Supplementary Methods

Circular dichroism spectroscopy

Ultraviolet circular dichroism spectra from 250 to 190 nm were recorded with a JASCO J-810 instrument. The spectra were measured using a 0.1-cm path length quartz cell. The concentration of Mso1p was 10 μM in ½ PBS buffer. The data are presented as the averages of five scans.

Supplementary Figure legends

Supplementary Figure S1. The localization of Mso1p-Sec1p complexes is affected by a defective Mss4p. (A) BiFC analysis of *MSS4* and *mss4ts* cells expressing YFP(C)-Mso1p and Sec1p-Venus(N). Scale bar 5 μm. (B) Quantification of the Mso1p-Sec1p BiFC signal localization in *MSS4* and *mss4ts* cells. The Mso1p-Sec1p BiFC localization was examined from a minimum of 50 individual cells/condition.

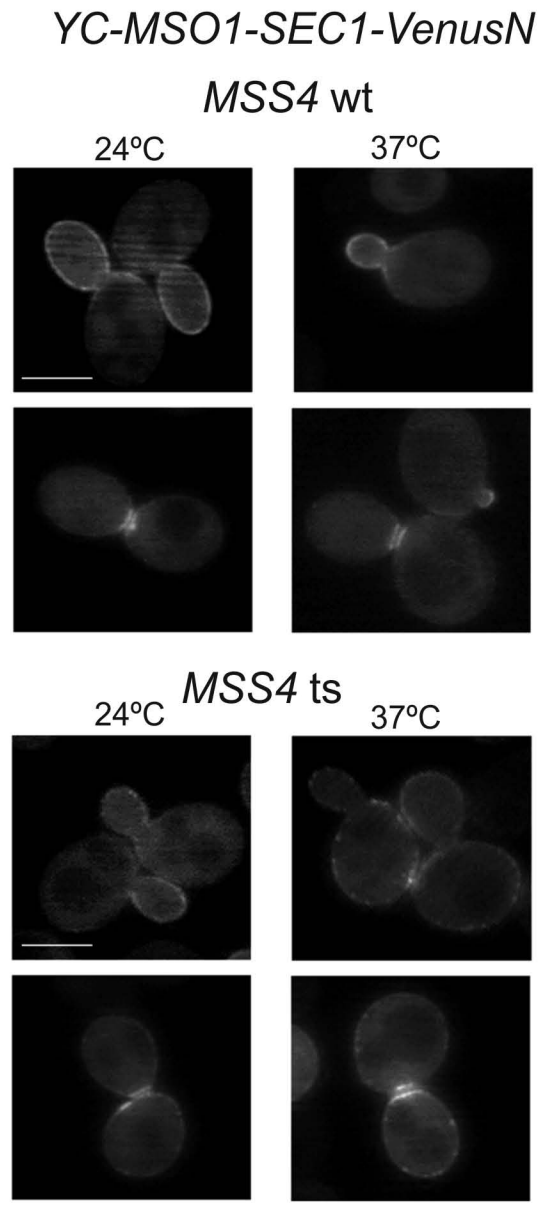
Supplementary Figure S2. The lipid specificity of Mso1p. Vesicle co-sedimentation assay reveals that Mso1p doesn't display phosphoinositide-specificity, but the membrane binding is enhanced by phoshoinositides. The final concentrations of proteins and lipids are 3 μM and 500 μM, respectively. The lipid composition is POPC:POPE:POPS:phosphoinositide=50:20:20:10. Error bars are standard deviation.

Supplementary Figure S3. Quenching of tryptophan (Trp16 in Mso1p) fluorescence by lipids brominated at positions 5, 6; 9, 10 and 11, 12. The concentrations of proteins and lipid were 0.3 μM and 200 μM, respectively. The lipid composition was POPC:POPE:POPS:PIP2= 50:20:20:10.

Supplementary Figure S4. Circular dichroic spectra of wild-type and mutant Mso1p. UV CD spectra from 250 to 190 nm were recorded with a 0.1-cm path length quartz cell. The concentration of Mso1p was 10 μM in ½ PBS buffer. The data represent the averages of five scans.

Supplementary Figure S1, Weber-Boyvat et al., 2012

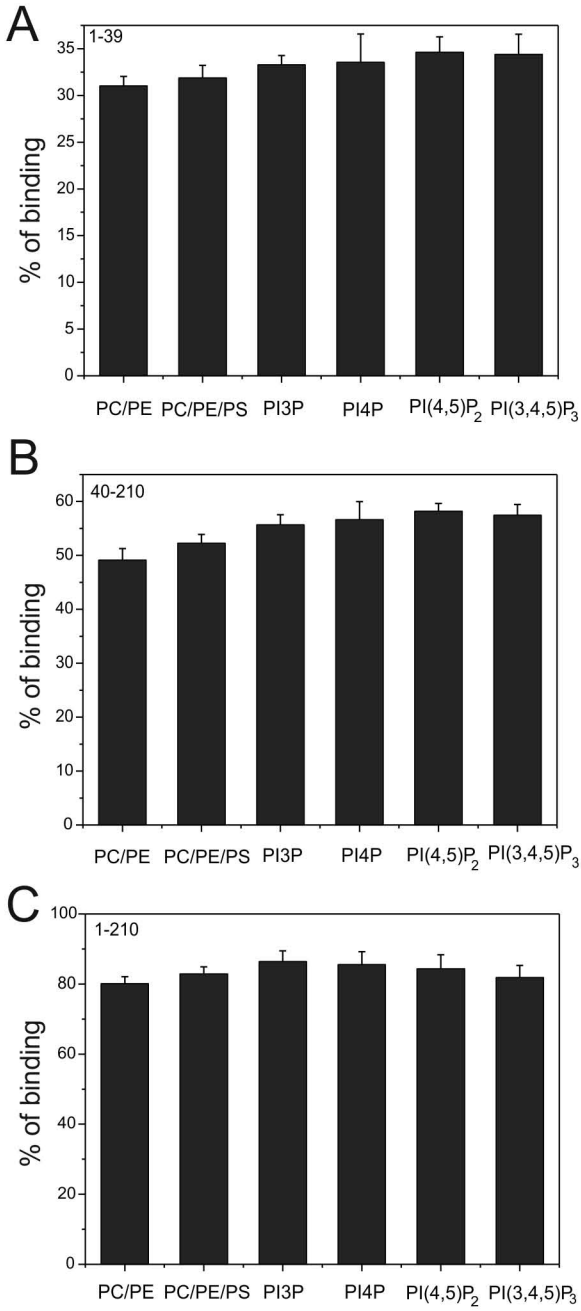
A



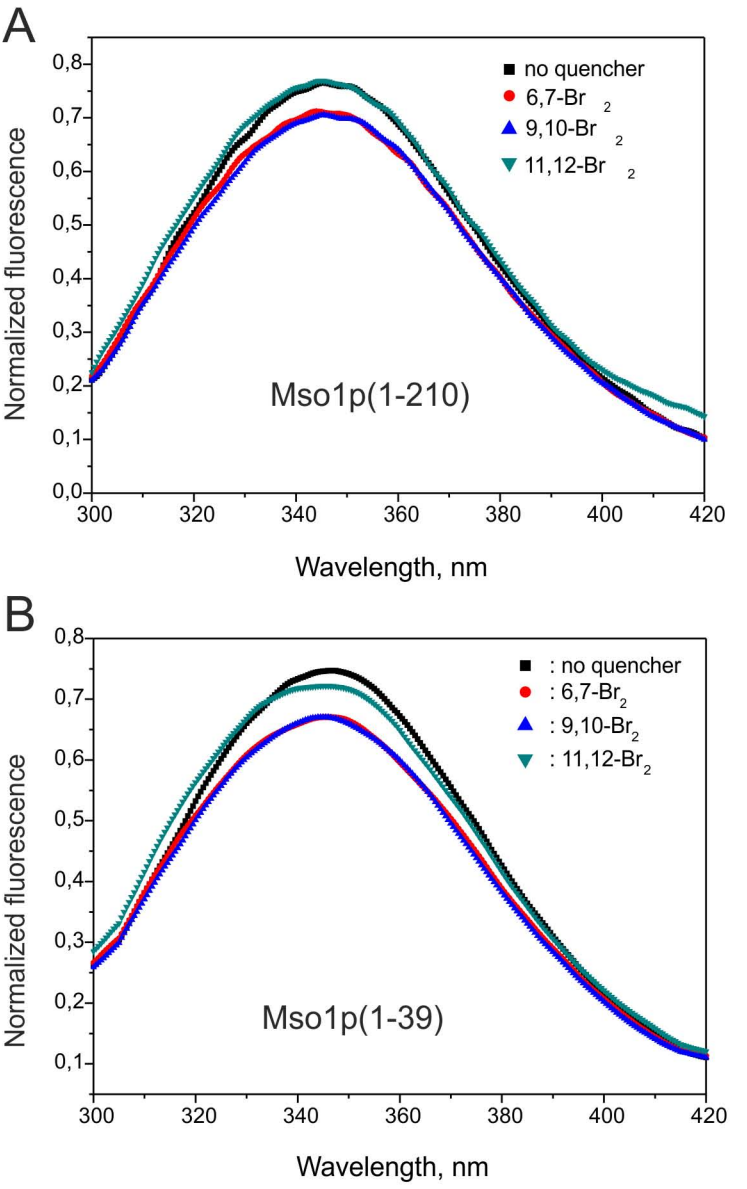
B

YCMSO1-SEC1VenusN	growing bud	septum	plasma membrane
MSS4 wt 24°C	0.74	0.23	0.03
MSS4 wt 37°C	0.72	0.23	0.05
mss4ts 24°C	0.62	0.17	0.21
mss4ts 37°C	0.35	0.13	0.52

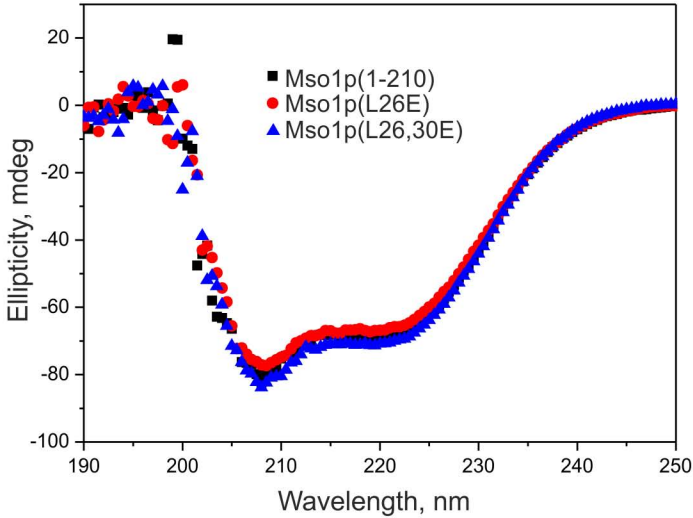
Supplementary Figure S2, Weber-Boyvat et al., 2012



Supplementary Figure S3, Weber-Boyvat et al., 2012



Supplementary Figure S4, Weber-Boyvat et al., 2012



Supplementary table S1

Strain	Genotype					Source
H304	*MAT***a** *leu2-3,112 ura3-52*					P. Novick
H2718	*MAT***a** *DON1-GFP-kanMX4 SPO21 LEU2 lys2 ura3 KanMx mso1::hphMX4*					M. Knop
H2719	*MAT*α *DON1-GFP-kanMX4 SPO21 leu2 ura3 LYS2 KanMx mso1::hphMX4*					M. Knop
H3742	*MAT*α *ura3-52 leu2-3,112 his3del200 trp1 exo70::HIS3 [exo70-38 CEN TRP1]*					W. Guo
H3751	*MAT***a** *ade5 his7 met10 trp1 ura3-52 cdc25-2*					Isakoff *et al*., 1998
H3788	*MAT***a** *leu2-3,112 ura3-52 mss4del::hphNT1 trp1::natNT2 [YCpMSS4-U]*					This study
Plasmid	**Name**	**Type**	**Promoter**	**Fragment**	**Marker**	**Source**
B704	pRS406	*Integr.*	-	-	*URA3*	Sikorski and Hieter. 1989
B1885	MSO1-Bluescript	-	*endog.*	*MSO1 wt*	amp	Knop *et al*., 2005
B1944	YEpMSO1(1-163)-U	*2μ*	*ADH1*	*MSO1(1-163)*	*URA3*	Knop *et al*., 2005
B1947	YEpMSO1(1-78)-U	*2μ*	*ADH1*	*MSO1(1-78)*	*URA3*	Knop *et al*., 2005
B1954	YIpMSO1	*Integr.*	*endog.*	*MSO1 wt*	*URA3*	Knop *et al*., 2005
B2308	pFA6-hphNTI	-	-	-	amp	Janke *et al*., 2004
B2918	YCpMSO1-VC-U	*CEN*	*ADH1*	*MSO1 wt*	*URA3*	Weber *et al*., 2010
B2930	YCpSEC1-VN-L	*CEN*	*ADH1*	*SEC1 wt*	*LEU2*	Weber *et al*., 2010
B3019	C-YN425ADH	*CEN*	*ADH*	-	*LEU2*	Skarp *et al*., 2008
B3022	pFA6-natNT2	-	-	-	amp	Janke *et al*., 2004
B3031	p416 MET YC-CDC42	*CEN*	*MET25*	*CDC42*	*URA3*	Cole et al., 2007
B3043	YCpYC-MSO1(136-210)-U	*CEN*	*MET25*	*MSO1(136-210)*	*URA3*	Weber *et al*., 2010
B3044	YCpYC-MSO1-U	*CEN*	*MET25*	*MSO1 wt*	*URA3*	Weber *et al*., 2010
B3247	YCpMSS4-U	*CEN*	*endog.*	*MSS4 wt*	*URA3*	Stefan et al., 2002
B3248	YCplac111mss4ts-102-L	*CEN*	*endog.*	*mss4 ts*	*LEU2*	Stefan et al., 2002
B3252	pGV251	*2μ*	*ADH 1*	-	*LEU2*	Isakoff *et al*., 1998
B3254	pGV253	*2μ*	*ADH1*	*PLCγ-PH*	*LEU2*	Isakoff *et al.,* 1998
B3269	YCp-VN-T	*CEN*	*ADH1*	*VenusN*	*TRP1*	This study
B3270	YCpSEC1-VN-T	*CEN*	*ADH1*	*SEC1 wt*	*TRP1*	This study
B3280	YCpMSS4-L	*CEN*	*endog.*	*MSS4 wt*	*LEU2*	This study
B3304	pGV251-MSO1	*2μ*	*ADH1*	*MSO1 wt*	*LEU2*	This study
B3305	pGV251-MSO1(136-210)	*2μ*	*ADH1*	*MSO1 (136-210)*	*LEU2*	This study
B3317	YEpYN-SEC4(Q79L)-L	*2μ*	*ADH1*	*SEC4(Q79L)*	*LEU2*	Weber-Boyvat *et al*.,2010
B3336	pJTAG-MSO1(40-210)	-	T7	*MSO1 (40-210)*	amp	This study
B3337	pJTAG-MSO1(59-210)	-	T7	*MSO1 (59-210)*	amp	This study
B3338	pJTAG-MSO1(136-210)	-	T7	*MSO1 (136-210)*	amp	This study
B3339	pJTAG-MSO1(1-135)	-	T7	*MSO1 (1-135)*	amp	This study
B3340	pJTAG-MSO1(1-58)	-	T7	*MSO1 (1-58)*	amp	This study
B3341	pJTAG-MSO1(1-39)	-	T7	*MSO1 (1-39)*	amp	This study
B3342	pJTAG-MSO1(1-210)	-	T7	*MSO1 (1-210)*	amp	This study
B3343	pGV251-MSO1(40-210)	*2μ*	*ADH1*	*MSO1 (40-210)*	*LEU2*	This study
B3344	pGV251-MSO1(59-210)	*2μ*	*ADH1*	*MSO1 (59-210)*	*LEU2*	This study
B3345	pGV251-MSO1(1-135)	*2μ*	*ADH1*	*MSO1 (1-135)*	*LEU2*	This study
B3346	pGV251-MSO1(1-58)	*2μ*	*ADH1*	*MSO1 (1-58)*	*LEU2*	This study
B3347	pGV251-MSO1(1-39)	*2μ*	*ADH1*	*MSO1 (1-39)*	*LEU2*	This study
B3352	YCpYC-MSO1(40-210)-U	*CEN*	*MET25*	*MSO1 (40-210)*	*URA3*	This study

B3353	YCpYC-MSO1(59-210)-U	*CEN*	*MET25*	*MSO1(59-210)*	*URA3*	Weber *et al*., 2010
B3354	YCpYC-MSO1(1-135)-U	*CEN*	*MET25*	*MSO1(1-135)*	*URA3*	Weber *et al*., 2010
B3355	YCpYC-MSO1(1-58)-U	*CEN*	*MET25*	*MSO1(1-58)*	*URA3*	Weber *et al*., 2010
B3356	YCpYC-MSO1(1-39)-U	*CEN*	*MET25*	*MSO1 (1-39)*	*URA3*	This study
B3410	MSO1(40-210)-Bluescript	-	*endog.*	*MSO1 (40-210)*	amp	This study
B3411	YIpMSO1(40-210)	*Integr.*	*endog.*	*MSO1 (40-210)*	*URA3*	This study
B3427	pGV251-MSO1(L26E)	*2µ*	*ADH1*	*MSO1 (L26E)*	*LEU2*	This study
B3428	pGV251-MSO1(L26.30E)	*2µ*	*ADH1*	*MSO1 (L26, 30E)*	*LEU2*	This study
B3429	pJTAG-MSO1(L26E)	-	-	*MSO1 (L26E)*	amp	This study
B3430	pJTAG-MSO1(L26,30E)	-	-	*MSO1 (L26, 30E)*	amp	This study
B3492	YEpMSO1-YN-L	*2µ*	*ADH1*	*MSO1 wt*	*LEU2*	This study
B3494	YCpYC-MSO1(L26E)-U	*CEN*	*MET25*	*MSO1 (L26E)*	*URA3*	This study
B3495	YCpYC-MSO1(L26,30E)-U	*CEN*	*MET25*	*MSO1 (L26, 30E)*	*URA3*	This study
B3502	pJTAG-Mint1PTB	-	-	*Mint1PTB*	amp	This study
B3503	pGV251-Mint1PTB	*2µ*	*ADH1*	*Mint1PTB*	*LEU2*	This study
B3505	pJTAG-MSO1(59-135)	-	-	*MSO1 (59-135)*	amp	This study
B3511	YIpMSO1(L26E)	*Integr.*	*endog.*	*MSO1 (L26E)*	*URA3*	This study
B3512	YIpMSO1(L26,30E)	*Interg*	*endog.*	*MSO1 (L26, 30E)*	*URA3*	This study
B3531	pJTAG-Mint1PTB(L473E)	-	-	*Mint1PTB(L473E)*	amp	This study
B3532	pJTAG-Mint1PTB(L473,474E)	-	-	*Mint1PTB(L473,474E)*	amp	This study
B3606	YEpMSO1(40-210)U	*2µ*	*ADH1*	*MSO1 (40-210)*	amp	This study

Plasmid	**Name**	**Species**	**Insert**	**Tag**	**Source**
B3691	rMunc18-1-VN	rat	rMunc18-1	Venus(N)	This study
B3693	YC-rMint1(full-length)	rat	rMint1(full-length)	Venus(C)	This study
B3694	YC-rMint1(MID+PTB)	rat	rMint1(MID+PTB)	Venus(C)	This study
B3695	YC-rMint1(PTB)	rat	rMint1(PTB)	Venus(C)	This study
B3696	mCherry-rMint1(full-length)	rat	Mint1(full-length)	mCherry	This study
B3712	mCherry-rMunc18-1	rat	rMunc18-1	mCherry	This study
B3717	YC-rMint1(MID)	rat	rMint1(MID)	Venus(C)	This study
B3750	YC-rMint1(full-length L473,474E)	rat	rMint1(full-length L473,474E)	Venus(C)	This study
B3751	YC-rMint1(MID+PTB L473,474E)	rat	rMint1(MID+PTB L473,474E)	Venus(C)	This study