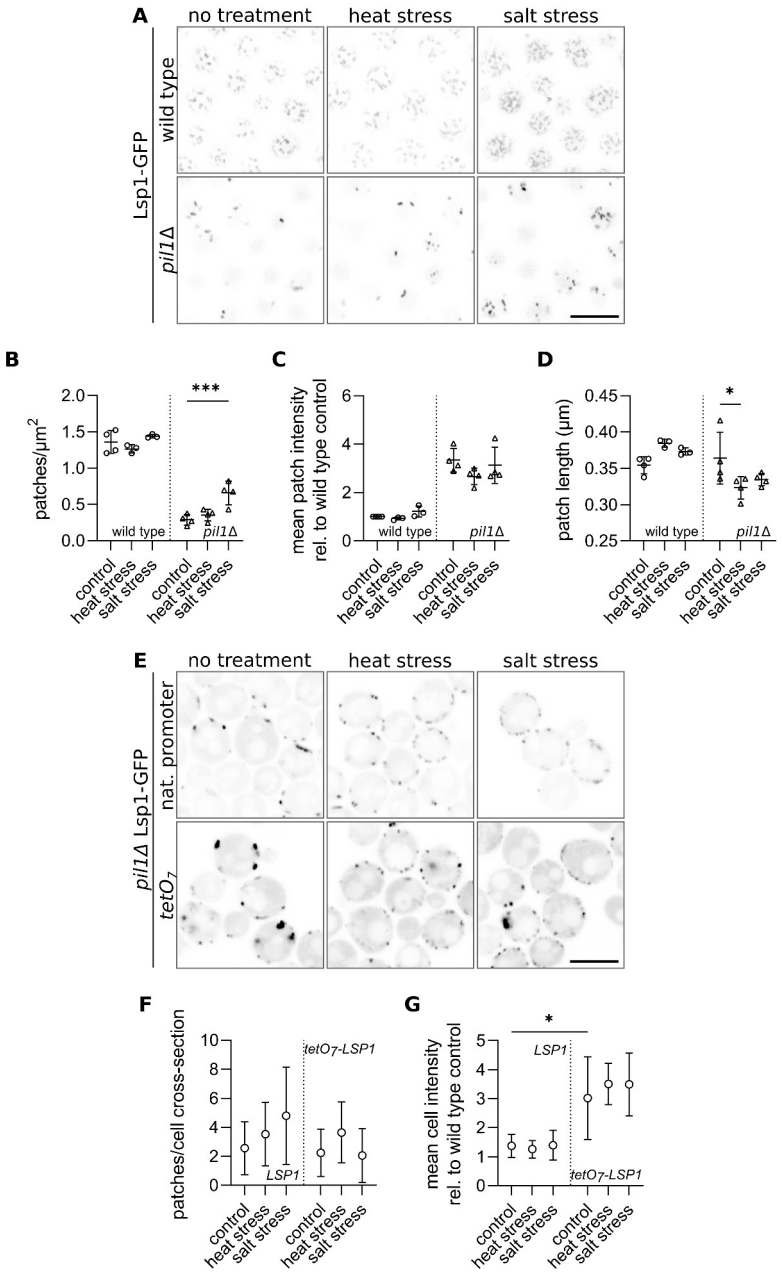

Fig. S1. Heat and salt stress induce the formation of Lsp1 foci in the plasma membrane. **A** Confocal fluorescence microscopy images (tangential sections) of *LSP1-GFP* expressing yeast cells grown exponentially for 4 hours at 28°C and subsequently shifted to 37°C for 2 hours or grown at 28°C in the presence of 1 M NaCl for 6 hours. Scale bar: 5 µm. **B-D** Quantification of the density of local Lsp1-GFP accumulations (patches)(B), mean patch intensity relative to the wild-type control (C) and patch length (D) in cells treated as in (A). Data are presented as mean ± SD from 4 biological replicates (circles – wild type, triangles – *pil1*Δ; 100–150 cells in each condition). **E** Confocal fluorescence microscopy images (transversal sections) of *pil1*Δ yeast cells expressing *LSP1-GFP* from either its native or *tetO7* promoter grown exponentially for 4 hours at 28°C and subsequently shifted to 37°C for 2 hours or grown at 28°C in the presence of 1 M NaCl for 6 hours. Scale bar: 5 µm. **F-G** Quantification of the number of local Lsp1-GFP accumulations (patches) per cell cross-section (F) and mean cell intensity relative to the wild-type control (G) in cells treated as in (E). Data are presented as mean ± SD from a single experiment (circles – wild type, triangles – *pil1*Δ; 100–150 cells in each condition). *tetO7-LSP1* indicates overexpression of *LSP1.* * – $P \leq 0.1$, *** – $P \leq 0.001$, two-way ANOVA.

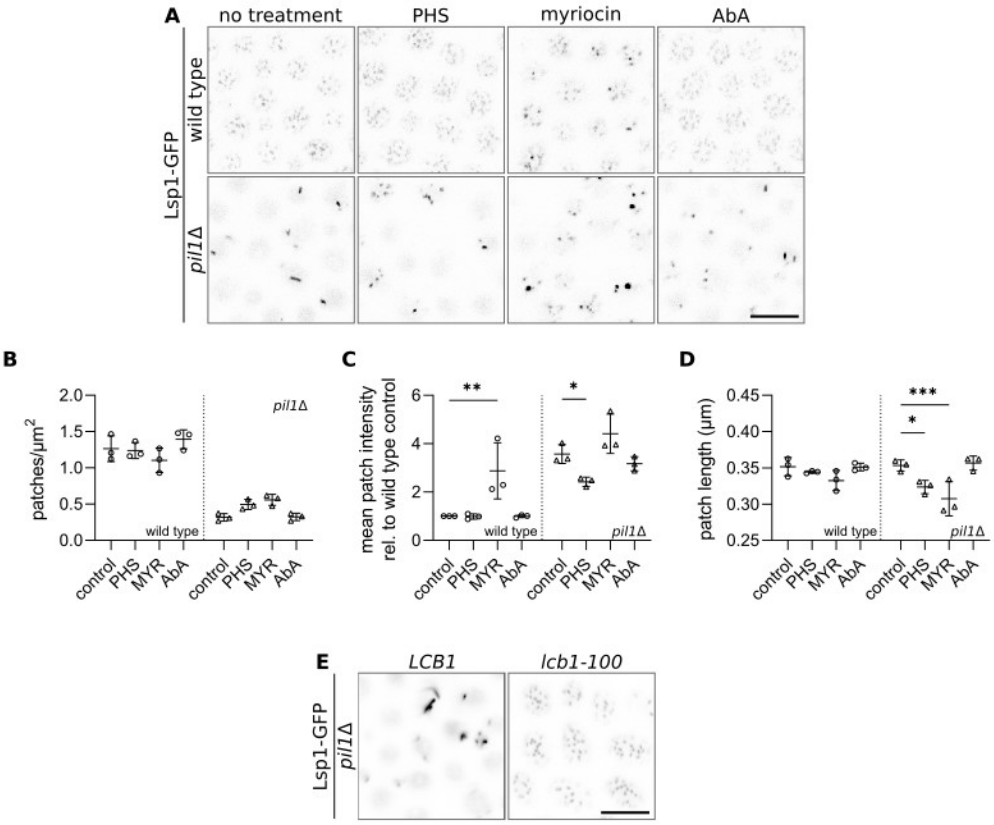

Fig. S2. Decrease in the activity of serine-palmitoyl transferase induces the formation of Lsp1 eisosomes in the plasma membrane; tangential sections.

A Confocal fluorescence microscopy images of *LSP1-GFP* expressing yeast cells (BY4742 background) grown exponentially for 4 hours at 28°C and treated with indicated chemicals for 2 hours. **B-D** Quantification of the density of local Lsp1-GFP accumulations (patches) (B), mean patch intensity relative to the wild-type control (C) and patch length (D) in cells treated as in (A). Data are presented as mean ± SD from 4 biological replicates (circles– wild type, triangles – pil1Δ; 150–200 cells in each condition). * – P ≤ 0.1, ** – P ≤ 0.01, *** – P ≤ 0.001, two-way ANOVA. E Confocal fluorescence microscopy images of LSP1-GFP expressing yeast cells (RH1800 background) grown exponentially for 5 hours at 25°C. PHS – phytosphingosine, 10 μM; MYR – myriocin, 10 μM; AbA –aureobasidin A, 1 μg/ml. Scale bars: 5 μm.

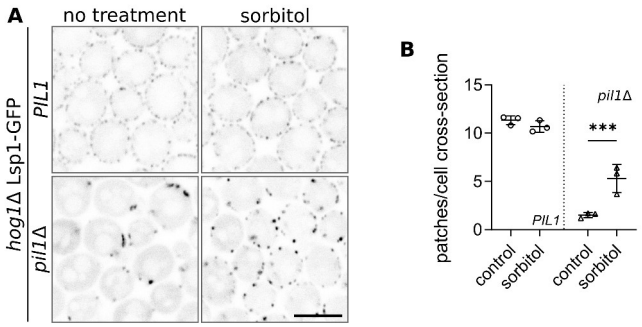

Fig. S3. Hog1 kinase is not required for Lsp1 eisosome formation in response to hyperosmotic stress
A Confocal fluorescence microscopy images (transversal sections) of *LSP1-GFP* expressing *hog1*Δ yeast cells grown exponentially for 6 hours at 28°C and treated with nothing or 1 M sorbitol for 25 minutes. Scale bar: 5 µm. **B** Quantification of the number of local Lsp1-GFP accumulations (patches) per cell cross-section in cells treated as in (E). Data are presented as mean ± SD from 3 biological replicates (circles – *hog1*Δ, triangles – *hog1*Δ*pil1*Δ; 170–230 cells in each condition). *** – $P \leq 0.001$, two-way ANOVA.

Table S1. Yeast strains used in the study.

Strain	Genotype	Source
BY4742, wild type	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*	*Euroscarf*
hog1Δ	BY4742; *hog1::kanMX4*	*Euroscarf*
nce102Δ	BY4742; *nce102::kanMX4*	*Euroscarf*
pil1Δ	BY4742; *pil1::kanMX4*	*Euroscarf*
pil1Δa	BY4741; *pil1::kanMX4*	*Euroscarf*
seg1Δ	BY4742; *seg1::kanMX4*	*Euroscarf*
nce102Δ pil1Δ	*BY4742 except MET15; pil1Δ nce102Δ*	This study
seg1Δ pil1Δ	*BY4742 except MET15; seg1Δ pil1Δ*	Vaskovicova et al., 2015
lsp1Δ pil1Δ	*BY4742; lsp1Δ pil1Δ*	This study
seg1Δ nce102Δ	*BY4742 except LYS2 met15Δ0; seg1Δ nce102Δ*	This study
seg1Δ nce102Δ pil1Δ	*BY4742 except MET15; seg1Δ nce102Δ seg1Δ*	This study
Y114	*pil1Δ nce102Δ*; *LSP1::GFP::LEU2* (YIp128)	This study
Y172	BY4742; *LSP1::GFP::LEU2* (YIp128)	Zahumensky et al., 2022
Y257	*pil1Δ*; *LSP1::GFP::LEU2* (YIp128)	This study
Y430	*seg1Δ pil1Δ*; *LSP1::GFP::LEU2* (YIp128)	This study
Y1199	BY4742; *LSP1::GFP::HIS3* (pKT128)	This study
Y1214	Y1199*; LSP1::GFP::G418* (pCM225)	This study
Y1219	Y1214*; pil1::pFA6a-natMX6*	This study
Y1313	*lsp1Δ pil1Δ*; *SEG1::GFP::LEU2* (YIp128)	This study
Y1315	*nce102Δ*; *LSP1::GFP::LEU2* (YIp128)	This study
Y1377	*seg1Δ*; *LSP1::GFP::LEU2* (YIp128)	This study
Y1393	*seg1Δ nce102Δ pil1Δ*; *LSP1::GFP::LEU2* (YIp128)	This study
Y1395	*seg1Δ nce102Δ*; *LSP1::GFP::LEU2* (YIp128)	This study
Y1417	*hog1Δ*; *LSP1::GFP::LEU2* (YIp128)	This study
Y1421	Y1417; *pil1::pFA6a-natMX6*	This study
RH1800, wild type	*MATα leu2Δ0 trp1Δ0 ura3Δ0 lys2Δ0 bar1-1*	H. Riezman
lcb1-100	RH1800; *lcb1-100*	H. Riezman
Y1233	RH1800; *LSP1::GFP::LEU2* (YIp128)	This study
Y1234	*lcb1-100*; *LSP1::GFP::LEU2* (YIp128)	This study
Y1238	Y1233; *pil1::pFA6a-natMX6*	This study
Y1239	Y1234; *pil1::pFA6a-natMX6*	This study

Table S2. Parent strains used for the construction of double and triple-deletion strains

final strain	***MATα strain***	***MATα strain***
lsp1Δpil1Δ	*pil1Δ LSP1::mRFP::LEU2*	*lsp1Δ PIL1::GFP::URA3*
seg1Δpil1Δ	*pil1Δ SEG1::GFP::LEU2*	*seg1Δ PIL1::mRFP::URA3*
nce102Δpil1Δ	*pil1Δ NCE102::mRFP::URA3*	*nce102Δ PIL1::GFP::LEU2*
seg1Δnce102Δ	*nce102Δ SEG1::GFP::URA3*	*seg1Δ NCE102::mRFP::LEU2*
seg1Δnce102Δpil1Δ	*pil1Δnce102Δ SEG1::GFP::LEU2*	*pil1Δseg1Δ NCE102::mRFP::URA3*

All listed strains were constructed in the course of this study; for details see Methods

Table S3. Differentially expressed genes in the *seg1Δnce102Δpil1Δ* mutant relative to the wild type. Yeast were cultured and processed as described in Methods. As significance thresholds, log 2 fold change (LFC) was set to 1, P-adjusted < 0.05.

Click here to download Table S3

Movie 1. Disintegration of eisosome remnants and formation of Lsp1 eisosomes following myriocin-induced inhibition of SPT

LSP1-GFP expressing yeast cells were grown exponentially for 4 hours at 28°C and treated with 10 µM myriocin at time t = 0 min. Z-stacks were recorded for 2 hours in 5 min increments. The obtained images were drift corrected and maximum intensity projections calculated in proprietary Zeiss ZEN software. Timestamp shows time following myriocin addition in HH:MM format. Scale bar: 5 µm.