Fig. S1. Protein amount of Lem2 drastically decreases within 10 hours after shut-off in the bqt4Δ lem2 shut-off cells.

Western blotting of Lem2 shut-off at several time points after induction by thiamine. The bqt4Δ lem2 shut-off cells were cultured in liquid EMMG at 30°C and lem2 shut-off was induced by adding thiamine into the medium. The cells were harvested at indicated times after shut-off (top) and western blotting was performed as described previously (Asakawa et al., 2014), with slight modifications. After NaOH/trichloroacetic acid precipitation, the pellets were resuspended in 2× Laemmli’s sample buffer. The protein extracts from to 5 × 10⁶ cells were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) on a 10% polyacrylamide gel. After SDS-PAGE, the proteins were transferred to a polyvinylidene fluoride (PVDF) membrane by wet transfer. The membrane was blocked with 5% skim milk and incubated with a rat monoclonal anti-HA antibody (50 ng/mL; 3F10, SIGMA-ALDRICH, MO, USA) for 3 hours at room temperature. The membrane was washed three times with PBS containing 0.05% Tween-20 and then incubated with horseradish peroxidase (HRP)-conjugated donkey anti-rat IgG (1:2000; GE Healthcare, IL, USA) overnight at 4°C. Bands were detected by chemiluminescence using a ChemiDocTM MP imaging system (Bio-Rad, CA, USA). α-tubulin served as a loading control and was detected by a mouse monoclonal anti-α-tubulin antibody (1:4000; DM1A, SIGMA-ALDRICH) and an HRP-conjugated sheep anti-mouse IgG antibody (1:2000; GE Healthcare, IL, USA). Longer exposure resulted in saturation for some bands, but enabled capturing weak bands on the same blot (“long exposure”). Molecular weights are indicated on the left.
Fig. S2. Elo2 suppresses the synthetic lethality of Lem2 and Bqt4 double deletion in the absence of Elo1.

Multicopy suppression of the bqt4Δ lem2 shut-off phenotype in the elo1Δ background. The elo1Δ bqt4Δ lem2 shut-off cells with multicopy plasmids containing the elo2+ gene (“elo2+”) or no gene (“control”) were precultured in liquid EMMG. The cells were plated on EMMG with (“+ Thiamine”, right) or without (“−”, left) thiamine in five-fold serial dilutions and incubated at 30°C for 3 days.
Aligned amino acid sequences and predicted secondary structures of *S. pombe*, *S. cerevisiae* and human fatty acid elongases. Red and orange boxes represent predicted α-helix and β-sheet regions, respectively. Blue characters represent predicted transmembrane regions. The alignment was performed using MEGA7 software (Kumer et al., 2016). The secondary structure prediction was predicted using PSIPRED (Daniel et al., 2013), and the transmembrane regions were predicted using TMHMM Server v. 2.0 (Krogh et al., 2001).
**Fig. S4.** The suppression of *bqt4Δ lem2* shut-off growth defect by increased expression of *elo2* under various promoters.

*elo2* gene was ectopically expressed from the *lys1* gene locus under the *adh1, 11, 13, 15* or *21* promoter in the *bqt4Δ lem2* shut-off strain. The *adh11, 13, 15* and *21* promoters are weaker versions of *adh1* with different TATA box sequences: “TATAAA”, “TATAT”, “TAAATATA”, and “TAAATA” instead of “TATAAATA” in the *adh1* promoter (gifts from Dr. T. Sakuno, The University of Tokyo) (Sakuno et al., 2009). The *bqt4Δ lem2* shut-off cells transformed with an empty vector (“control”) and *lem2* (“*lem2P-lem2*”) represent a negative and a positive control, respectively. The cells were cultured on EMMG plate in the absence (“-”, left) or presence (“+ Thiamine”, right) of thiamine in five-fold serial dilutions, and incubated at 30°C for 3 days (“-”) or 4 days (“+ Thiamine”). The *elo2* overexpression under the *adh1* promoter was extremely toxic in *lem2* ON cells (“*adh1P-elo2*”, left). However, the *adh1* promoter showed the highest suppression of all *adh1* derivative promoters when *lem2* was shut-off (right).
Fig. S5. Cytoplasm proteins also leaked into the nucleus when nuclear proteins leaked out.

A montage of time-lapse images (top) and the graph of the nuc:cyt ratio of GFP and mRFP fluorescence intensity (bottom) of the bqt4Δ lem2 shut-off cell expressing mRFP-GST-NLS and Rna1-GFP. mRFP-GST-NLS was expressed under adh1 promoter. Chromosomal fusion of GFP to rna1+ were performed using a PCR-based gene targeting method (Bähler et al., 1998). The bqt4Δ lem2 shut-off cells were cultured in EMMG with thiamine for 8 hours before the observation to shut off lem2+. Nuclear and cell shapes were recognized according to the nuclear mRFP-GST-NLS and the cytoplasmic Rna1-GFP fluorescent images, respectively, and fluorescence intensities were quantified on a single focal plane image in each time point. The bar represents 5 μm.
Fig. S6. Nuclear protein leakage does not occur in the elo2 shut-off cells.

Snapshot images of the elo2 shut-off cells (left) and the quantification of cells showing nuclear protein leakage (right). The elo2 shut-off cells expressing GFP-GST-NLS and Ish1-mCherry were cultured in EMMG with ("+ Thiamine") and without ("-" ) thiamine liquid medium for 24 hours and were observed under a fluorescence microscope. Single section images processed by denoising and 3D deconvolution are displayed at higher brightness to show the GFP fluorescence signals in the cytoplasm. The bars represent 5 μm. Cells with higher GFP fluorescence intensity in the cytoplasm than in the background were counted as "leaking" cells. The numbers under the bars indicate the number of cells examined.
Table S1. Yeast strains used in this study

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<td>YK312</td>
<td>S. pombe</td>
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<td>h' ura4-D18 lys1'::nmt81P-lem2::GFP lym2Δ::karb bqt4Δ::hph elo1Δ::nat pFL20-elo2'</td>
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<td>YK730</td>
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<td>YK732</td>
<td>S. pombe</td>
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<td>S. pombe</td>
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<td>This study</td>
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<td>YK744</td>
<td>S. pombe</td>
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<td>This study</td>
<td>S4</td>
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<td>YK882</td>
<td>S. pombe</td>
<td>h' nat1-GFP::nat aur1'::adh1P-mRFP-GST-NLS leu1'::nmt81P-FLAG-lem2::IAA17-HA lym2Δ::karb bqt4Δ::hph lys1'::pYC36</td>
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<tr>
<td>YK934</td>
<td>S. pombe</td>
<td>h' ura4-D18 leu1'::adh1P-GFP-GST-NLS ish1-mCherry::ura4' aur1'::nmt41P-FLAG-elo2-2-HA elo2Δ::kar ly1'::pYC36</td>
<td>This study</td>
<td>S6</td>
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## Table S2. Plasmids used in this study

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
<th>Construction</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>pHA203</td>
<td>lys1-N nmt81P-lem2-2-GFP-nmt1T</td>
<td>leu2&quot; ORF fused by GFP(s65I) was amplified by PCR and inserted between the BamHI and BglII sites of pCST5 (nmt81P version of pCST3, Chikashige et al. 2004).</td>
<td>This study</td>
</tr>
<tr>
<td>pYK206</td>
<td>lys1-N nmt81P-FLAG-lem2-AIA17-HA-nmt1T</td>
<td>leu2&quot; ORF fused by IAA17 coding sequence was amplified and inserted between the SalI and BamHI sites of pCST5-FLAG-HA (nmt81P version of pCST3-FLAG-HA, Hirano et al. 2018).</td>
<td>This study</td>
</tr>
<tr>
<td>pFL20</td>
<td>ars stb URA3*</td>
<td></td>
<td>Rosson and Lacroute 1983</td>
</tr>
<tr>
<td>pYK299</td>
<td>ars stb URA3* lem2*</td>
<td>lem2&quot; (-991 to +2990 from the beginning of the protein coding sequence) was amplified from S. pombe genome DNA and inserted at the BamHI site of pFL20.</td>
<td>This Study</td>
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<tr>
<td>pYK300</td>
<td>ars stb URA3* bqt4*</td>
<td>bqt4&quot; (-1000 to +2000 from the beginning of the protein coding sequence) was amplified from S. pombe genome DNA and inserted at the BamHI site of pFL20.</td>
<td>This Study</td>
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<tr>
<td>pYK94</td>
<td>ars stb URA3* Chl:280819-2817505</td>
<td>Screened plasmid from S. pombe genome library (pTN-F1).</td>
<td>This Study</td>
</tr>
<tr>
<td>pYK97</td>
<td>ars stb URA3* elo2*</td>
<td>elo2&quot; (-497 to +2281 from the beginning of the protein coding sequence) was amplified from S. pombe genome DNA and inserted at the BamHI site of pFL20.</td>
<td>This Study</td>
</tr>
<tr>
<td>pYK99</td>
<td>ars stb URA3* elo1*</td>
<td>elo1&quot; (-1109 to +2608 from the beginning of the protein coding sequence) was amplified from S. pombe genome DNA and inserted at the BamHI site of pFL20.</td>
<td>This Study</td>
</tr>
<tr>
<td>pYK224</td>
<td>leu1-N nmt81P-FLAG-lem2-AIA17-HA-nmt1T</td>
<td>leu1-N sequence was amplified from pYC28 (Chikashige et al. 2017) and inserted at the NotI site of pYK206 (NotI sites of pYK206 were deleted).</td>
<td>This study</td>
</tr>
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<td>pYC36</td>
<td>lys1-N</td>
<td></td>
<td>Chikashige et al. 2004</td>
</tr>
<tr>
<td>pYK139</td>
<td>lys1-N nda3P-elo2*-nmt1T</td>
<td>elo2&quot; ORF was amplified from S. pombe cDNA and inserted between BamHI and BglII site of pCSTnda (nda3P version of pCST3).</td>
<td>This study</td>
</tr>
<tr>
<td>pYK203</td>
<td>aur1&quot; nmt41P-FLAG-elo2-HA-nmt1T</td>
<td>elo2&quot; ORF was digested from pYK139 (between the BamHI and BglII sites) and inserted between the BamHI and BglII sites of pYK179 (a derivative of pCST3-FLAG-HA harboring aur1&quot; and nda3P instead of lys1-N and nmt1P).</td>
<td>This study</td>
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<tr>
<td>pYK147</td>
<td>lys1-N adh11P-nmt1T</td>
<td>nmt1P of pCST3 (between the PstI and SaI sites) was exchanged for adh11P.</td>
<td>This study</td>
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<tr>
<td>pYK207</td>
<td>lys1-N adh11P-GFP-nmt1T</td>
<td>GFP(s65I) was inserted between the BamHI and BglII sites of pYK147.</td>
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</tr>
<tr>
<td>pYK262</td>
<td>lys1-N adh11P-ELOV1-1-GFP-nmt1T</td>
<td>H. sapiens ELOVL1 ORF was amplified by PCR from HA-ELOV1 plasmid (Ohno et al. 2010) and inserted at the SaI site of pYK207.</td>
<td>This study</td>
</tr>
<tr>
<td>pYK263</td>
<td>lys1-N adh11P-ELOV2-2-GFP-nmt1T</td>
<td>H. sapiens ELOVL2 ORF was amplified by PCR from HA-ELOV2 plasmid (Ohno et al. 2010) and inserted at the SaI site of pYK207.</td>
<td>This study</td>
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<tr>
<td>pYK264</td>
<td>lys1-N adh11P-ELOV3-1-GFP-nmt1T</td>
<td>H. sapiens ELOVL3 ORF was amplified by PCR from HA-ELOV3 plasmid (Ohno et al. 2010) and inserted at the SaI site of pYK207.</td>
<td>This study</td>
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<tr>
<td>pYK265</td>
<td>lys1-N adh11P-ELOV4-4-GFP-nmt1T</td>
<td>H. sapiens ELOVL4 ORF was amplified by PCR from HA-ELOV4 plasmid (Ohno et al. 2010) and inserted at the SaI site of pYK207.</td>
<td>This study</td>
</tr>
<tr>
<td>pYK266</td>
<td>lys1-N adh11P-ELOV5-5-GFP-nmt1T</td>
<td>H. sapiens ELOVL5 ORF was amplified by PCR from HA-ELOV5 plasmid (Ohno et al. 2010) and inserted at the SaI site of pYK207.</td>
<td>This study</td>
</tr>
<tr>
<td>pYK267</td>
<td>lys1-N adh11P-ELOV6-6-GFP-nmt1T</td>
<td>H. sapiens ELOVL6 ORF was amplified by PCR from HA-ELOV6 plasmid (Ohno et al. 2010) and inserted at the SaI site of pYK207.</td>
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<tr>
<td>pYK268</td>
<td>lys1-N adh11P-ELOV7-7-GFP-nmt1T</td>
<td>H. sapiens ELOVL7 ORF was amplified by PCR from HA-ELOV7 plasmid (Ohno et al. 2010) and inserted at the SaI site of pYK207.</td>
<td>This study</td>
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<td>pYK254</td>
<td>lys1-N adh11P-EL01-1-GFP-nmt1T</td>
<td>S. cerevisiae ELO1 ORF was amplified by PCR from S. cerevisiae genome DNA and inserted at the BamHI site of pYK207 (the downstream BamHI site was deleted).</td>
<td>This study</td>
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Table S2. Plasmids used in this study (cont.)

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<th>Name</th>
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<td>pYK255</td>
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<td>S. cerevisiae ELO2 ORF was amplified by PCR from S. cerevisiae genome DNA and inserted at the BamHI site of pYK207 (the downstream BamHI site was deleted).</td>
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<tr>
<td>pYK256</td>
<td>lys1-N adh11P-ELO3-GFP-nmt1T</td>
<td>S. cerevisiae ELO3 ORF was amplified by PCR from S. cerevisiae genome DNA and inserted at the BamHI site of pYK207 (the downstream BamHI site was deleted).</td>
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<td>pYK159</td>
<td>lys1-N adh11P-elo2-GFP-nmt1T</td>
<td>elo2 ORF was amplified by PCR from S. pombe cDNA and inserted at the BamHI site of pYK207 (the downstream BamHI site was deleted).</td>
<td>This study</td>
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<td>pYK321</td>
<td>lys1-N adh11P-elo1-GFP-nmt1T</td>
<td>elo1 ORF was amplified by PCR from S. pombe cDNA and inserted at the BamHI site of pYK207 (the downstream BamHI site was deleted).</td>
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<td>pAKNF313</td>
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<td>Ohno et al. 2010</td>
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<td>pYK130</td>
<td>CEN/ARS HIS3 TDH3P-3FLAG-elo2</td>
<td>elo2 ORF was amplified by PCR from S. pombe cDNA and inserted between the BamHI and SacI sites of pAKNF313.</td>
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<td>elo1 ORF was amplified by PCR from S. pombe cDNA and inserted between the BamHI and SacI sites of pAKNF313.</td>
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<td>CEN/ARS HIS3 TDH3P-3FLAG-ELO3</td>
<td>S. cerevisiae ELO3 ORF was amplified by PCR from S. cerevisiae genome DNA and inserted between the BamHI and SacI sites of pAKNF313.</td>
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<td>pYK153</td>
<td>lys1-N adh11P-elo2-nmt1T</td>
<td>elo2 ORF was amplified by PCR from S. pombe cDNA and inserted between the BamHI and BglII sites of pYK147.</td>
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<tr>
<td>pYK350</td>
<td>lys1-N adh11P-elo2-H168A-nmt1T</td>
<td>pYK153 was amplified using mutated primers (5'-CCATTGCTACGCCCATGGTATTACGGCTCTCATTG-3', 5'-ATGGGCGTAGCAATGGAGAAAGGCAAGGGTTTC-3') to induce H168A (CAC → GCC) mutation.</td>
<td>This study</td>
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<td>pYC19</td>
<td>aur1r</td>
<td></td>
<td>Chikashige et al. 2004</td>
</tr>
<tr>
<td>pYK204</td>
<td>leu1-N adh1P-GFP-GST-NLS-nmt1T</td>
<td>nmt1P and lys1-N of pCST3 were exchanged for adh1P and leu1-N respectively. GFPs65I fused by GST and Xenopus laevis nucleoplasmin NLS was inserted between the BamHI and BglII sites.</td>
<td>This study</td>
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<tr>
<td>pYK276</td>
<td>aur1' adh11P-elo2'-nmt1T</td>
<td>elo2' ORF was digested from pYK153 (between the BamHI and BglII sites) and inserted between the BamHI and BglII sites of pYK171 (aur1' version of pYK147).</td>
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<td>pYK152</td>
<td>lys1-N adh1P-elo2'-nmt1T</td>
<td>elo2' ORF was digested from pYK153 (between the BamHI and BglII sites) and inserted between the BamHI and BglII sites of pYK146 (adh1P version of pYK147).</td>
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<td>elo2' ORF was digested from pYK153 (between the BamHI and BglII sites) and inserted between the BamHI and BglII sites of pYK148 (adh13P version of pYK147).</td>
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<td>pNF38</td>
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<td>Tange et al. 2016</td>
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<td>aur1' adh1P-GFP-GST-NLS-nmt1T</td>
<td>nmt1P and lys1-N of pCST3 were exchanged for adh1P and aur1' respectively. mRFP fused by GST and Xenopus laevis nucleoplasmin NLS was inserted between the BamHI and BglII sites.</td>
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Table S3. Selected m/z values and collision energy settings for detection of phytoceramides in LC-MS/MS analysis.

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<th>Phytoceramide species</th>
<th>Precursor ion (Q1) [M+H]^+</th>
<th>Product ion (Q3)</th>
<th>Collision energy (eV)</th>
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<td>t18:0-dsC16:0</td>
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<td>300.2</td>
<td>25</td>
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<tr>
<td>t20:0-C16:0</td>
<td>584.3</td>
<td>328.2</td>
<td>25</td>
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<tr>
<td>t20:0-C18:0</td>
<td>612.3</td>
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<td>25</td>
</tr>
<tr>
<td>t20:0-C20:0</td>
<td>640.3</td>
<td>328.2</td>
<td>30</td>
</tr>
<tr>
<td>t20:0-C22:0</td>
<td>668.3</td>
<td>328.2</td>
<td>30</td>
</tr>
<tr>
<td>t20:0-C24:0</td>
<td>696.3</td>
<td>328.2</td>
<td>35</td>
</tr>
<tr>
<td>t20:0-C26:0</td>
<td>724.3</td>
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</tr>
<tr>
<td>t20:0-hC16:0</td>
<td>600.3</td>
<td>328.2</td>
<td>25</td>
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<tr>
<td>t20:0-hC18:0</td>
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<td>t20:0-hC26:0</td>
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Movie 1. Time-lapse observation of nuclear protein leakage in living cells of the *bqt4Δ lem2* shut-off strain.

Movie associated with Fig. 7. Cells of the *bqt4Δ lem2* shut-off strain expressing GFP-GST-NLS were cultured in EMMG liquid medium and observed under a fluorescent microscope immediately after addition of thiamine. Images were collected every 5 minutes at 3 focal sections up to 24 hours. Unprocessed single-section images in every time points are shown in the movie. The number at upper-left indicates the elapsed time in hours:minutes.
Supplementary References


