 patches for each strain. (\*\*) denotes p=0.005 via student's t-test. (B) Ede1-GFP dynamics are defective in the *sla1ΔSR* mutant (SL6898 & SL6899 combined data) as compared to wild-type cells (SL6897). "% Mobile Patches" indicates % Ede1 patches (n=100) found at the start of the movie that completed internalization in the first 8 min of the movie. (C) Abp1-RFP lifetime is delayed in *sla1ΔSR* (SL6616) as compared to wild-type cells (multiple strains) and overexpression of *LAS17* (YEp-LAS17) further extends Abp1 patch lifetime. n≥27 patches for each strain. P values from student's t test: (\*\*\*) p<0.0001 comparing wild-type to *sla1ΔSR*; (\*\*) p<0.005 comparing *sla1ΔSR* cells with and without *LAS17* overexpression. (D) Overexpression of *LAS17* (YEp-LAS17) had no affect on Pan1-GFP lifetimes in wild-type cells (SL5425) or in *scd5-PP1Δ2* cells (SL5429). n≥27 patches for each strain. Error bars in A,C,D are standard deviations.

**Table S1. *Saccharomyces cerevisiae* strains**

Strain	Genotype	Source <sup>a</sup>
SL1462	<i>MATa leu2 ura3-52 trp1 his3-Δ200</i>	
SL1463	<i>MATα leu2 ura3-52 trp1 his3-Δ200</i>	
SL4128	<i>MATa/α leu2 ura3-52/ura3-53 trp1/trp1 his3-Δ200/his3-Δ200 scd5-Δ::TRP1/SCD5</i>	
SL4609	<i>MATa leu2 trp1 ura3-52 his3-Δ200 GAL2 scd5-PP1Δ2</i>	
SL4610	<i>MATα leu2 trp1 ura3-52 his3-Δ200 GAL2 scd5-PP1Δ2</i>	
SL4702	<i>MATa leu2 ura3-52 trp1 his3-Δ200 scd5-Δ::TRP1 pJSC31[CEN, LEU2, GFP-SCD5]</i>	
SL4706	<i>MATa leu2 ura3-52 trp1 his3-Δ200 scd5-Δ::TRP1 pCC545[CEN, LEU2, SCD5]</i>	
SL4823	<i>MATa leu2 ura3-52 trp1 his3-Δ200 scd5-Δ::TRP1 pJSC9[CEN, LEU2, scd5-PP1Δ2]</i>	
SL4851	<i>MATa leu2 ura3-52 trp1 his3-Δ200 ent2-Δ::HIS3 scd5-Δ::TRP1 pBW56[CEN, URA3, GFP-ENT2] pCC545[CEN, LEU2, SCD5]</i>	
SL4852	<i>MATa leu2 ura3-52 trp1 his3- Δ200 ent2-Δ::HIS3 scd5-Δ::TRP1 pBW56[CEN, URA3, GFP-ENT2] pJSC9[CEN, LEU2, scd5-PP1Δ2]</i>	
SL5265	<i>MATα leu2 trp1 ura3 his3-Δ200 BBC1-GFP::TRP1 ABP1-RFP::KanMX6 scd5-PP1Δ2</i>	
SL5302	<i>MATα leu2 trp1 ura3 his3-Δ200 scd5-Δ::TRP1 ABP1-RFP::KanMX6 pJSC31 [CEN, LEU2, GFP-SCD5]</i>	
SL5303	<i>MATa leu2 trp1 ura3 his3-Δ200 scd5-Δ::TRP1 ABP1-RFP::KanMX6 pJSC32 [CEN, LEU2, GFP-scd5- PP1Δ2]</i>	
SL5326	<i>MATa leu2 trp1 ura3-52 his3-Δ200 ABP1-RFP::KanMX6 scd5-PP1Δ2</i>	
SL5328	<i>MATa leu2 trp1 ura3 his3 lys-2-801 LAS17-GFP::HIS3 scd5-PP1Δ2</i>	
SL5354	<i>MATα leu2 trp1 ura3 his3-Δ200 clc1Δ::HIS3 pTMN37 [CEN, URA3, GFP-CLC1]</i>	
SL5356	<i>MATα leu2 trp1 ura3 his3-Δ200 clc1Δ::HIS3 scd5- PP1Δ2 pTMN37 [CEN, URA3, GFP-CLC1]</i>	
SL5411	<i>MATα leu2 trp1 ura3-52 his3-Δ200 SLA1-GFP::HIS3 ABP1-RFP::KanMX6 scd5-PP1Δ2</i>	
SL5412	<i>MATa leu2 trp1 ura3-52 his3-Δ200 SLA1-GFP::HIS3 ABP1-RFP::KanMX6</i>	
SL5425	<i>MATα leu2 trp1 ura3-52 his3-Δ200 PAN1-GFP::HIS3 ABP1-RFP::KanMX6</i>	
SL5429	<i>MATα leu2 trp1 ura3-52 his3-Δ200 PAN1-GFP::HIS3 ABP1-RFP::KanMX6 scd5-PP1Δ2</i>	
SL5436	<i>MATα leu2 ura3-52 trp1 his3- Δ200 ent2-Δ::HIS3 ABP1-RFP::KanMX6 pBW56[CEN, URA3, GFP-ENT2]</i>	
SL5440	<i>MATa leu2 ura3-52 trp1 his3-Δ200 ent1-Δ::LEU2 ABP1-RFP::KanMX6 scd5-PP1Δ2 pBW54[CEN, URA3, GFP-ENT1]</i>	
SL5441	<i>MATa leu2 ura3-52 trp1 his3-Δ200 yap1801-Δ::HIS3 ABP1-RFP::KanMX6 scd5-PP1Δ2 pGFP-YAP1801[CEN, URA3, GFP-YAP1801]</i>	
SL5480	<i>MATa leu2 ura3-52 trp1 his3-Δ200 ent1-Δ::LEU2 ABP1-RFP::KanMX6 pBW54[CEN, URA3, GFP-ENT1]</i>	
SL5481	<i>MATa leu2 ura3-52 trp1 his3- Δ200 ent2-Δ::HIS3 ABP1-RFP::KanMX6 scd5-PP1Δ2 pBW56[CEN, URA3, GFP-ENT2]</i>	
SL5482	<i>MATa leu2 ura3-52 trp1 his3-Δ200 yap1801-Δ::HIS3 ABP1-RFP::KanMX6 pGFP-YAP1801[CEN, URA3, GFP-YAP1801]</i>	
SL5580	<i>MATa ade2 ade3::CMD1 lys his3 leu2 trp1 ura3 met15 cmd1Δ::HIS3 bar::LYS2 pMYO5-RFP[URA], pVRP1-GFP [LEU]</i>	
SL5755	<i>MATa leu2 trp1 ura3-52 his3-Δ200 lys2-801 EDE1-GFP::HisMX6 ABP1-RFP::KanMX6</i>	
SL5839	<i>MATα leu2 trp1 ura3-52 his3-Δ200 GAL2 SLA2-GFP::HisMX6 SLA1-RFP::KanMX</i>	
SL5840	<i>MATα leu2 trp1 ura3-52 his3-Δ200 GAL2 SLA2-GFP::HisMX6 SLA1-RFP::KanMX6</i>	
SL5887	<i>MATa leu2 trp1 ura3-52 his3-Δ200 GAL2 SLA2-GFP::HisMX6 SLA1-RFP::KanMX6 scd5-PP1Δ2</i>	
SL5928	<i>MATa leu2 trp1 ura3-52 his3-Δ200 GAL2 SLA2-GFP::TRP1 ABP1-RFP::KanMX6</i>	

SL6023	<i>MAT<math>\alpha</math> leu2 trp1 ura3-52 his3-<math>\Delta</math>200 SLA2-GFP::TRP1 ABP1-RFP::KanMX6 scd5-PP1<math>\Delta</math>2</i>	
SL6024	<i>MAT<math>\alpha</math> leu2 trp1 ura3-52 his3-<math>\Delta</math>200 SLA2-GFP::TRP1 ABP1-RFP::KanMX6 scd5-PP1<math>\Delta</math>2</i>	
SL6026	<i>MAT<math>\alpha</math> leu2 trp1 ura3-52 his3-<math>\Delta</math>200 SLA2-GFP::TRP1 ABP1-RFP::KanMX6</i>	
SL6029	<i>MAT<math>\alpha</math> leu2 trp1 ura3-52 his3-<math>\Delta</math>200 EDE1-GFP::HisMX6 ABP1-RFP::KanMX6 scd5-PP1<math>\Delta</math>2</i>	
SL6032	<i>MAT<math>\alpha</math> leu2 trp1 ura3-52 his3-<math>\Delta</math>200 EDE1-GFP:: HisMX6 ABP1-RFP::KanMX6</i>	
SL6039	<i>MAT<math>\alpha</math> leu2 trp1 ura3-52 his3-<math>\Delta</math>200 GAL2 END3-GFP::TRP1 ABP1-RFP::KanMX6</i>	
SL6041	<i>MAT<math>\alpha</math> leu2 trp1 ura3-52 his3-<math>\Delta</math>200 GAL2 END3-GFP::TRP1 scd5-PP1<math>\Delta</math>2</i>	
SL6141	<i>MAT<math>\alpha</math> leu2 ura3-52 his3-<math>\Delta</math>200 TRP1 PAN1-GFP::HIS3 SLA1-RFP::KanMX6</i>	
SL6142	<i>MAT<math>\alpha</math> leu2 ura3-52 his3-<math>\Delta</math>200 TRP1 PAN1-GFP::HIS3 SLA1-RFP::KanMX6 scd5-PP1<math>\Delta</math>2</i>	
SL6175	<i>MAT<math>\alpha</math> leu2 trp1 ura3-52 his3-<math>\Delta</math>200 SLA2-GFP::TRP1 ABP1-RFP::KanMX6 scd5-PP1<math>\Delta</math>2 pMYO5-RFP[CEN, URA3]</i>	
SL6179	<i>MAT<math>\alpha</math> leu2 trp1 ura3-52 his3-<math>\Delta</math>200 SLA2-GFP::TRP1 END3-CHERRY::HisMX6</i>	
SL6181	<i>MAT<math>\alpha</math> leu2 trp1 ura3-52 his3-<math>\Delta</math>200 SLA2-GFP::TRP1 END3-CHERRY::HisMX6 scd5-PP1<math>\Delta</math>2</i>	
SL6216	<i>MAT<math>\alpha</math> leu2 trp1 ura3-52 his3-<math>\Delta</math>200 sla1-<math>\Delta</math>SR::HisMX6</i>	
SL6310	<i>MAT<math>\alpha</math> leu2 trp1 ura3-52 his3-<math>\Delta</math>200 sla1-<math>\Delta</math>SR-GFP::HisMX6 ABP1-RFP::KanMX6</i>	
SL6365	<i>MAT<math>\alpha</math> leu2 ura3-52 trp1 his3-<math>\Delta</math>200 yap1802-<math>\Delta</math>::HIS3 ABP1-RFP::KanMX6 pGFP-YAP1802[CEN, URA3]</i>	
SL6366	<i>MAT<math>\alpha</math> leu2 ura3-52 trp1 his3-<math>\Delta</math>200 yap1802-<math>\Delta</math>::HIS3 ABP1-RFP::KanMX6 scd5-PP1<math>\Delta</math>2 pGFP-YAP1802[CEN, URA3]</i>	
SL6616	<i>MAT<math>\alpha</math> leu2 ura3-52 trp1 his3-<math>\Delta</math>200 Pan1-GFP::HIS3 ABP1-RFP::KanMX6 sla1-<math>\Delta</math>SR::NatMX6</i>	
SL6897	<i>MAT<math>\alpha</math> leu2 trp1 ura3-52 his3-<math>\Delta</math>200 EDE1-GFP::HisMX6 ABP1-RFP::KanMX6</i>	
SL6898	<i>MAT<math>\alpha</math> leu2 trp1 ura3-52 his3-<math>\Delta</math>200 EDE1-GFP::HisMX6 ABP1-RFP::KanMX6 sla1<math>\Delta</math>SR:HisMX6</i>	
SL6899	<i>MAT<math>\alpha</math> leu2 trp1 ura3-52 his3-<math>\Delta</math>200 EDE1-GFP::HisMX6 ABP1-RFP::KanMX6 sla1<math>\Delta</math>SR:HisMX6</i>	
DDY2736	<i>MAT<math>\alpha</math> leu2-3,112 ura3-52 his3-<math>\Delta</math>200 lys-2-801 LAS17-GFP::HIS3</i>	(Kaksonen et al., 2005)
SCMIG844	<i>MAT<math>\alpha</math> leu2 trp1 ura3 his3-<math>\Delta</math>200 BBC1-GFP::TRP1 ABP1-RFP::KanMX6</i>	Gift from Maribel Geli

<sup>a</sup>Strains are from this lab and generated for this study, except where indicated. The integrated *scd5* PP1 binding site allele (*scd5-PP1 $\Delta$ 2*) was generated as described previously for *scd5- $\Delta$ 338* (Henry et al., 2002) using pJSC24 (see below) linearized with BlnI and transformed into SL4128. C-terminal XFP tagged strains were generated for live cell microscopy by integrative homologous recombination using methods described in (Wach et al., 1997; Longtine et al., 1998). The *sla1 $\Delta$ SR* and *sla1 $\Delta$ SR-GFP* were made by inserting *HIS3MX6* or *GFP:HIS3MX6* into the *SLA1* locus truncating *SLA1* after codon 850.

**Table S2. Plasmids**

<b>Construct</b>	<b>Description</b>	<b>Source</b>
pCC545	<i>Plasmid contains SCD5 in pRS315 (CEN, LEU2)</i>	Gift from Clarence Chan
pTMN37	<i>Plasmid contains GFP-CLC1 [CEN, URA3]</i>	(Newpher et al., 2005)
pBW54	<i>Plasmid contains GFP-Ent1 [CEN, URA3]</i>	(Watson et al., 2001)
pBW56	<i>Plasmid contains GFP-ENT2 [CEN, URA3]</i>	(Watson et al., 2001)
pGFP-YAP1801	<i>Plasmid contains GFP-YAP1801 in pRS416 [CEN, URA3]</i>	Gift from Beverly Wendland
pGFP-YAP1802	<i>Plasmid contains YAP1802 in pRS416 [CEN, URA3]</i>	Gift from Beverly Wendland
pMyo5-RFP	<i>Plasmid contains MYO5-RFP [CEN4, URA3]</i>	(Grotsch et al., 2010)
pTmn59	<i>The DNA coding region for Sla1 (856-1244 a.a.) was generated by PCR and cloned in frame into pGEX4-T1 digested with BamH1 and Sal1</i>	
pGEX-4T1	<i>GST expression Vector</i>	GE Healthcare
pLR1-HA-426	<i>His3Mx6-HA selectable marker was introduced into the DNA coding region for PAN1 (after the LR1 region) in pJSC65 to generate pLR1-HA-426 [2<math>\mu</math>, URA3, HIS3MX, pan1-LR1-HA]</i>	
pRS426	<i>Plasmid contains [2<math>\mu</math>, URA3]</i>	(Sikorski and Hieter, 1989)
pYAp1802-426	<i>Plasmid contains YAP1802 in pRS426 [2<math>\mu</math>, URA3]</i>	Gift from Beverly Wendland
pSR1 (END3)	<i>Plasmid contains END3 in pRS426 [2<math>\mu</math>, URA3]</i>	Gift from Howard Riezman
pENT1	<i>Plasmid contains ENT1 in pRS426 [2<math>\mu</math>, URA3]</i>	Gift from Beverly Wendland
pENT2	<i>Plasmid contains ENT2 in pRS426 [2<math>\mu</math>, URA3]</i>	Gift from Beverly Wendland
pGFP-YAP1801-416	<i>Plasmid contains YAP1801 in pRS416 [CEN, URA3]</i>	Gift from Beverly Wendland
pRJC2	<i>His3Mx6 selectable marker was introduced into the DNA coding region for Sla1 (850 a.a.) in pJSC66 [2<math>\mu</math>, URA3, SLA1] to generate pRJC2 [2<math>\mu</math>, URA3, HIS3MX6, SLA1<math>\Delta</math>SR]</i>	
pRJC6	<i>A Agel cut pJSC66 was filled in by klenow fragment and religated to generate pRJC6 [2<math>\mu</math>, URA3, SLA1-SR]</i>	
pJSC2	<i>BamH1-Sal1 fragment was moved from pKRH20 (pGBD-scd5-<math>\Delta</math>645; (Henry et al., 2002) into (GST) expression vector, pTB338 [CEN, LEU2, GAL1-GST from Michael Hall], to generate an in frame fusion between GST and SCD5 codons 1-227 under control of the GAL1 promoter (pJSC1). Then the remainder of SCD5 was reconstituted by inserting Xba1-Sph1 fragment (contains codons 188 through the end of the ORF) into pJSC1 to generate pJSC2 [CEN, LEU2 pGAL<sub>1,10</sub>-GST-SCD5]</i>	
pJSC9	<i>Plasmid contains scd5-PP1<math>\Delta</math>2 in pRS315 [CEN, LEU]</i>	(Chang et al., 2002)
pJSC24	<i>A BamH1-ClaI fragment of JSC9 was subcloned into BamH1+ClaI cut pAC10 (Henry et al. 2002) to generate pJSC24 [URA3, YIP5-scd5-PP1<math>\Delta</math>2]</i>	This Study
pJSC31	<i>Plasmid contains GFP-SCD5 in pRS315 (CEN, LEU2)</i>	(Chang et al., 2002)



pJSC32	<i>pJSC32 (GFP-scd5-PP1Δ2) was constructed by gap-repairing a SpeI-cut pJSC31 with 2.5-kb AflII-PstI fragments of pJSC9 (CEN, LEU2, scd5-PP1Δ2)</i>	
pJSC63	<i>A SpeI + BlnI cut pJSC2 was gap-repaired with 2.5-kb AflII-PstI fragments of pJSC9 to generate pJSC63 [CEN, LEU2 pGAL<sub>1,10</sub>-GST-scd5-PP1Δ2]</i>	
pJSC65	<i>A PvuI fragment of pRS425-PAN1 was gap repaired into SacI cut pRS426 to generate pJSC65 [2μ, URA3, PAN1]</i>	
pJSC66	<i>A PvuI fragment of pRS313-SLA1 was gap repaired into SacI cut pRS426 to generate pJSC66 [2μ, URA3, SLA1]</i>	
YEplac181	<i>Plasmid contains [2μ, LEU2]</i>	(Gietz and Sugino, 1988)
pAM155	<i>Plasmid contains LAS17 in YEplac181 [2μ, LEU2]</i>	(Naqvi et al., 1998)
<p><sup>a</sup>Plasmids are from this lab and generated for this study, except where indicated. pJSC24 (YIp5-<i>scd5-PP1Δ2</i>) was generated by subcloning a BamH1-ClaI fragment from pJSC9 (described in (Chang et al., 2002)) into YIp5. For construction of pJSC63 (<i>CEN, LEU2, pGAL1:GST-scd5-PP1Δ2</i>), SpeI-BlnI cut pJSC2 (Chang et al., 2002) was gap-repaired with a 2.5-kb AflII-PstI <i>scd5-PP1Δ2</i> fragment from pJSC9. pJSC32 (<i>CEN, LEU2, GFP-scd5-PP1Δ2</i>) was constructed by gap-repairing a SpeI-cut pJSC31 (Chang et al., 2006) with a 2.5-kb AflII-PstI <i>scd5-PP1Δ2</i> fragment from pJSC9. pTMN69 (<i>GST-sla1-SR</i>) for bacteria expression of the SR domain was made by PCR amplifying <i>SLA1</i> codons 856-1244 and subcloning into BamH1-SalI digested pGEX4-T1. pJSC66 (<i>2u, URA3, SLA1</i>) was generated by gap repair of a PvuI fragment from pRS313-<i>SLA1</i> (from D. Drubin) into SacI cut pRS426. To generate pRJC2 (<i>2u, URA3, HIS3MX6, sla1ΔSR</i>) a <i>HIS3MX6</i> cassette with flanking <i>SLA1</i> sequence was integrated into pJSC66 truncating <i>SLA1</i> after codon 850. To generate pRJC6 (<i>2u, URA3, sla1-SR</i>), pJSC66 was cut with AgeI, sticky-ends were filled in with Klenow fragment and the plasmid was religated, deleting <i>SLA1</i> codons 56 to 856. A PvuI fragment of pRS425-<i>PAN1</i> (B. Wendland) was gap repaired into SacI cut pRS426 to generate pJSC65 (<i>2u, URA3, PAN1</i>). In order to generate pLR1-HA-426 (<i>2u, URA3, pan1-LR1-HA:HIS3MX6</i>) a HA<sub>3</sub>-<i>HIS3MX6</i> cassette with flanking <i>PAN1</i> sequence was integrated into pJSC65, truncating <i>PAN1</i> after codon 383 with an in frame HA<sub>3</sub> tag.</p>		

**Table S3. Endocytic factor dynamics in wild-type and *scd5* mutant**

<b>PROTEIN</b>		<b>SCD5</b>	<b><i>scd5-PP1Δ2</i></b>
<b>Ede1</b>	Lt	78.2	>240
	SD	29.6	N/A
	P value		N/A
	N value	46	N/A
<b>Sla2</b>	Lt	43.8	171.9
	SD	13.0	68.0
	P value		P<0.0001
	N value	50	46
<b>Ent2</b>	Lt	44.8	126.8
	SD	15.1	14.3
	P value		P<0.0001
	N value	26	30
<b>Ent1</b>	Lt	31.3	33.9
	SD	6.9	9.2
	P value		N.S.
	N value	30	28
<b>Yap1802</b>	Lt	37.8	100.9
	SD	10.6	32.7
	P value		P<0.0001
	N value	30	29
<b>Yap1801</b>	Lt	30.9	95.9
	SD	8.1	35.4
	P value		P<0.0001
	N value	30	28
<b>Sla1</b>	Lt	33.8	52.3
	SD	6.5	14.3
	P value		P<0.0001
	N value	31	31
<b>Pan1</b>	Lt	36.9	69.8
	SD	7.3	20.3
	P value		P<0.0001
	N value	30	31
<b>End3</b>	Lt	34.3	68.3
	SD	6.8	22.1
	P value		P<0.0001
	N value	47	30.0

<b>Scd5</b>	Lt	27.7	40.9
	SD	5.2	10.9
	P value		P<0.0001
	N value	30	30
<b>Las17</b>	Lt	34.1	48.2
	SD	10.3	12.1
	P value		P<0.0001
	N value	30	30
<b>Bbc1</b>	Lt	13.5	13.5
	SD	2.9	2.2
	P value		N.S
	N value	30	34
<b>Abp1</b>	Lt	15.7	20.9
	SD	3.9	6.1
	P value		P<0.0001
	N value	181	118
<b>Myo5</b>	Lt	12.5	12.6
	SD	3.5	3.3
	P value		N.S
	N value	30	30

**Table S4.**  
**Endocytic factor patch to cytosol (P/C) ratios**

<b>PROTEIN</b>		<b>SCD5</b>	<b>scd5-PP1Δ2</b>
<b>Ede1</b>	P/C	4.9	3.8
	SD	1.2	1.7
	P value		N.S.
	N value	30	30
<b>Sla2</b>	P/C	3.5	2.9
	SD	1.0	1.1
	P value		N.S.
	N value	30	30
<b>Pan1</b>	P/C	3.5	2.8
	SD	1.5	1.2
	P value		N.S.
	N value	30	30
<b>End3</b>	P/C	3.9	2.7
	SD	1.5	1.1
	P value		P=0.0007

	N value	30	30
Sla1	P/C	4.6	1.6
	SD	0.9	0.3
	P value		P<0.0001
	N value	26	26

**Table S5. Endocytic factor patch density (PD)**

PROTEIN		SCD5	scd5-PP1D2
Sla2	PD	0.497	0.677
	SD	0.097	0.137
	P value		P< 0.0001
	N value	30	27
Pan1	PD	0.392	0.42
	SD	0.11	0.089
	P value	30	N.S.
	N value	30	30
End3	PD	0.399	0.323
	SD	0.121	0.094
	P value		N.S.
	N value	30	30
Sla1	PD	0.416	0.175
	SD	0.113	0.078
	P value		P< 0.0001
	N value	30	30

### Supplemental References

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