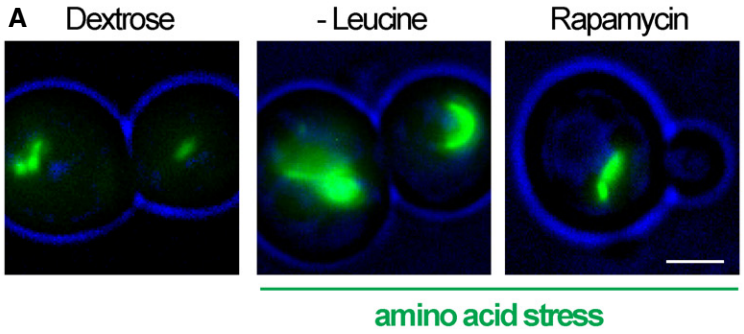
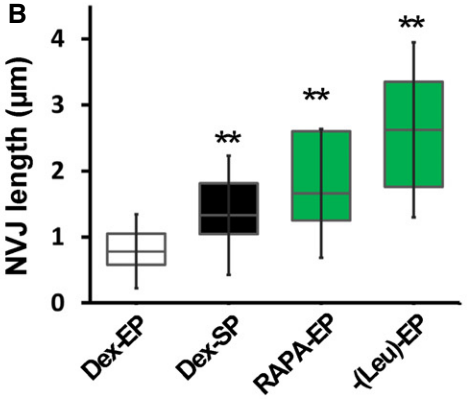
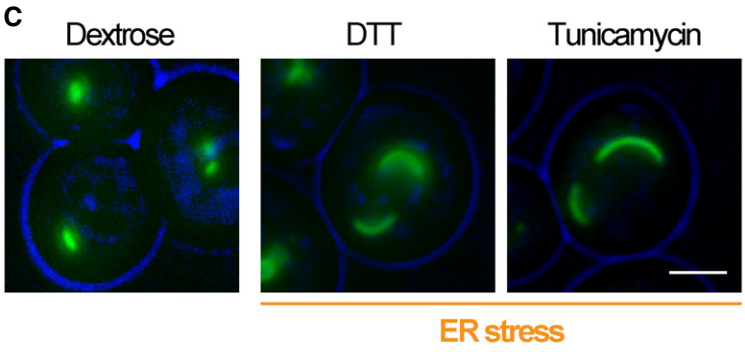
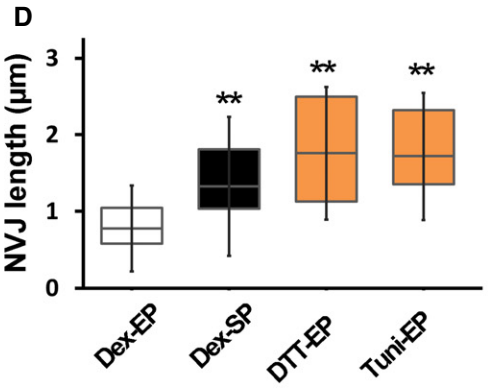
Expanded View Figures









E

Environmental transitions that lead to stress	Average NVJ size (pixels^2)
Temperature Shock	
Heat shock	13.70
Cold shock	13.25
pH Extremes	
Acid	12.30
Alkali	14.96
Starvation	
Stationary Phase	17.58
Non-fermentable carbon	
Acetate	21.40
Diverse Drug Treatments	
Cerulenin	13.08
Terbinafine	13.82
Dinitrophenol (DNP)	12.52

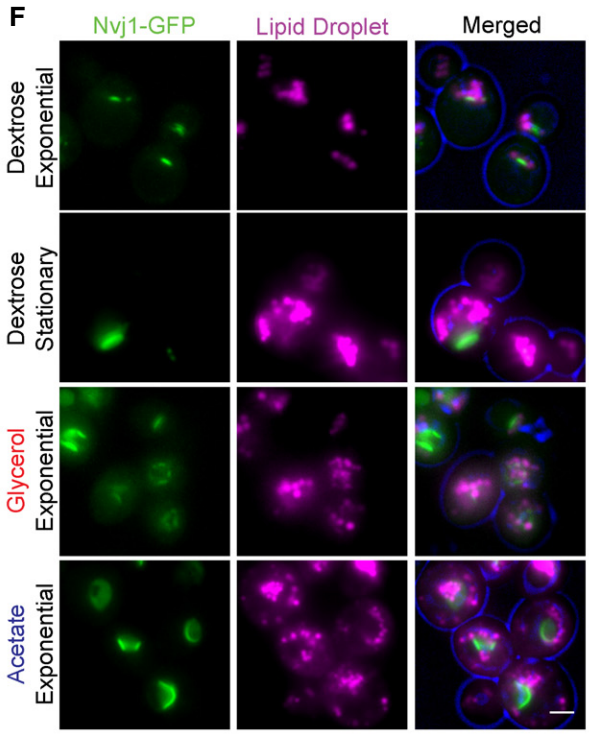
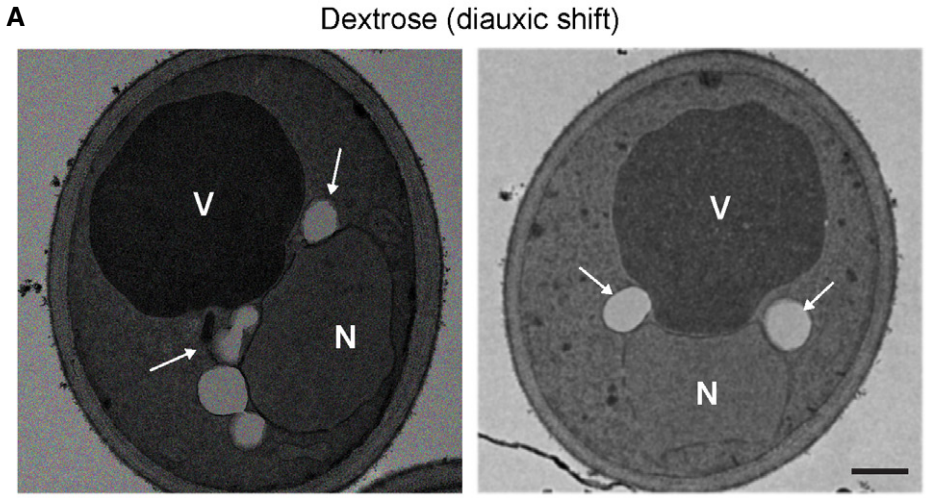


Figure EV1.

Figure EV1. NVJ contact sites respond to nutrient stress.

A Light microscopy for cells expressing chromosomally tagged Nvj1-GFP imaged in amino acid stress. Scale bar, 2 μm.
B Quantification of the NVJ length (μm) in (A) (box plots of median and range, $n > 50$ cells, $^{**}P < 0.005$, Student's t-test).
C Light microscopy for cells expressing chromosomally tagged Nvj1-GFP imaged in DTT and tunicamycin. Scale bar, 2 μm.
D Quantification of the NVJ length (μm) in (C) (box plots of median and range, $n > 50$ cells, $^{**}P < 0.005$, Student's t-test).
E Summary table for different stress conditions and their effect on NVJ size based on visual screening. Values are in pixels^2.
F Light microscopy of LDs in yeast expressing chromosomally tagged Nvj1-GFP grown in different conditions. Scale bar, 2 μm.



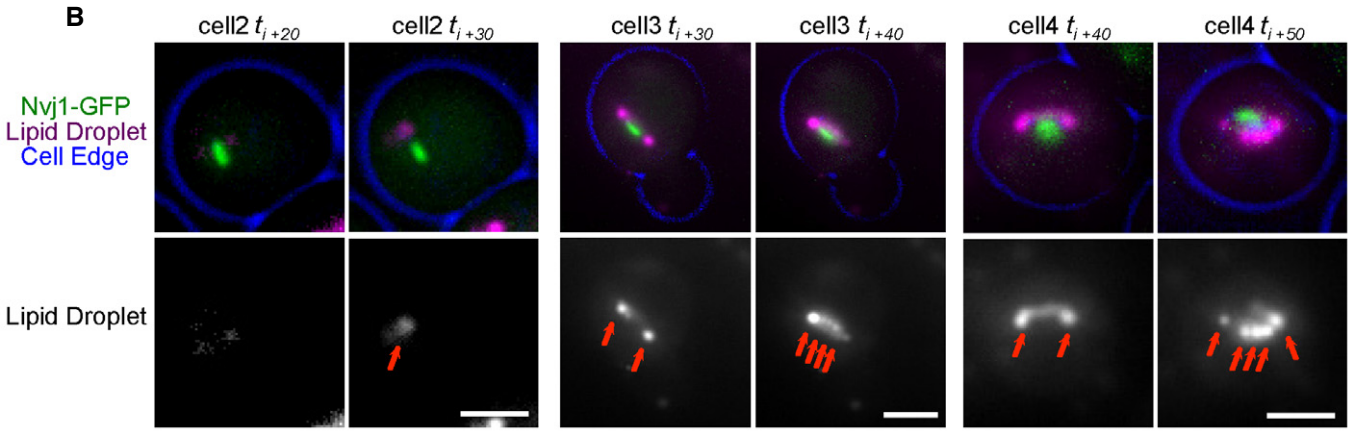


Figure EV2. The NVJ is a site for LD formation.

A TEM of WT yeast grown at diauxic shift. Arrows indicate LDs. Scale bar, 0.5 μm. N, nucleus; V, vacuole.
B Single cells (cell 1, 2, and 3) imaged at different times after cerulenin washout (t_i). LDs (arrows) visualized by AutoDOT staining and NVJ visualized by Nvj1-GFP. Scale bar, 2 μm.

Figure EV3. Mdm1 enriches at the NVJ where LD form.

A Sequential optical sections of yeast with Mdm1-GFP and Nvj1-mCherry. Arrows indicate sites for Mdm1-GFP enrichment. Scale bar, 2 μm.
B 3D stacking of optical sections and line trace plot. Arrows indicate sites for Mdm1-GFP enrichment.
C Light microscopy of yeast mildly over-expressing Mdm1-GFP, with LDs and vacuole labeled using AutoDOT and FM4-64 dye, respectively. Right: Line trace for light microscopy image (yellow line). Scale bar, 2 μm. N, nucleus; V, vacuole.
D Neutral lipids TLC for WT, *nvj1*Δ, and *mdm1*Δ yeast quantified in Fig 4E. SE, sterol esters; TAG, triacylglycerides; FFA, free fatty acids; S, sterols.
E Top: Dual-labeling imaging for Mdm1-GFP and Faa1-mCherry. Scale bar, 2 μm. Bottom: Line trace for light microscopy (top, yellow line). N, nucleus; V, vacuole.
F Light microscopy for chromosomally tagged Faa1-GFP in exponential phase. Arrows indicate the ER network. Scale bar, 2 μm. N, nucleus; V, vacuole.
G Quantification of Faa1-GFP localization in different growth conditions. EP, exponential phase; DS, diauxic shift.

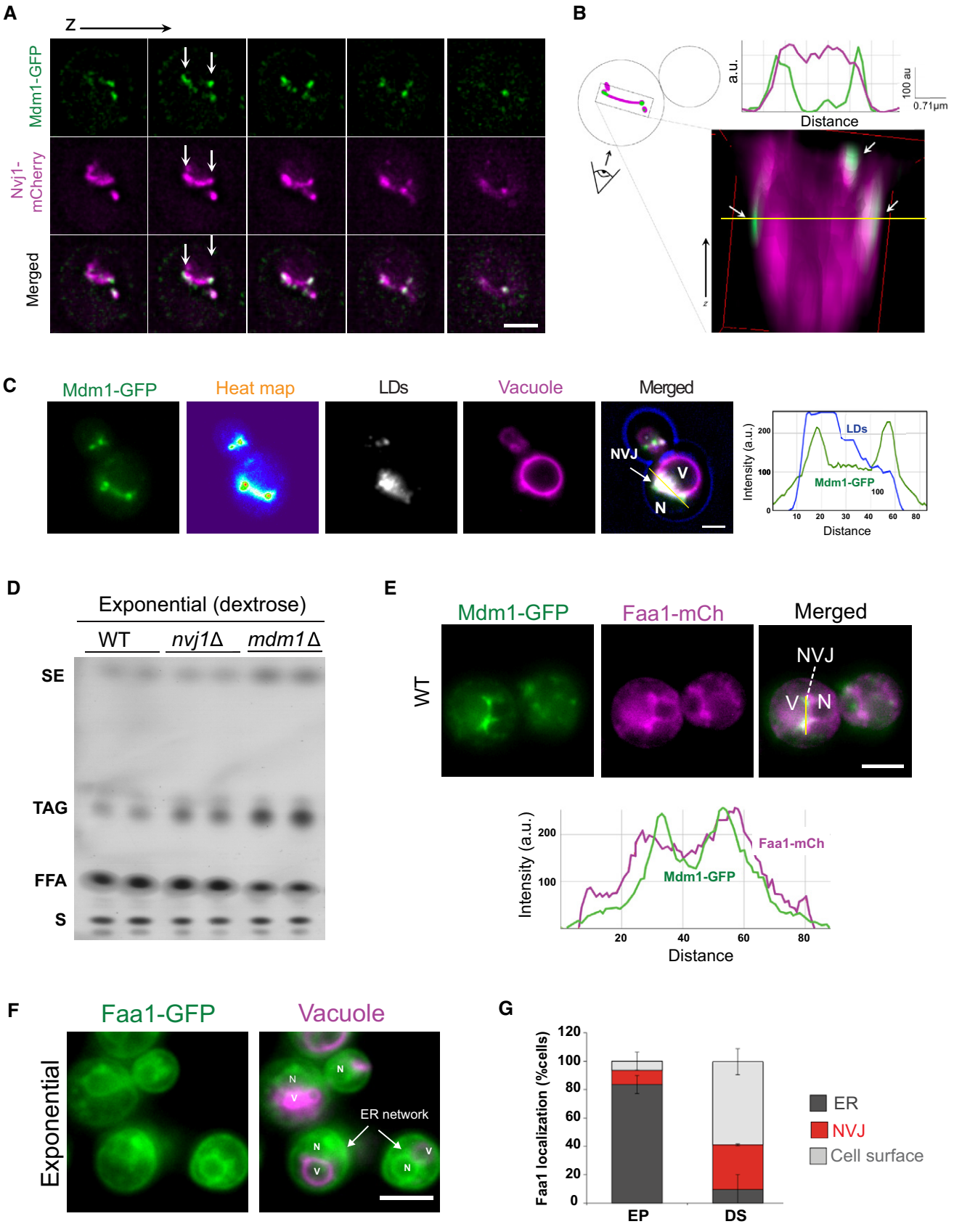


Figure EV3.

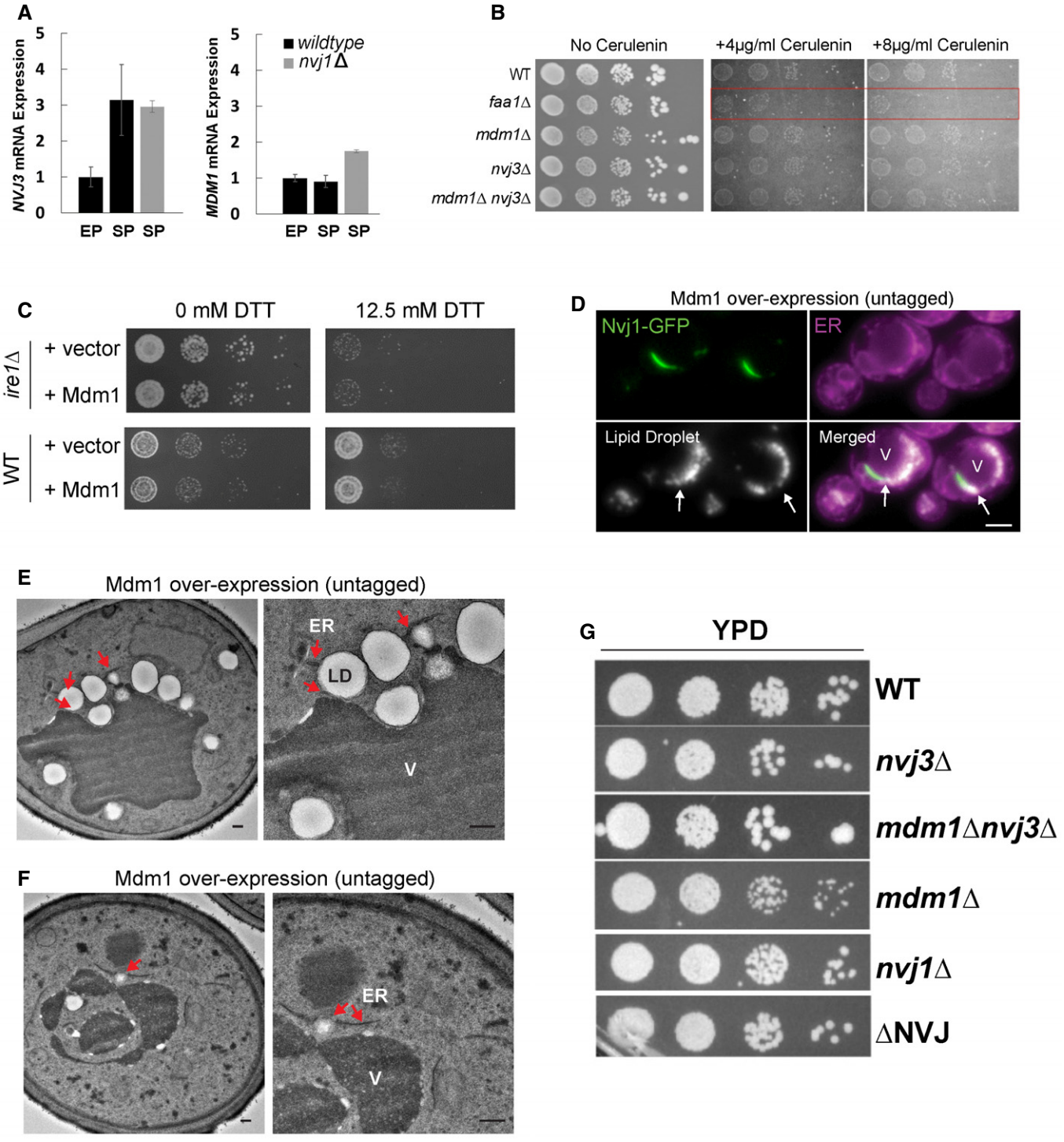


Figure EV4. Mdm1 modulates LD formation at the ER-vacuole contact sites.

A qRT–PCR showing expression levels of Mdm1 and Nvj3 in *nvj1*Δ yeast cultured in EP and SP (mean ± SEM, $n = 3$ independent cultures per condition, ***$P < 0.001$, Student's *t*-test). EP, exponential phase; SP, stationary phase.

B Plating assay of WT, *faa1*Δ *mdm1*Δ, *nuj3*Δ, and *mdm1*Δ*nvj3*Δ on plates containing different concentrations of cerulenin.

C Growth assays on DTT-containing plates for both wild-type yeast and *ire1*Δ over-expressing Mdm1.

D Light microscopy of cells over-expressing untagged Mdm1 show increase LDs that cluster at the NVJ (arrows, visualized by Nvj1-GFP). Scale bar, 2 μm. V, vacuole.

E, F TEM of WT cells over-expressing untagged Mdm1. Arrows indicate LDs associated with LDs near the vacuole. Scale bar, 0.5 μm. V, vacuole.

G Plating assay for yeast lacking different NVJ proteins.

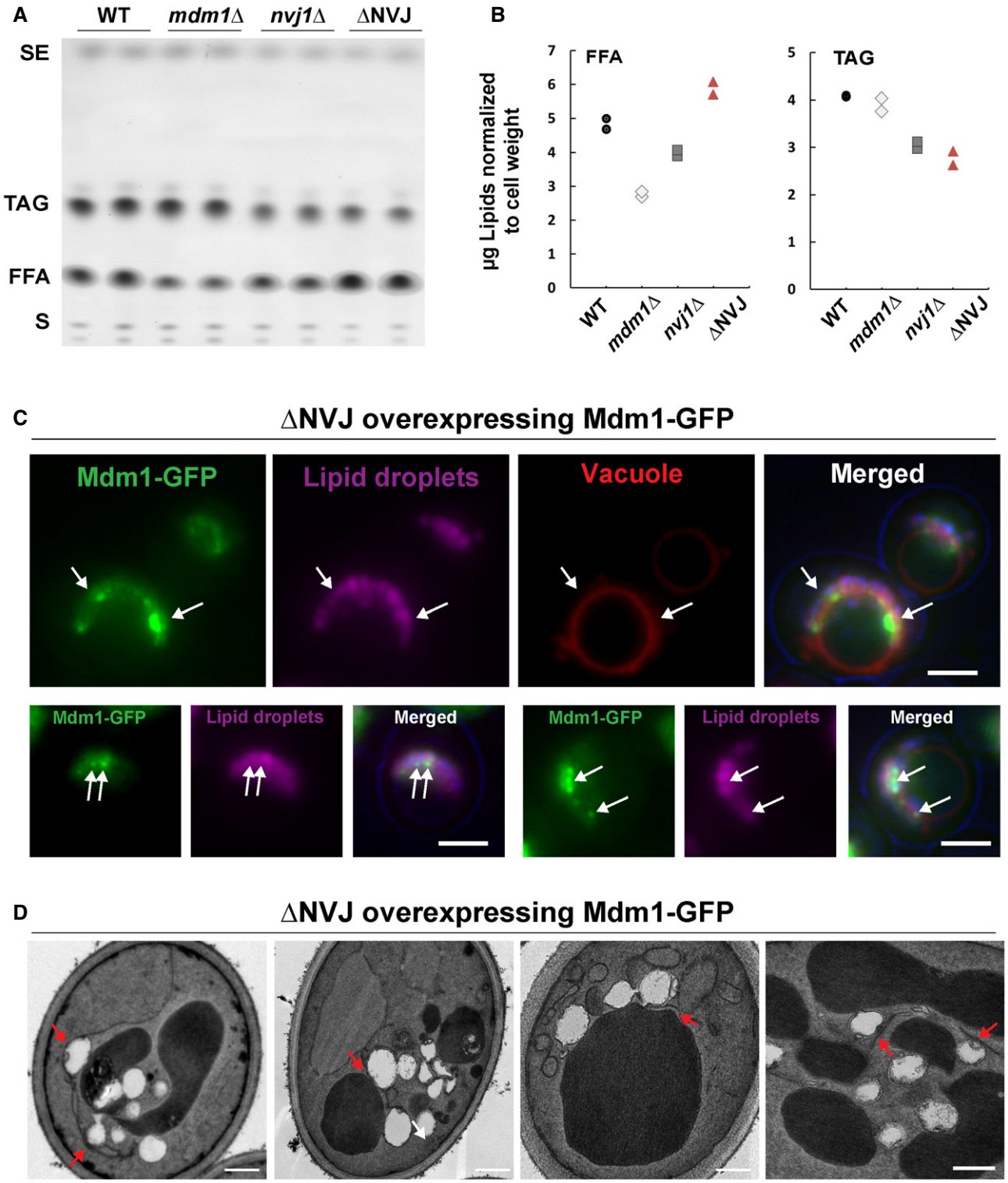


Figure EV5. Mdm1 marks sites for LD formation.

A TLC of neutral lipids for WT and mutant yeast fed oleic acid overnight quantified in Fig 7C. SE, sterol esters; TAG, triacylglycerides; FFA, free fatty acids; S, sterols.
B Quantification of FFA and TAG for TLC in (A). The absolute values here correspond to the ratio graphed in Fig 7C.
C Light microscopy of NVJΔ yeast over-expressing Mdm1-GFP. LDs are stained with AutoDOT. Arrows point to co-localization of LDs and Mdm1-GFP. Scale bar, 2 µm.
D Electron micrographs of NVJΔ yeast over-expressing Mdm1-GFP. Arrows indicate ER wrapping LDs near the vacuole. Scale bar, 0.5 µm.