

Expanded View Figures

Figure EV1. Yeast tetraspanners define subdomains in MCC/eisosomes—Supplement.

- A TIRFM images showing colocalization of indicated tetraspanners (C-terminally endogenously fused to GFP (cyan)) with the MCC/eisosome marker Pil1-RFP (magenta).
- B Pearson correlation coefficients for colocalization shown in (A).
- C Lateral distribution of tetraspanners from (A) quantified using the network factor.
- D Linearized profiles of WT cells expressing endogenous mNeGr fusions to indicated Sur7 tetraspanners and Lsp1 (cyan) together with Pil1-RFP (magenta). Symbol graph represents quantification of radial distances between indicated protein pairs. Dotted lines indicate the 25 nm bracket representing the estimated resolution of peak fitting.
- E Linearized profiles of WT cells expressing Sur7-GFP from the *PMA1* promoter (cyan) and Pil1-RFP (magenta) stained with 5 µg/ml filipin (magenta/cyan). Symbol graph represents quantification of radial distances between indicated pairs. Dotted lines indicate the 25 nm bracket representing the estimated resolution of peak fitting.
- F Co-Immunoprecipitation of different Sur7 tetraspanner pairs. The indicated GFP-tagged bait proteins were overexpressed from the GPD promoter and were pulled down with anti-GFP antibody. The prey protein Sur7-3xHA was expressed under the *PMA1* promoter. Western blot probed with antibodies directed against HA and GFP. I: Input, IP: Co-IP with anti-GFP, C: Control IP with unspecific IgG.

Data information: (B, C) Boxplots (interquartile range (box), min to max spread (whiskers) and median values (line in boxes)), ANOVA with Dunnett's multiple comparison test, $n = 29\text{--}49$ cells from three experiments (B), $n = 59\text{--}150$ cells from three experiments (C). (D, E) Symbol graph (error bars: SD), ANOVA with Tukey's multiple comparison test, $n > 119$ (D) and $n > 152$ (E) measurements from three experiments each. P -values: *** $P < 0.01$. Scale bars: 1 µm.

Source data are available online for this figure.

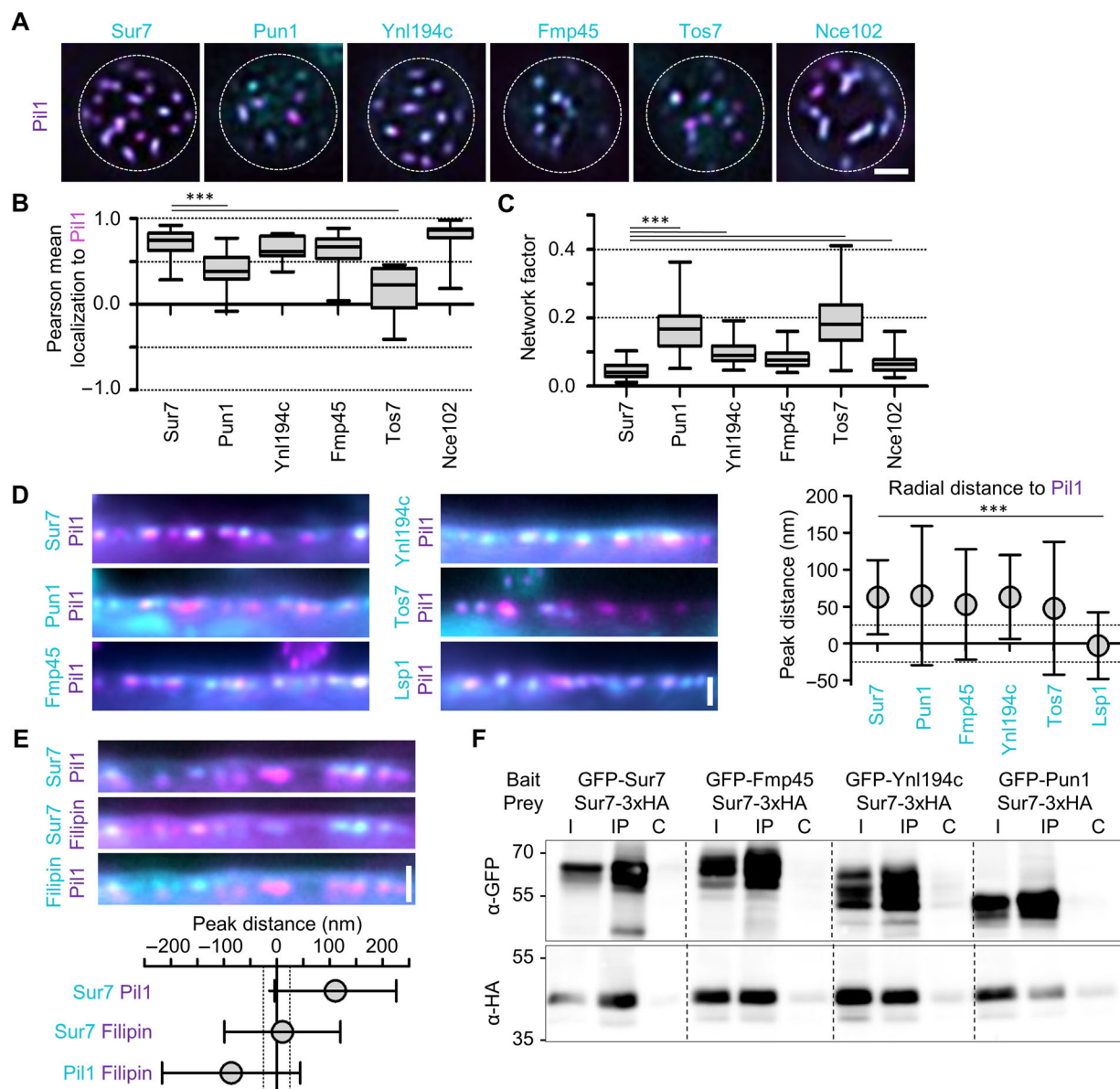


Figure EV1.

Figure EV2. Super-resolution imaging of Sur7 tetraspanners—Supplement.

- A STED images of indicated tetraspanners expressed from the *PMA1* promoter and fused to the Halo tag.
 B Two-color STED and confocal images of WT cells expressing endogenously tagged Sur7-Halo (STED, magenta) and Pil1-mNeGr (confocal, cyan).
 C Effect of indicated lipid perturbations on lateral distribution of Sur7-mNeGr.
 D Lateral distribution of Sur7-mNeGr quantified by the network factor in strains shown in (C).

Data information: (D) Boxplot (interquartile range (box), min to max spread (whiskers) and median values (line in boxes)), ANOVA with Dunnett's multiple comparison test, $n = 30$ –227 cells from three experiments. P -values: *** $P < 0.01$. Scale bars: 1 μ m, 200 nm (zoom).

Source data are available online for this figure.

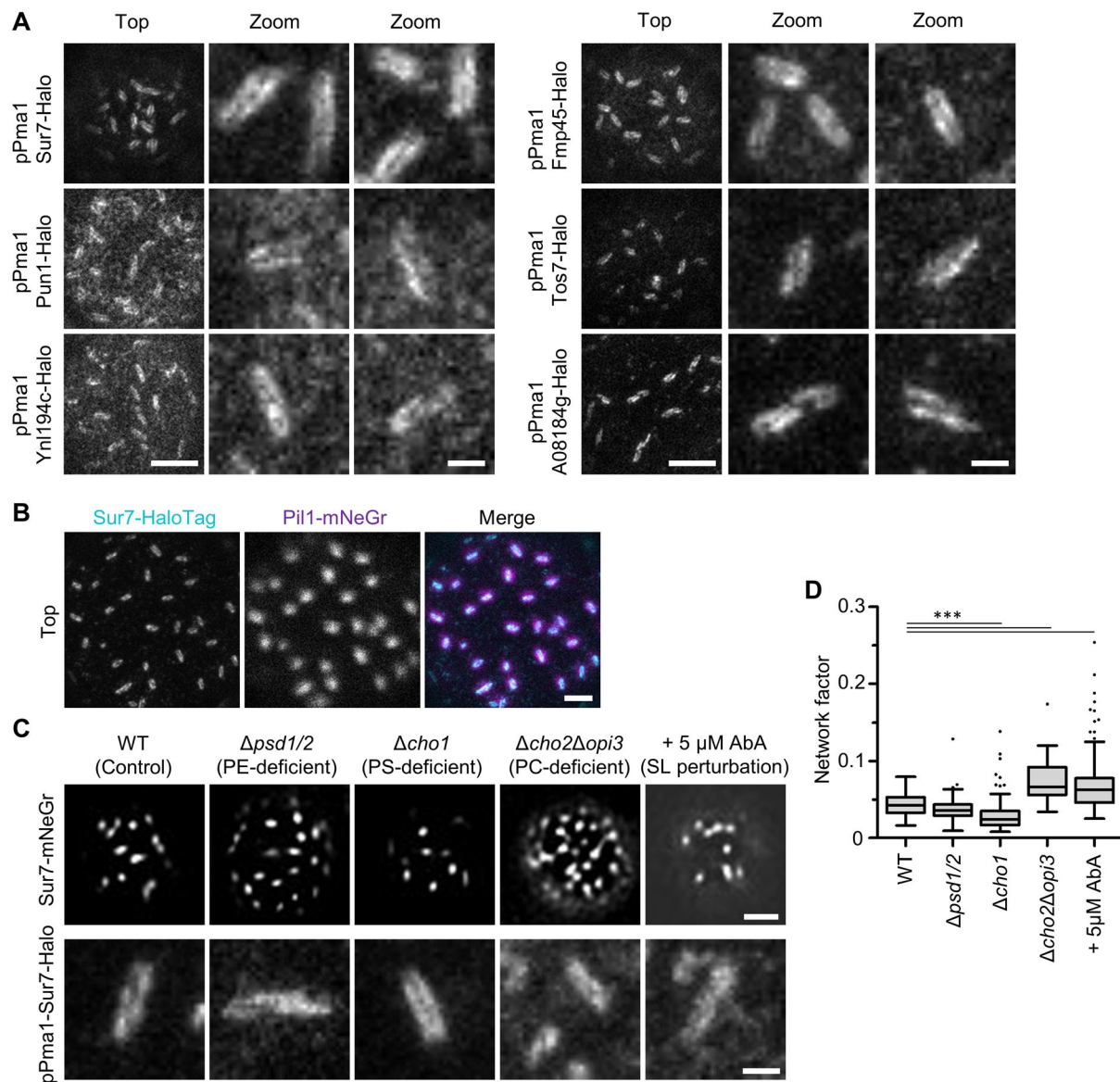


Figure EV2.

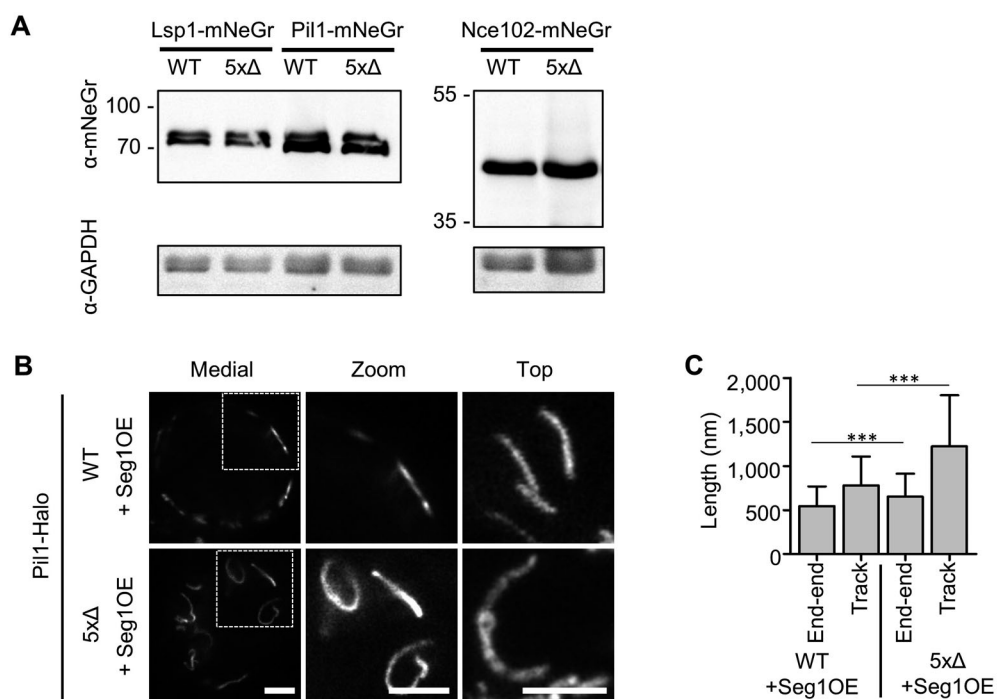


Figure EV3. Sur7 tetraspanners prevent closure of MCC/eisosome furrows—Supplement.

A Western blot analysis depicting the expression of endogenous mNeGr fusions to Lsp1, Pil1 and Nce102 in WT and 5xΔ cells.

B STED microscopy of endogenously tagged Pil1-Halo in WT and 5xΔ cells that overexpress Seg1 from the GPD promoter (OE).

C Quantification of track length for Pil1-Halo positive structures in (B).

Data information: (C) Bar graph (error bars: SD), unpaired *t*-test, *n* = 53–175 tracks from three experiments. *P*-values: ****P* < 0.01. Scale bars: 1 μm. Source data are available online for this figure.

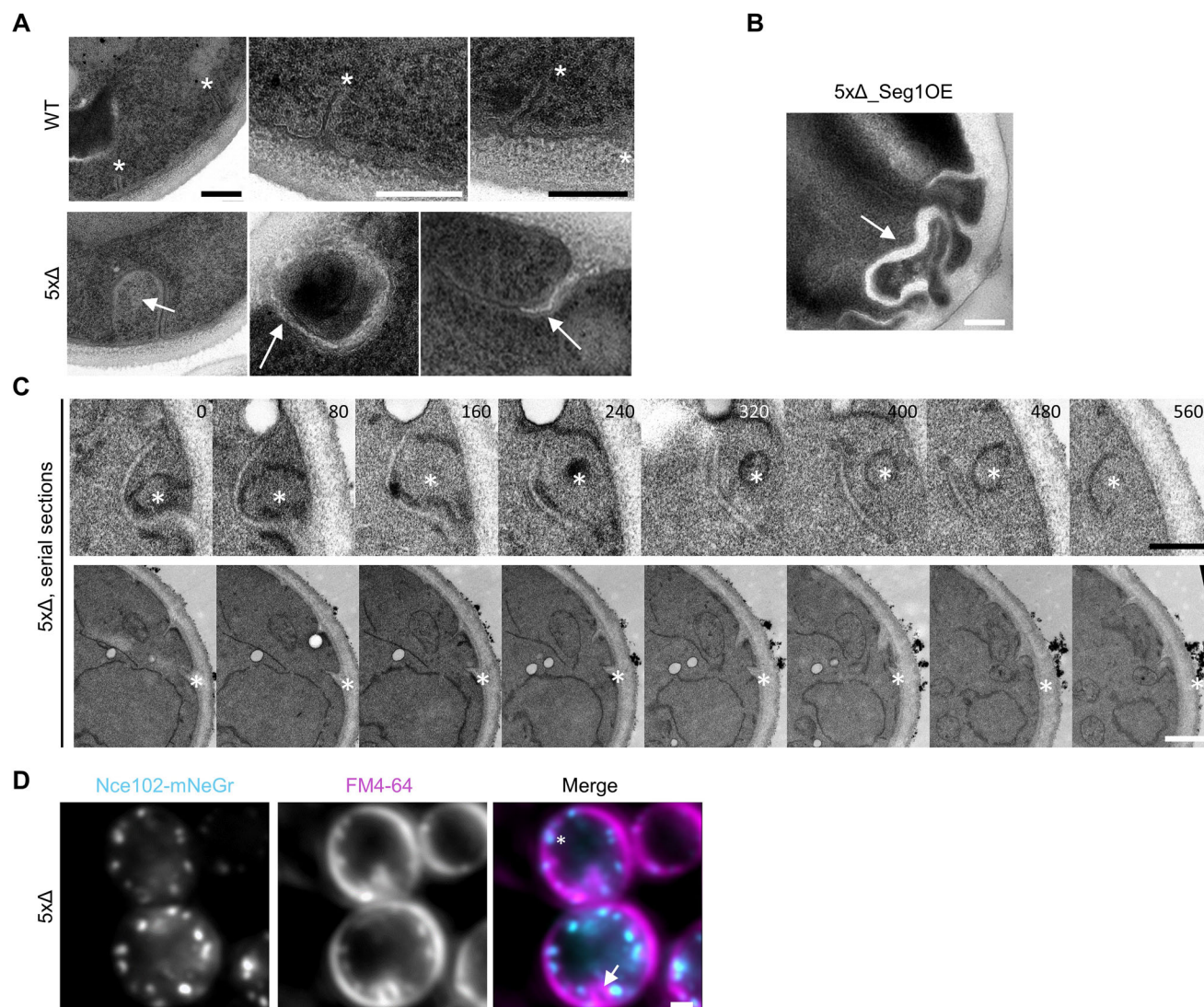


Figure EV4. Sur7 tetraspanners prevent closure of MCC/eisosome furrows – Supplement.

- A** TEM micrographs of ultrathin sections of WT and 5xΔ. Asterisks indicate perpendicular sections through MCC/eisosome furrows. Arrows indicate abnormal PM invaginations.
- B** TEM micrograph of ultrathin sections of a 5xΔ cell overexpressing Seg1 from the GPD promoter (OE). The arrow indicates an abnormal PM invagination.
- C** Exemplary serial TEM sections of 5xΔ cells illustrating the sheet-like invaginations filled with electron translucent cell wall material. Asterisks indicate positions of invaginations. Sample depth is indicated in nm.
- D** 5xΔ cells expressing Nce102-mNeGr (cyan) and labeled with the membrane dye FM4-64 (magenta). Asterisk indicates a Nce102-positive tube that is labeled by FM4-64. Arrow indicates a large FM4-64 positive invagination that does not contain Nce102.

Data information: Scale bars: 200 nm (A–C), 1 μm (D).

Source data are available online for this figure.

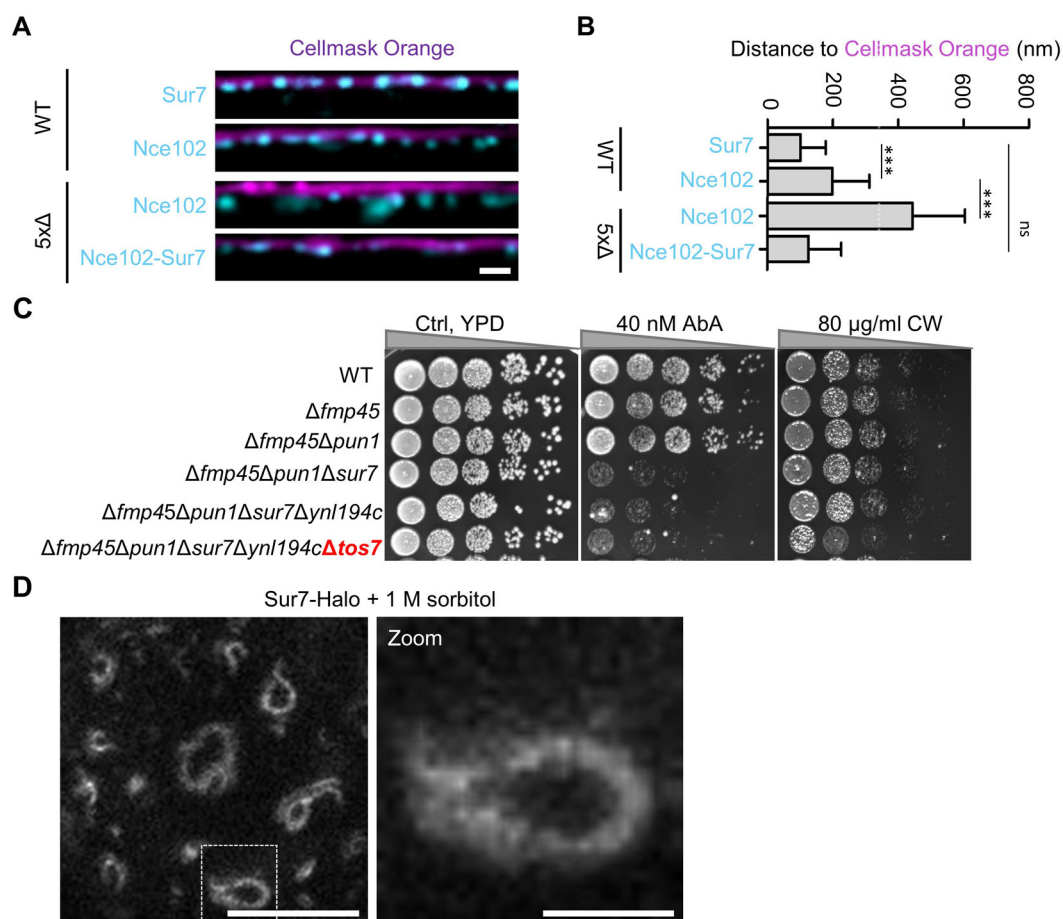


Figure EV5. Mechanistic basis of Sur7 function in subdomain organization—Supplement.

- A Linearized profiles of WT cells expressing indicated GFP fusions (cyan) stained with the PM dye Cellmask Orange (magenta).
- B Bar graph representing quantification of the radial distances between indicated protein and Cellmask Orange in (A).
- C Growth assays with successive deletions of Sur7 tetraspanners. Cells were grown on YPD plates containing 40 nM AbA or 40 μg/ml calcofluor white (CW) at 30°C for 48 h.
- D STED image of WT cells expressing Sur7-Halo from the *PMA1* promoter after addition of 1 M sorbitol for 5 min.

Data information: (B) Bar graph (error bars: SD), ANOVA with Tukey's multiple comparison test, $n > 133$ measurements from three experiments. P -values: *** $P < 0.01$, ns: not significant. Scale bars: 1 μm, 200 nm (zoom).

Source data are available online for this figure.