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Supplemental material

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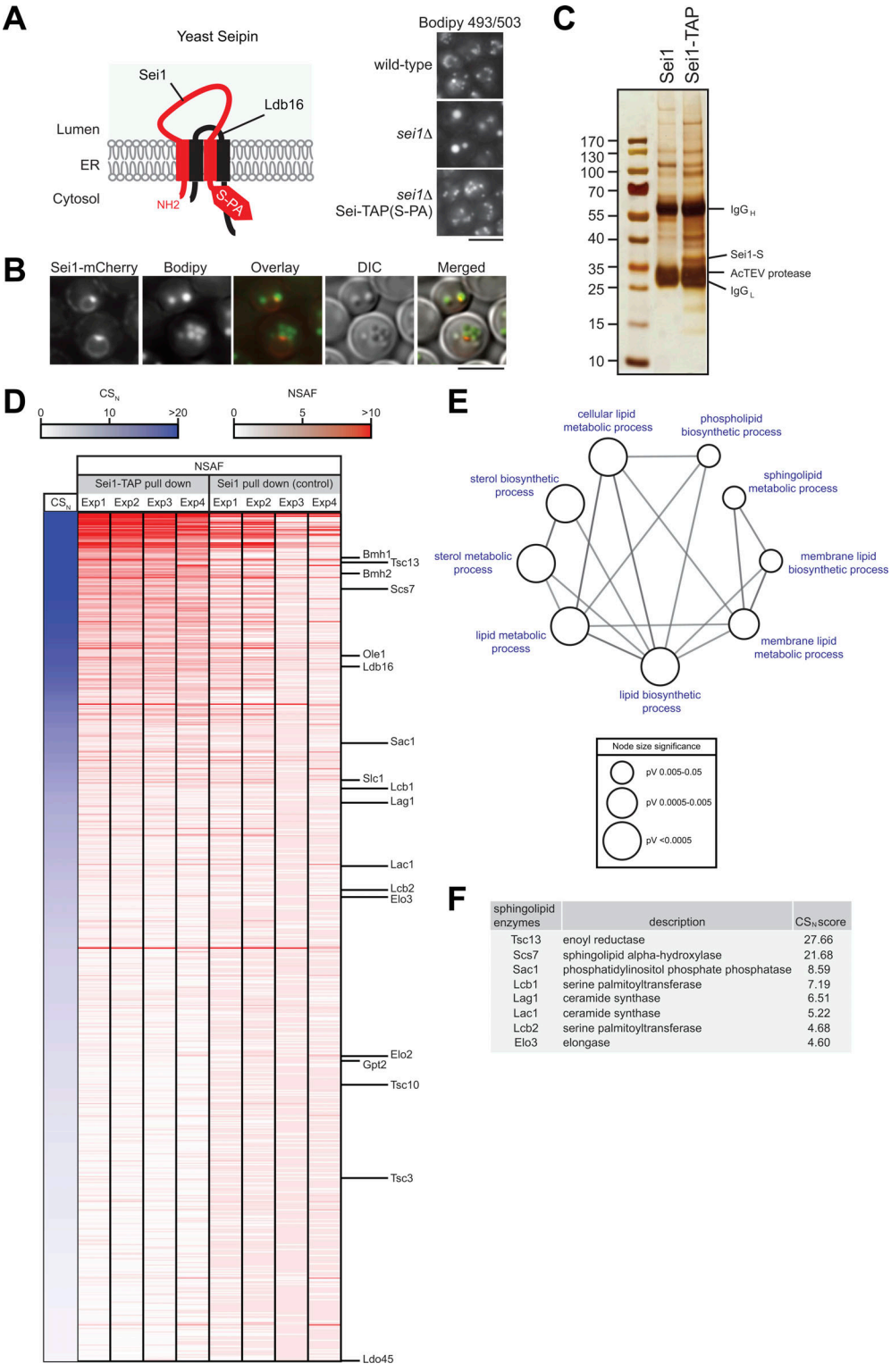
Provided online are two tables. Table S1 lists the strains, plasmids, and primers used in this study. Table S2 lists the complete set of data for the Sei1-TAP purification screen.



sphingolipid enzymes	description	CS_N score
Tsc13	enoyl reductase	27.66
Scs7	sphingolipid alpha-hydroxylase	21.68
Sac1	phosphatidylinositol phosphate phosphatase	8.59
Lcb1	serine palmitoyltransferase	7.19
Lag1	ceramide synthase	6.51
Lac1	ceramide synthase	5.22
Lcb2	serine palmitoyltransferase	4.68
Elo3	elongase	4.60

Figure S1. **Putative seipin-interacting proteins revealed by large-scale Sei1-TAP purification. (A)** Left: Schematic representation of Sei1-TAP and Ldb16, together as yeast seipin, in the ER membrane. Right: LDs in wild-type, *sei1Δ*, and the *GPD* promoter-driven Sei1-TAP expression strain were stained by Bodipy 493/503, followed by fluorescence microscopy. Scale bar, 5 µm. **(B)** Cells harboring Sei1-mCherry driven by the *GPD* promoter were grown in SCD medium and their LDs stained with Bodipy 493/503, followed by fluorescence microscopy. Scale bar, 5 µm. **(C)** TAP purification from Sei1 and Sei1-TAP strains were resolved by SDS-PAGE, followed by silver staining. **(D)** Summary of the putative Sei1-interacting proteins with $CS_{(N)} \geq 1$ as described in Materials and methods. Selected sphingolipid enzymes and proteins previously shown to interact with seipin were labeled. **(E)** Proteins pulled down with Sei1-TAP showing $CS_{(N)} \geq 1$ were analyzed by ClueGO to show GO/pathway terms. Nine high-confidence Sei1-interacting proteins enriched in lipid metabolism pathways are displayed. The lines represent GO-term relationships, and the node size represents the term enrichment significance. **(F)** List of sphingolipid biosynthetic enzymes found in D with $CS_{(N)} \geq 3$.

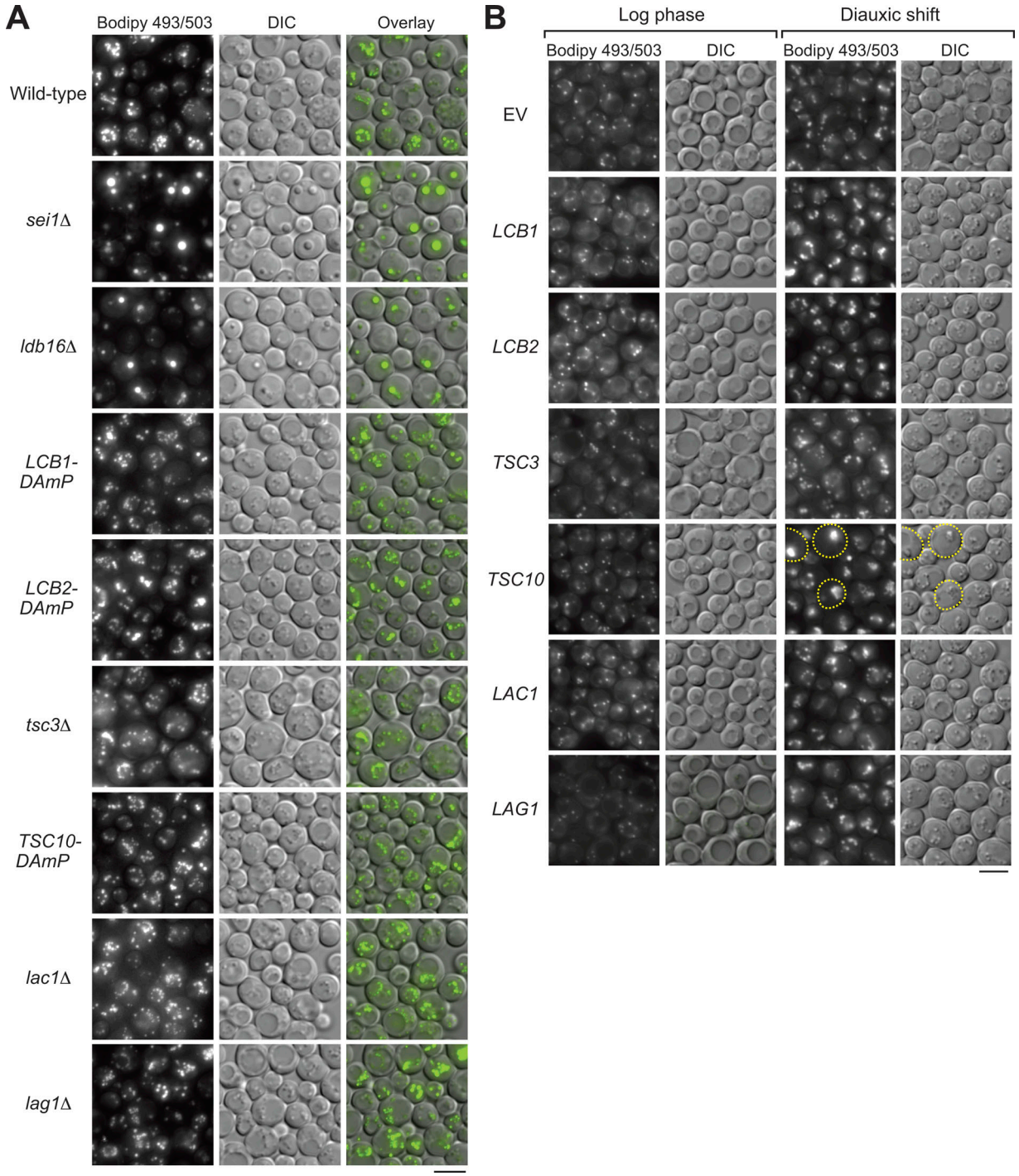


Figure S2. **The LD phenotypes of cells with altered sphingolipid enzymes. (A)** Yeast strains as indicated were grown in SCD medium to the diauxic shift and their LDs stained with Bodipy 493/503, followed by microscopy. Fluorescence images are the maximum-intensity projection of 10 optical sections spaced at 0.5 µm. Scale bar, 5 µm. **(B)** Wild-type cells transformed with *pRS426* (empty vector [EV]), *pRS426-LCB1*, *pRS426-LCB2*, *pRS426-TSC3*, *pRS426-TSC10*, *pRS426-LAC1*, or *pRS426-LAG1* were grown in SCD medium to log (OD 0.6–0.8) or diauxic shift stage (OD 1.8) and their LDs stained with Bodipy 493/503, followed by fluorescence microscopy. Cells displaying aggregated LDs were circled in yellow. Scale bar, 5 µm.

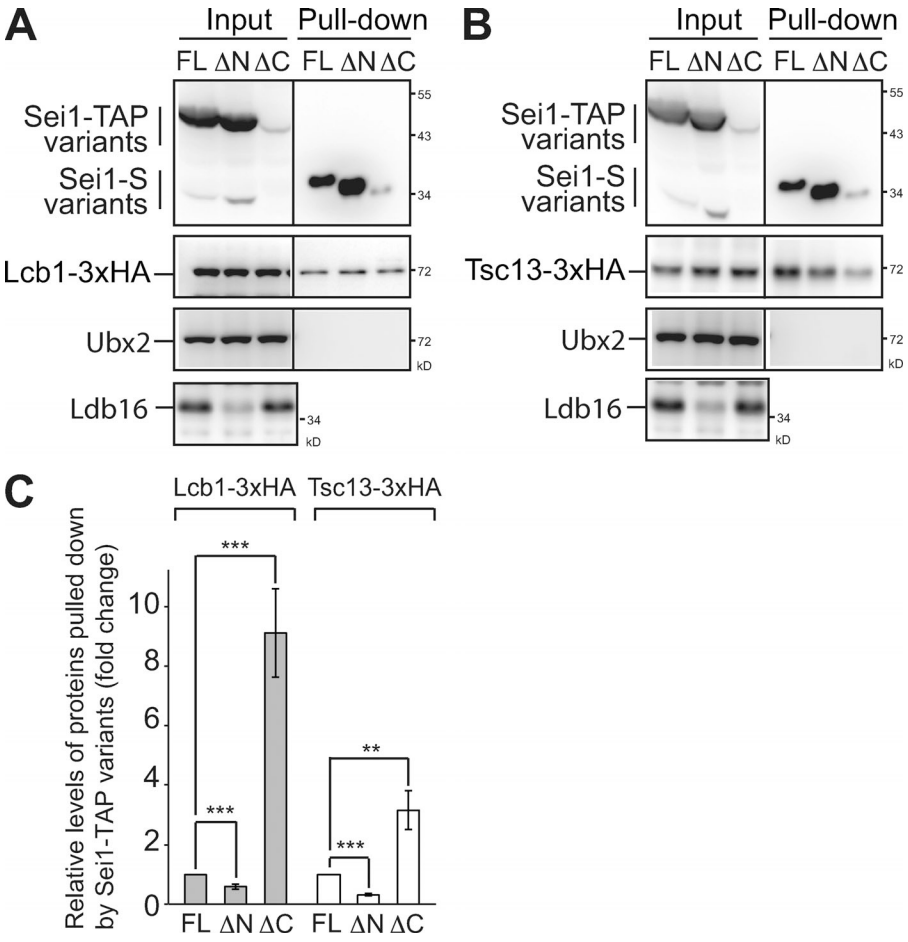


Figure S3. **Lcb1-3xHA and Tsc13-3xHA levels pulled down by Sei1-TAP deleted of N- or C-terminus. (A)** Cells expressing Lcb1-3xHA and full-length (FL), N-terminal deletion (ΔN), or C-terminal deletion (ΔC) of Sei1-TAP were grown in SCD medium to log phase and lysed by spheroplasting. The cleared lysates (input) underwent a pull-down assay with IgG Sepharose, followed by TEV protease cleavage (pull-down). Input and pull-down fractions were examined by Western blot analysis with anti-S, anti-HA, anti-Ubx2, and anti-Ldb16 antibodies. **(B)** Same as A, except that cells expressed Tsc13-3xHA instead of Lcb1-3xHA. **(C)** After being normalized to Sei1-S in the pull-down factions, the levels of Lcb1-3xHA (A) and Tsc13-3xHA (B) in the pull-down factions were compared with that of full length and plotted as fold change. Data are mean ± SD from three independent experiments. **, $P < 0.01$; ***, $P < 0.001$.