Moreira et al., http://www.jcb.org/cgi/content/full/jcb.201202097/DC1

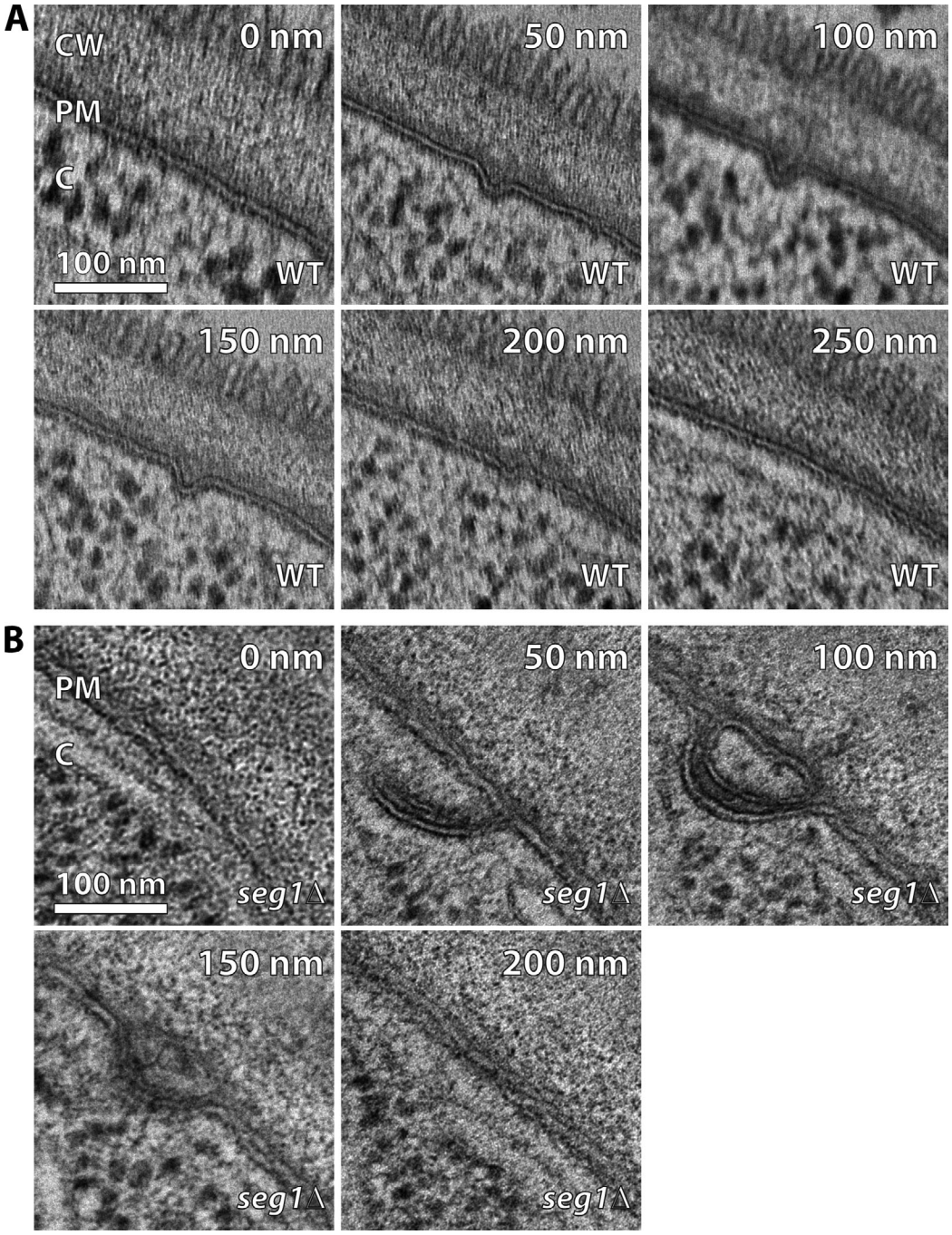

Figure S1. **Plasma membrane morphology in wild-type and *seg1Δ* cells.** (A) Electron micrographs of sequential 50-nm sections from a wild-type (WT) cell following a plasma membrane furrow ~200 nm long. (B) As in A, but following a large, irregular plasma membrane invagination in a *seg1*Δ cell. CW, cell wall; PM, plasma membrane; C, cytoplasm.

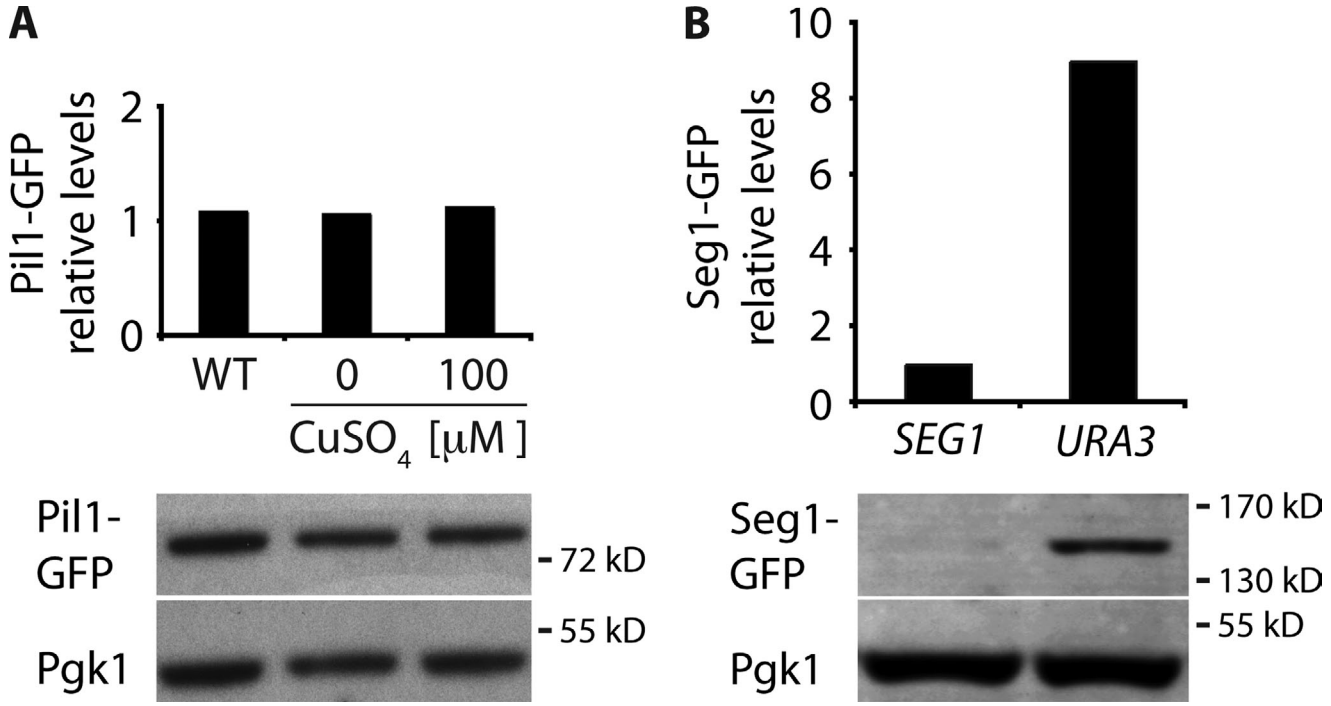

Figure S2. **Pil1-GFP and Seg1-GFP levels in Seg1 overexpression strains.** (A) Pil1-GFP levels are unaffected by Seg1 overexpression. Western blots and quantification of Pil1-GFP levels relative to Pgk1 in cells expressing Pil1-GFP (WT) and cells additionally expressing Seg1 from the *CUP1* promoter are shown. The latter were grown overnight in the presence of 0 or 100 μM $CuSO_4$. Pil1-GFP levels were normalized to WT. (B) Seg1-GFP expression from the *URA3* locus produces elevated levels. Western blots and quantification of Seg1-GFP levels relative to Pgk1 in cells expressing Seg1-GFP from the *SEG1* locus (*SEG1*) and cells lacking endogenous Seg1 but expressing Seg1-GFP from the *URA3* locus (*URA3*) are shown. The experiments shown in A and B were each performed once.

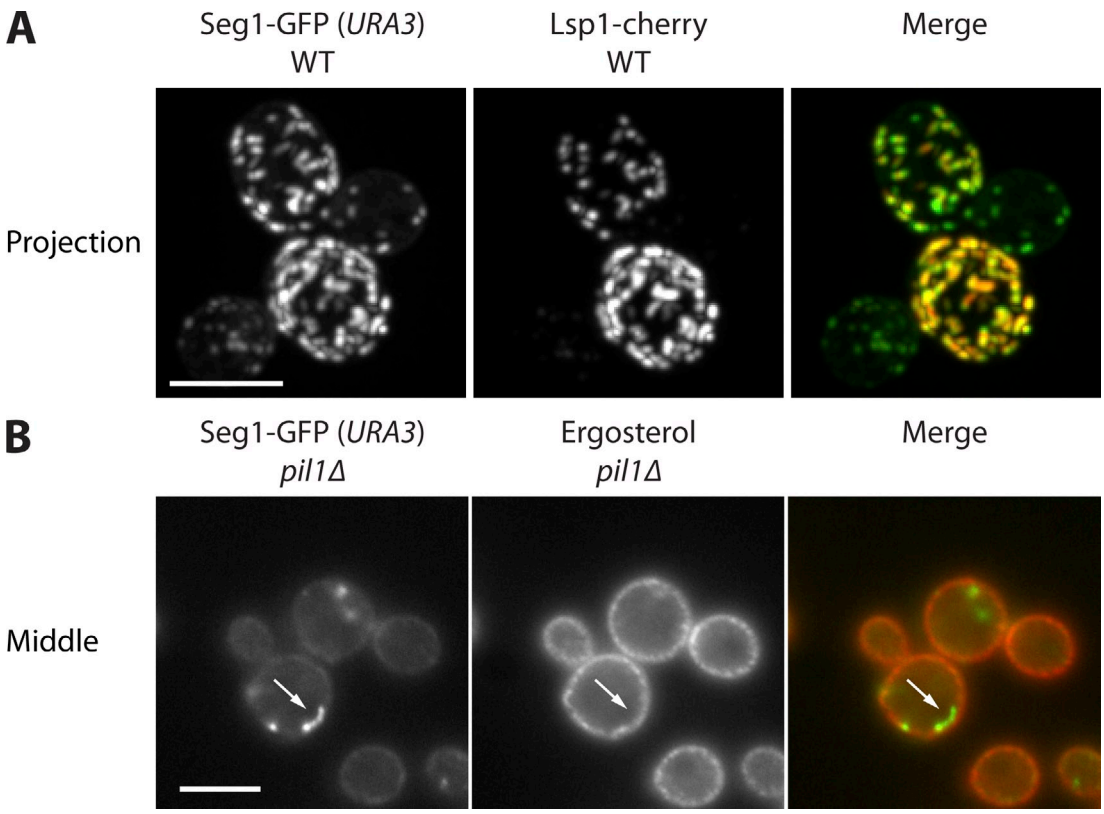

Figure S3. **Localization of Lsp1 and ergosterol to Seg-GFP rods.** (A) Projections from confocal stacks of wild-type (WT) cells expressing Lsp1-cherry, lacking endogenous Seg1, and expressing Seg1-GFP from the *URA3* locus. (B) Epifluorescence images of *pil1*Δ cells lacking endogenous Seg1, expressing Seg1-GFP from the *URA3* locus, and stained with filipin to visualize ergosterol. The arrows indicate a Seg1-GFP rod. Bars, 5 µm.

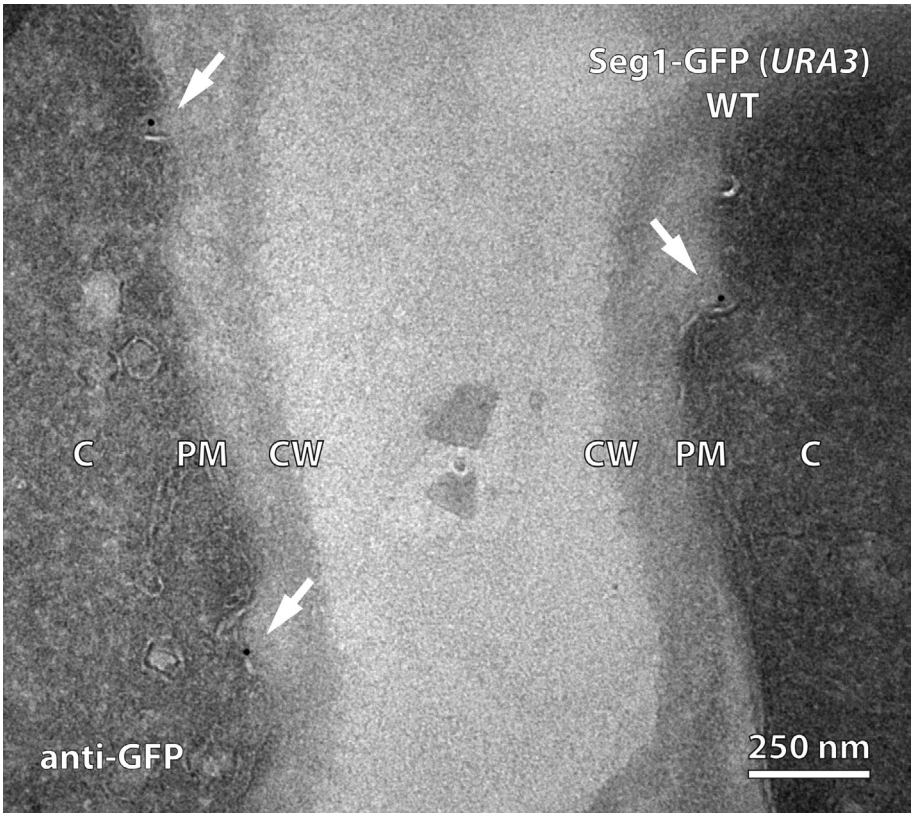

Figure S4. **Immunogold labeling of GFP in cells overexpressing Seg1-GFP.** Electron micrographs of two adjacent wild-type (WT) cells lacking endogenous Seg1, expressing Seg1-GFP from the *URA3* locus, and labeled with anti-GFP antibody and gold-conjugated protein A. Arrows indicate gold particles. Note that all gold particles are found in plasma membrane invaginations. CW, cell wall; PM, plasma membrane; C, cytoplasm.

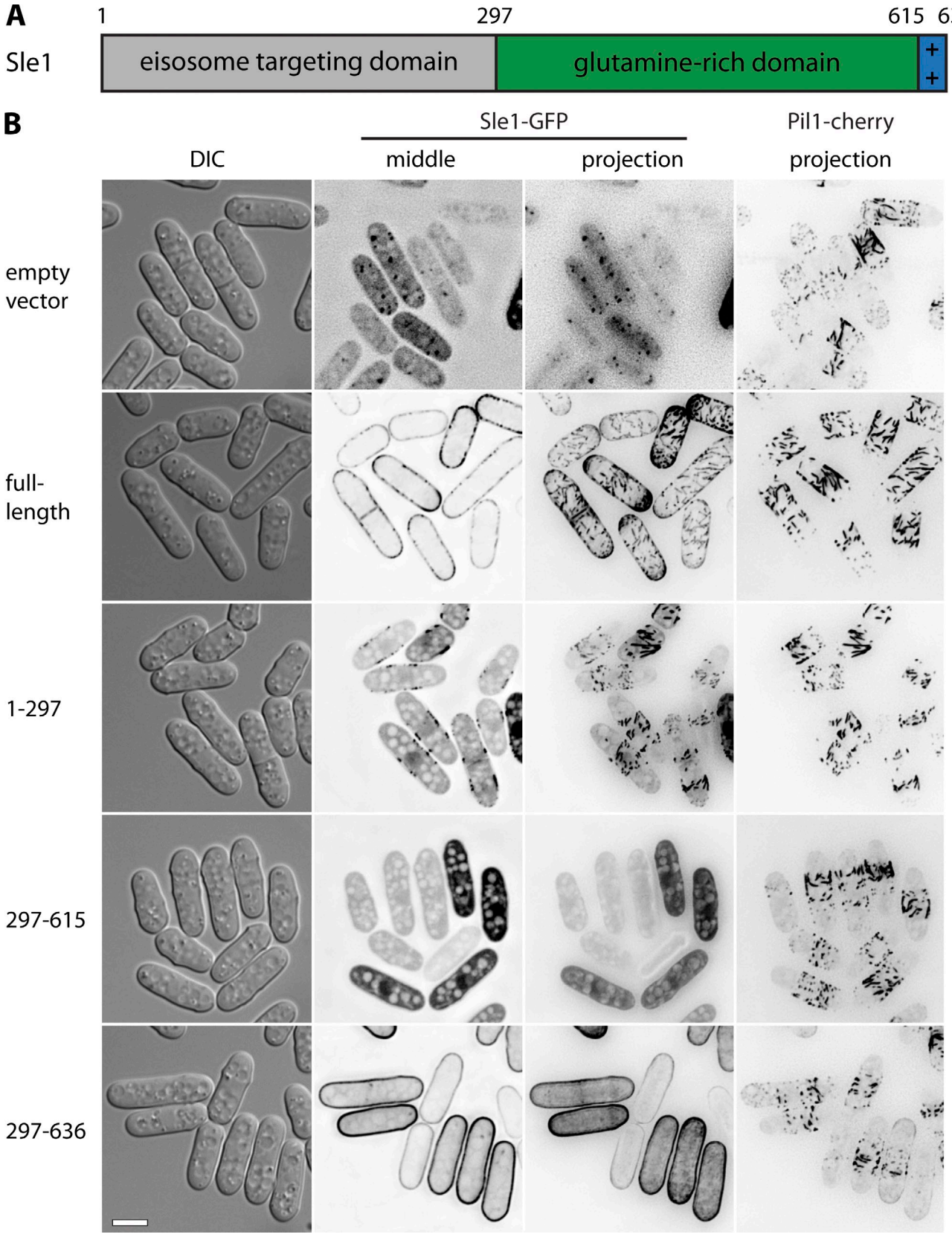

Figure S5. **Domain structure and eisosome targeting of Sle1/SPAC1A6.07.** (A) Schematic of the domain structure of *S. pombe* Sle1/SPAC1A6.07. Plus signs denote the polybasic C terminus. (B) Differential interference contrast, middle focal plane, and maximum projection images from confocal stacks of *pil1-cherry sle1Δ* cells expressing the indicated Sle1-GFP constructs. Projections are from deconvolved z planes in the top half of cells. Bar, 5 µm.

Table S1. ***S. cerevisiae* strains used in this study**

Strain	Genotype
CRY1	*MATα ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*
CRY2	*MATa ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*
TWY110[a]	*MATa PIL1-GFP::HIS3*
TWY113[a]	*MATα LSP1-GFP::HIS3*
TWY70[a]	*MATa his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*
TWY1118	*MATa SEG1-TEV-GFP::HIS3 his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*
KEM120	*MATa PIL1-GFP::HIS3 seg1Δ::NAT^R*
KEM121	*MATa seg1Δ::NAT^R*
KEM18	*MATα pil1Δ::LEU2*
KEM122	*MATα SEG1-GFP::HIS3 PIL1-cherry::KAN^R*
KEM130	*MATα SEG1-GFP::HIS3*
KEM124	*MATα SEG1-GFP::HIS3 pil1Δ::LEU2*
KEM134	*MATα SEG1-GFP::HIS3 pil1Δ::LEU2 lsp1Δ::NAT^R*
KEM153	*MATα seg1Δ942-GFP::TRP1*
KEM154	*MATα seg1Δ942-GFP::TRP1 PIL1-cherry::KAN^R*
KEM159	*MATα seg1Δ942-GFP::TRP1 pil1Δ::KAN^R lsp1Δ::HIS3*
KEM161	*MATa PIL1-GFP::HIS3 seg1Δ942::KAN^R*
KEM126	*MATa PIL1-GFP::HIS3 NAT^R::P$_{CUP1}$-SEG1*
KEM127	*MATα NAT^R::P$_{CUP1}$-SEG1-GFP::HIS3*
KEM128	*MATa ura3::SEG1-GFP seg1Δ::NAT^R*
KEM132	*MATa ura3::SEG1-GFP seg1Δ::NAT^R pil1Δ::LEU2*
KEM135	*MATa ura3::SEG1-GFP seg1Δ::NAT^R pil1Δ::LEU2 lsp1Δ::KAN^R*
KEM141	*MATa ura3::SEG1-GFP seg1Δ::NAT^R PIL1-cherry::KAN^R*
KEM148	*MATa ura3::SEG1-GFP LSP1-cherry:KAN^R*
KEM146	*MATa ura3::SEG1-GFP seg1Δ::NAT^R pil1Δ::LEU2 LSP1-cherry::KAN^R*

All strains used in this study were derived from CRY1 or CRY2. These strains are wild-type W303 (ATCC 201238 and 201240). The only exception is TWY1118, which was derived from TWY70. TWY70 is wild-type BY4742 (ATCC 201389).
[a]Walther et al., 2006.

Reference

Walther, T.C., J.H. Brickner, P.S. Aguilar, S. Bernales, C. Pantoja, and P. Walter. 2006. Eisosomes mark static sites of endocytosis. *Nature*. 439:998–1003. http://dx.doi.org/10.1038/nature04472