SUPPLEMENTAL TABLES

Table S1. Plasmids used in this study

Plasmid	Description	Ref
Yep13-GFP-Bem3	GFP-Bem3, 2μ *LEU2*	(Caviston et al., 2003)
pRS316-HA-mRFP-cSNC1	HA-mRFP-cSnc1, CEN *URA3*	(Robinson et al., 2006)
PHO5pr-RFP-Vps21	RFP-Vps21 expressed from *PHO5* promoter, CEN *TRP1*	(Markgraf et al., 2009)
pRS416-Cherry-Vps4^{E233Q}	Cherry-Vps4^{E233Q} expressed from Vps21promoter, CEN *URA3*	(Davies et al., 2010)
pRS315-GFP-Sec4	GFP-Sec4, CEN *LEU2*	(Calero et al., 2003)
pRS315-GFP-Rga1	GFP-Rga1, CEN *LEU2*	(Caviston et al., 2003)
pRS315-Cdc24-GFP	GFP-Cdc24,CEN *LEU2*	Erfei Bi Lab
Gic1-GFPx3	*GIC1* in pRS316-P*(a derivative of pRS316) with triple GFP tag inserted in frame with construct. CEN *URA3* variant	(Takahashi and Pryciak, 2007)
pGAL1.416-Bem3-GFP	GFP-Bem3 expressed from a GAL1 promoter, CEN *URA3*	(Knaus et al., 2007)
pYES2.1/V5-HIS-TOPO-Sec15	Sec15 expressed from the GAL1 promoter, 2μ *URA3*	This study
pYES2.1/V5-HIS-TOPO-Sro7	Sro7 expressed from the GAL1 promoter, 2μ *URA3*	This study
pRS315-GFP-Sec4^{Q79L}	GFP- Sec4^{Q79L}, CEN *LEU2*	This study
pRS315-GFP-Sec4^{S29N}	GFP- Sec4^{S29N}, CEN *LEU2*	This study
Yep13-GFP-Bem3^{PHm}	GFP-Bem3$^{R644S, R645S, K647D}$ 2μ *LEU2*	This study
Yep13-GFP-Bem3^{K1003A}	GFP-Bem3^{K1003A} 2μ *LEU2*	This study
Yep13-GFP-Bem3PX mutant	GFP-Bem3 $^{Y524W, R578S, L580W, F581M}$ 2μ *LEU2*	This study
PX-PH domain-His6	pET28a HIS$_6$-BEM3$^{491-774}$	This study
Yep13-HA-Bem3	HA-Bem3, 2μ *LEU2*	This study
pGAL1.426-Bem3-HA-His6	Bem3 expressed from GAL1 promoter, 2μ *URA3*	This study

pAD54-RFP-SEC4	RFP-Sec4 expressed from the ADH promoter, 2μ *LEU2*	(Aronov and Gerst, 2004)
pYES2.1/V5-HIS-TOPO-CaBem3	CaBem3 expressed from the GAL1 promoter, 2μ *URA3*	This study
pGAL1.426-Bem3^{K1003A}-HA-His6	Bem3 ^{K1003A} expressed from GAL1 promoter, 2μ *URA3*	This study

Table S2. Strains used in this study		
Name	**Genotype**	**Source**
W303	Mat***a*** *ade2-1 his3-1 leu2-3112 trp1-1 ura3-1 can1-100*	Laboratory Strain
SEY6210	*MAT***a** *leu2-3,112 ura3-52 his3-200 trp1-901 lys2-801 suc2-9*	Laboratory Strain
BY4741	*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Invitrogen
RH4344 *(rcy1Δ)*	*Mat*a *yjl204c::kanMX his4 leu2 ura3 lys2 bar1*	(Wiederkehr et al., 2000)
RLY 3090	Mat***a*** *BEM3-GFP::HIS5 his3Δ1;leu2Δ0;met15Δ0;ura3Δ0*	(Huh et al., 2003)
*vps29*Δ	*vps29::kanMX* in W303	This study
Bem3-GFP (Diploid)	*MAT*a/α *his3Δ1/his3Δ1 leu2Δ0/leu2Δ0 lys2Δ0/lys2Δ0 met15Δ0/ met15Δ0 ura3Δ0/ura3Δ0* transformed with Yep13-GFP-Bem3	This study
*sla2*Δ [see Note1]	*MAT**a** sla2:: HisMX leu2-3,112 ura3-52 his3-200 trp1-901 lys2-801 suc2-9*	(Stefan et al., 2005)
BWY2595 (ent1Δ, ent2Δ, yap1801Δ, yap1802Δ) [see Note2]	*Matα leu2-3 ura3-52 his3-Δ200 trp1-Δ901 lys2-801 suc2-Δ9 ent1::LEU2 ent2::HIS3 yap1801::HIS3 yap1802::LEU2+ pBW0778 (ent1ENTH domain, CEN)*	Wendland lab
*bem3*Δ	*MAT**a** bem3::kanMX his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Hazbun Lab
DAO2C	Mat***a*** *ura3, leu2, met1,cdc3-6*	Haarer Lab
sec4-8	Mat***a*** sec4-8 ura 3-52	Novick Lab

Note1: *sla2Δ*: Sla2 knockout strain defective for endocytosis and proper actin cytoskeleton organization. Formation of actin comet tails associated with endocytic sites in these cells has been reported. (Kaksonen et. al., *Cell* **115**, 475–48 (2003).

Note2: Quadruple mutant strain with deletions of ENT1, ENT2, YAP1801, and YAP1802. The ENTH domain of Ent1 is expressed from a plasmid to maintain viability. These cells are defective in endocytosis and are inviable at 37^0C.

Table S3. Antibodies used in this study				
Host	**Antigen**	**Clone**	**Source**	**Dilution**
Mouse	HIS_6	6XHIS	Clontech, CA	1:5000
Mouse	HA	HA.11	Covance, NJ	1:2000
Rabbit	Bgl2	9937F	Schekman Lab	1:10,000
Mouse	Pma1	40B7	Encor Biotechnology Inc., FL	1:5000

SUPPLEMENTAL REFERENCES

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SUPPLEMENTARY FIGURE LEGENDS

Figure S1. (A) *sla2Δ* cells expressing GFP-Bem3 and RFP-tagged Ede1/Abp1, **(B**) GFP-Bem3-expressing *ent1Δ/ent2Δ /yap1801Δ/yap1802Δ* cells or **(C)** WT cells expressing GFP-Rga1, GFP-Cdc24 and GFP**x**3-Gic1 from their respective endogenous promoters, were imaged at 100X using a FITC filter. Scale bars: 5µm

Figure S2. GFP-Bem3 was expressed from the indicated promoters and copy numbers in W303 WT cells (except RL 3090=endogenous promoter, single copy) and grown overnight at 30°C in the appropriate selective media containing 2% glucose and imaged at 100X using a FITC filter. High levels of Bem3 expression from the *GAL1* promoter were achieved by growing cells in 2% galactose containing media for 4 hours prior to imaging. Arrows and arrowheads point to Bem3 at polarized cap and intracellular Bem3-containing compartments, respectively. Scale bar: 5µm

Figure S3. Intracellular compartments marked by Yep13-GFP-Bem3 were visualized in W303 WT cells expressing empty vector (EV) control or overexpressing Ypt31^{N126I} from a *GAL1* promoter. Cells were grown overnight in galactose containing selective media at 30°C and imaged at 100X using a FITC filter. The total area of Bem3-containing compartments is significantly larger in cells overexpressing Ypt31^{N126I} compared to EV control. The total area of the Bem3-containing compartment present within a cell was measured using ImageJ (see material and methods) and plotted as a function of bud/mother area ratio. Scale bar: 5µm

Figure S4. (A) Wild-type W303 cells expressingGFP-Bem3 were grown at 24°C (permissive temperature) overnight or shifted to 37°C for 6h (restrictive temperature) before imaging at 100X using a FITC filter. Quantification of the Bem3-compartment area was performed using ImageJ. No significant

difference in Bem3-compartment area was observed when wild-type cells were grown at the restrictive temperature.

(B) Cells overexpressing GFP-Bem3^{K1003A} (mutant unable to bind Cdc42) were imaged at 100X using a FITC filter and DIC.

(C) Cells expressing Sec4-GFP and transformed with either Bem3 full-length, Bem3 PX-PH fragment or empty vector were imaged at 100X using a FITC filter.

Figure S5. (A) Wild-type W303 cells expressing GFP-Sec4 and *Candida albicans* CaBem3 from a *GAL1* promoter were grown overnight in media containing 2% glucose at 30°C with shaking at 250 RPM, transferred to 2% galactose containing selective media for 4h and imaged at 100X using a FITC filter. Arrows point to clustered GFP-Sec4. Scale bar: 5µm. (B) *sec4-10* cells expressing GFP-Bem3 were imaged at 100X using a FITC filter.

Supplementary Movie 1. Dynamics of the Bem3-containing compartment.

W303 yeast cells transformed with Yep13-GFP-Bem3 (2µ, *LEU2*) were grown overnight in selective media, spotted on media-embedded agarose beds the next morning and imaged with a FITC filter using a 100X objective at 10 second intervals. The cell outline is marked. Movie playback rate: 7 frames/second. Scale bar: 5µm.

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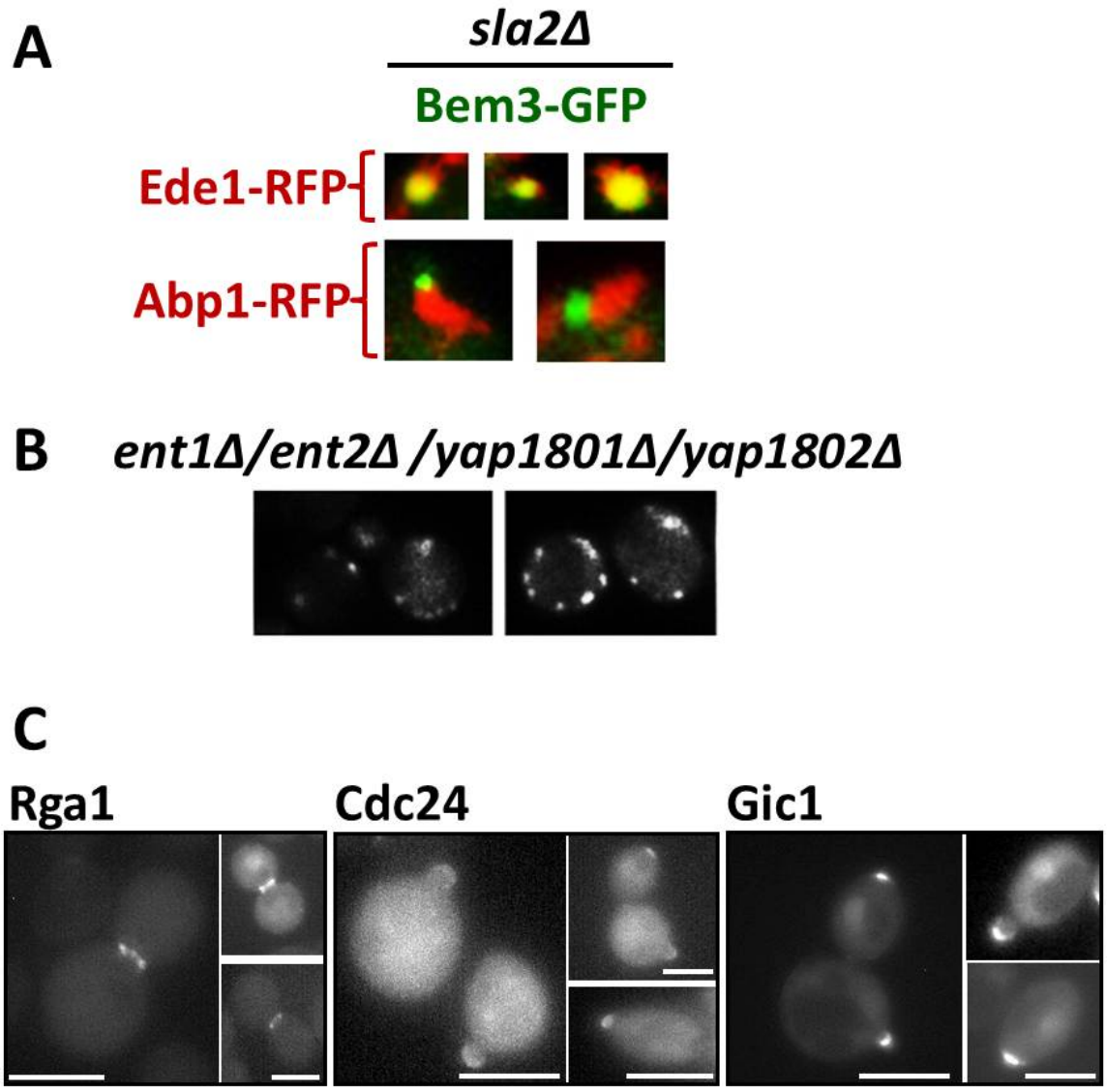
Supplementary Figure 1



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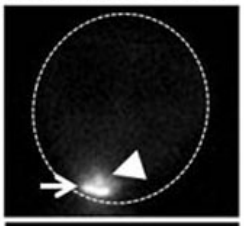
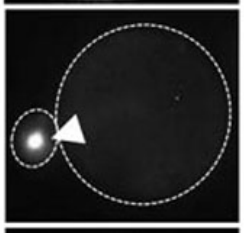
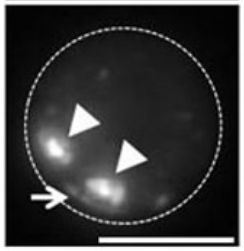
Supplementary Figure 2

Promoter	Copy number	Bem3-GFP
Endogenous	Single	
Endogenous	High	
GAL1	High	

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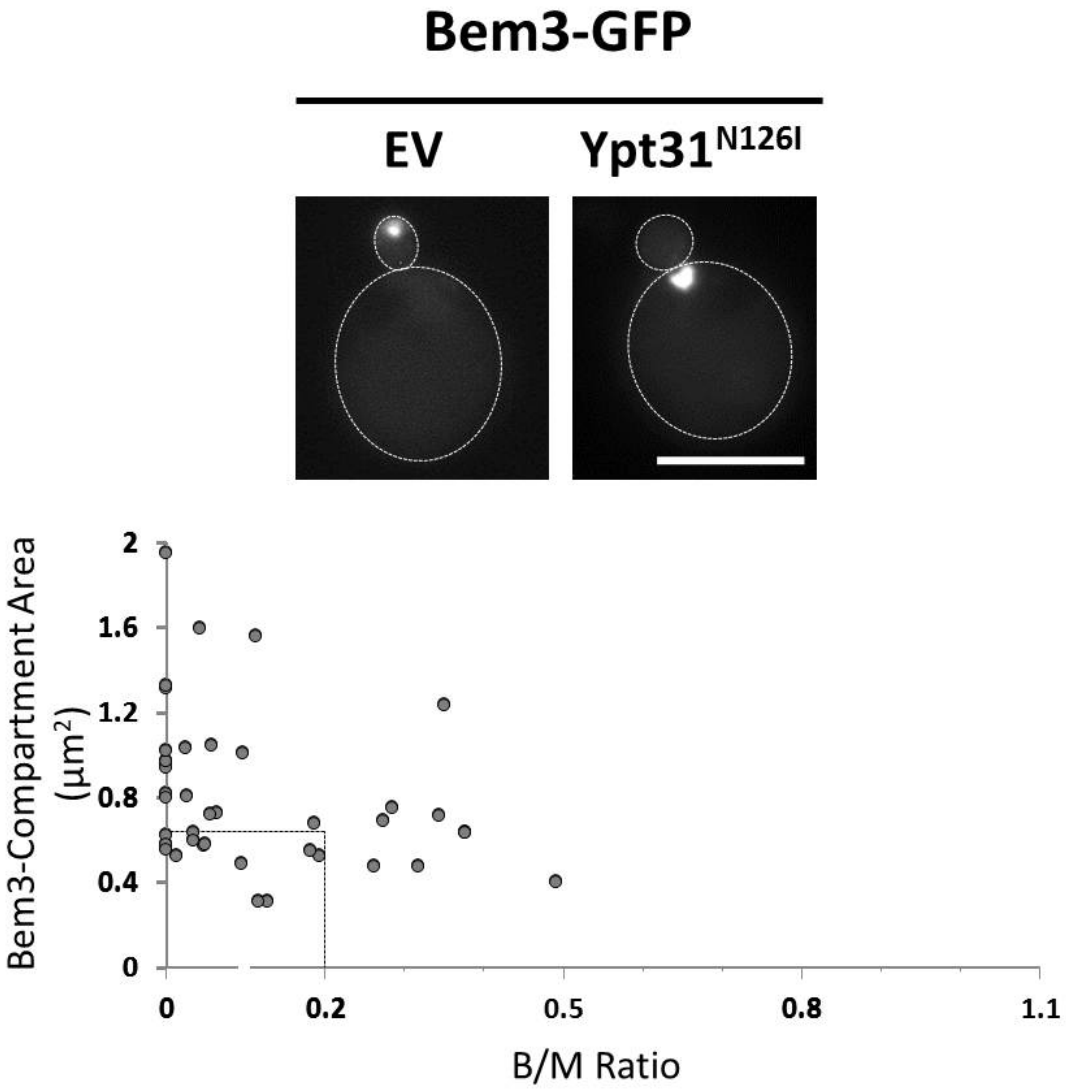
Supplementary Fig. 3



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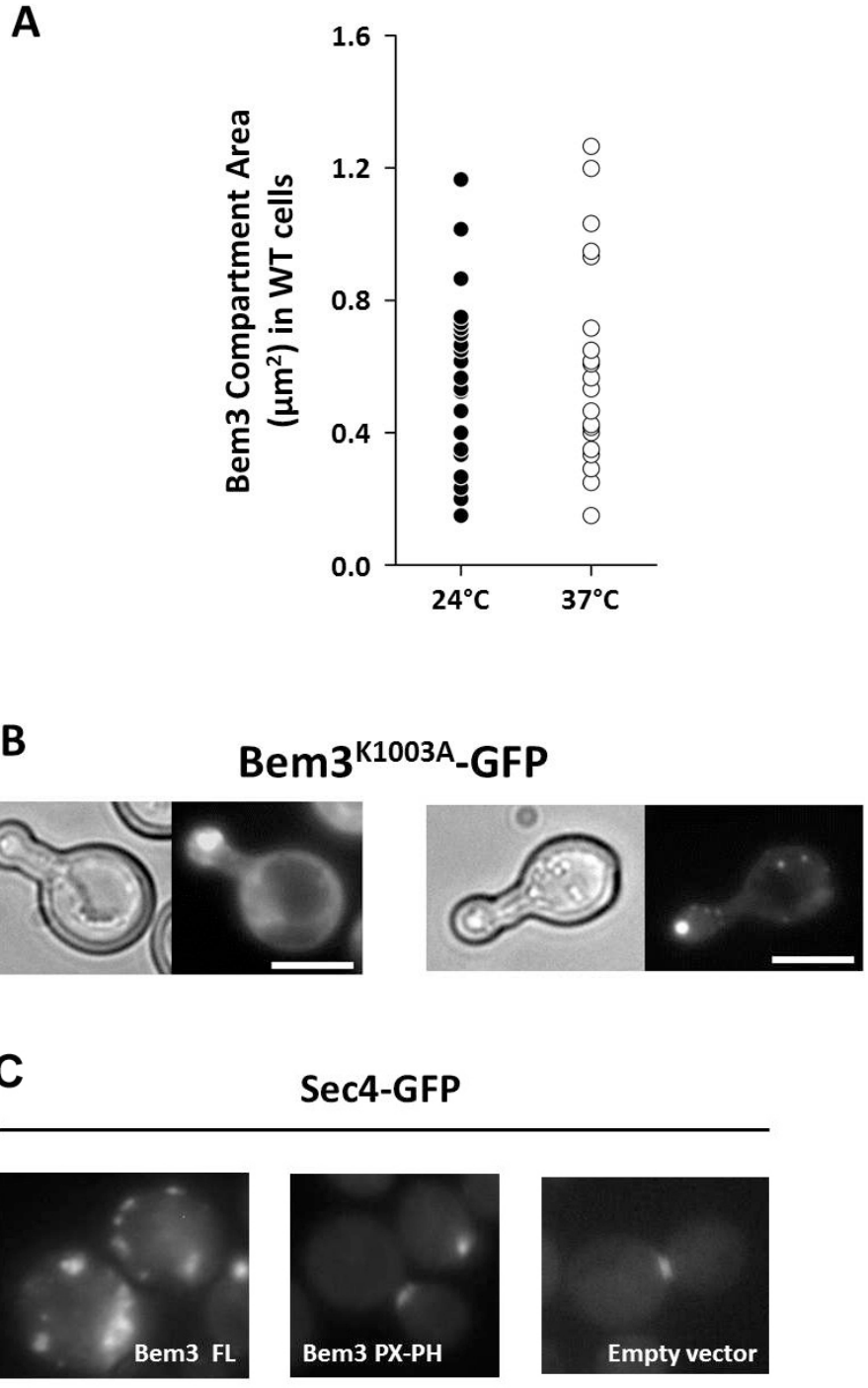
Supplementary Figure 4



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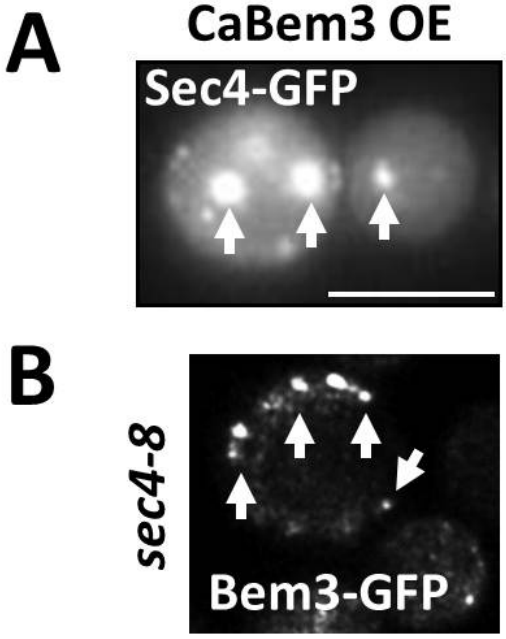
Supplementary Figure 5



Movie 1. Dynamics of the Bem3-containing compartment. W303 yeast cells transformed with Yep13-GFP-Bem3 (2μ, *LEU2*) were grown overnight in selective media, spotted on media-embedded agarose beds the next morning and imaged with a FITC filter using a 100× objective at 10 second intervals. The cell outline is marked. Movie playback rate: 7 frames/second. Scale bar: 5 μm.