Murley et al., http://www.jcb.org/cgi/content/full/jcb.201502033/DC1

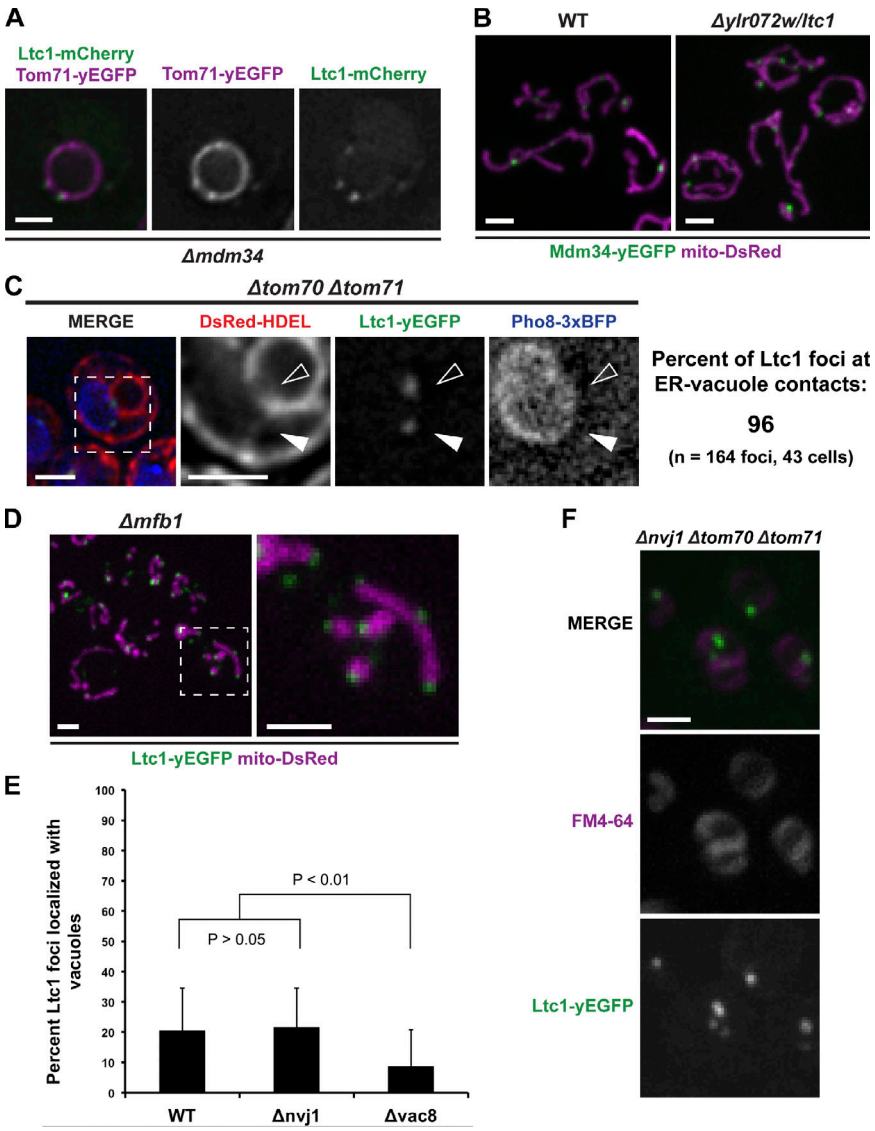

Figure S1. **Characterization of Ltc1 localization in cells.** (A) Ltc1 localization to mitochondria is ERMES independent. *Δmdm34* cells expressing Ltc1-yEmCherry and Tom71-yEGFP were grown to mid-log phase and imaged as described in "Fluorescence microscopy." Ltc1 foci predominately localize with Tom71 (87.5%; *n* = 27 cells), similar to Ltc1 localization to ER–mitochondria contacts in WT cells (Fig. 1 E). (B) ERMES foci formation is independent of Ltc1. WT and *Δltc1* cells expressing Mdm34-yEGFP and mtDsRed were grown to mid-log phase and imaged as described in "Fluorescence microscopy." (C) Nonmitochondrial Ltc1 foci localize to ER–vacuole contact sites in *Δtom70 Δtom71* cells. *Δtom70 Δtom71* cells expressing Ltc1-yEGFP, ER-targeted DsRed (DsRed-HDEL), and the vacuole marker Pho8-3×BFP were grown to mid-log phase and imaged as described in "Fluorescence microscopy." The dashed boxed marks a region enlarged and separated into grayscale images for each channel to the right of the merged imaged. Open arrowheads indicate NVJs; closed arrowheads indicate other ER–vacuole contact sites. (D) Ltc1-yEGFP localization is independent of Mfb1. *Δmfb1* cells expressing Ltc1-yEGFP and mtDsRed were grown to mid-log phase and imaged as described in "Fluorescence microscopy." Images represent a maximum intensity z-projection. The dashed box denotes the enlarged region shown to the right. (E and F) Localization of Ltc1-yEGFP to vacuoles is not dependent on Nvj1. WT, *Δvac8*, *Δnvj1*, and *Δnvj1 Δtom70 Δtom71* cells expressing Ltc1-yEGFP were grown overnight in YPD media with 1 µM FM4-64 dye to label vacuoles and imaged as described in "Fluorescence microscopy." Localization of Ltc1-yEGFP in E was assessed in 3-µm sections from the center of cells. The proportion of Ltc1-yEGFP foci localized to vacuoles per cell is plotted, and *n* = 110, 155, and 82 cells analyzed in WT, *Δnvj1*, and *Δvac8*, respectively. Vacuoles were fragmented (more than five vacuoles per cell) in a significant proportion of *Δvac8* cells and occupied a large fraction of cell volume that precluded an analysis of the significance of Ltc1-yEGFP localization. Error bars represent the standard error. P-values were calculated using one-way ANOVA with Turkey's honest significant difference. Bars, 2 µm.

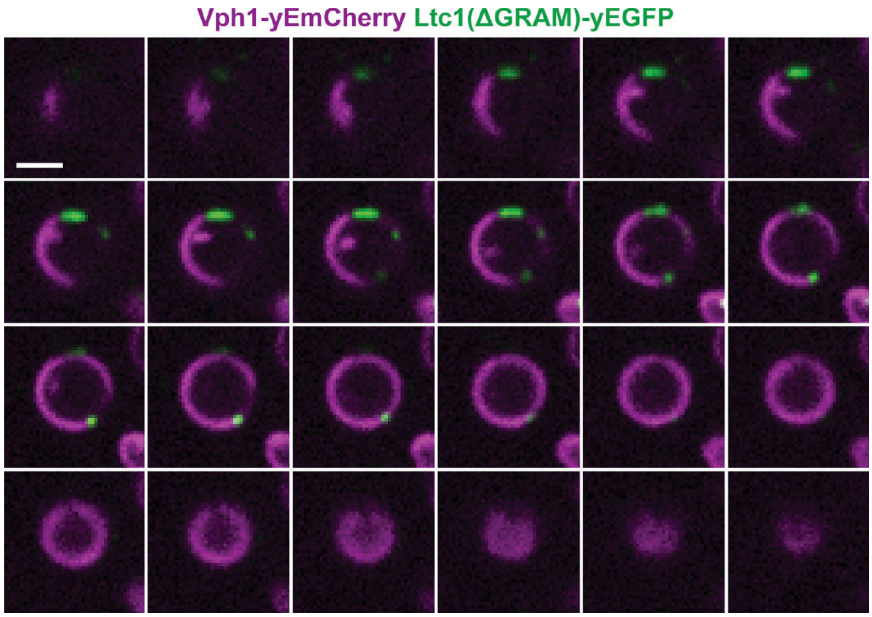

Figure S2. **Additional example of Ltc1(ΔGRAM) localization relative to Vph1-yEmcherry.** Depicted is a z-series montage of a yeast cell expressing Ltc1(ΔGRAM)-yEGFP Vph1-yEmCherry. Cells were grown to mid-log phase in synthetic dextrose medium for 12 generations and imaged as described in "Fluorescence microscopy." Bar, 2 µm.

Table S1, included as a separate Excel file, lists strains used in this study.