Appendix

Shortening of membrane lipid acyl chains compensates for phosphatidylcholine deficiency in choline-auxotroph yeast

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Appendix Figure S1. Phenotypes of evolved 2n-1 cho2opi3 suppressors

(A) 2D-TLC analysis of total lipid extracts of coS#4 cells cultured in SD C+I+ and C-I+; ori, origin; NL, neutral lipids.

(B) Quantification of lipid droplet size in wild type, cho2opi3, coS#3 and S#4 after culture to mid-log phase in SD C-I+ as indicated. Lipid droplet relative size was determined as a percentage of total cell area in 15 2D projection images. Details are described in Materials and Methods; error bars represent the SD (n=15); ★ represents P-value < 0.0002 vs. wild type; * represents P-value <0.02 vs. S#3 without choline, as determined by Student’s t-test.

(C) cho2opi3 suppressors do not grow on the non-fermentable carbon source glycerol. Ten-fold serial dilutions of 1 OD600 unit/mL of the strains indicated were spotted on SG C-I+ plates, and incubated at 30°C for 10 d.

(D) Choline supplementation induces endoduplication of chr XV in aneuploid co S#3. Growth phenotype on SD C- and C+ (4 d at 30°C) and absolute copy number of chr I, IV, VI, IX and XV as determined by qPCR and FACS after culturing co S#3 in SD C+ for the number of days indicated with daily passage to fresh medium at OD600 0.05. Data are presented as mean value from 2 assays using primers complementary to non-coding regions on the left and right arm of each chr, respectively, with the individual values indicated.
Appendix Figure S2. Molecular species profiles of membrane lipids

Molecular species profiles (mol % of class) of (A) glycerolipid classes and (B) sphingolipid classes (sum of carbon atoms in the long chain base and fatty acyl chain : sum of double bonds in the long chain base and fatty acyl chain ; sum of hydroxyl groups in the long chain base and fatty acyl chain) in the strains indicated, cultured in in SD C\(^+/\). Molecular species contributing at least 2% of a class are depicted. Error bars depict SD (n=3).
Appendix Figure S3. Aneuploidy of cho2opi3lro1 pLRO1 suppressors

Absolute copy numbers of chr I, IV, VI, IX and XV in 4 cho2opi3lro1 pLRO1 suppressor clones and the haploid cho2opi3lro1 pLRO1 parent compared to wild type. Data are presented as mean value from 2 assays using primers complementary to non-coding regions on the left and right arm of each chr, respectively, with the individual values indicated.

Appendix Figure S4. Free fatty acid content and DGA1

(A) TLC analysis of neutral lipids shows accumulation of free fatty acids (FFA) in co S(2n-1) cultured without choline; STE, sterolester.

(B) Loss of 1 copy of DGA1 does not rescue the choline auxotrophy of the co diploid strain. Serial dilution experiment comparing haploid wild type and cho2opi3 to 2 clones of heterozygous diploid co/co DGA1/dga1 on SD C+/- after incubation for 3 d at 30°C.
Appendix Table S1. GO-BP enrichment for transcripts changing in PC-depleted cho2opi3 and in all 3 PC-free aneuploid cho2opi3 suppressors tested (S#3, S#4 and S#5)

Changes in transcript levels were used for enrichment analysis based on a fold-change cutoff of 1.7. Transcripts with decreased expression in the aneuploid cho2opi3 suppressors encoded by chromosome XV were omitted from the analysis. GO term enrichment was considered significant with a p-value less than 0.01 (after Bonferroni correction). Enriched GO terms were summarized by the REVIGO software using a cutoff value C of 0.5 (Supek et al., 2011).

Transcripts with increased expression: cho2opi3 vs. WT

| Gene Ontology – Biological Process                                         | Corrected p-value | Cluster frequency | Background frequency |
|--------------------------------------------------------------------------|--------------------|------------------|----------------------|
| oxidation-reduction process (GO:00551114)                                | 1.08E-43           | 120/420 (28.6%)  | 442/6439 (6.86%)     |
| generation of precursor metabolites and energy (GO:0006091)               | 1.88E-33           | 71/420 (16.9%)   | 193/6439 (3%)        |
| ATP metabolic process (GO:0046034)                                       | 4.03E-32           | 49/420 (11.7%)   | 90/6439 (1.4%)       |
| drug metabolic process (GO:0017144)                                      | 2.53E-27           | 78/420 (18.6%)   | 281/6439 (4.36%)     |
| small molecule metabolic process (GO:0044281)                            | 2.49E-26           | 133/420 (31.7%)  | 764/6439 (11.9%)     |
| nucleobase-containing small molecule metabolic process (GO:0055086)      | 7.39E-19           | 68/420 (16.2%)   | 289/6439 (4.49%)     |
| carbohydrate derivative metabolic process (GO:1901135)                   | 3.50E-13           | 70/420 (16.7%)   | 381/6439 (5.92%)     |
| carbohydrate metabolic process (GO:0005975)                              | 4.06E-11           | 52/420 (12.4%)   | 254/6439 (3.94%)     |
| transmembrane transport (GO:0055085)                                     | 1.61E-10           | 73/420 (17.4%)   | 457/6439 (7.1%)      |
| response to oxidative stress (GO:0006979)                                | 9.49E-08           | 31/420 (7.38%)   | 128/6439 (1.99%)     |
| protein folding (GO:0006457)                                             | 1.64E-06           | 28/420 (6.67%)   | 119/6439 (1.85%)     |
| phosphorus metabolic process (GO:0006793)                                | 2.11E-06           | 88/420 (21%)     | 724/6439 (11.2%)     |
| tricarboxylic acid metabolic process (GO:0072350)                        | 2.27E-06           | 14/420 (3.33%)   | 31/6439 (0.481%)     |
| response to abiotic stimulus (GO:0009628)                                | 3.06E-05           | 34/420 (8.1%)    | 186/6439 (2.89%)     |
| sulfur compound metabolic process (GO:0006790)                           | 1.11E-04           | 27/420 (6.43%)   | 134/6439 (2.08%)     |
| cofactor metabolic process (GO:0051186)                                  | 1.19E-04           | 40/420 (9.52%)   | 253/6439 (3.93%)     |
| response to chemical (GO:0042221)                                       | 2.68E-04           | 67/420 (16%)     | 551/6439 (8.56%)     |
| primary alcohol metabolic process (GO:0034308)                           | 3.48E-04           | 9/420 (2.14%)    | 17/6439 (0.264%)     |
| intron homing (GO:0006314)                                               | 2.17E-03           | 6/420 (1.43%)    | 8/6439 (0.124%)      |
| cellular aldehyde metabolic process (GO:0006081)                         | 4.24E-03           | 14/420 (3.33%)   | 52/6439 (0.808%)     |

Transcripts with decreased expression: cho2opi3 vs. WT

| Gene Ontology – Biological Process                                      | Corrected p-value | Cluster frequency | Background frequency |
|-------------------------------------------------------------------------|--------------------|------------------|----------------------|
| cellular amino acid metabolic process (GO:0006520)                     | 2.89E-13           | 40/233 (17.2%)   | 249/6439 (3.87%)     |
| ribonucleoprotein complex biogenesis (GO:0022613)                      | 2.40E-05           | 47/233 (20.2%)   | 566/6439 (8.79%)     |
| small molecule metabolic process (GO:0044281)                          | 2.88E-05           | 57/233 (24.5%)   | 764/6439 (11.9%)     |
| Gene Ontology – Biological Process                                      | Corrected p-value | Cluster frequency | Background frequency |
|------------------------------------------------------------------------|-------------------|-------------------|----------------------|
| oxidation-reduction process (GO:0055114)                               | 1.43E-05          | 16/49 (32.7%)     | 442/6439 (6.86%)     |
| sulfur amino acid biosynthetic process (GO:0000097)                     | 1.21E-04          | 6/49 (12.2%)      | 41/6439 (0.637%)     |
| small molecule metabolic process (GO:0044281)                           | 1.08E-03          | 18/49 (36.7%)     | 764/6439 (11.9%)     |
| sulfur compound metabolic process (GO:0006790)                          | 1.41E-03          | 8/49 (16.3%)      | 134/6439 (2.08%)     |
| fatty acid catabolic process (GO:0009062)                               | 3.19E-03          | 4/49 (8.16%)      | 20/6439 (0.311%)     |

Transcripts with increased expression: aneuploid suppressors co S#3, #4, #5 vs. WT

| Gene Ontology – Biological Process                                      | Corrected p-value | Cluster frequency | Background frequency |
|------------------------------------------------------------------------|-------------------|-------------------|----------------------|
| response to pheromone triggering conjugation with cellular fusion (GO:0000749) | 2.75E-06          | 11/106 (10.4%)    | 64/6439 (0.994%)     |
| cellular amino acid biosynthetic process (GO:0008652)                   | 2.69E-04          | 17/106 (16%)      | 131/6439 (2.03%)     |
| one-carbon metabolic process (GO:0006730)                               | 4.27E-03          | 5/106 (4.72%)     | 18/6439 (0.28%)      |
| small molecule metabolic process (GO:0044281)                           | 5.05E-03          | 29/106 (27.4%)    | 764/6439 (11.9%)     |
### Appendix Table S2. Primers used in this study

| Construct | Oligos |
|-----------|--------|
| **OPI3-LEU2**<sup>1</sup> | **Forward** 5’CAGAGCCATAAACAGCAATTGAAGCAAACGAATAGCGTGTAAGATGCAAGAGTTCG3’<br>**Reverse** 5’GCATAGGCTTCAAACATATAGAATATAGTAAGAGGACACCCCTCCTCTTGCTCAATATTA3’ |
| **OPI3-LEU2** control PCR | **A** 5’TGGCTTCCGTATGAGCAGGGT3’<br>**D** 5’ACTGAGAAGAAATTCG3’ |
| **LRO1-HIS3**<sup>2</sup> | **Forward** 5’TCTCTAAATAACGCGATGAGGAAGACGTCATAGTAACAGCCGTAATCTGTGCGTCGAC3’<br>**Reverse** 5’CTTGGAAATAATACACGGATGGATAGTGAGTCAATGTCGGATCGAGCTACGCTG3’ |
| **LRO1-HIS3** control PCR | **A** 5’AAATGGAATGCGGCAAGAATG3’<br>**B** 5’CGCAGATGCGCTGATGGGATGGTATCTTACGCTGAC3’<br>**C** 5’AAATGACATCTAGGCTGCTG3’<br>**D** 5’ACTGACGACAAAGCCATACCTC3’ |
| **DGA1-HIS3**<sup>2</sup> | **Forward** 5’CGACAGTGGGCTCATAGGCTGATGGGATGGTATCTTACGCTGAC3’<br>**Reverse** 5’AGCTGACGACAAAGCCATACCTC3’ |
| **DGA1-HIS3** control PCR | **A** 5’GGAGTACCTTACACACCGGGG3’<br>**D** 5’GGAGTACCTTACACACCGGGG3’ |
| **POX1-HIS3**<sup>2</sup> | **Forward** 5’TGACACATTTAAGCCCAAGAAGGTG3’<br>**D** 5’TGACACATTTAAGCCCAAGAAGGTG3’ |
| **POX1-HIS3** control PCR | **A** 5’GAGGTACTCCGAGCAGGAATGGG3’<br>**B** 5’GACGTTCGTTCGACTGATGA3’<br>**C** 5’TAATGACATCCTAGTGCAG3’<br>**D** 5’TGGGTTCTCTTAAAGCCGCA3’ |
| **CEN15:pGal1-HIS3** control PCR | **A** 5’TCAACCAACCTCAAACCTCAG3’<br>**B** 5’GACGTTCGTTCGACTGATGA3’<br>**C** 5’TTGTGACGCTTCCATCAG3’<br>**D** 5’TGGGTTCTCTTAAAGCCGCA3’ |
| **gRNA**<sup>2</sup> | **Forward** 5’TGCAGCTTCTCTCCGCGTAAACACCTCTCCTCAGTAAAATGATCGGGAATCAATACG3’<br>**Reverse** 5’TGGTGAATACGGACTAGCCCTTTAATTTTAAATCTAGCTAAACACCCAGCTATGATGCTTCTC3’ |
| **Repair DNA**<sup>3</sup> | **Forward** 5’TTCCTTGTCTTTAGCCTCCACATCTTCCAACACACGATTACCAGG3’<br>**Reverse** 5’TGGTGAATACGGACTAGCCCTTTAATTTTAAATCTAGCTAAACACCCAGCTATGATGCTTCTC3’ |
| **ACC1-acc1<sup>N1446H</sup>** | **Forward** 5’TCGTCGGTCCTATCGTTCGCTG3’<br>**Reverse** 5’ACGAGATTGCAACGCAAGA3’<br>**Sequencing** 5’AGAAGATGCGCAGATACATC3’ |

<sup>1</sup>The underlined sequences correspond to nucleotides upstream (forward) and downstream (reverse complementary) of the gene to be deleted.
The underlined sequences present the ACC1 target sequence (that is followed by the PAM [NGG] on the chromosome); the flanking sequences overlap with both sides of the linearized pMEL16 backbone. The nucleotide in bold indicates the nucleotide to be edited.

The nucleotide in bold indicates the edited nucleotide.