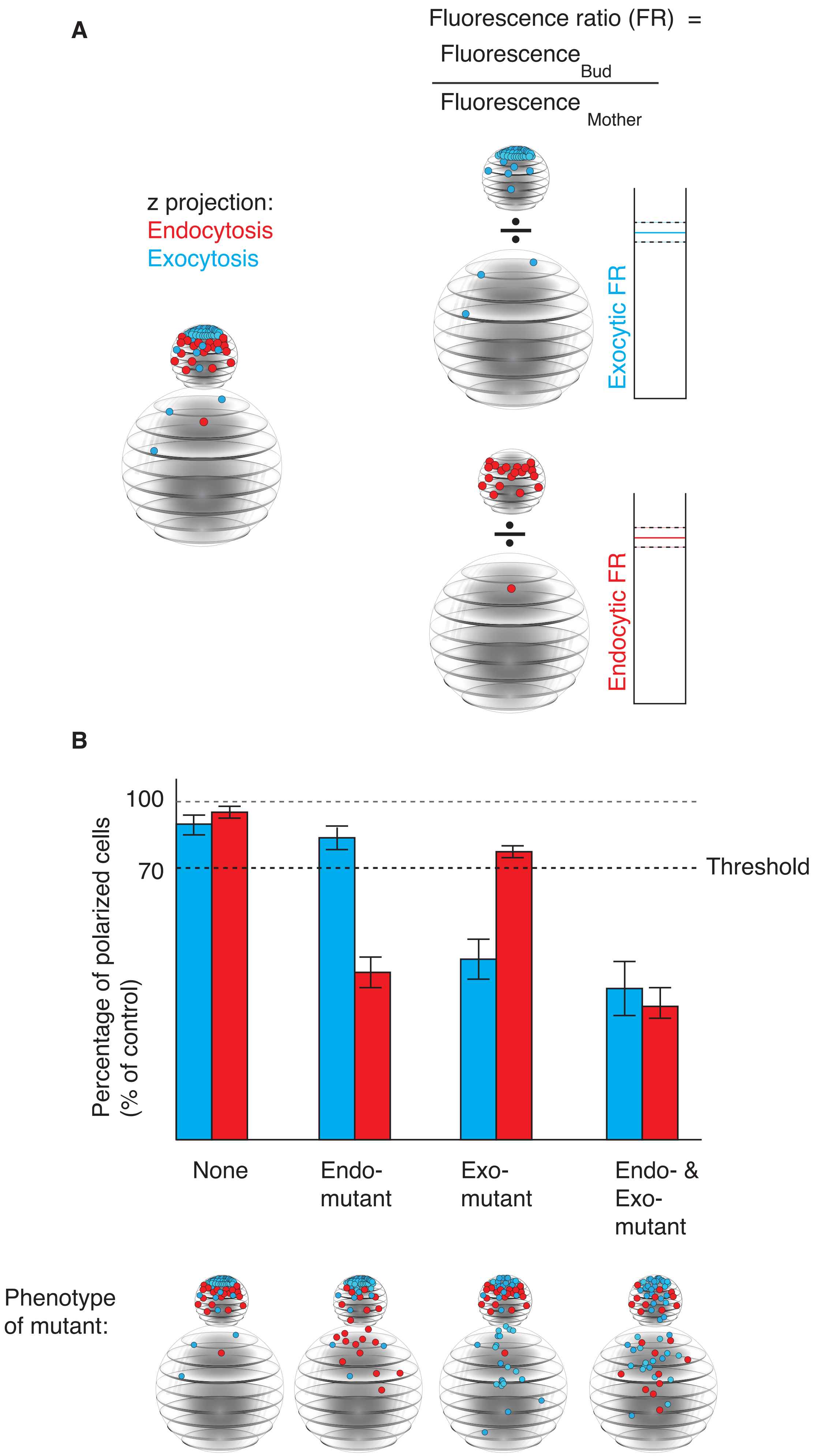
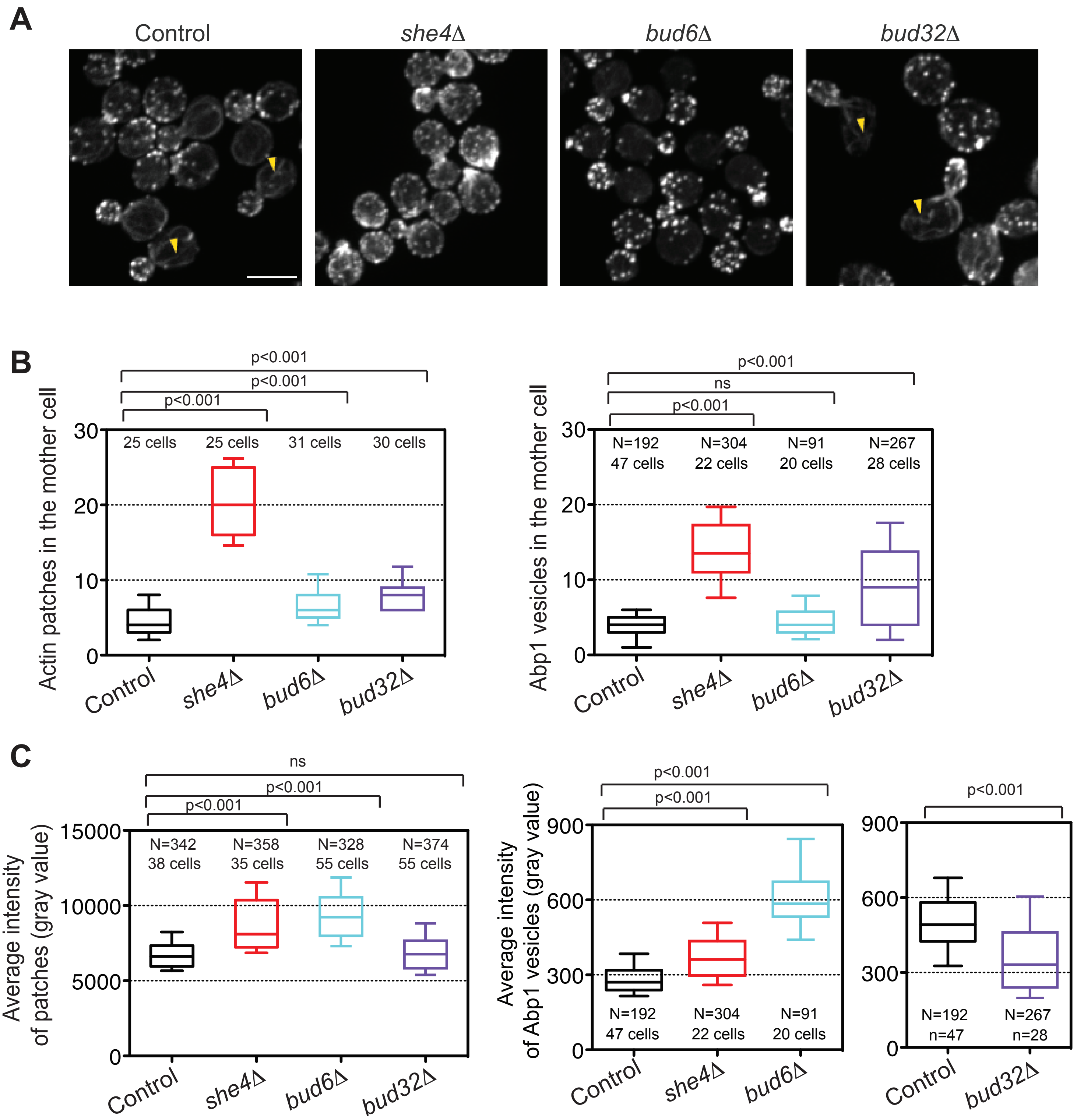
Supplemental Materials

Molecular Biology of the Cell

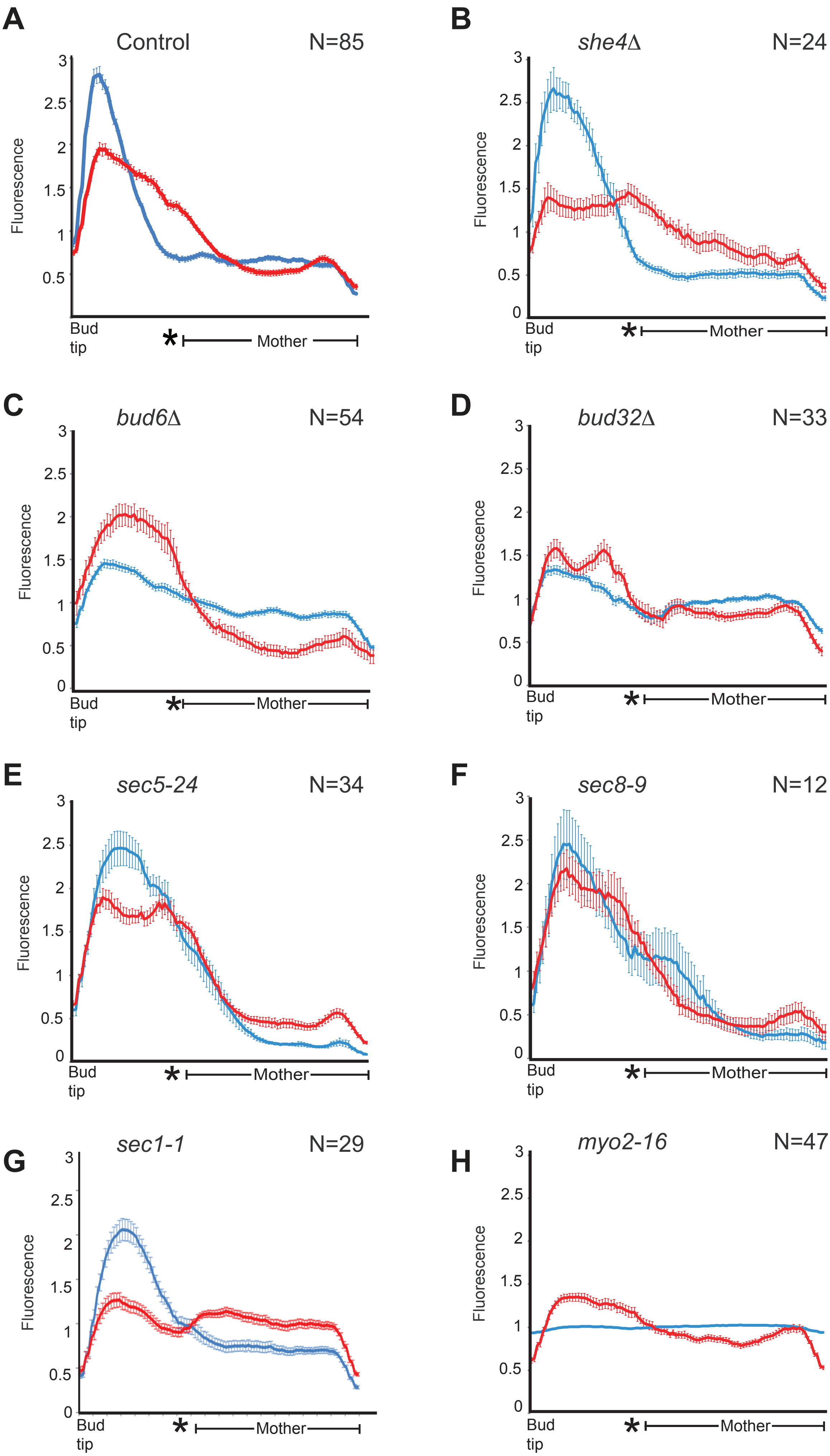
Jose et al.



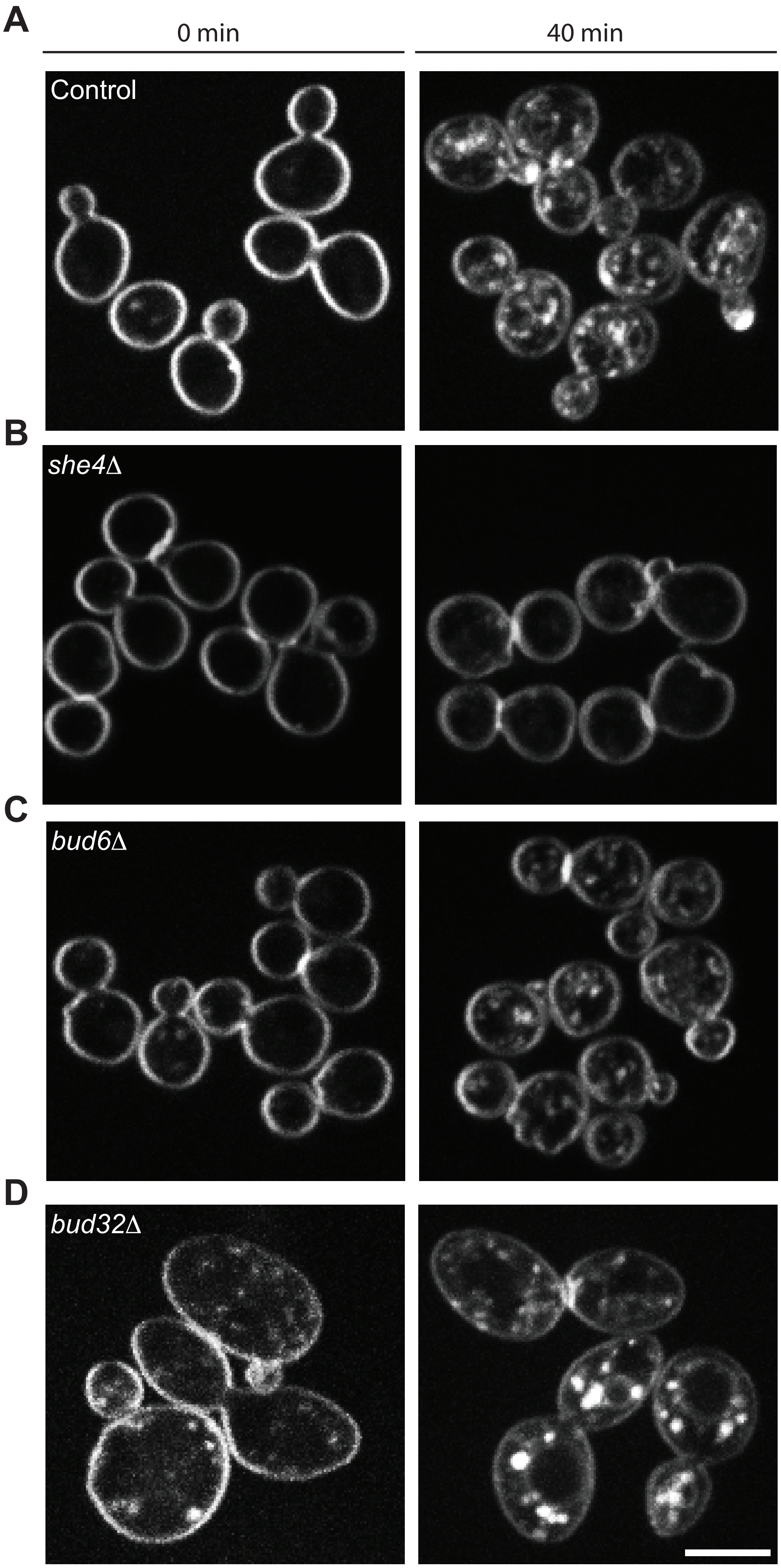
Jose et al. Figure S1



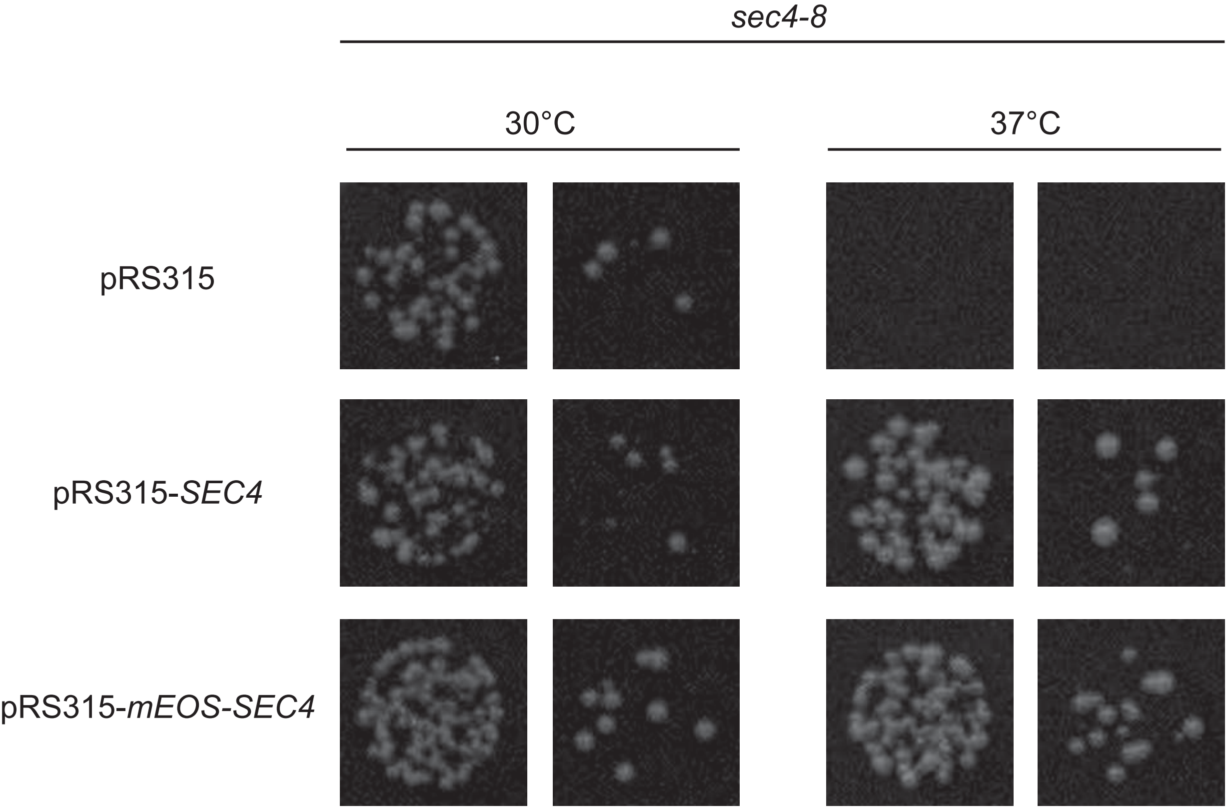
Jose et. al. Figure S2



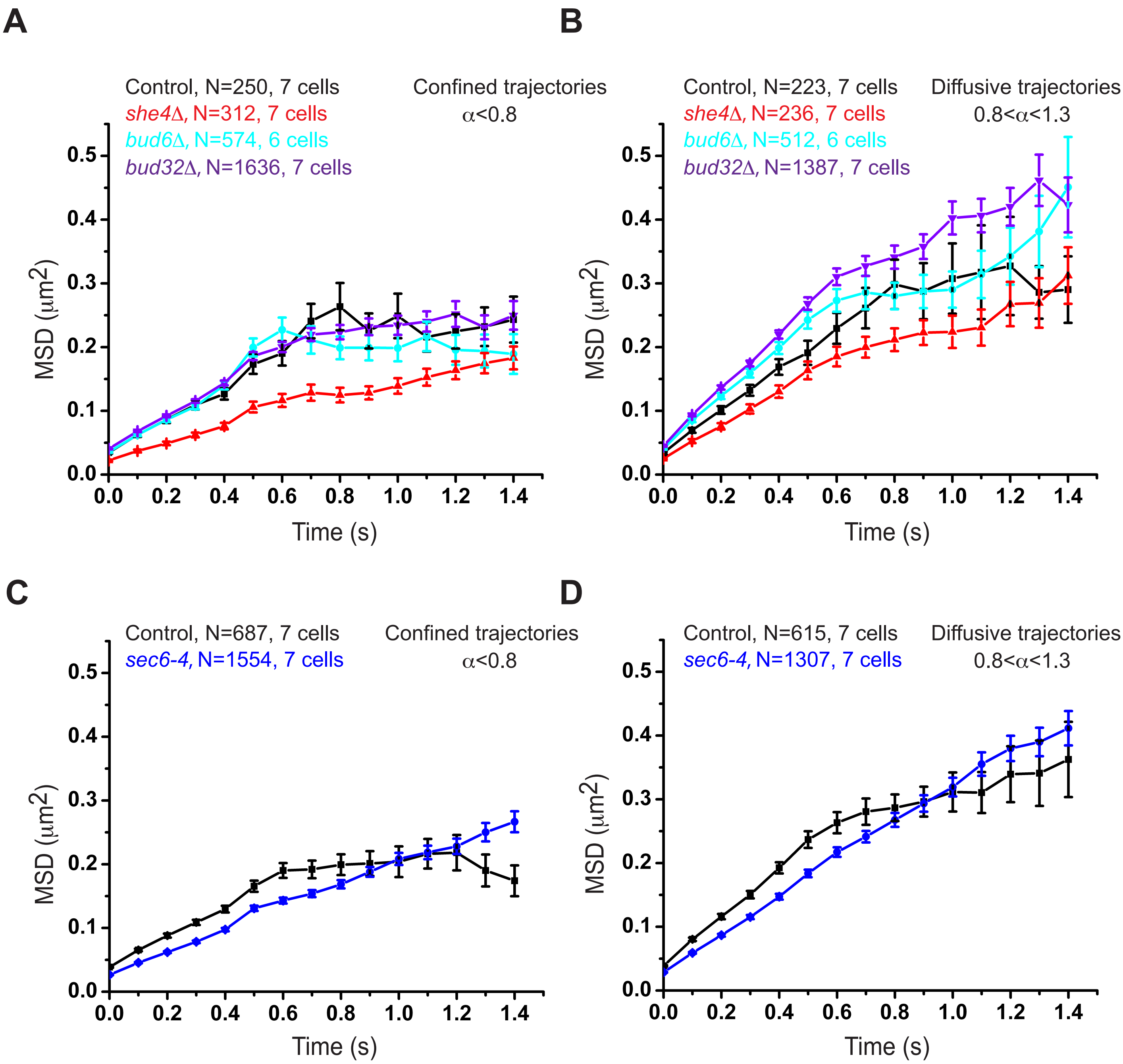
Jose et al. Figure S3



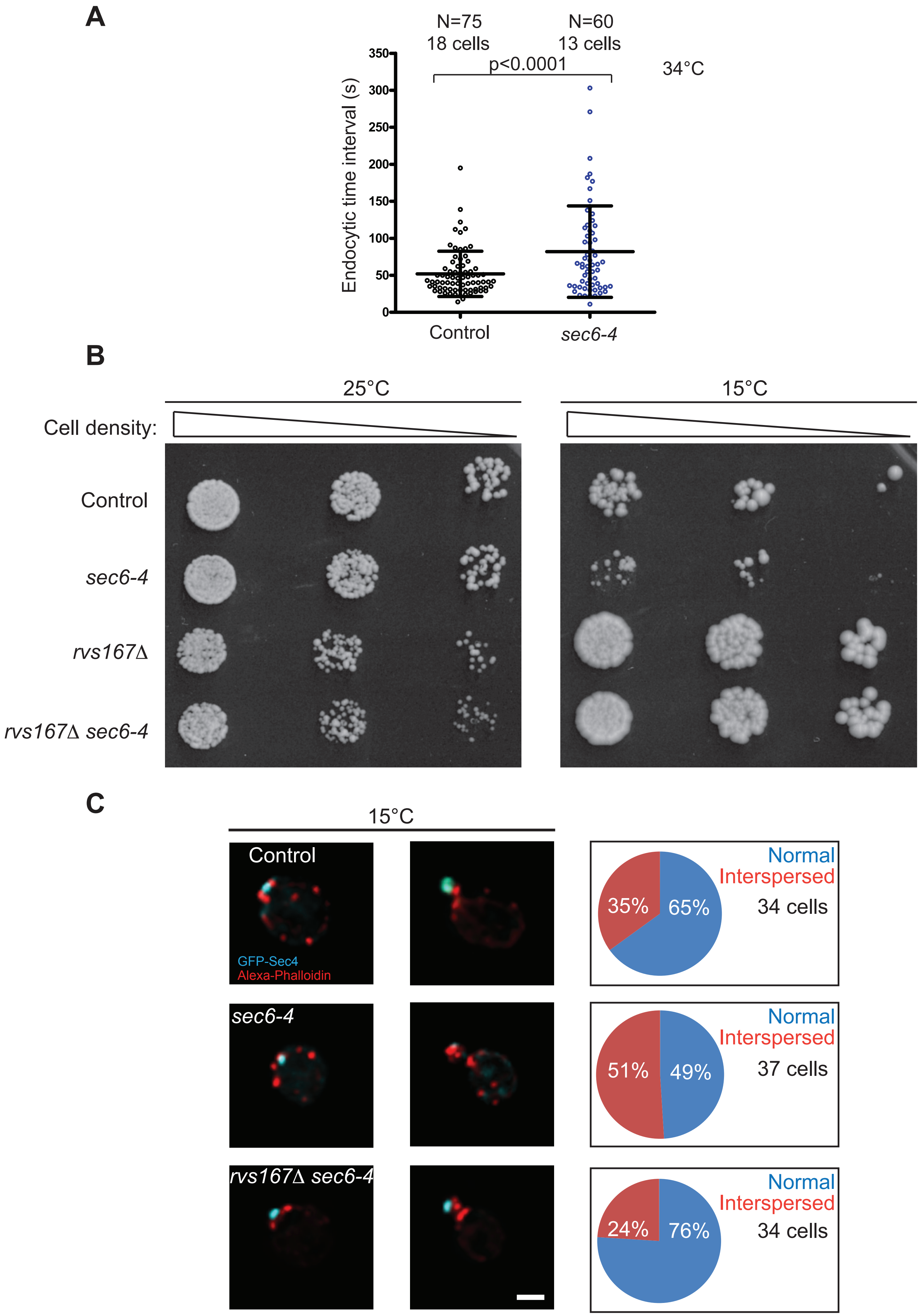
Jose et. al. Figure S4



Jose et al. Figure S5



Jose et al. Figure S6



Jose et. al. Figure S7

Table S1. List of all the yeast strains used for the candidate screen.

Mutant	Systematic Name	Genotype
*sec6-4***	YIL068C	MATa, sec6-4::HIS3, his3-11, leu2-3,112, trp1-1, ura3-52, ade2-1, can1-100, GAL+, bar1
*cdc42-6***	YLR229C	MATα, leu2-3,112 cdc42-6
*sec7-4***	YLR229C	MATa, his3-118, ura3-52, leu2-3, 112, sec7-4
*cdc24ts***	YAL041W	MATa, leu2-3,112, trp1-1, ade2-1, can1-100, GAL+, bar1, cdc24 3xHA::Kl URA
*sec10-2***	YLR166C	MATa, ura3-52, sec10-2
*sec15-1***	YGL233W	MATa, ura3-52, sec15-1
*sec5-24***	YDR166C	MATa, ura3-52, sec5-24
*sec8-9***	YPR055W	MATa, ura3-52, sec8-9
*sec3-5***	YER008C	MATa, ura3-52, sec3-5
*exo70-38***	YJL085W	MATα, his3-^200, leu2-3,112, trp1-1, ura3-52, exo70::HIS3
*mss4-102***	YDR208W	MATα, leu2-3, 112, ura3-52, his3-Δ200, trp1-Δ901, lys2-801, suc2-Δ9, mss4Δ::HIS3Mx6
*stt4-4***	YLR305C	MATα, leu2-3, 112, ura3-52, his3-Δ200, trp1-Δ901, lys2-801, suc2-Δ9, stt4Δ::HIS3
*pik1-83***	YNL267W	MATα, leu2-3, 112, ura3-52, his3-Δ200, trp1-Δ901, lys2-801, suc2-Δ9, pik1Δ::HIS3
*sec1-1***	YDR164C	MATα, ura3-52, leu2-Δ1, trp1-Δ63, ade2-101, sec1-1
*sec33-1***	YDL145C	MATα, leu2,2-112, ura3-52, sec33-1
*sec27-1***	YGL137W	MATα, leu2, ura3, trp1, HIS+, sec27-1
*ret1-3***	YDL145C	MATα, leu2, ura3, trp1, HIS+, ret1-1
*ret2-1***	YFR051C	MATα, leu2, ura3, trp1, HIS+, ret2-1
*glc7-10**	YER133W	MATa, ade2-1, his3-11, leu2-3, 112, ura3-1, can1-100, ssd1-d2, glc7::LEU2, trp1-1::glc7-10::TRP1, GAL+

*glc7-12**	YER133W	MATa, ade2-1, his3-11, leu2-3, 112, ura3-1, can1-100, ssd1-d2, glc7::LEU2, trp1-1::glc7-12::TRP1, GAL+
*cdc3-18***	YLR314C	MATa, his3-11, leu2-3, 112, trp1-1, ura3-52, ade2-1, can1-100, GAL+, bar1, cdc3-18
*cdc12-6***	YHR107C	MATa, his3-11, leu2-3, 112, trp1-1 ura3-52, ade2-1, can1-100, GAL+, bar1, cdc12-6
*cdc5-1***	YMR001C	MATα, his3-11, leu2-3, 112, trp1-1, ura3-52, ade2-1, can1-100, GAL+, cdc5-1
*cdc20-1***	YGL116W	MATα, ura3-1, leu2-3, 112, trp1-1, his3-11, ade2-1, can1-100, cdc20-1
*cdc14-1***	YFR028C	MATa, his3-11, 15, leu2-3, 112, trp1-1, ura3-1, ade2-1, can1-100, GAL+, bar1, cdc14-1
*cdc14-3**	YFR028C	MATa, ura3-1, leu2-3, 112, trp1-1, his3-11, 15, ade2-1, can1-100, cdc14-3
*myo2-66***	YOR326W	MATα, his3-Δ200, ura3-52, myo2-66
*myo2-16***	YOR326W	MATa, ade2, his3, leu2, lys2, ura3, bar1::URA3, myo2-16::HIS3
*rho1-4***	YPR165W	MATa, ura3, trp1, ade2, lys2, his3, leu2, GAL-, rho1Δ::HIS3, ade3::rho1-4::LEU2
*rho1-11**	YPR165W	MATa, ura3, trp1, ade2, lys2, his3, leu2, GAL-, rho1Δ::HIS3, ade3::rho1-11::LEU2
*rho1-2***	YPR165W	MATa, ura3, trp1, ade2, lys2, his3, leu2, GAL-, rho1Δ::HIS3, ade3::rho1-2::LEU2
*rho1-10***	YPR165W	MATa, ura3, trp1, ade2, lys2, his3, leu2, GAL-, rho1Δ::HIS3, ade3::rho1-10::LEU2
*cdc1-1**	YDR182W	MATa, his3^1, leu2^0, met15^0, ura3^0, SSD1-V
*sec2-41***	YNL272C	"
*cdc25-1***	YLR310C	"
*cog2-1**	YGR120C	"
*sec20-1***	YDR498C	"
*act1-120***	YFL039C	"
*cog3-1***	YER157W	"

*sed5-1***	YLR026C	"
*bos1-1***	YLR078C	"
*sly1-ts***	YDR189W	"
*smc1-2**	YFL008W	"
*sec12-1***	YNR026C	"
*alg2-1***	YGL065C	"
*sec24-20***	YIL109C	"
*lst8-6***	YNL006W	"
*tor2-21***	YKL203C	"
*sec14-3***	YMR079W	"
*gab1-1***	YLR459W	"
*pan1-4***	YIR006C	"
*cmd1-8***	YBR109C	"
*smc3-42**	YJL074C	"
scc2-4	YDR180W	"
*cdc11-1***	YJR076C	"
*arp3-D11A***	YJR065C	"
*kap95-E126K***	YLR347C	"
*sec63-1***	YOR254C	"
*ipl1-2***	YPL209C	"
*cdc11-2***	YJR076C	"
*lcb2-2***	YDR062W	"
*scd5-PPID2***	YOR329C	"
*smc2-8***	YFR031C	"
*lcb1-10***	YMR296C	"
*lcb2-19***	YDR062W	"
*cdc48-4601**	YDL126C	"

*cdc34-1***	YDR054C	"
*cdc34-2***	YDR054C	"
*sda1-2**	YGR245C	"
*pkc1-ts***	YBL105C	"
*las17-13***	YOR181W	"
*taf4-18***	YMR005W	"
*arp2-3***	YDL029W	MATa, his3^200, leu2^1, lys2-801, ura3-52, arp2-3::URA3
*arp2-7***	YDL029W	MATa, his3^200, leu2^1, lys2-801, ura3-52, arp2-7::URA3
cho1Δ	YER026C	MATa, his3-11, leu2-3,112, trp1-1, ade2-1, can1-100, GAL+, bar1, CHO1::URA3
bar1Δ	YIL015W	MATa, his3^1, leu2^0, met15^0, ura3^0, SSD1-V
rho2Δ	YNL090W	"
rho4Δ	YKR055W	"
bem3Δ	YPL115C	"
bem4Δ	YPL161C	"
rgd1Δ	YBR260C	"
rdi1Δ	YDL135C	"
rom1Δ	YGR070W	"
rom2Δ	YLR371W	"
end3Δ	YNL084C	"
*sla1Δ***	YBL007C	"
abp1Δ	YCR088W	"
rvs161Δ	YCR009C	"
sac6Δ	YDR129C	"
*cap1Δ***	YKL007W	"
bbc1Δ	YJL020C	"

rvs167Δ	YDR388W	"
*pea2Δ**	YER149C	"
*grr1Δ***	YJR090C	"
*elm1Δ***	YKL048C	"
myo4Δ	YAL029C	"
nap1Δ	YKR048C	"
hof1Δ	YMR032W	"
*bud6Δ***	YLR319C	"
fig4Δ	YNL325C	"
cdc10Δ	YCR002C	"
shs1Δ	YDL225W	"
*cla4Δ***	YNL298W	"
msb2Δ	YGR014W	"
boi1Δ	YBL085W	"
boi2Δ	YER114C	"
arf3Δ	YOR094W	"
arf1Δ	YDL192W	"
bem1Δ	YBR200W	"
rga1Δ	YOR127W	"
rga2Δ	YDR379W	"
arf2Δ	YDL137W	"
gts1Δ	YGL181W	"
mon1Δ	YGL124C	"
syt1Δ	YPR095C	"
afi1Δ	YOR129C	"
ypt7Δ	YML001W	"
ypt31Δ	YER031C	"

ypt32Δ	YGL210W	"
ypt52Δ	YKR014C	"
ypt53Δ	YNL093W	"
gyp5Δ	YPL249C	"
msb3Δ	YNL293W	"
msb4Δ	YOL112W	"
*ypt51Δ***	YOR089C	"
ypt6Δ	YLR262C	"
ypt10Δ	YBR264C	"
ypt11Δ	YNL304W	"
bud7Δ	YOR299W	"
bud1Δ	YGR152C	"
bud2Δ	YKL092C	"
bud3Δ	YCL014W	"
bud4Δ	YJR092W	"
bud14Δ	YAR014C	"
akr2Δ	YOR034C	"
ent4Δ	YLL038C	"
erg3Δ	YLR056W	"
ldb19Δ	YOR322C	"
snc1Δ	YAL030W	"
snc2Δ	YOR327C	"
vps21Δ	YOR089C	"
*drs2Δ***	YAL026C	"
inp54Δ	YOL065C	"
mon2Δ	YNL297C	"
rod1Δ	YOR018W	"

slm2Δ	YNL047C	"
*tfp3Δ***	YPL234C	"
vma4Δ	YOR332W	"
kin1Δ	YDR122W	"
osh3Δ	YHR073W	"
osh4Δ	YPL145C	"
osh7Δ	YHR001W	"
sds24Δ	YBR214W	"
yck1Δ	YHR135C	"
*doa4Δ***	YDR069C	"
lsb5Δ	YCL034W	"
*yap1801Δ***	YHR161C	"
bzz1Δ	YHR114W	"
dnf2Δ	YDR093W	"
*cup5***	YEL027W	"
slm6	YBR266C	"
vma8	YEL051W	"
vma10	YHR039C-A	"
kap120	YPL125W	"
dnm1Δ	YLL001W	"
*whi2Δ***	YOR043W	"
bud20Δ	YLR074C	"
kin2Δ	YLR096W	"
osh1Δ	YAR042W	"
sur7Δ	YML052W	"
swf1Δ	YDR126W	"
*bni1Δ***	YNL271C	"

gyl1Δ	YMR192W	"
rax1Δ	YOR301W	"
bud22Δ	YMR014W	"
*ckb2Δ***	YOR039W	"
*cka2Δ***	YOR061W	"
arp1Δ	YHR129C	"
*zds1Δ***	YMR273C	"
*eeb1Δ***	YPL095C	"
*eht1Δ***	YBR177C	"
*gic1Δ***	YHR061C	"
*hkr1Δ***	YDR420W	"
*mnn1Δ***	YER001W	"
*pct1Δ***	YGR202C	"
atg5Δ	YPL149W	"
*bmh2Δ***	YDR099W	"
rta1Δ	YGR213C	"
sbh1Δ	YER087C-B	"
sem1Δ	YDR363W-A	"
ste20Δ	YHL007C	"
*atg12Δ**	YBR217W	"
*can1Δ***	YEL063C	"
*shm1Δ***	YBR263W	"
*atf1Δ**	YOR377W	"
*dnf3Δ***	YMR162C	"
*faa4Δ***	YMR246W	"
*plb1Δ***	YMR008C	"
*shm2Δ***	YLR058C	"

*tgl5Δ***	YOR081C	"
*erg6Δ**	YML008C	"
*she4Δ***	YOR035C	"
*rrt8Δ**	YOL048C	"
*ylr046cΔ**	ylr046c	"
*alg8Δ***	YOR067C	"
arg82Δ	YDR173C	"
*arv1Δ***	YLR242C	"
*bud8Δ***	YLR353W	"
*chs5Δ**	YLR330W	"
*ent5Δ***	YDR153C	"
*inp53Δ***	YOR109W	"
*rcy1Δ***	YJL204C	"
*sph1Δ**	YLR313C	"
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*did4Δ***	YKL002W	"
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myo3Δ	YKL129C	"
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*cdc50Δ**	YCR094W	"
*mnn11Δ**	YJL183W	"
*msb1Δ**	YOR188W	"
*atg26Δ**	YLR189C	"

*mnn5Δ**	YJL186W	"
mnn6Δ	YPL053C	"
*smy1Δ**	YKL079W	"
akr1Δ	YDR264C	"
aly1Δ	YKR021W	"
aly2Δ	YJL084C	"
bit61Δ	YJL058C	"
*csr1Δ**	YLR380W	"
*csr2Δ**	YPR030W	"
*fld1Δ***	YLR404W	"
myo5Δ	YMR109W	"
*orm2Δ**	YLR350W	"
*ste2Δ**	YFL026W	"
*yck2Δ**	YNL154C	"
ysc84Δ	YHR016C	"
avo2Δ	YMR068W	"
bsp1Δ	YPR171W	"
ede1Δ	YBL047C	"
inp51Δ	YIL002C	"
ppa1Δ	YHR026W	"
*rtn1Δ**	YDR233C	"
*sac1Δ**	YKL212W	"
*see1Δ**	YIL064W	"
svl3Δ	YPL032C	"
*tsc11Δ**	YER093C	"
vma13Δ	YPR036W	"
*yap1802Δ**	YGR241C	"

*shr5Δ**	YOL110W	"
*bck1Δ**	YJL095W	"
*cos10Δ***	YNR075W	"
*ehd3**	YDR036C	"
*faa1Δ**	YOR317W	"
*gvp36Δ***	YIL041W	"
*ldb17Δ**	YDL146W	"
*pkh2Δ**	YOL100W	"
*rav1Δ**	YJR033C	"
*srt1Δ***	YMR101C	"
*vma6Δ***	YLR447C	"
*ydl162cΔ**	YDL162C	"
*bud27Δ***	YFL023W	"
*dpp1Δ**	YDR284C	"
*ent3Δ**	YJR125C	"
*gpt2Δ**	YKR067W	"
*hfd1Δ**	YMR110C	"
*kcs1Δ***	YDR017C	"
*lsb6Δ***	YJL100W	"
*osh6Δ**	YKR003W	"
*rog3Δ**	YFR022W	"
*spo14Δ**	YKR031C	"
*sro7Δ***	YPR032W	"
*tgl3Δ**	YMR313C	"
*ark1Δ**	YNL020C	"
*ast1Δ**	YBL069W	"
*ecm21Δ**	YBL101C	"

*ent1Δ**	YDL161W	"
*inp52Δ**	YNL106C	"
*orm1Δ**	YGR038W	"
*osh2Δ**	YDL019C	"
*pdr16Δ**	YNL231C	"
*slc1Δ**	YDL052C	"
*sro77Δ**	YBL106C	"
*tcb2Δ***	YNL087W	"
*tpm1Δ***	YNL079C	"
*akl1Δ***	YBR059C	"
*bnr1Δ**	YIL159W	"
*ddi1Δ**	YER143W	"
dnf1Δ	YER166W	"
*erf2Δ**	YLR246W	"
*frm2Δ***	YCL026C-A	"
*lsp1Δ***	YPL004C	"
*nma111Δ**	YNL123W	"
*pet10Δ**	YKR046C	"
*pil1Δ**	YGR086C	"
*slg1Δ**	YOR008C	"
*tor1Δ**	YJR066W	"
*vph1Δ**	YOR270C	"
*vrp1Δ***	YLR337C	"
*alg9Δ**	YNL219C	"
bmh1Δ	YER177W	"
*pug1Δ**	YER185W	"
*siw14Δ**	YNL032W	"

*slm1Δ**	YIL105C	"
stv1Δ	YMR054W	"
*tpm2Δ***	YIL138C	"
*vma2Δ***	YBR127C	"
*axl2Δ***	YIL140W	"
*ayr1Δ**	YIL124W	"
*bem2Δ***	YER155C	"
*bud23Δ**	YCR047C	"
*bud32Δ***	YGR262C	"
*chc1Δ***	YGL206C	"
*ckb1Δ**	YGL019W	"
*gic2Δ**	YDR309C	"
*grh1Δ**	YDR517W	"
*lpp1Δ**	YDR503C	"
*mnn9Δ**	YPL050C	"
*tep1Δ**	YNL128W	"
*tgl1Δ**	YKL140W	"
*vps34Δ***	YLR240W	"
*ymr210wΔ**	ymr210w	"
bud17Δ	YNR027W	"
*mnn2Δ**	YBR015C	"
*mnn10Δ***	YDR245W	"
*per1Δ**	YCR044C	"
*pkh1Δ**	YDR490C	"
*pmr1Δ**	YGL167C	"
*tfp1Δ***	YDL185W	"
*ykl091cΔ**	ykl091c	"

*zds2Δ**	YML109W	"
apq12Δ	YIL040W	"
*bud25Δ**	YER014C-A	"
*cka1Δ**	YIL035C	"
*clc1Δ***	YGR167W	"
*cyb5Δ**	YNL111C	"
*dga1Δ**	YOR245C	"
*fab1Δ**	YFR019W	"
mnn4Δ	YKL201C	"
*mnn8Δ***	YEL036C	"
*pdr5Δ**	YOR153W	"
*ydl109cΔ**	YDL109C	"
sac7Δ	YDR389W	"
mid2Δ	YLR332W	"
abp140Δ	YOR239W	"
wsc1Δ	YOR008C	"
mkk1Δ	YOR231W	"
mkk2Δ	YPL140C	"
tus1Δ	YLR425W	"
lrg1Δ	YDL240W	"
pxl1Δ	YKR090W	"
bag7Δ	YOR134W	"
*bro1Δ***	YPL084W	"
*pep12Δ***	YOR036W	"
mlc2Δ	YPR188C	"
*sla2Δ***	YNL243W	"

** Mutants identified from qualitative analysis as strongly altering the spatial organization of endo- and exocytosis. *Subtle mutants identified from qualitative analysis. " denotes similar to previous.

Table S2. A two-tailed Student t-test confirms the statistical relevance of mutant phenotypes determined from the thresholding-based quantitative analysis of the screen data.

Mutant	*p-value endo*	*p-value exo*	*Mutant*	*p-value endo*	*p-value exo*	*Mutant*	*p-value endo*	*p-value exo*
abp140 Δ	///////	0.0147	*cyb5 Δ*	///////	0.038	*rho1-2*	<0.0001	<0.0001
act1-120	<0.0001	<0.0001	*did4 Δ*	0.48	///////	*rho1-4*	0.15	0.0007
akl1 Δ	0.0009	///////	*dnf3 Δ*	///////	0.0001	*rho3-1*	///////	0.0046
alg2-1	<0.0001	<0.0001	*doa4 Δ*	///////	0.0019	*sac7 Δ*	///////	0.0043
anp1 Δ	///////	0.015	*drs2 Δ*	///////	0.028	*scd5 Δ*	///////	<0.0001
arp2 Δ	<0.0001	<0.0001	*elm1 Δ*	<0.0001	///////	*sda1-2*	///////	<0.0001
arp3-D11A	///////	0.025	*frm2 Δ*	///////	0.0005	*sla2 Δ*	<0.0001	///////
art5 Δ	0.0095	0.037	*gab1-1*	0.988	0.012	*sec1-1*	0.0001	///////
atg26 Δ	0.0080	///////	*glc7-10*	///////	0.0004	*sec12-1*	<0.0001	<0.0001
ayr1 Δ	0.0037	///////	*ipl1-2*	0.055	<0.0001	*sec14-3*	///////	<0.0001
bem2 Δ	0.035	///////	*kap95-E126K*	0.014	<0.0001	*sec15-1*	0.19	///////
bni1 Δ	///////	0.037	*kcs1 Δ*	///////	0.0043	*sec2-41*	///////	0.0002
bos1-1	<0.0001	<0.0001	*las17-13*	0.0052	<0.0001	*sec20-1*	<0.0001	<0.0001
bro1 Δ	///////	<0.0001	*lcb1-10*	<0.0001	<0.0001	*sec24-20*	0.35	<0.0001
bud23 Δ	///////	0.024	*lcb2-19*	<0.0001	<0.0001	*sec27-1*	///////	<0.0001
bud25 Δ	0.024	<0.0001	*lcb2-2*	///////	<0.0001	*sec5-24*	0.016	///////
bud32 Δ	0.021	<0.0001	*lpp1 Δ*	///////	0.013	*sec63-1*	///////	<0.0001
bud6 Δ	///////	<0.0001	*lrg1 Δ*	0.0027	///////	*sec6-4*	///////	0.012
cap1 Δ	///////	0.0002	*lst8-6*	///////	0.025	*sec7-4*	///////	<0.0001

cdc1-1	///////	0.0015	*mkk2 Δ*	0.00096	0.0736	*sed5-1*	<0.0001	<0.0001
cdc11-2	///////	<0.0001	*mnn10 Δ*	0.307	0.0148	*she4 Δ*	0.0057	///////
cdc12-6	<0.0001	<0.0001	*mss4-102*	0.032	///////	*sly1-ts*	0.0021	<0.0001
cdc14-1	0.28	<0.0001	*myo2-16*	///////	<0.0001	*smc1-259*	///////	<0.0001
cdc20-1	0.0031	<0.0001	*myo2-66*	0.0008	<0.0001	*smc2-8*	///////	0.0001
cdc24-ts	0.050	<0.0001	*pan1-4*	0.0057	0.0042	*sph1 Δ*	<0.0001	///////
cdc25-1	///////	<0.0001	*pep12 Δ*	///////	0.0034	*stt4-4*	<0.0001	///////
cdc3-18	///////	0.0003	*pik1-83*	///////	<0.0001	*taf4-18*	///////	<0.0001
cdc42-6	<0.0001	<0.0001	*pkc1-1*	0.0027	0.0002	*tcb2 Δ*	///////	0.042
cdc5-1	///////	<0.0001	*pmr1 Δ*	///////	<0.0001	*tor2-21*	0.0008	<0.0001
cla4 Δ	///////	0.016	*rcy1 Δ*	///////	0.041	*tpm1 Δ*	///////	<0.0001
clc1 Δ	///////	0.0074	*ret1-3*	///////	<0.0001	*vma1 Δ*	///////	0.0009
cmd1-8	///////	<0.0001	*ret1/sec33-1*	0.0008	<0.0001	*vps34 Δ*	///////	0.0003
cog2-1	///////	0.0036	*ret2-1*	///////	<0.0001	*vrp1 Δ*	0.043	///////
cog3-1	<0.0001	<0.0001	RHO1 control	///////	<0.0001	*zds2 Δ*	0.0063	///////
cos10 Δ	///////	0.0059						

Table S3. A two-tailed Mann-Whitney test confirms that the global median diffusion of exocytic vesicles is significantly different between the control and mutants.

	she4Δ N=1722	*bud6Δ* N=3684	*bud32Δ* N=8117
Control N=1584	***	**	***
she4Δ		***	***
bud6Δ			***

N refers to the number of vesicle trajectories analyzed. *** $p<0.0001$, ** $p=0.002$

Supplemental Material

Figure S1. A quantitative imaging-based strategy to identify mutants affecting the organization of endo- and exocytic domains.

(A) A schematic showing z-axis projections of endocytic (red) and exocytic (cyan) domains in a budding yeast cell. The fluorescence ratio (FR) for endo- and exocytic domains was calculated for individual small-budded cells and the median and confidence intervals for each mutant were calculated. The FR, reflecting the endo- and exocytic polarity states of individual mutant cells, was compared to these medians. (B) The polarization of endo- and exocytic domains is statistically analyzed at a population level for each strain. The data shown illustrates, from left to right, mutants that do not significantly affect the organization of domains (with a number of polarized cells higher than 70% of the control strain), and mutants affecting endocytosis, exocytosis, or localization of both domains.

Figure S2. Actin is perturbed differentially in mutants affecting endo- and exocytosis.

(A) Maximum intensity projections of steady state images of fixed control and mutant cells in log phase labelled with Alexa546-Phalloidin. Normally oriented actin cables in control cells and mis-oriented cables in *bud32Δ* mutants are indicated by arrows. Scale bar: 5µm. **(B) The left chart displays the number of Alexa546 phalloidin-labelled actin patches in control, *she4Δ*, *bud6Δ* and *bud32Δ* cells. The right chart shows Abp1-RFP-marked endocytic vesicles**

in the same strains. (C) Comparison of the distribution of the average fluorescence intensity of Alexa546 phalloidin-labelled actin patches between control, *she4Δ*, *bud6Δ* and *bud32Δ* cells. The right charts show the intensity of Abp1-RFP-marked endocytic vesicles in the same strains. Note that two charts are shown for the mutants, since they were imaged at different times. The median (band within the box) and 10-90 percentile values (whiskers) are shown in the plot.

Figure S3. Distinct mutants display unique endo- and exocytic fluorescence intensity distributions along the mother - bud axis.

Endo- (red) and exocytic (cyan) fluorescence distributions (y axis) are shown along the bud - mother cell axis (x axis). The mutants and number of cells used to generate the distributions are indicated. *myo2-16*, *sec5-24* and *sec8-9* are shown after shifting the cells to the restrictive temperature (34°C). Fluorescence levels are normalized to the total fluorescence, so that completely depolarized cells yield a flat distribution at y=1. Asterisks indicate the position of the average bud neck along the x axis. Error bars show the SD.

Figure S4. she4Δ and bud32Δ mutants are defective in endocytosis-based FM4-64 internalization.

Maximum intensity projections of images obtained from control cells (A), *she4Δ* (B), *bud6Δ* (C) and *bud32Δ* mutants (D) in log phase using a spinning disc confocal microscope immediately, and 40 minutes after FM4-64 labelling. Scale bar: 5µm.

Figure S5. The mEOS-Sec4 construct is functional.

The mEos-Sec4 fusion protein was verified to be functional by a growth assay. The *sec4-8* mutant allele was transformed with an empty plasmid (pRS315, top), a plasmid encoding *SEC4* with its native promoter and terminator (pRS315-*SEC4*, middle) and a plasmid expressing mEos fused to the amino terminus of Sec4 (pRS315-mEOS-*SEC4*, bottom). The temperature sensitivity of *sec4*-8 at 37°C was rescued by pRS315-*SEC4* and pRS315-mEOS-*SEC4*, but not by pRS315 alone.

Figure S6. The MSD curves of distinct mEOS-Sec4 exocytic vesicle sub-populations display differences in mobility in *she4Δ*, *bud6Δ*, *bud32Δ* and *sec6-4* mutants compared to control cells.

The average MSD curves for the confined (A) and diffusive (B) vesicle sub-populations in control cells (black), *she4Δ* (red), *bud6Δ* (cyan) and *bud32Δ* (purple) mutants. The average MSD curves for the confined (C) and diffusive (D) vesicle sub-populations in control cells (black) and *sec6-4* (blue) mutants. N indicates the number of trajectories.

Figure S7. The *sec6-4* mutant displays a perturbed endocytic signature at the restrictive temperature of 34ºC, while down regulating endocytosis in the *sec6-4* exocyst mutant suppresses its growth defect at 15ºC.

(A) A scatter dot plot showing the time intervals between consecutive endocytic events in polarized unbudded control cells (black) and *sec6-4* mutants (blue) after shifting the cells to 34ºC for 1 hour. The black bars indicate the mean and SD over N events. (B) A genetic interaction suggests a functional link between the endocytic and exocytic pathways, where deletion of the endocytic gene *RVS167* (*rvs167Δ*) suppresses the growth defect of the exocyst mutant *sec6-4* at 15°C. (C) The organization of endo- and exocytic zones is similar to control cells in the *sec6-4 rvs167Δ* double mutant at 15ºC. Scale bar: 2µm. The percentage of cells displaying normal and interspersed endo- and exocytic distributions are displayed as pie charts.

Table S1. Yeast strains used in the mutant screen.

Table S2. Statistical significance of the mutant phenotypes identified by the screen.

Table S3. A two-tailed Mann-Whitney test indicates that the median instantaneous diffusion of exocytic vesicles is significantly different between the control and mutants.