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Supplementary Materials for

Organelle luminal dependence of (+)strand RNA virus replication reveals a hidden druggable target

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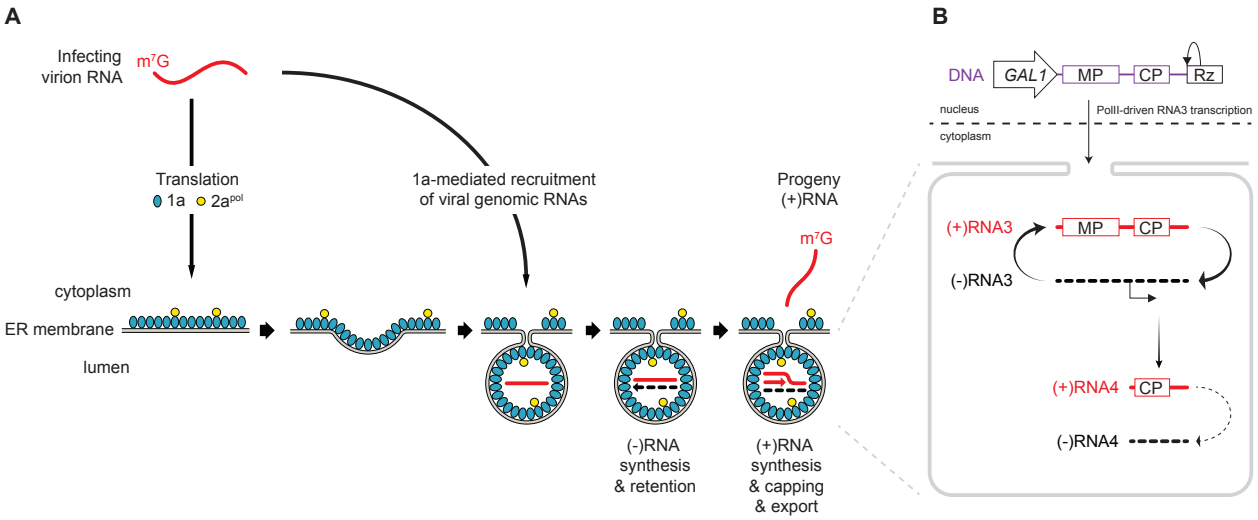

fig. S1. Model for BMV RNA replication complex assembly and function. (**A**) 1a as the master organizer of BMV RNA replication complex assembly. Please see Introduction for additional details. (**B**) Schematic of BMV genomic RNA replication and subgenomic mRNA transcription pathways in 1a-induced replication compartments. Positive-strand subgenomic RNA4 is initiated from an internal promoter (bent arrow) in negative-strand RNA3. Negative-strand RNA4 is a dead-end product of positive-strand RNA4 templates. In the experiments presented here, these viral RNA synthesis pathways were initiated from an initial RNA3 template provided by transcription from a DNA plasmid, and driven by DNA-directed co-expression of viral RNA replication proteins 1a and $2a^{pol}$. MP (movement protein) and CP (capsid protein) designate RNA3 open reading frames.

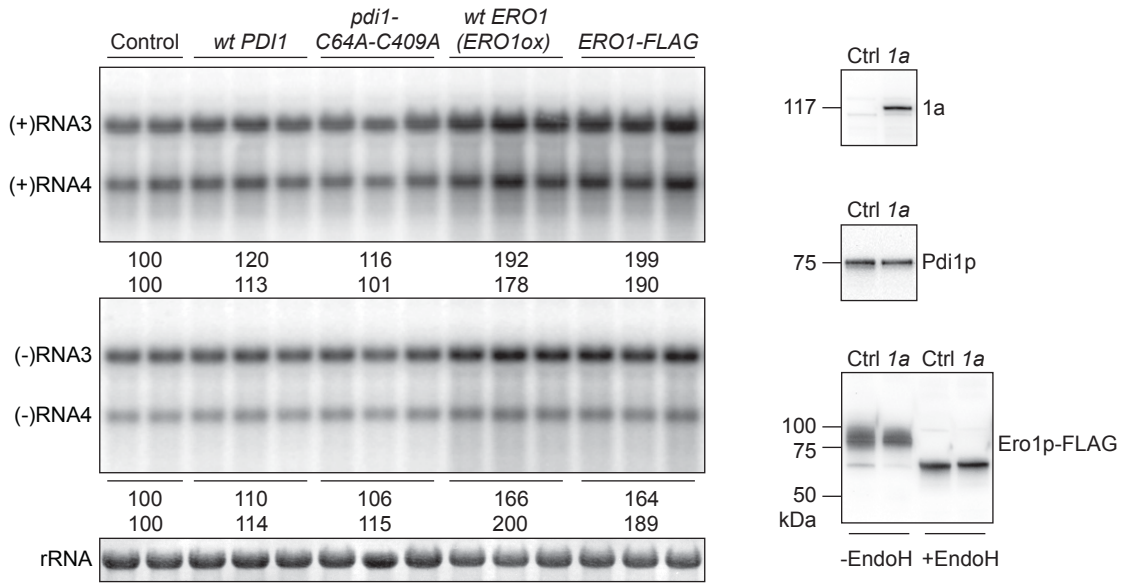

fig. S2. Ero1p-FLAG stimulates BMV RNA replication and is glycosylated in control and 1a-expressing cells. Wt yeast strain YPH500 was transformed with plasmids encoding 1a, $2a^{pol}$, RNA3, and either an empty plasmid (Control) or plasmids encoding either wt *PDI1*, inactive *pdi1-C64A-C409A* mutant (*49*), wt *ERO1 (ERO1ox),* or *ERO1-FLAG*. *PDI1* encodes protein disulfide isomerase which transfers disulfides from Ero1p to substrate proteins in the ER lumen (*50*) and overexpressing either wt or mutant form of Pdi1p had little effect on BMV RNA replication. Total RNA was extracted and accumulation of RNA3 and RNA4 of both polarities was measured by Northern blotting. 25*S* rRNA serves a loading control. Total protein was extracted from yeast expressing Ero1p-FLAG alone, or with 1a. The samples then were subjected to endoglycosidase H (EndoH) treatment. Glycosylated Ero1p-FLAG migrates slower than deglycosylated Ero1p-FLAG (hypothetical molecular weight (Da) 66027.51, without any signal sequence cleavage) (*16, 17*).

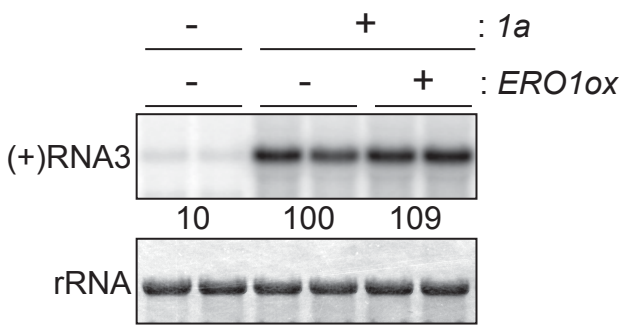

fig. S3. Effect of *ERO1* overexpression on 1a-increased RNA3 accumulation. Northern blot analysis of (+)RNA3 isolated from yeast cells expressing RNA3 only, with 1a, and with 1a plus Ero1p. 25*S* rRNA serves as a loading control.

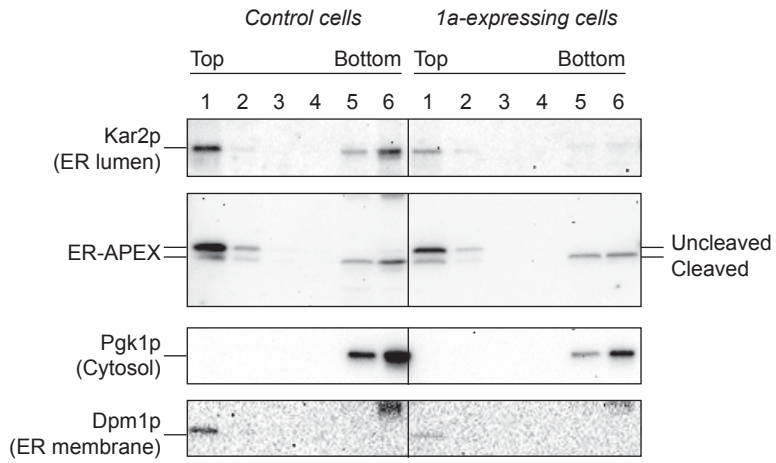

fig. S4. Subcellular fractionation analysis of ER-APEX. Lysates of yeast cells expressing ER-APEX alone, or with 1a were loaded under flotation gradients. After centrifugation, six fractions were collected from the top to the bottom of the gradient. Each fraction was analyzed by Western blotting using antibodies against indicated proteins (*34*). Note that the distribution patterns of Kar2p and ER-APEX are similar in control and 1a-expressing cells, and that those proteins, especially those with hydrophobic signal sequences cleaved, are also present in the bottom fractions due to partial leaking of free secretory proteins from the ER lumen upon cell disruption (*51*).

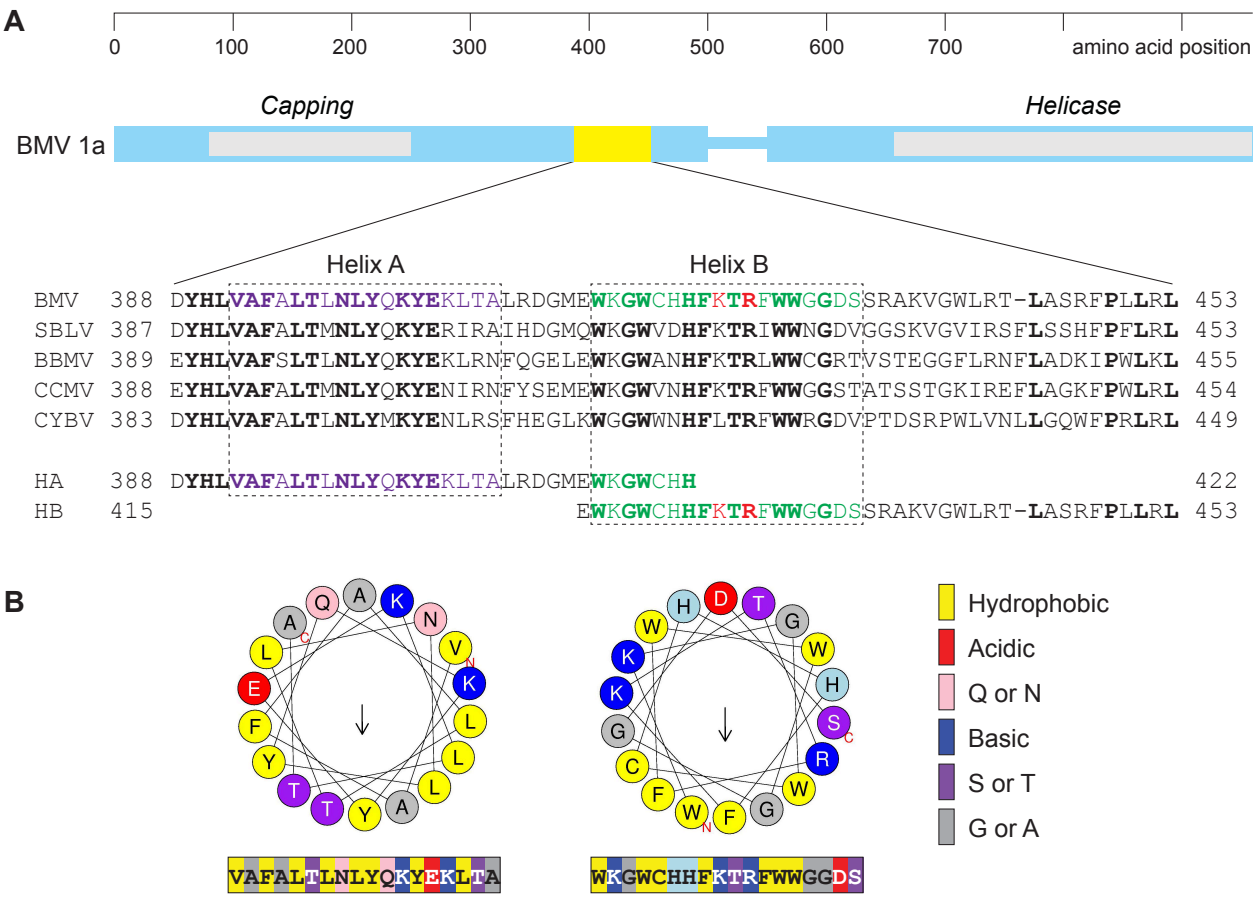

fig. S5. Evolutionarily conserved helix A and B sequences. (**A**) The upper schematic represents the BMV 1a protein, with conserved enzymatic domains shown as gray shaded areas. The sequence alignment below the schematic compares the BMV 1a sequence with analogous 1a sequences of other *Bromoviridae* members, showing strong evolutionary conservation of two amphipathic sequences. Purple letters indicate helix A, the previously-characterized, primary 1a-ER interaction site (*34*). Green letters indicate a second amphipathic helix, helix B, predicted by Ahola and Karlin (*35*). At the bottom, HA and HB correspond to the two peptide sequences used in Fig. 3. The two red letters in helix B indicate the amino acid residues, K424 and R426, whose mutations give rise to the viroporin-defective 1a mutant phenotypes shown in Fig. 4. (**B**) Helical wheel representations of helix A and B sequences

plotted by HeliQuest (*52*). Arrows represent the magnitude and direction of the hydrophobic moment, i.e., the mean vector sum of the hydrophobicities of the side chains of each helix.