Figure EV1. An inducible system for ER membrane biogenesis.
Sec63-mNeon images of cortical sections of cells containing the estradiol-inducible artificial transcription factor GEM (SSY2328) and cells additionally containing ino2* under the control of the GAL promoter (SSY1402). Cells were untreated or treated with 800 nM estradiol for 6 h.
Source data are available online for this figure.
Figure EV2. Deletion of ICE2 impairs UPR signaling during ER stress.

A  Flow cytometric measurements of GFP levels of WT and Δice2 cells containing the transcriptional UPR reporter (SSY2306, 2312). Cells were treated with 1 μg/ml tunicamycin for the times indicated. Data were normalized to untreated WT cells. Mean ± s.e.m., n = 3 biological replicates. Asterisks indicate statistical significance compared with the corresponding value in WT cells, as judged by a two-tailed Student’s t-test assuming equal variance. An exception was the test against the normalized value for WT cells, for which a two-tailed Student’s t-test with unequal variance was applied. **P < 0.01; n.s., not significant.

B, C  Flow cytometric measurements of GFP levels of WT and Δice2 cells containing the HAC1 mRNA splicing reporter (SSY2309, 2313). Cells were treated with 8 mM DTT (B) or 1 μg/ml tunicamycin (C) for the times indicated. Data were normalized to untreated WT cells. Mean ± s.e.m., n = 3 biological replicates. Asterisks indicate statistical significance compared with the corresponding value in WT cells, as judged by a two-tailed Student’s t-test assuming equal variance. Exceptions were the tests against the normalized values for WT cells, for which a two-tailed Student’s t-test with unequal variance was applied. *P < 0.05; **P < 0.01; n.s., not significant.

Source data are available online for this figure.
Figure EV3. Absence of lipid droplets has no effect on ER expansion in WT or Δice2 cells.

A Growth assays of untreated WT, Δice2, ΔLD, and ΔLD Δice2 cells (SSY2228, 2229, 2230, 2256). Numbers represent areas under the curves and serve as growth indices. Mean ± s.e.m., n = 3 biological replicates. ΔLD, Δlipid droplet.

B, C Quantification of peripheral ER structures in WT, Δice2, ΔLD, and ΔLD Δice2 cells harboring the inducible system (SSY2598, 2599, 2600, 2601), which were untreated or treated with either 800 nM estradiol for 6 h (B) or 8 mM DTT for 1 h (C). Bars are the mean percentage of cell cortex covered by tubules (purple) or sheets (green), n = 3 biological replicates. Upper error bars are s.e.m. for the sum of tubules and sheets, and lower error bars are s.e.m. for sheets. Asterisks indicate statistical significance, as judged by a two-tailed Student’s t-test assuming equal variance. *P < 0.05; **P < 0.01; n.s., not significant.

D Lipidomic analysis of WT, Δice2, Δnem1, Δice2 Δnem1, Δspo7, and Δice2 Δspo7 cells (SSY1404, 2356, 2482, 2484, 2481, 2483). Mean ± s.e.m., n = 4 biological replicates. Asterisks indicate statistical significance compared with WT cells, as judged by a two-tailed Student’s t-test assuming equal variance. *P < 0.05; **P < 0.01. The data are the same as in Fig 5C and D but are shown as lipid-to-protein ratios in µg measured lipid per g total protein.

Source data are available online for this figure.
Figure EV3.
Figure EV4. Abundance of Ice2, Spo7, and Nem1.

A Western blot of HA from total membranes prepared from WT, ΔIce2, and ICE2-overexpressing and ΔSpo7 cells containing Nem1-HA (SSY2913, 2914, 2915, 2945). An unspecific band served as a loading control.

B Western blot of HA from total cell membranes from WT cells (SSY122) and cells expressing Ice2-HA, Spo7-HA, or Nem1-HA (SSY2421, 2910, 2913). Dpm1 served as a loading control.

C Western blot of HA from total membranes prepared from WT cells (SSY122), WT, ΔIce2, and ICE2-overexpressing cells containing Spo7-HA (SSY2910, 2911, 2912), and WT, ΔIce2, and ICE2-overexpressing cells containing Nem1-HA (SSY2913, 2914, 2915). Sec61 served as a loading control. o/e, overexpression.

D Images of cells expressing endogenously tagged Ice2-mScarlet and Sei1-mNeon (SSY3318) and stained with monodansylpentane to highlight lipid droplets. Arrows indicate foci containing Ice2.

Source data are available online for this figure.
Figure EV4.
Figure EV5. Phosphorylation status of pah1(7A).
Western blot of HA from WT and Δice2 cells in which PAH1 was replaced with PAH1-HA or pah1(7A)-HA as indicated (SSY2841, SSY2842, SSY2970).
Source data are available online for this figure.