Supporting Information

Diaz et al. 10.1073/pnas.1011105107

SI Experimental Procedures

For fatty acid (FA) analysis, total fatty acids from 10 optical densities at 600 nm (OD600) of yeast were extracted and were converted to methyl esters as described (1). FA species were separated according to chain length and degree of saturation by gas-liquid chromatography and were identified by retention time. The molar amount of each species was measured by using flame ionization detector (1).

1. Miyazaki M, Kim HJ, Man WC, Ntambi JM (2001) Oleoyl-CoA is the major de novo product of stearoyl-CoA desaturase 1 gene isoform and substrate for the biosynthesis of the Harderian gland 1-alkyl-2,3-diacylglycerol. *J Biol Chem* 276:39455–39461.

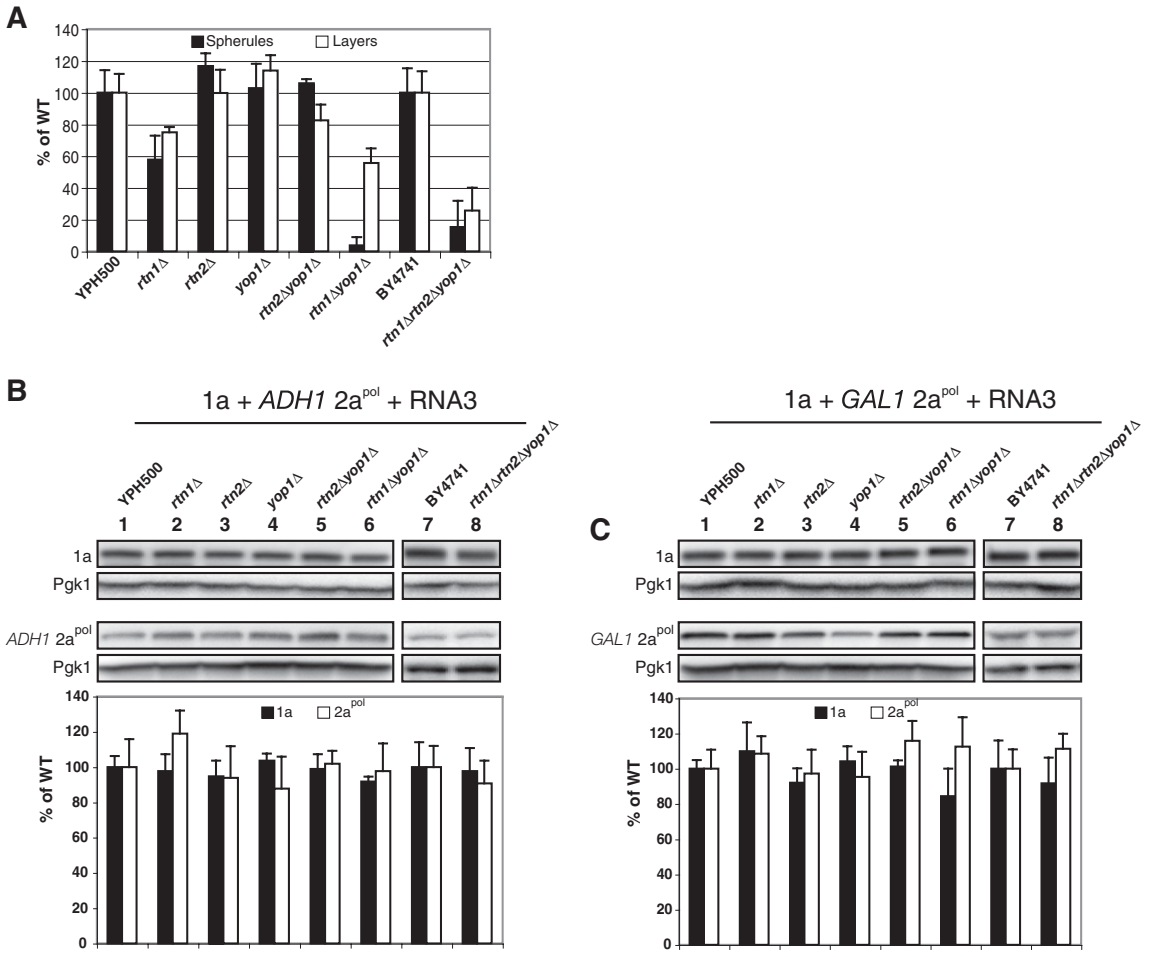

Fig. S1. BMV 1a and 2a levels remain constant in absence of RHPs, whereas negative-strand RNA synthesis is inhibited. (*A*) Detection of negative-strand BMV RNA synthesis in yeast coexpressing 1a, RNA3, and either *ADH1* $2a^{pol}$ (spherules) or *GAL1* $2a^{pol}$ (layers). Total RNA extracts were obtained from wt YPH500 yeast (lane 1), *rtn1*Δ (lane 2), *rtn2*Δ (lane 3), *yop1*Δ (lane 4), *rtn2*Δ*yop1*Δ (lane 5), *rtn1*Δ*yop1*Δ (lane 6), wt BY4741 (lane 7), or *rtn1*Δ*rtn2*Δ*yop1*Δ (lane 8) yeast expressing indicated BMV components. Accumulation of negative-strand subgenomic RNA4 was detected by Northern blotting using probes specific to BMV RNAs. Equal loading of total RNA was verified by probing for 18S ribosomal RNA. Accumulation of BMV 1a and *ADH1* $2a^{pol}$ (*B*) or *GAL1* $2a^{pol}$ (*C*) in wt, single-, double- or triple-deletion yeast cells was measured by Western blot analysis. Total proteins were extracted from equal numbers of yeast cells and analyzed by SDS/PAGE. Equal loading of total protein was verified by measuring Pgk1p levels. All values represent the mean of at least three independent experiments.

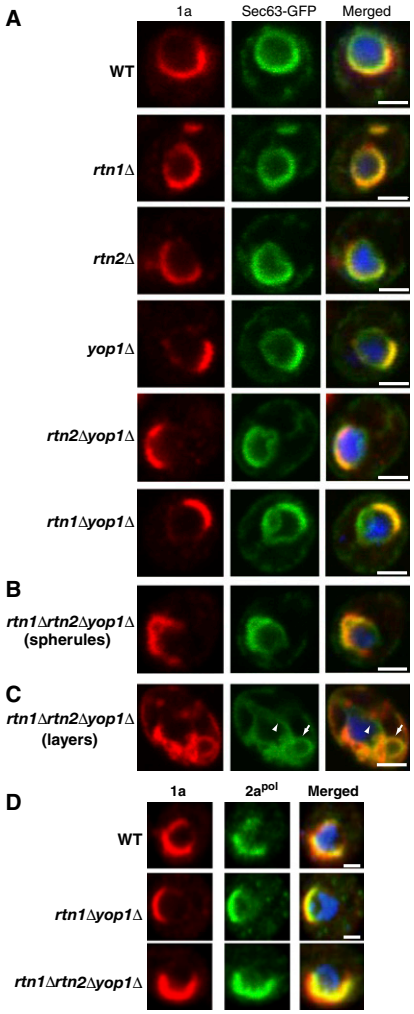

Fig. S2. BMV 1a retains ER membrane localization in reticulon deletion strains. (*A*) Confocal fluorescence images of wt, single-, and double-deletion mutant yeast cells coexpressing the ER marker Sec63-GFP and 1a plus $2a^{pol}$. Representative images for 1a (*Left*; red), Sec63-GFP (*Center*; green), and merged signals (*Right*) are shown. DNA was stained with DAPI (blue). (*B*) *rtn1*Δ*rtn2*Δ*yop1*Δ yeast cells coexpressing the ER marker Sec63-GFP and 1a plus *ADH1* $2a^{pol}$ (spherules). (*C*) *rtn1*Δ*rtn2*Δ*yop1*Δ yeast cells coexpressing the ER marker Sec63-GFP and 1a plus *GAL1* $2a^{pol}$ (layers). Arrowhead indicates nuclear membrane, whereas arrow points out 1a signal extending away from the nuclear membrane. (D) Localization of $2a^{pol}$ in WT, *rtn1*Δ, or *rtn1*Δ*yop1*Δ yeast. Representative images for 1a (*Left*; red), $2a^{pol}$ (*Center*; green), and merged signals (*Right*) are shown. DNA was stained with DAPI (blue). (Scale bars: 2 μm.)

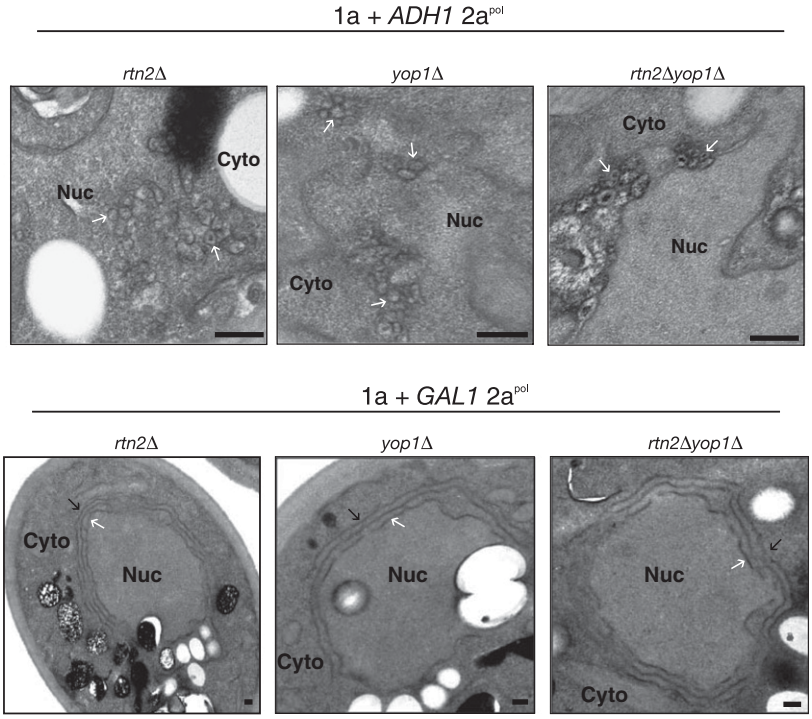

Fig. S3. BMV-induced replication compartments in RHP deletion strains. Representative electron micrographs of *rtn2*Δ, *yop1*Δ, or *rtn2*Δ*yop1*Δ yeast cells expressing BMV-induced spherules (1a + *ADH1* $2a^{pol}$; *Upper*) or double-membrane layers (1a + *GAL1* $2a^{pol}$; *Lower*). Nuc, nucleus; Cyto, cytoplasm. (Scale bars: 200 nm.)

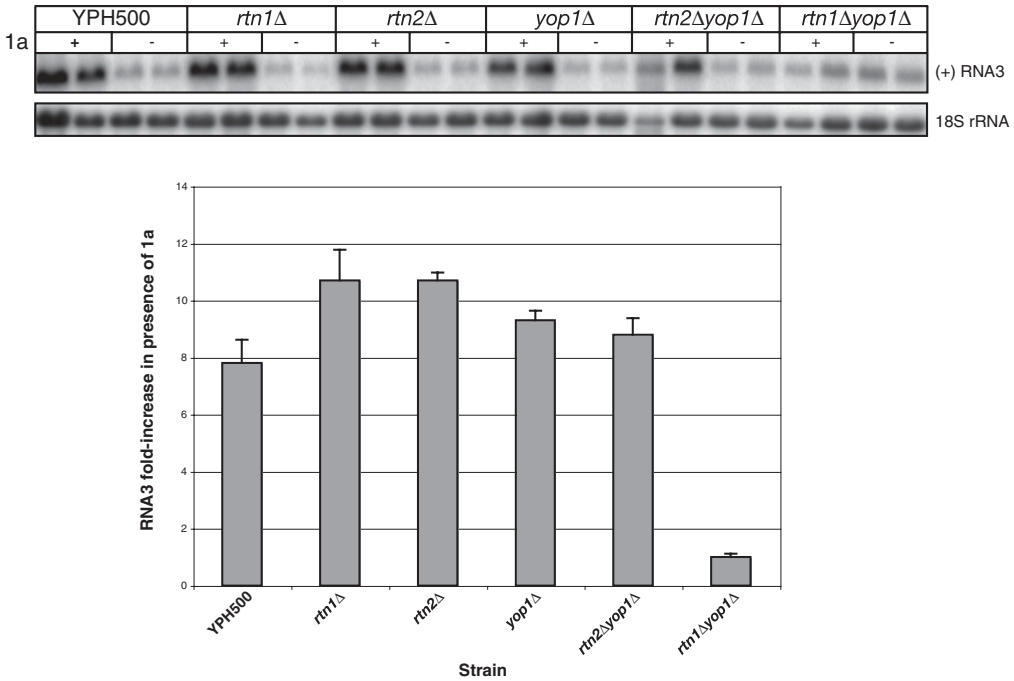

Fig. S4. BMV 1a fails to stabilize RNA3 in *rtn1*Δ*yop1*Δ yeast. Shown is the Northern blot analysis of 1a stimulation of RNA3 accumulation in the absence of $2a^{pol}$. RNA3 levels in the presence and absence of 1a were measured in wt and single- and double-deletion mutant yeast. Fold increase in the presence of 1a is indicated. Equal loading of total RNA was verified by probing for 18S ribosomal RNA.

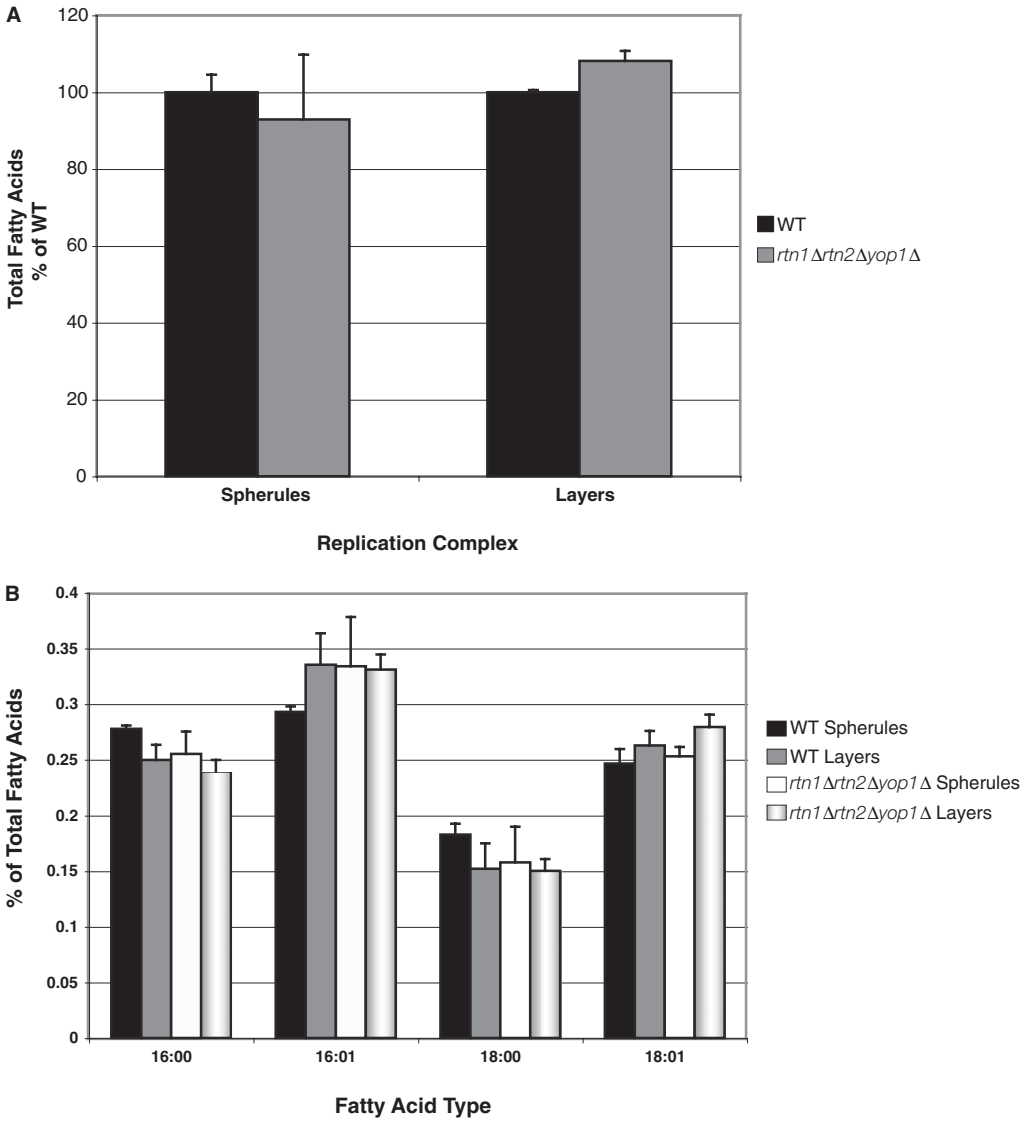

Fig. S5. Yeast fatty acid levels in WT and reticulon triple deletion strain. Total accumulation and composition of yeast fatty acids (FAs) in yeast expressing BMV induced replication compartments in the presence or absence of the reticulons and Yop1p. WT yeast and mutant yeast were grown in defined, galactose-containing medium to midlog phase. Total FAs were extracted, converted to methyl esters, and separated by chain length and degree of saturation by using gas-liquid chromatography. The molar amount of 16:0, 16:1, 18:0, and 18:1 FA was measured by using flame ionization detector and was normalized by using 15:0 and the OD_{600} of each yeast culture. (*A*) Total FA per cell, shown as a percentage of the total FA per cell of wt yeast expressing 1a alone (spherules) and 1a plus *GAL1* $2a^{pol}$ (layers). (*B*) Relative levels of 16:0, 16:1, 18:0, and 18:1 FA in each sample, as a molar percentage of total FA. Yeast genotype (WT or *rtn1*Δ*rtn2*Δ*yop1*Δ) and the presence of spherules or layers is indicated. Each histogram shows the averages of three experiments and SDs.

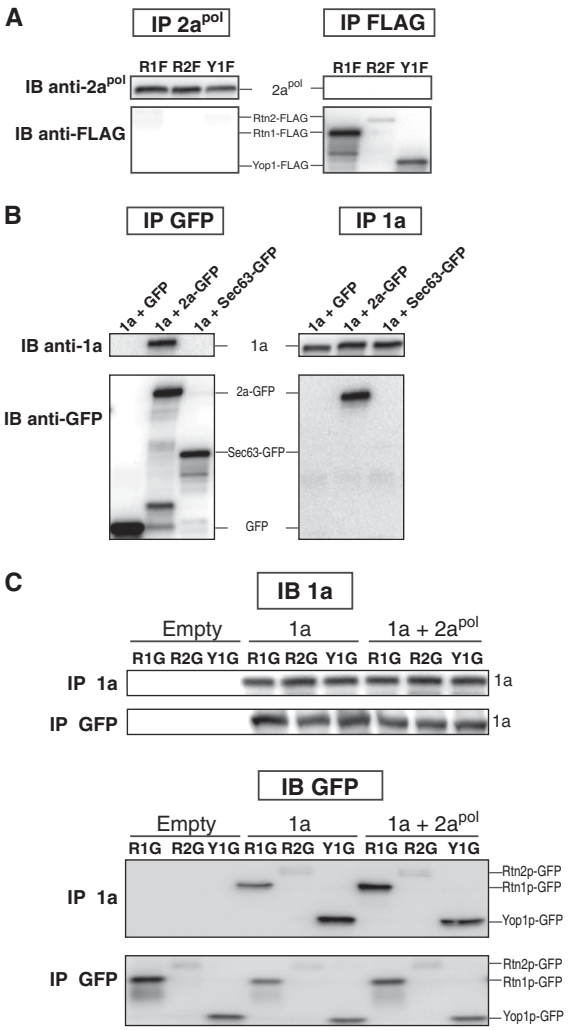

Fig. S6. The 1a–RHP interaction is specific. (*A*) Yeast were transformed with plasmids encoding the FLAG-tagged RHPs and *GAL1* 2a^{pol}. Cells were lysed, and the cleared lysates were subjected to immunoprecipitation by using anti-2a or anti-FLAG antibodies (IP 2a and IP FLAG, respectively). The resulting immunoprecipitates were analyzed on 4–15% SDS/PAGE and immunoblotted by using anti-2a or anti-FLAG antibodies. The positions of 2a, Rtn1p-FLAG, Rtn2p-FLAG, and Yop1p-FLAG are shown. (*B*) Control experiment to rule out membrane-dependence of 1a–RHP interaction. Wt yeast expressing free GFP, 2a^{pol}-GFP, or Sec63p-GFP and BMV 1a were lysed, and cleared lysates were subjected to immunoprecipitation by using anti-1a or anti-GFP antibodies (IP 1a and IP GFP). Reactions were analyzed on 4–15% SDS/PAGE and immunoblotted by using anti-1a or anti-GFP antibodies. (*C*) Wt yeast or yeast cells expressing Rtn1p-GFP, Rtn2p-GFP, or Yop1p-GFP from their endogenous promoters and chromosomal loci were transformed with empty plasmids, BMV 1a alone, or 1a plus *GAL1* 2a^{pol} (layers). Yeast cells were processed as described above. The resulting immunoprecipitates were analyzed by SDS/PAGE and immunoblotted by using anti-1a or anti-GFP antibodies. The positions of 1a, Rtn1p-GFP, Rtn2p-GFP, Yop1p-GFP, and GFP are shown on the right.

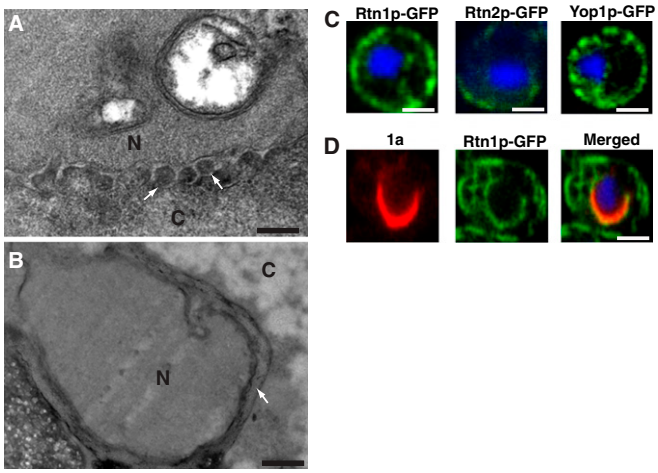

Fig. S7. Fusing GFP to reticulon and Yop1 proteins inhibits spherule formation. (*A*) EM of yeast expressing 1a and wt Rtn1p. Arrow points to spherular structures. (Scale bar: 200 nm.) (*B*) EM of yeast expressing Rtn1-GFP from its endogenous promoter and chromosomal loci transformed BMV 1a. Arrow points to layer-like structure surrounding the nucleus. (Scale bar: 200 nm.) Localization of GFP-tagged reticulon and Yop1 proteins in yeast transformed with empty plasmids (*C*) or BMV 1a (red) (*D*). DNA was stained with DAPI (blue). (Scale bars: 2 μm.)

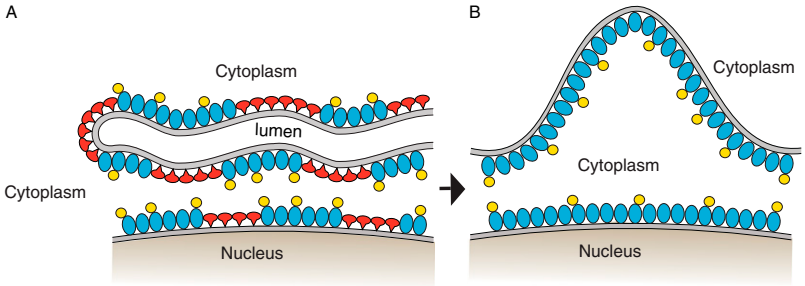

Fig. S8. Models for the potential role of RHPs in the formation and/or maintenance of BMV induced replication compartments. (*A*) Just as they stabilize ER tubules, RHPs might help to initiate each new layer by forming arc-like scaffolds to stabilize the positively curved ends (half tubules) where each perinuclear ER layer folds over to begin the next outer layer. Because RHPs were also recruited to the intermembrane spaces, the self-interacting 1a and RHP proteins might cluster in the concave and convex membrane regions, respectively, neutralizing each other's induced membrane curvature to yield relatively flat sheets. (*B*) The absence of RHPs produces aberrant single layers with prominent, negatively curved bulges consistent with unbridled 1a-induced curvature.