SUPPLEMENTAL INFORMATION FOR MAIN FIGURES

Figure 1. **Aneuploidy Increases Sphingolipid Biosynthesis**

(A) For simplicity, we only depicted genes included in our studies. A complete set of genes that regulate sphingolipid synthesis in yeast can be found in several reviews (Montefusco et al., 2014). SPT is composed of 2 subunits: *LCB1* (*YMR296C*) and *LCB2* (*YDR062W*). LCB4 (*YOR171C*) = LCB kinase. LCB3 (*YJL134W*) = LCB-P phosphatase. *DPL1* (*YDR294C*) = LCB-P lyase. *YPC1* (*YBR183W*) and *YDC1* (*YPL087W*) = ceramidase. *LAG1 (YHL003C*) and *LAC1* (*YKL008C*)= ceramide synthase. *AUR1* (*YKL004W*) = inositol phosphorylceramide synthase. *CSG2* (*YBR036C*) = required for mannosylation of inositolphosphorylceramide.

(B) 10-fold serial dilutions of cells were incubated at 30ºC for 2-3 days. Strains plated from top to bottom are: top plate: E113 (WT), E88 (Dis I), E89 (Dis II), E91 (Dis IV), E92 (Dis V), E93 (Dis VIII), and E94 (Dis IX); second plate: E113 (WT), E96 (Dis X), E97 (Dis XI), E98 (Dis XII), E100 (Dis XIII), E101 (Dis XIV), E102 (Dis XV), and E103 (Dis XVI); third plate: E113 (WT), E104 (Dis XI + XV), E105 (Dis XI + XVI), E106 (Dis I + VI + XIII), E108 (Dis VIII + XIV); bottom plate: E113 (WT), E507 (YAC I), E508 (YAC II), E509 (YAC III), E510 (YAC IV), and E511 (YAC V).

(C) Left panel: quantification of the viability of cells treated with myriocin. Cell densities were quantified from high resolution tiff images of cell plated on agar using image J (https://imagej.nih.gov/ij/index.html). 3 biological replicates are shown in Figures 1B, 3E, and S3B. Right panel: doubling times of: E113 (WT), E88 (Dis I), E89 (Dis II), E91 (Dis IV), E92 (Dis V), E93 (Dis VIII), E94 (Dis IX), E96 (Dis X), E97 (Dis XI), E98 (Dis XII), E100 (Dis XIII), E101 (Dis XIV), E102 (Dis XV), and E103 (Dis XVI), with or without myriocin.

(D) 10-fold serial dilutions of cells were incubated at 30ºC for 2-3 days. Strains plated from top to bottom are: top plate top plate: E113 (WT), E88 (Dis I), E89 (Dis II), E91 (Dis IV), E92 (Dis V), E93 (Dis VIII), and E94 (Dis IX); second plate: E113 (WT), E96 (Dis X), E97 (Dis XI), E98 (Dis XII), E100 (Dis XIII), E101 (Dis XIV), E102 (Dis XV), and E103 (Dis XVI); third plate: E113 (WT), E104 (Dis XI + XV), E105 (Dis XI + XVI), E106 (Dis I + VI + XIII), E108 (Dis VIII + XIV); bottom plate: E113 (WT), E507 (YAC I), E508 (YAC II), E509 (YAC III), E510 (YAC IV), and E511 (YAC V). Note: disome XI, which harbors 2 copies of, the cerulenin target gene *FAS1* displayed resistance to this drug.

(E) 10-fold serial dilutions of cells were incubated at 30ºC for 2-3 days. Strains plated from top to bottom: E113 (WT), E533 (*cdc4-1*), E534 (*cdc15-2*), E535 (*cdc28-13*), and E93 (Dis VIII).

(F) Relative levels of LCB and ceramides relative to E113 (WT) for strains (left to right): E88 (Dis I), E89 (Dis II), E91 (Dis IV), E92 (Dis V), E93 (Dis VIII), E94 (Dis IX), E96 (Dis X), E97 (Dis XI), E98 (Dis XII), E100 (Dis XIII), E101 (Dis XIV), E102 (Dis XV), and E103 (Dis XVI). See

Table S1 for $\log_2$ ratios values.
(G) No additional information.

Figure 2. Aneuploid Cells Require Increase Serine Biosynthesis for Their Survival
(A) No additional information.
(B) Data obtained from (Dephoure et al., 2014).
(C) Growth curves of E192 (WT) and E567 (*ser2*Δ) (left), E194 (Dis II) and E569 (Dis II-*ser2*Δ) (right).
(D) Strains harboring *SER2*: E192 (WT), E193 (Dis I), E194 (Dis II), E195 (Dis IV), E196 (Dis V), E197 (Dis VIII), E198 (Dis IX), E199 (Dis X), E200 (Dis XI), E201 (Dis XII), E202 (Dis XIII), E203 (Dis XIV), E204 (Dis XV), E205 (Dis XVI). Strains harboring *ser2*Δ: E567 (*ser2*Δ), E568 (Dis I-*ser2*Δ), E569 (Dis II- *ser2*Δ), E570 (Dis IV-*ser2*Δ), E571 (Dis V-*ser2*Δ), E572 (Dis VIII-*ser2*Δ), E573 (Dis IX-*ser2*Δ), E574 (Dis X-*ser2*Δ), E575 (Dis XI-*ser2*Δ), E576 (Dis XII-*ser2*Δ), E577 (Dis XIII-*ser2*Δ), E578 (Dis XIV-*ser2*Δ), E579 (Dis XV-*ser2*Δ), E580 (Dis XVI-*ser2*Δ).
(E) Growth curves of E567 (*ser2*Δ) (left) and E569 (Dis II-*ser2*Δ) (right).
(F) Doubling times of E567 (*ser2*Δ), E568 (Dis I-*ser2*Δ), E569 (Dis II- *ser2*Δ), E570 (Dis IV-*ser2*Δ), E571 (Dis V-*ser2*Δ), E572 (Dis VIII-*ser2*Δ), E573 (Dis IX-*ser2*Δ), E574 (Dis X-*ser2*Δ), E575 (Dis XI-*ser2*Δ), E576 (Dis XII-*ser2*Δ), E577 (Dis XIII-*ser2*Δ), E578 (Dis XIV-*ser2*Δ), E579 (Dis XV-*ser2*Δ), and E580 (Dis XVI-*ser2*Δ).

Figure 3. Aneuploid Cells Use Serine for the Synthesis of Sphingolipids
(A) Strains used: WT (E567), disome VIII (E572), and disome XIV (E578).
(B-C) Strains use: E567 (*ser2*Δ), E568 (Dis I-*ser2*Δ), E569 (Dis II- *ser2*Δ), E570 (Dis IV-*ser2*Δ), E571 (Dis V-*ser2*Δ), E572 (Dis VIII-*ser2*Δ), E573 (Dis IX-*ser2*Δ), E574 (Dis X-*ser2*Δ), E575 (Dis XI-*ser2*Δ), E576 (Dis XII-*ser2*Δ), E577 (Dis XIII-*ser2*Δ), E578 (Dis XIV-*ser2*Δ), E579 (Dis XV-*ser2*Δ), and E580 (Dis XVI-*ser2*Δ).
(D) Linear fit performed with GraphPad Prism7.
(E) Proliferative capability cell with wild type copy of *SER2* and cells harboring *ser2*Δ in the presence of myriocin. Strains used (top to bottom): E192 (WT), E567 (*ser2*Δ), E193 (Dis I), E568 (Dis I-*ser2*Δ), E194 (Dis II), E569 (Dis II- *ser2*Δ), E195 (Dis IV), E570 (Dis IV-*ser2*Δ), E192 (WT), E196 (Dis V), E571 (Dis V-*ser2*Δ), E197 (Dis VIII), E572 (Dis VIII-*ser2*Δ), E198 (Dis IX), E573 (Dis IX-*ser2*Δ), E192 (WT), E199 (Dis X), E574 (Dis X-*ser2*Δ), E200 (Dis XI), E575 (Dis XI-*ser2*Δ), E201 (Dis XII), E576 (Dis XII-*ser2*Δ), E192 (WT), E202 (Dis XIII), E577 (Dis XIII-*ser2*Δ), E203 (Dis XIV), E578 (Dis XIV-*ser2*Δ), E204 (Dis XV), E579 (Dis XV-*ser2*Δ), E205 (Dis

XVI), E580 (Dis XVI-*ser2*Δ).

Figure 4. Reduced Ceramide Synthesis Improves the Fitness of Aneuploid Cells

(A) Strains harboring *LAG1* are marked with *CaURA3* to match the deletions: E192 (WT), E193 (Dis I), E194 (Dis II), E195 (Dis IV), E196 (Dis V), E198 (Dis IX), E199 (Dis X), E200 (Dis XI), E201 (Dis XII), E202 (Dis XIII), E203 (Dis XIV), E204 (Dis XV), and E205 (Dis XVI). Strains harboring *lag1*Δ: E413 (*lag1*Δ), E414 (Dis I-*lag1*Δ), E415 (Dis II-*lag1*Δ), E416 (Dis IV-*lag1*Δ), E417 (Dis V-*lag1*Δ), E419 (Dis IX-*lag1*Δ), E420 (Dis X-*lag1*Δ), E421 (Dis XI-*lag1*Δ), E908 (Dis XII-*lag1*Δ), E423 (Dis XIII-*lag1*Δ), E424 (Dis XIV-*lag1*Δ), E425 (Dis XV-*lag1*Δ), and E426 (Dis XVI-*lag1*Δ). Note: *LAG1* is located on chromosome VIII therefore disome VIII was omitted from this analysis.

(B) Strains harboring *LCB3* are marked with *CaURA3* to match the deletions: E192 (WT), E193 (Dis I), E194 (Dis II), E195 (Dis IV), E196 (Dis V), E197 (Dis VIII), E198 (Dis IX), E200 (Dis XI), E201 (Dis XII), E202 (Dis XIII), E203 (Dis XIV), E204 (Dis XV), and E205 (Dis XVI). Strains harboring *lcb3*Δ: E445 (WT-*lcb3*Δ), E446 (Dis I-*lcb3*Δ), E447 (Dis II-*lcb3*Δ), E448 (Dis IV-*lcb3*Δ), E449 (Dis V-*lcb3*Δ), E450 (Dis VIII-*lcb3*Δ), E451 (Dis IX-*lcb3*Δ), E453 (Dis XI-*lcb3*Δ), E454 (Dis XII-*lcb3*Δ), E455 (Dis XIII-*lcb3*Δ), E456 (Dis XIV-*lcb3*Δ), E457 (Dis XV-*lcb3*Δ), and E458 (Dis XVI-*lcb3*Δ). Note: *LCB3* is located on chromosome X therefore disome X was omitted from this analysis.

(C) Strains harboring LCB4 are marked with CaURA3 to match deletions: E192 (WT), E193 (Dis I), E194 (Dis II), E195 (Dis IV), E196 (Dis V), E197 (Dis VIII), E198 (Dis IX), E199 (Dis X), E200 (Dis XI), E201 (Dis XII), E202 (Dis XIII), E203 (Dis XIV), and E205 (Dis XVI). Strains harboring lcb4Δ: E460 (*lcb4*Δ), E461 (Dis I-*lcb4*Δ), E462 (Dis II-*lcb4*Δ), E463 (Dis IV-*lcb4*Δ), E464 (Dis V-*lcb4*Δ), E474 (Dis VIII-*lcb4*Δ), E466 (Dis IX-*lcb4*Δ), E497 (Dis X-*lcb4*Δ), E468 (Dis XI-*lcb4*Δ), E469 (Dis XII-*lcb4*Δ), E470 (Dis XIII-*lcb4*Δ), E471 (Dis XIV-*lcb4*Δ), and E473 (Dis XVI-*lcb4*Δ).

Figure 5. Effects of Shifting the Balance between Ceramide and Long-chain Bases in Aneuploid Strains.

(A) Strains harboring *CSG2* are: E192 (WT), E193 (Dis I), E195 (Dis IV), E196 (Dis V), E197 (Dis VIII), E198 (Dis IX), E199 (Dis X), E200 (Dis XI), E201 (Dis XII), E202 (Dis XIII), E203 (Dis XIV), E204 (Dis XV), E205 (Dis XVI). Strains harboring *csg2*Δ: E910 (*csg2*Δ), E911 (Dis I-*csg2*Δ), E913 (Dis IV-*csg2*Δ), E914 (Dis V-*csg2*Δ), E915 (Dis VIII-*csg2*Δ), E916 (Dis IX-*csg2*Δ), E917 (Dis X-*csg2*Δ), E918 (Dis XI-*csg2*Δ), E919 (Dis XII-*csg2*Δ), E920 (Dis XIII-*csg2*Δ), E921 (Dis XIV-*csg2*Δ), E922 (Dis XV-*csg2*Δ), and E923 (Dis XVI-*csg2*Δ).

(B) No additional information.

Figure 6. Loss of *LCB3* Remodels Membrane Protein Composition and Promotes RNA biosynthesis.

Gene expression of disomes and disome harboring *ubp6*Δ was obtained from (Dephoure et al., 2014). Strains analyzed in this study are the disome *lcb3*Δ: E445 (WT-*lcb3*Δ), E446 (Dis I-*lcb3*Δ), E447 (Dis II-*lcb3*Δ), E448 (Dis IV-*lcb3*Δ), E449 (Dis V-*lcb3*Δ), E450 (Dis VIII-*lcb3*Δ), E451 (Dis IX-*lcb3*Δ), E453 (Dis XI-*lcb3*Δ), E455 (Dis XIII-*lcb3*Δ), E456 (Dis XIV-*lcb3*Δ), E457 (Dis XV-*lcb3*Δ). Average $\log_2$ ratio was calculated for the 13 disomes, 13 disomes-*ubp6*Δ and 10 disomes-*lcb3*Δ. Stress response consists of genes whose averages > 0.2 (upregulated genes) and < -0.2 in all strains (downregulated genes). Cluster 1 (attenuated by *lcb3*Δ) consists of genes whose averages are > 0.2 in the disomes and between 0.2 and -0.2 in the disomes-*lcb3*Δ. Cluster 2 (downregulated by *lcb3*Δ) consists of genes whose averages are greater than 0.2 in the disomes and < -0.2 in the disomes-*lcb3*Δ. Cluster 3 (upregulated by *lcb3*Δ) consists of genes whose averages are between -0.2 and 0.2 in the disomes and > 0.2 in the disomes-*lcb3*Δ. Cluster 4 (attenuated by *lcb3*Δ) consists of genes whose averages are between < -0.2 in the disomes and between -0.2 and 0.2 in the disomes-*lcb3*Δ.

Figure 7.

(A) No additional information.

(B) Upregulated and downregulated clusters consist of proteins whose average is greater than 0.2 and lower that -0.2, respectively, only in the disomes-*lcb3*Δ.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Yeast strains and growth conditions

All stains are derivatives of W303 (E187) and are listed below. All aneuploid strains used in this study were subjected to comparative genomic hybridization (CGH) to ensure that the additional chromosome was present in its entirety. All deletions were first introduced in wild type cells by transformation and backcrossed into the disomes. One liter of synthetic medium consists of 1.7 grams of yeast nitrogen base without amino acids or ammonium sulfate, 4.8 grams of ammonium sulfate, and 2 grams of amino acid mix, which consist of equal amounts of Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, Iso, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Tyr, Val, adenine and uracil. Duplicated chromosomes are marked with HIS3 and KanMX6 cassette and the presence of the extra chromosome is ensured by selection in medium lacking His and the addition of

kanamycin (200 µg/ml).

Rationale for performing analysis of 13 out of 16 possible disomic yeast strains.
Disomic yeast strains were generated by a chromosome transfer strategy described in Torres et al. (2007)(Torres et al., 2007). Cells disomic for chromosomes III and VII were not obtained because the MAT locus and the CYH2 locus located on chromosome III and VII, respectively, are required for selection steps during chromosome transfer procedure. Cell disomic for chromosome VI could not be generated as two copies of ACT1 and TUB2 seem to cause lethally (Anders et al., 2009).

Global lipodomics of yeast Cells.
The lipid compositions of total cell extracts were determined by quantitative shotgun lipidomic analysis as previously described Ejsing et al. (2009)(Ejsing et al., 2009; Klose et al., 2012). Briefly, samples were mixed with 30 µl of internal lipid standard mixture, providing a total spike of 24 pmol DAG 17:0-17:0, 22 pmol PA 17:0-14:1, 41 pmol PE 17:0-14:1, 41 pmol PS 17:0-14:1, 42 pmol PC 17:0-14:1, 40 pmol PI 17:0-14:1, 14 pmol CL 15:0-15:0-15:0-16:1, 22 pmol ceramide 18:0;3/18:0;0, 37 pmol IPC 18:0;2/26:0;0, 36 pmol MIPC 18:0;2/26:0;0, 31 pmol M(IP)2C 18:0;2/26:0;0, and 57 pmol cholesterol-D7. Samples were subsequently subjected to two-step lipid extraction (1 ml of solvent in each step) executed at 4°C. The lower organic phases were collected and evaporated in a vacuum evaporator at 4°C. Lipid extracts were dissolved in 100 µl chloroform/methanol (1 : 2; vol/vol) and analyzed by mass spectrometry using a LTQ Orbitrap XL (Thermo Fisher Scientific) equipped with a robotic nanoflow ion source TriVersa NanoMate (Advion Biosciences). PA, PS, PE, PC, CL, PI, IPC, MIPC and M(IP)2C, DAG and lysolipid species were monitored by negative ion mode FT MS analysis, whereas TAG and ceramide species were monitored by positive ion mode FT MS analysis.

Quantification of LCB and LCB-P using quantitative HPLC.
Trichloroacetic acid (TCA) was added to the cells (10 OD_{600} units) to a final concentration of 5%, and samples were incubated on ice for 5 min. Cells were harvested by centrifugation and washed once with 10 ml ice-cold 5% TCA, followed by three washes in 10 ml ice-cold water. Samples were frozen at −80°C until further processing. The sphingoid bases were extracted and converted to fluorescent aminoquinoline derivatives, which were separated and analyzed by HPLC as described Lester and Dickson (2001)(Lester and Dickson, 2001). The concentration were calculated from the “Peak Area” (this the area of the fluorescent signal from the HPLC)

data shown above the “Conc” data. The “area under the peaks” was converted to molar concentration by injecting a standard: 100 pmoles of PHS that was derivatized with the AQC reagent which gives the fluorescent signal that is recorded as the peak area (conversion factor 0.28). The standard was injected at the start and at the end of the sample run and the average area was used to calculate the concentration of the LCB and LCBP peaks. 3 biological triplicates for each disome was analyzed.

Serine palmitoyltransferase enzymatic activity measurements. SPT activity was measured using methods described by Harmon et al. (Harmon et al., 2013) and Rutti et al. (Rutti et al., 2009). Briefly, 3 x 10^5 of exponentially growing yeast cells were used for each assay. After breaking cells with bead beading in 500 µl of cold 50 mM HEPES/NaOH (pH 8.1) and 1 mM EDTA, cell debris and glass beads were removed by centrifugation at 10,000 g for 5 minutes in 4°C. To obtain microsomes, the supernatant were centrifugated at 100,000 g for 30 minutes in 4°C. Microsomes were incubated in 200 µl of reaction buffer containing 50 mM HEPES/NaOH (pH 8.1), 50 µM pyridoxy phosphate, 100 µM palmitoyl-CoA, 5 mM L-serine and 0.5 mM L-[U-C^{14}]serine (50 µCi/ml) at 37°C for 60 minutes. C^{14}-labelled lipids were extracted by addition of 0.5 ml of methanol/KOH:$CHCl_3$ (4:1) with vortexing. After sequential additions of 0.5 ml of $CHCl_3$, 0.5 mL of 0.2 mN NH_4OH and 100 µl of 2 N NH_4OH, mix them and centrifuge at 12,000 g for 1 min at room temperature. The lower phase ($CHCl_3$) was washed twice with 0.9 ml of 0.2 mN NH_4OH and transferred in new tube. The $CHCl_3$ was dried under N_2 gas flow and resuspended in 5 mL of scintillation cocktail. The radioactivity from the isolated lipids was determined by scintillation counter.

Serine Intake and ceramide extraction

5 x 10^6 cells were inoculated in synthetic media containing 1 mM Serine (0.1 µCi/ml of L-[U-C^{14}]serine). To measure the initial serine concentration, 200 µl of cells were spun down at 13k x g for 10 minutes and 100 µl of media was taken for C^{14} measurements by scintillation counter. Cells were grown for 24 hours at 30°C and the radioactivity in the media was measured again. Initial minus final measurements were divided by the number of cells generated for each strain . These experiment were performed in triplicates. To measure the incorporation of serine into ceramides, 1.5 ml of cells were treated with 5% TCA and incubated on ice for 10 minutes and washed three times with cold H_2O. Ceramides were extracted as in Dickson et al. (1997)(Dickson et al., 1997). Briefly, cells were resuspended in 600 µL chloroform:methanol (1:1) followed by addition of 1 volume glass beads and vortexed 5 x 30 seconds with ice in

between. Lysates were incubated at 60-65 °C for 60 minutes. After centrifugation, supernatants were treated with 150 µL 0.5N NaOH in methanol at 37 °C for 30 minutes. Solution was neutralized with 90 µL glacial acetic acid and blow down with N_2. To remove phospholipids, pellets were treated with 500 µL monomethylamine reagent and incubated at 50 °C for 40 minutes. After drying them with N_2, ceramides were resuspended in 500 µL chloroform:methanol (1:1) and add 250 µL H_2O. After washing the lipids twice with 250 µL alkaline H_2O, 200 µL of lipids were used to measure radioactivity.

TMT-labeling

100 µg total peptide from each strain was resuspended in 100 µl of 0.2 M Hepes (pH 8.5). TMT six-plex reagents (0.8 mg per vial) (Thermo Fisher) were resuspended in 41 µl of anhydrous ACN and 10 µl of each reagent was added to each sample. Reactions were allowed to proceed at room temperature for 1 hour, after which they were quenched by the addition of 8 µl of 5% hydroxylamine for 15 min and then acidified by the addition of 16 µl neat FA. Reaction products from all six differentially labeled samples were combined and 1 ml of 1% FA was added before desalting on a 200-mg tC18 Sep-Pak. Eluted peptides were dried in a SpeedVac and stored at –20°C.

Peptide Fractionation

TMT-labeled peptides were separated by high-pH reverse-phase HPLC (Wang et al., 2011). 600 µg of six-plex labeled peptides were resuspended in 250 µl buffer A (5% ACN, 10 mM NH_4HCO_3, pH 8) and separated on a 4.6 mm x 250 mm 300Extend-C18, 5 µm column (Agilent) using a 50 min gradient from 18% to 38% buffer B (90% acn, 10 mM NH_4HCO_3, pH 8) at a flow rate of 0.8 ml/min. Fractions were collected over 45 min at 28 sec intervals beginning 5 min after the start of the gradient in a 96-well plate and lyophilized. Fractions were resuspended in 30 µl 1% FA and pooled into 12 samples of four fractions each (only 48 of 96 fractions were used) by combining fractions 1/25/49/73, 3/27/51/75, 5/29/53/77, 7/31/55/79, 9/33/57/81, 11/35/59/83, 14/38/62/86, 16/40/64/88, 18/42/66/90, 20/44/68/92, 22/46/70/94, 24/48/72/96 into glass vial inserts. This pooling strategy serves to minimize peptide overlap between fractions. The pooled samples were dried down and resuspended in 25 µl of 5% FA.

LC-MS/MS Analysis

For TMT experiments, 2-4 µl of each fraction was analyzed on a LTQ Orbitrap Velos mass spectrometer (Thermo Fisher Scientific) equipped with an Accela 600 quaternary pump (Thermo

Fisher Scientific) and a Famos Microautosampler (LC Packings). Peptides were separated with a gradient of 6 to 24% ACN in 0.125% FA over 150 min and detected using a data-dependent Top10-MS2/MS3 "multi-notch" method (Ting et al., 2011) [and McAlister in press]. For each cycle, one full MS scan was acquired in the Orbitrap at a resolution of 30,000 or 60,000 at m/z = 400 with automatic gain control (AGC) target of 2×10^6. Each full scan was followed by the selection of the most intense ions, up to 10, for collision-induced dissociation (CID) and MS2 analysis in the linear ion trap for peptide identification and subsequent higher-energy collisional dissociation (HCD) and MS3 analysis in the Orbitrap for quantification of the TMT reporter ions. AGC targets of 4×10^3 and 2×10^4 were used for MS2 and MS3 scans, respectively. Ions selected for MS2 analysis were excluded from reanalysis for 90 sec. Ions with +1 or unassigned charge were also excluded from analysis. A single MS3 scan was performed for each MS2 scan selecting the most intense ions from the MS2 for fragmentation in the HCD cell. The resultant fragment ions were detected in the orbitrap at a resolution of 7500. Maximum ion accumulation times were 1000 ms for each full MS scan, 150 ms for MS2 scans, and 250 ms for MS3 scans.

Database Searching and Filtering

MS/MS spectra were matched to peptide sequences using SEQUEST v.28 (rev. 13) (Eng et al., 1994) and a composite database containing the translated sequences of all predicted open reading frames of Saccharomyces cerevisiae (http://downloads.yeastgenome.org) and its reversed complement. Search parameters allowed for two missed cleavages, a mass tolerance of 20 ppm, a static modification of 57.02146 Da (carboxyamidomethylation) on cysteine, and dynamic modifications of 15.99491 Da (oxidation) on methionine. For TMT samples a static modification of 229.16293 Da on peptide amino termini and lysines was added.

Peptide spectral matches were filtered to 1% FDR using the target-decoy strategy (Elias and Gygi, 2007) combined with linear discriminant analysis (LDA) (Huttlin et al., 2010) using the SEQUEST Xcorr and ΔCn' scores, precursor mass error, observed ion charge state, and the number of missed cleavages. LDA models were calculated for each LC-MS/MS run with peptide matches to forward and reversed protein sequences as positive and negative training data. The data were further filtered to control protein-level FDRs. Protein scores were derived from the product of all LDA peptide probabilities, sorted by rank, and filtered to 1% FDR. The FDR of the remaining peptides fell markedly after protein filtering. Further filtering based on the quality of quantitative measurements (see below) resulted in a final protein FDR < 1% for all experiments. Remaining peptide matches to the decoy database as well as contaminating proteins (e.g. human keratins) were removed from the final data set.

Peptide quantification

For TMT experiments raw reporter ion intensities were denormalized by multiplying with the ion accumulation times for each MS3 scan and corrected for isotopic overlap between reporter ions by using empirically derived values. We required each peptide to have denormalized reporter ion intensities ≥ 20 for the zero time point and at least four of six TMT channels.

Protein quantification

In all experiments, protein ratios were normalized to account for small variations in cell mixing by recentering the $\log_2$ protein abundance ratio distributions over zero using the assumption that most proteins are present at a one-to-one ratio. Proteins coded on the duplicated chromosomes, which are more abundant in the disomes were excluded when calculating this normalization factor. For TMT experiments, relative protein abundances were calculated as the weighted average of all peptides from each protein using the ratio of the summed reporter ion intensities in each channel. Ratios for both experiments were $\log_2$-transformed for all subsequent analysis.

Gene Expression Arrays

Total RNA was isolated from cells frozen on filters. Filters were incubated for 1 hour at 65°C in lysis buffer (10 mM EDTA, 0.5% SDS, and 10 mM Tris, pH 7.5) and acid phenol. The aqueous phase was further extracted twice with an equal volume of chloroform using phase lock gel (Eppendorf). Total RNA was then ethanol precipitated and further purified over RNeasy columns (Qiagen). RNA quality was checked using the Bioanalyzer RNA Nano kit, and 325 ng was used for microarray labeling with the Agilent Low RNA Input Fluorescent Linear Amplification Kit. Reactions were performed as directed except using half the recommended reaction volume and one quarter the recommended Cy-CTP amount. Dye incorporation and yield were measured with a Nanodrop spectrophotometer. Equal amounts of differentially labeled control and sample cRNA were combined such that each sample contained at least 2.5 pmol dye. Samples were mixed with control targets, fragmented, combined with hybridization buffer, and hybridized to a microarray consisting of 60mer probes for each yeast open reading frame (Agilent). Microarrays were rotated at 60°C for 17 hours in a hybridization oven (Agilent). Arrays were then washed according to the Agilent SSPE wash protocol, and scanned on an Agilent scanner. The image was processed using the default settings with Agilent Feature Extraction software. All data analysis was performed using the resulting $\log_2$ ratio data, and filtered for spots called as

significantly over background in at least one channel. The accession number for the microarray data is GSE93762.

Strains Utilized in this Study

Wild-type

Strain Number	Genotype
E187	*MATa, ade2-1, leu2-3, ura3, trp1-1, his3-11, 15, can1-100, GAL, psi+*
E188	*MATα, ade2-1, leu2-3, ura3, trp1-1, his3-11, 15, can1-100, GAL, psi+*

Parental Aneuploid Strains

Strain	Dis	Relevant Genotype
E113	WT	*MATa, ade1::HIS3, lys2::KanMX6*
E88	I	*MATa, ade1::HIS3, ade1::KanMX6*
E89	II	*MATa, lys2::HIS3, lys2::KanMX6*
E91	IV	*MATa, trp1::HIS3, trp1::KanMX6*
E92	V	*MATa, can1::HIS3, intergenic region (187520-187620) between YER015W and YER016W::KanMX6*
E93	VIII	*MATa, intergenic region (119778-119573) between YHR006W and YHR007C::HIS3, intergenic region (119778-119573) between YHR006W and YHR007C::KanMX6*
E94	IX	*MATa, intergenic region (341900-34200) between YIL009W and YIL008W::HIS3, intergenic region (341900-34200) between YIL009W and YIL008W::KanMX6*
E96	X	*MATa, intergenic region (322250-322350) between YJL061W and YJL060W::HIS3, intergenic region (322250-322350) between YJL061W and YJL060W::KanMX6*
E97	XI	*MATa, intergenic region (430900-431000) between YKL006C-A and YKL006W::HIS3, intergenic region (430900-431000) between YKL006C-A and YKL006W::KanMX6*
E98	XII	*MATa, ade16::HIS3, ade16::KanMX6*
E100	XIII	*MATa, intergenic region (309200-309300) between YMR017W and YMR018W::HIS3, intergenic region (309200-309300) between YMR017W and YMR018W::KanMX6*

E101	XIV	*MATa, intergenic region (622880-622980) between YNL005C and YNL004W::HIS3, intergenic region (622880-622980) between YNL005C and YNL004W::KanMX6*
E102	XV	*MATa, leu9::HIS3, leu9::KanMX6*
E103	XVI	*MATa, met12::HIS3, met12::KanMX6*

Double disomes

Strain	Dis	Relevant Genotype
E104	XI + XV	*MATa, met14::HIS3, met14::KanMX6.* See Torres et al,. 2007
E105	XI + XVI	*MATa, met12::HIS3, met12::KanMX6.* See Torres et al,. 2007
E106	I + VI + XIII	*MATa, met10::HIS3, met10::KanMX6.* See Torres et al,. 2007
E108	VIII + XIV	*MATa, intergenic region (622880-622980) between YNL005C and YNL004W::HIS3, intergenic region (622880-622980) between YNL005C and YNL004W::KanMX6.* See Torres et al,. 2007

URA+ strains used for doubling times comparisons. These strains are marker matched with strains harboring gene deletions.

Strain	Dis	Relevant Genotype
E192	WT	*MATa, ade1::HIS3, lys2::KanMX6 , intergenic region (181900-182000) between YFR016C and YFR017C:: CaURA3*
E193	I	*MATa, ade1::HIS3, ade1::KanMX6 , intergenic region (181900-182000) between YFR016C and YFR017C:: CaURA3*
E194	II	*MATa, lys2::HIS3, lys2::KanMX6 , intergenic region (181900-182000) between YFR016C and YFR017C:: CaURA3*
E195	IV	*MATa, trp1::HIS3, trp1::KanMX6 , intergenic region (181900-182000) between YFR016C and YFR017C:: CaURA3*
E196	V	*MATa, can1::HIS3, intergenic region (187520-187620) between YER015W and YER016W::KanMX6 , intergenic region (181900-182000) between YFR016C and YFR017C:: CaURA3*
E197	VIII	*MATa, intergenic region (119778-119573) between YHR006W and YHR007C::HIS3, intergenic region (119778-119573) between YHR006W and YHR007C::KanMX6 , intergenic region (181900-182000) between YFR016C and YFR017C:: CaURA3*

E198	IX	*MATa, intergenic region (341900-34200) between YIL009W and YIL008W::HIS3, intergenic region (341900-34200) between YIL009W and YIL008W::KanMX6 , intergenic region (181900-182000) between YFR016C and YFR017C:: CaURA3*
E199	X	*MATa, intergenic region (322250-322350) between YJL061W and YJL060W::HIS3, intergenic region (322250-322350) between YJL061W and , intergenic region (181900-182000) between YFR016C and YFR017C:: CaURA3YJL060W::KanMX6 , intergenic region (181900-182000) between YFR016C and YFR017C:: CaURA3*
E200	XI	*MATa, intergenic region (430900-431000) between YKL006C-A and YKL006W::HIS3, intergenic region (430900-431000) between YKL006C-A and YKL006W::KanMX6*
E201	XII	*MATa, ade16::HIS3, ade16::KanMX6 , intergenic region (181900-182000) between YFR016C and YFR017C:: CaURA3*
E202	XIII	*MATa, intergenic region (309200-309300) between YMR017W and YMR018W::HIS3, intergenic region (309200-309300) between YMR017W and YMR018W::KanMX6 , intergenic region (181900-182000) between YFR016C and YFR017C:: CaURA3*
E203	XIV	*MATa, intergenic region (622880-622980) between YNL005C and YNL004W::HIS3, intergenic region (622880-622980) between YNL005C and YNL004W::KanMX6 , intergenic region (181900-182000) between YFR016C and YFR017C:: CaURA3*
E204	XV	*MATa, leu9::HIS3, leu9::KanMX6 , intergenic region (181900-182000) between YFR016C and YFR017C:: CaURA3*
E205	XVI	*MATa, met12::HIS3, met12::KanMX6 , intergenic region (181900-182000) between YFR016C and YFR017C:: CaURA3*

GFP strains used in competition experiments

Strain	**Dis**	**Relevant Genotype**
E276	WT	*MATa, ade1::HIS3, lys2::KanMX6, PGK1::PGK1-EGFP-CaURA3*
E277	I	*MATa, ade1::HIS3, ade1::KanMX6, PGK1::PGK1-EGFP-CaURA3*
E278	II	*MATa, lys2::HIS3, lys2::KanMX6, PGK1::PGK1-EGFP-CaURA3*
E279	IV	*MATa, trp1::HIS3, trp1::KanMX6, PGK1::PGK1-EGFP-CaURA3*

E280	V	*MATa, can1::HIS3, intergenic region (187520-187620) between YER015W and YER016W::KanMX6, PGK1::PGK1-EGFP-CaURA3*
E281	VIII	*MATa, intergenic region (119778-119573) between YHR006W and YHR007C::HIS3, intergenic region (119778-119573) between YHR006W and YHR007C::KanMX6, PGK1::PGK1-EGFP-CaURA3*
E481	VIII	*MATalpha, intergenic region (119778-119573) between YHR006W and YHR007C::HIS3, intergenic region (119778-119573) between YHR006W and YHR007C::KanMX6, PGK1::PGK1-EGFP-CaURA3*
E282	IX	*MATa, intergenic region (341900-34200) between YIL009W and YIL008W::HIS3, intergenic region (341900-34200) between YIL009W and YIL008W::KanMX6, PGK1::PGK1-EGFP-CaURA3*
E483	X	*MATalpha, ura2::KAN, ura2::HIS3, PGK1::PGK1-EGFP-CaURA3*
E283	XI	*MATa, intergenic region (430900-431000) between YKL006C-A and YKL006W::HIS3, intergenic region (430900-431000) between YKL006C-A and YKL006W::KanMX6, PGK1::PGK1-EGFP-CaURA3*
E284	XII	*MATa, ade16::HIS3, ade16::KanMX6, PGK1::PGK1-EGFP-CaURA3*
E285	XIII	*MATa, ura5::HIS3, ura5::KanMX6, PGK1::PGK1-EGFP-CaURA3*
E486	XIII	*MATalpha, ura5::HIS3, ura5::KanMX6, PGK1::PGK1-EGFP-CaURA3*
E286	XIV	*MATa, intergenic region (622880-622980) between YNL005C and YNL004W::HIS3, intergenic region (622880-622980) between YNL005C and YNL004W::KanMX6, PGK1::PGK1-EGFP-CaURA3*
E487	XIV	*MATalpha, intergenic region (622880-622980) between YNL005C and YNL004W::HIS3, intergenic region (622880-622980) between YNL005C and YNL004W::KanMX6, PGK1::PGK1-EGFP-CaURA3*
E287	XV	*MATa, leu9::HIS3, leu9::KanMX6, PGK1::PGK1-EGFP-CaURA3*
E288	XVI	*MATa, met12::HIS3, met12::KanMX6, PGK1::PGK1-EGFP-CaURA3*
E489	XVI	*MATalpha, met12::HIS3, met12::KanMX6, PGK1::PGK1-EGFP-CaURA3*

***SVF1* deletions**

Strain	**Dis**	**Relevant Genotype**
E290	WT	*MATa, ade1::HIS3, lys2::KanMX6, svf1::CaURA3*
E291	I	*MATa, ade1::HIS3, ade1::KanMX6, svf1::CaURA3*
E292	II	*MATa, lys2::HIS3, lys2::KanMX6, svf1::CaURA3*

	IV	Not generated because SVF1 is on chromosome IV.
E293	V	*MATa, can1::HIS3, intergenic region (187520-187620) between YER015W and YER016W::KanMX6, svf1::CaURA3*
E294	VIII	*MATa, intergenic region (119778-119573) between YHR006W and YHR007C::HIS3, intergenic region (119778-119573) between YHR006W and YHR007C::KanMX6, svf1::CaURA3*
E295	IX	*MATa, intergenic region (341900-34200) between YIL009W and YIL008W::HIS3, intergenic region (341900-34200) between YIL009W and YIL008W::KanMX6, svf1::CaURA3*
E296	X	*MATa, ura2::HIS3, ura2::KanMX6, svf1::CaURA3*
E297	XI	*MATa, intergenic region (430900-431000) between YKL006C-A and YKL006W::HIS3, intergenic region (430900-431000) between YKL006C-A and YKL006W::KanMX6, svf1::CaURA3*
E298	XII	*MATa, ade16::HIS3, ade16::KanMX6, svf1::CaURA3*
E299	XIII	*MATa, ura5::HIS3, ura5::KanMX6, svf1::CaURA3*
E300	XIV	*MATa, intergenic region (622880-622980) between YNL005C and YNL004W::HIS3, intergenic region (622880-622980) between YNL005C and YNL004W::KanMX6, svf1::CaURA3*
E301	XV	*MATa, leu9::HIS3, leu9::KanMX6, svf1::CaURA3*
E302	XVI	*MATa, met12::HIS3, met12::KanMX6, svf1::CaURA3*

***LCB3* deletions**

Strain	**Dis**	**Relevant Genotype**
E445	WT	*MATa, ade1::HIS3, lys2::KanMX6, lcb3::CaURA3*
E446	I	*MATa, ade1::HIS3, ade1::KanMX6, lcb3::CaURA3*
E447	II	*MATa, lys2::HIS3, lys2::KanMX6, lcb3::CaURA3*
E448	IV	*MATa, trp1::HIS3, trp1::KanMX6, lcb3::CaURA3*
E449	V	*MATa, can1::HIS3, intergenic region (187520-187620) between YER015W and YER016W::KanMX6, lcb3::CaURA3*
E450	VIII	*MATa, intergenic region (119778-119573) between YHR006W and YHR007C::HIS3, intergenic region (119778-119573) between YHR006W and*

		YHR007C::KanMX6, lcb3::CaURA3
E451	IX	*MATa, intergenic region (341900-34200) between YIL009W and YIL008W::HIS3, intergenic region (341900-34200) between YIL009W and YIL008W::KanMX6, lcb3::CaURA3*
E452	X	*MATalpha, intergenic region (322250-322350) between YJL061W and YJL060W::HIS3, intergenic region (322250-322350) between YJL061W and YJL060W::KanMX6, LCB3/lcb3::CaURA3*
E453	XI	*MATa, intergenic region (430900-431000) between YKL006C-A and YKL006W::HIS3, intergenic region (430900-431000) between YKL006C-A and YKL006W::KanMX6, lcb3::CaURA3*
E454	XII	*MATa, ade16::HIS3, ade16::KanMX6, lcb3::CaURA3*
E455	XIII	*MATa, intergenic region (309200-309300) between YMR017W and YMR018W::HIS3, intergenic region (309200-309300) between YMR017W and YMR018W::KanMX6, lcb3::CaURA3*
E456	XIV	*MATalpha, intergenic region (622880-622980) between YNL005C and YNL004W::HIS3, intergenic region (622880-622980) between YNL005C and YNL004W::KanMX6, lcb3::CaURA3*
E457	XV	*MATa, leu9::HIS3, leu9::KanMX6, lcb3::CaURA3*
E458	XVI	*MATalpha, met12::HIS3, met12::KanMX6, lcb3::CaURA3*

***LCB4* deletions**

Strain	Dis	Relevant Genotype
E460	WT	*MATa, ade1::HIS3, lys2::KanMX6, lcb4::CaURA3*
E461	I	*MATa, ade1::HIS3, ade1::KanMX6, lcb4::CaURA3*
E462	II	*MATa, lys2::HIS3, lys2::KanMX6, lcb4::CaURA3*
E463	IV	*MATa, trp1::HIS3, trp1::KanMX6, lcb4::CaURA3*
E464	V	*MATa, can1::HIS3, intergenic region (187520-187620) between YER015W and YER016W::KanMX6, lcb4::CaURA3*
E474	VIII	*MATalpha, intergenic region (119778-119573) between YHR006W and YHR007C::HIS3, intergenic region (119778-119573) between YHR006W and*

		YHR007C::KanMX6, lcb4::CaURA3
E466	IX	*MATa, intergenic region (341900-34200) between YIL009W and YIL008W::HIS3, intergenic region (341900-34200) between YIL009W and YIL008W::KanMX6, lcb4::CaURA3*
E697	X	*MATalpha, intergenic region (322250-322350) between YJL061W and YJL060W::HIS3, intergenic region (322250-322350) between YJL061W and YJL060W::KanMX6, lcb4::CaURA3*
E468	XI	*MATa, intergenic region (430900-431000) between YKL006C-A and YKL006W::HIS3, intergenic region (430900-431000) between YKL006C-A and YKL006W::KanMX6, lcb4::CaURA3*
E469	XII	*MATa, ade16::HIS3, ade16::KanMX6, lcb4::CaURA3*
E470	XIII	*MATalpha, intergenic region (309200-309300) between YMR017W and YMR018W::HIS3, intergenic region (309200-309300) between YMR017W and YMR018W::KanMX6, lcb4::CaURA3*
E471	XIV	*MATa, intergenic region (622880-622980) between YNL005C and YNL004W::HIS3, intergenic region (622880-622980) between YNL005C and YNL004W::KanMX6, lcb4::CaURA3*
E472	XV	*MATa, leu9::HIS3, leu9::KanMX6, LCB4/lcb4::CaURA3*
E473	XVI	*MATa, met12::HIS3, met12::KanMX6, lcb4::CaURA3*

***LAG1* deletions**

Strain	**Dis**	**Relevant Genotype**
E413	WT	*MATa, ade1::HIS3, lys2::KanMX6, lag1::CaURA3*
E414	I	*MATa, ade1::HIS3, ade1::KanMX6, lag1::CaURA3*
E415	II	*MATa, lys2::HIS3, lys2::KanMX6, lag1::CaURA3*
E416	IV	*MATa, trp1::HIS3, trp1::KanMX6, lag1::CaURA3*
E417	V	*MATa, can1::HIS3, intergenic region (187520-187620) between YER015W and YER016W::KanMX6, lag1::CaURA3*
	VIII	*Not generated because LAG1 is on chr VIII*
E419	IX	*MATa, intergenic region (341900-34200) between YIL009W and YIL008W::HIS3, intergenic region (341900-34200) between YIL009W and YIL008W::KanMX6, lag1::CaURA3*

E420	X	*MATa, intergenic region (322250-322350) between YJL061W and YJL060W::HIS3, intergenic region (322250-322350) between YJL061W and YJL060W::KanMX6, lag1::CaURA3*
E421	XI	*MATa, intergenic region (430900-431000) between YKL006C-A and YKL006W::HIS3, intergenic region (430900-431000) between YKL006C-A and YKL006W::KanMX6, lag1::CaURA3*
E908	XII	*MATa, ade16::HIS3, ade16::KanMX6, lag1::CaURA3*
E423	XIII	*MATalpha, intergenic region (309200-309300) between YMR017W and YMR018W::HIS3, intergenic region (309200-309300) between YMR017W and YMR018W::KanMX6, lag1::CaURA3*
E424	XIV	*MATa, intergenic region (622880-622980) between YNL005C and YNL004W::HIS3, intergenic region (622880-622980) between YNL005C and YNL004W::KanMX6, lag1::CaURA3*
E425	XV	*MATalpha, leu9::HIS3, leu9::KanMX6, lag1::CaURA3*
E426	XVI	*MATa, met12::HIS3, met12::KanMX6, lag1::CaURA3*

***DPL1* deletions**

Strain	**Dis**	**Relevant Genotype**
E610	WT	*MATa, ade1::HIS3, lys2::KanMX6, dpl1::CaURA3*
E596	I	*MATa, ade1::HIS3, ade1::KanMX6, dpl1::CaURA3*
E597	II	*MATa, lys2::HIS3, lys2::KanMX6, dpl1::CaURA3*
	IV	*Not generated, DPL1 is on chr IV*
E599	V	*MATa, can1::HIS3, intergenic region (187520-187620) between YER015W and YER016W::KanMX6, dpl1::CaURA3*
E600	VIII	*MATa, intergenic region (119778-119573) between YHR006W and YHR007C::HIS3, intergenic region (119778-119573) between YHR006W and YHR007C::KanMX6, dpl1::CaURA3*
E601	IX	*MATa, intergenic region (341900-34200) between YIL009W and YIL008W::HIS3, intergenic region (341900-34200) between YIL009W and YIL008W::KanMX6, l dpl1::CaURA3*
E602	X	*MATalpha, intergenic region (322250-322350) between YJL061W and YJL060W::HIS3, intergenic region (322250-322350) between YJL061W and*

		YJL060W::KanMX6, dpl1::CaURA3
E603	XI	*MATa, intergenic region (430900-431000) between YKL006C-A and YKL006W::HIS3, intergenic region (430900-431000) between YKL006C-A and YKL006W::KanMX6, dpl1::CaURA3*
E604	XII	*MATa, ade16::HIS3, ade16::KanMX6, dpl1::CaURA3*
E605	XIII	*MATalpha, intergenic region (309200-309300) between YMR017W and YMR018W::HIS3, intergenic region (309200-309300) between YMR017W and YMR018W::KanMX6, dpl1::CaURA3*
E606	XIV	*MATa, intergenic region (622880-622980) between YNL005C and YNL004W::HIS3, intergenic region (622880-622980) between YNL005C and YNL004W::KanMX6, dpl1::CaURA3*
E607	XV	*MATa, leu9::HIS3, leu9::KanMX6, dpl1::CaURA3*
E608	XVI	*MATa, met12::HIS3, met12::KanMX6, dpl1::CaURA3*

***CSG2* deletions**

Strain	**Dis**	**Relevant Genotype**
E910	WT	*MATa, ade1::HIS3, lys2::KanMX6, csg2::CaURA3*
E911	I	*MATalpha, ade1::HIS3, ade1::KanMX6, csg2::CaURA3*
	II	Not generated because CSG2 is on chromosome II.
E913	IV	*MATa, trp1::HIS3, trp1::KanMX6, csg2::CaURA3*
E914	V	*MATa, can1::HIS3, intergenic region (187520-187620) between YER015W and YER016W::KanMX6, csg2::CaURA3*
E915	VIII	*MATa, intergenic region (119778-119573) between YHR006W and YHR007C::HIS3, intergenic region (119778-119573) between YHR006W and YHR007C::KanMX6, csg2::CaURA3*
E916	IX	*MATa, intergenic region (341900-34200) between YIL009W and YIL008W::HIS3, intergenic region (341900-34200) between YIL009W and YIL008W::KanMX6, I csg2::CaURA3*
E917	X	*MATalpha, intergenic region (322250-322350) between YJL061W and YJL060W::HIS3, intergenic region (322250-322350) between YJL061W and YJL060W::KanMX6, csg2::CaURA3*
E918	XI	*MATa, intergenic region (430900-431000) between YKL006C-A and*

		YKL006W::HIS3, intergenic region (430900-431000) between YKL006C-A and YKL006W::KanMX6, csg2::CaURA3
E919	XII	*MATa, ade16::HIS3, ade16::KanMX6, csg2::CaURA3*
E920	XIII	*MATa, intergenic region (309200-309300) between YMR017W and YMR018W::HIS3, intergenic region (309200-309300) between YMR017W and YMR018W::KanMX6, csg2::CaURA3*
E921	XIV	*MATa, intergenic region (622880-622980) between YNL005C and YNL004W::HIS3, intergenic region (622880-622980) between YNL005C and YNL004W::KanMX6, csg2::CaURA3*
E922	XV	*MATalpha, leu9::HIS3, leu9::KanMX6, csg2::CaURA3*
E923	XVI	*MATalpha, met12::HIS3, met12::KanMX6, csg2::CaURA3*

***YPC1* deletions**

Strain	**Dis**	**Relevant Genotype**
E619	WT	*MATa, ade1::HIS3, lys2::KanMX6, ypc1::CaURA3*
E620	I	*MATa, ade1::HIS3, ade1::KanMX6, ypc1::CaURA3*
E624	VIII	*MATa, intergenic region (119778-119573) between YHR006W and YHR007C::HIS3, intergenic region (119778-119573) between YHR006W and YHR007C::KanMX6, ypc1::CaURA3*
E628	XII	*MATa, ade16::HIS3, ade16::KanMX6, ypc1::CaURA3*
E632	XVI	*MATa, met12::HIS3, met12::KanMX6, ypc1::CaURA3*

***SER2* deletions**

Strain	**Dis**	**Relevant Genotype**
E567	WT	*MATa, ade1::HIS3, lys2::KanMX6, ser2::CaURA3*
E568	I	*MATa, ade1::HIS3, ade1::KanMX6, ser2::CaURA3*
E569	II	*MATa, lys2::HIS3, lys2::KanMX6, ser2::CaURA3*
E570	IV	*MATa, trp1::HIS3, trp1::KanMX6, ser2::CaURA3*
E571	V	*MATa, can1::HIS3, intergenic region (187520-187620) between YER015W and YER016W::KanMX6, ser2::CaURA3*
E572	VIII	*MATa, intergenic region (119778-119573) between YHR006W and YHR007C::HIS3, intergenic region (119778-119573) between YHR006W and*

		YHR007C::KanMX6, ser2::CaURA3
E573	IX	*MATa, intergenic region (341900-34200) between YIL009W and YIL008W::HIS3, intergenic region (341900-34200) between YIL009W and YIL008W::KanMX6, I ser2::CaURA3*
E 574	X	*MATalpha, intergenic region (322250-322350) between YJL061W and YJL060W::HIS3, intergenic region (322250-322350) between YJL061W and YJL060W::KanMX6, ser2::CaURA3*
E575	XI	*MATa, intergenic region (430900-431000) between YKL006C-A and YKL006W::HIS3, intergenic region (430900-431000) between YKL006C-A and YKL006W::KanMX6, ser2::CaURA3*
E576	XII	*MATa, ade16::HIS3, ade16::KanMX6, ser2::CaURA3*
E577	XIII	*MATa, intergenic region (309200-309300) between YMR017W and YMR018W::HIS3, intergenic region (309200-309300) between YMR017W and YMR018W::KanMX6, ser2::CaURA3*
E578	XIV	*MATa, intergenic region (622880-622980) between YNL005C and YNL004W::HIS3, intergenic region (622880-622980) between YNL005C and YNL004W::KanMX6, ser2::CaURA3*
E579	XV	*MATa, leu9::HIS3, leu9::KanMX6, ser2::CaURA3*
E580	XVI	*MATalpha, met12::HIS3, met12::KanMX6, ser2::CaURA3*

***SER1* deletions**

Strain	**Dis**	**Relevant Genotype**
E582	WT	*MATa, ade1::HIS3, lys2::KanMX6, ser1::CaURA3*
E583	I	*MATa, ade1::HIS3, ade1::KanMX6, ser1::CaURA3*
E584	II	*MATa, lys2::HIS3, lys2::KanMX6, ser1::CaURA3*
E585	IV	*MATalpha, trp1::HIS3, trp1::KanMX6, ser1::CaURA3*
E586	V	*MATalpha, can1::HIS3, intergenic region (187520-187620) between YER015W and YER016W::KanMX6, ser1::CaURA3*
E587	VIII	*MATa, intergenic region (119778-119573) between YHR006W and YHR007C::HIS3, intergenic region (119778-119573) between YHR006W and YHR007C::KanMX6, ser1::CaURA3*
E588	IX	*MATa, intergenic region (341900-34200) between YIL009W and YIL008W::HIS3,*

		intergenic region (341900-34200) between YIL009W and YIL008W::KanMX6, I ser1::CaURA3
E589	X	*MATalpha, intergenic region (322250-322350) between YJL061W and YJL060W::HIS3, intergenic region (322250-322350) between YJL061W and YJL060W::KanMX6, ser1::CaURA3*
E590	XI	*MATa, intergenic region (430900-431000) between YKL006C-A and YKL006W::HIS3, intergenic region (430900-431000) between YKL006C-A and YKL006W::KanMX6, ser1::CaURA3*
E591	XII	*MATa, ade16::HIS3, ade16::KanMX6, ser1::CaURA3*
E592	XIII	*MATa, intergenic region (309200-309300) between YMR017W and YMR018W::HIS3, intergenic region (309200-309300) between YMR017W and YMR018W::KanMX6, ser1::CaURA3*
E593	XIV	*MATalpha, intergenic region (622880-622980) between YNL005C and YNL004W::HIS3, intergenic region (622880-622980) between YNL005C and YNL004W::KanMX6, ser1::CaURA3*
	XV	*Not generated, SER1 is on chr XV*
E594	XVI	*MATa, met12::HIS3, met12::KanMX6, ser1::CaURA3*

YAC strains containing human DNA from the Y chromosome

E507	YAC I	*ade1::HIS3, lys2::KAN /YAC-1.* Approximate size 850 kb(Foote et al., 1992; Torres et al., 2007).
E508	YAC II	*ade1::HIS3, lys2::KAN /YAC-2*. Approximate size 670 kb(Foote et al., 1992; Torres et al., 2007).
E509	YAC III	*ade1::HIS3, lys2::KAN /YAC-3*. Approximate size 620 kb(Foote et al., 1992; Torres et al., 2007).
E510	YAC IV	*ade1::HIS3, lys2::KAN /YAC-4*. Approximate size 580 kb(Foote et al., 1992; Torres et al., 2007).
E511	YAC V	*ade1::HIS3, lys2::KAN /YAC-5.* Approximate size 450 kb(Foote et al., 1992; Torres et al., 2007).

Cell cycle mutants (kindly provided by Jennifer Benanti)

E533	*cdc4-1*	*MATa, ade2-1, his3-11, leu2-3,112 trp1-1 ura3-1 can1-100, cdc4-1*
E534	*cdc15-2*	*MATa, ade2-1, his3-11, leu2-3,112 trp1-1 ura3-1 can1-100, cdc15-2*
E535	*cdc28-13*	*MATa, ade2-,1 his3-11, leu2-3,112 trp1-1 ura3-1 can1-100, cdc28-13*

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Figure S1 - Related to Figure 1

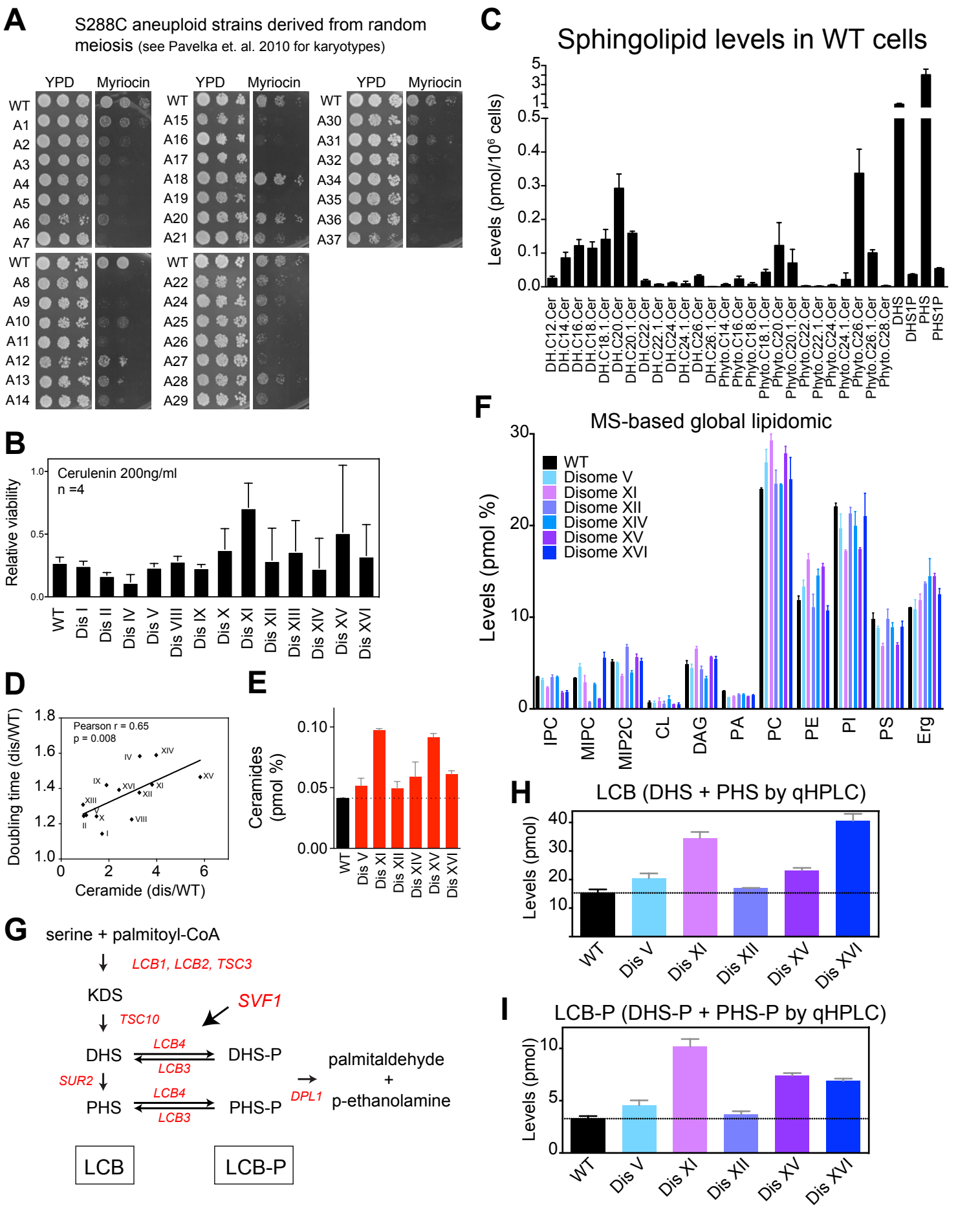


Figure S1. Aneuploidy Increases Sphingolipid Biosynthesis. Related to Figure 1.

(A) 10-fold serial dilutions of cells were incubated at 30ºC for 2-3 days with or without myriocin (0.5 ug/ml). A18 and A20 show minimal sensitivity to myriocin. See Supplementary Figure 4 in Pavelka N. et al. (2010) for karyotypes.

(B) Quantification of the viability of cells treated with 200 ng/ml cerulenin relative to untreated cells. Cell densities were quantified from high resolution tiff images of cell plated on agar using image J (https://imagej.nih.gov/ij/index.html).

(C) Levels of LCB and ceramides in wild type cells (E113) quantified by LC-LC/MS. Error bars represent SD of 3 biological replicates. These data represent the reference lipid levels for Figure 1F and 1G.

(D) Linear correlation of ceramide increases and size of the extra chromosome in the disomes. Fitting was performed using the GraphPad Prism7 software.

(E) Ceramide levels in WT and 6 disomes. Strains form left to right: E113 (WT), E92 (Dis V), E97 (Dis XI), E98 (Dis XII), E101 (Dis XIV), E102 (Dis XV), and E103 (Dis XVI).

(F) Global lipodomics analysis of WT and 6 disomes. IPC, inositol-phosphorylceramide, MIPC, mannosyl-IPC; $M(IP)_2C$, mannosyl-diinositol-phosphorylceramide. CL, cardiolipin; DAG, diacylglycerol; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; Erg, ergosterol. Six disomes varying on the degree of their phenotypes and size of the extra chromosomes were chosen to perform global lipid analysis. Two biological replicates were performed and compared to WT. Strains form left to right: E113 (WT), E92 (Dis V), E97 (Dis XI), E98 (Dis XII), E101 (Dis XIV), E102 (Dis XV), and E103 (Dis XVI). See Table S1 for % molar and $\log_2$ ratio values.

(G) Schematic of the LCBs metabolism in yeast. *SVF1* (*YDR346C*) = survival factor 1. *SUR2* (*YDR297W*) = sphinganine C4-hydroxylase. KDS = ketodihydrosphinganine.

(H) Levels of DHS and PHS in E113 (WT), E92 (Dis V), E97 (Dis XI), E98 (Dis XII), E102 (Dis XV), and E103 (Dis XVI). Error bars represent +/- SD of 3 independent measurements.

(I) Levels of DHS-P and PHS-P in E113 (WT), E92 (Dis V), E97 (Dis XI), E98 (Dis XII), E102 (Dis XV), and E103 (Dis XVI). Error bars represent +/- SD of 3 independent measurements.

Figure S2 - Related to Figure 2

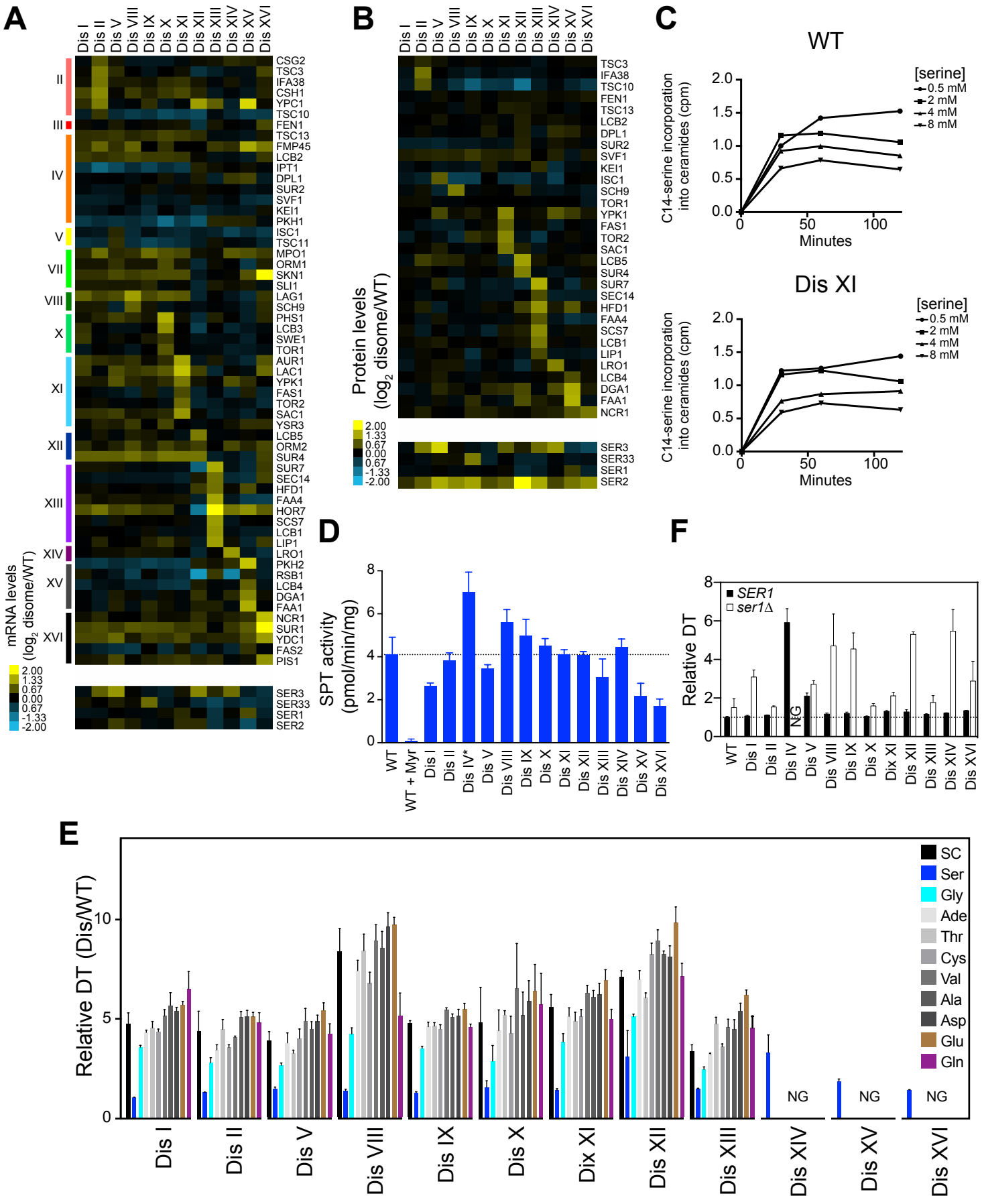


Figure S2. Aneuploid Cells Require Increase Serine Biosynthesis for Their Survival. Related to Figure 2.

(A) mRNA (left heat map) and (B) protein (right heat map) expression levels of genes that regulate sphingolipid (top) and serine (bottom) biosynthesis in the disomes relative to WT (log_2 ratio (disome/wt)). Data was obtained from Dephoure *et al*. (2014)(Dephoure et al., 2014). Columns represent experiments and rows represent genes.

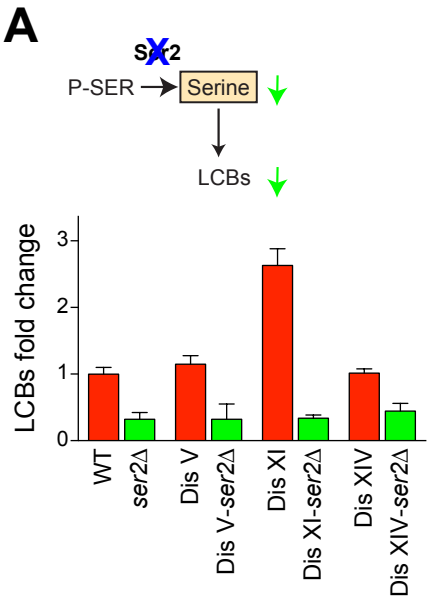
(C) Kinetics of the serine palmitoyltransferase (SPT) enzymatic activity in wild type cells (E113) and disome XI (E97) are very similar. K_m for serine does not change in disome XI compared to wild type cells.

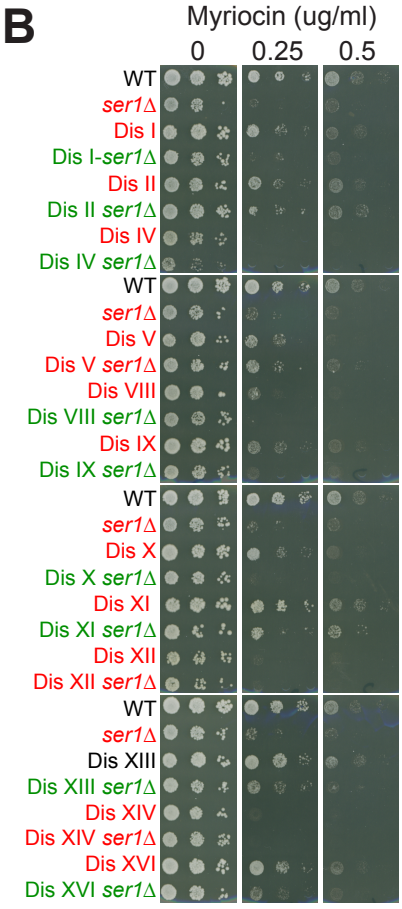
(D) Quantification of SPT enzymatic activity in wild type cells and the disomes. Error bars represent +/- SD of 3 independent measurements. WT + 200 ng/ml myriocin showed no SPT activity. Asterisk (*) indicates that disome IV cells show significant increase in SPT activity because they harbor two copies of the *LCB2* gene which encodes for the catalytic subunit of SPT. Strains used: E113 (WT), E88 (Dis I), E89 (Dis II), E91 (Dis IV), E92 (Dis V), E93 (Dis VIII), E94 (Dis IX), E96 (Dis X), E97 (Dis XI), E98 (Dis XII), E100 (Dis XIII), E101 (Dis XIV), E102 (Dis XV), and E103 (Dis XVI).

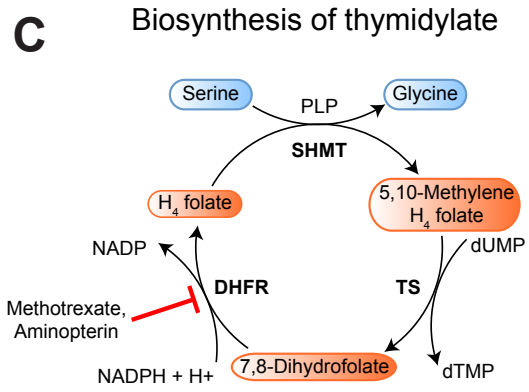
(E) Doubling times of disomes harboring *ser2*Δ in medium containing 1 mM (synthetic complete, SC) or 5 mM of respective amino acid or adenine. Error bars = +/- SD, n = 3. Black arrows point to strains that did not grow in culture. Error bars = +/- SD, n = 3 biological replicates. Strains used: E568 (Dis I-*ser2*Δ), E569 (Dis II- *ser2*Δ), E570 (Dis IV-*ser2*Δ), E571 (Dis V-*ser2*Δ), E572 (Dis VIII-*ser2*Δ), E573 (Dis IX-*ser2*Δ), E574 (Dis X-*ser2*Δ), E575 (Dis XI-*ser2*Δ), E576 (Dis XII-*ser2*Δ), E577 (Dis XIII-*ser2*Δ), E578 (Dis XIV-*ser2*Δ), E579 (Dis XV-*ser2*Δ), and E580 (Dis XVI-*ser2*Δ). NG = no growth.

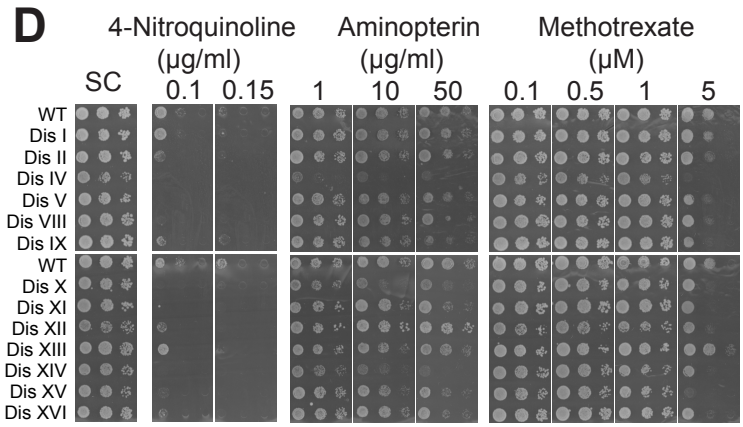
(F) Doubling times of disomes (black bars) and disomes harboring s*er1*Δ (open bars) relative to WT. Error bars represent +/- SD of 3 biological replicates. Cells were grown at 30ºC in synthetic medium. NG = no growth. Strains harboring *SER1*: E192 (WT), E193 (Dis I), E194 (Dis II), E195 (Dis IV), E196 (Dis V), E197 (Dis VIII), E198 (Dis IX), E199 (Dis X), E200 (Dis XI), E201 (Dis XII), E202 (Dis XIII), E203 (Dis XIV), E205 (Dis XVI). Strains harboring *ser1*Δ: E582 (*ser1*Δ), E583 (Dis I-*ser1*Δ), E584 (Dis II- *ser1*Δ), E585 (Dis IV-*ser1*Δ), E586 (Dis V-*ser1*Δ), E587 (Dis VIII-*ser1*Δ), E588 (Dis IX-*ser1*Δ), E589 (Dis X-*ser1*Δ), E590 (Dis XI-*ser1*Δ), E591 (Dis XII-*ser1*Δ), E592 (Dis XIII-*ser1*Δ), E593 (Dis XIV-*ser1*Δ), and E594 (Dis XVI-*ser1*Δ).

Figure S3 - Related to Figure 3









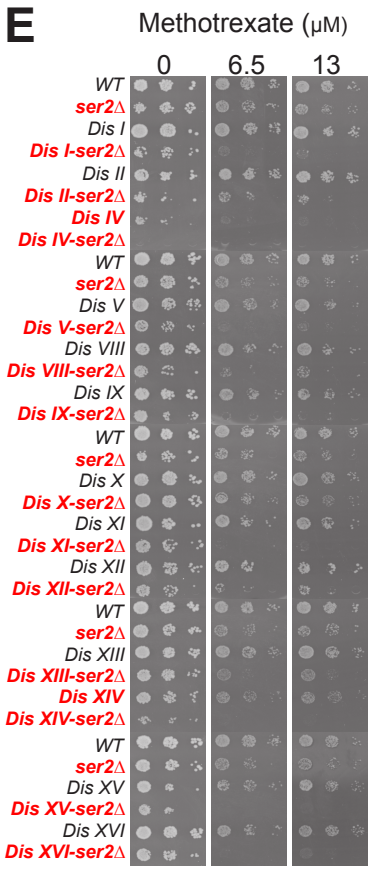


Figure S3. Aneuploid Cells Use Serine for the Synthesis of Sphingolipids. Related to Figure 3.
(A) Fold change of LCBs (DHS, PHS, DHS-P, and PHS-P) in WT, disome V, disome XI, and disome XIV. n = 3. Three pair of disomes were chosen to examine LCB changes upon loss of SER2. E192 (WT), E567 (ser2Δ), E196 (Dis V), E571 (Dis V-ser2Δ), E200 (Dis XI), E575 (Dis XI-ser2Δ), E203 (Dis XIV), E578 (Dis XIV-ser2Δ).
(B) Proliferative capability cell with wild type copy of *SER1* and cells harboring *ser1*Δ in the presence of myriocin. Strains used (top to bottom): E192 (WT), E582 (*ser1*Δ), E193 (Dis I), E583 (Dis I-*ser1*Δ), E194 (Dis II), E584 (Dis II- *ser1*Δ), E195 (Dis IV), E585 (Dis IV-*ser1*Δ), E192 (WT), E196 (Dis V), E586 (Dis V-*ser1*Δ), E197 (Dis VIII), E587(Dis VIII-*ser1*Δ), E198 (Dis IX), E588 (Dis IX-*ser1*Δ), E192 (WT), E199 (Dis X), E589 (Dis X-*ser1*Δ), E200 (Dis XI), E590 (Dis XI-*ser1*Δ), E201 (Dis XII), E591 (Dis XII-*ser1*Δ), E192 (WT), E202 (Dis XIII), E592 (Dis XIII-*ser1*Δ), E203 (Dis XIV), E593 (Dis XIV-*ser1*Δ), E205 (Dis XVI), E594 (Dis XVI-*ser1*Δ).
(C) Schematic of the biosynthesis of dTMP from serine. SHMT = serine hydroxymethyltransferase, TS = thymidylate synthase, DHFR = dihydrofolate reductase.
(D) Proliferative capability of WT and disomes in the presence of 4-NQ, aminopterin and methotrexate. Top plate: E113 (WT), E88 (Dis I), E89 (Dis II), E91 (Dis IV), E92 (Dis V), E93 (Dis VIII), E94 (Dis IX), and E96 (Dis X); bottom plate: E113 (WT), E97 (Dis XI), E98 (Dis XII), E100 (Dis XIII), E101 (Dis XIV), E102 (Dis XV), and E103 (Dis XVI).
(E) Proliferative capability of cells with wild type copy of *SER2* and cells harboring *ser2*Δ in the presence of methotrexate. Strains used (top to bottom): E192 (WT), E567 (*ser2*Δ), E193 (Dis I), E568 (Dis I-*ser2*Δ), E194 (Dis II), E569 (Dis II-*ser2*Δ), E195 (Dis IV), E570 (Dis IV-*ser2*Δ), E192 (WT), E196 (Dis V), E571 (Dis V-*ser2*Δ), E197 (Dis VIII), E572 (Dis VIII-*ser2*Δ), E198 (Dis IX), E573 (Dis IX-*ser2*Δ), E192 (WT), E199 (Dis X), E574 (Dis X-*ser2*Δ), E200 (Dis XI), E575 (Dis XI-*ser2*Δ), E201 (Dis XII), E576 (Dis XII-*ser2*Δ), E192 (WT), E202 (Dis XIII), E577 (Dis XIII-*ser2*Δ), E203 (Dis XIV), E578 (Dis XIV-*ser2*Δ), E204 (Dis XV), E579 (Dis XV-*ser2*Δ), E205 (Dis XVI), E580 (Dis XVI-*ser2*Δ).

Figure S4 - Related to Figure 4

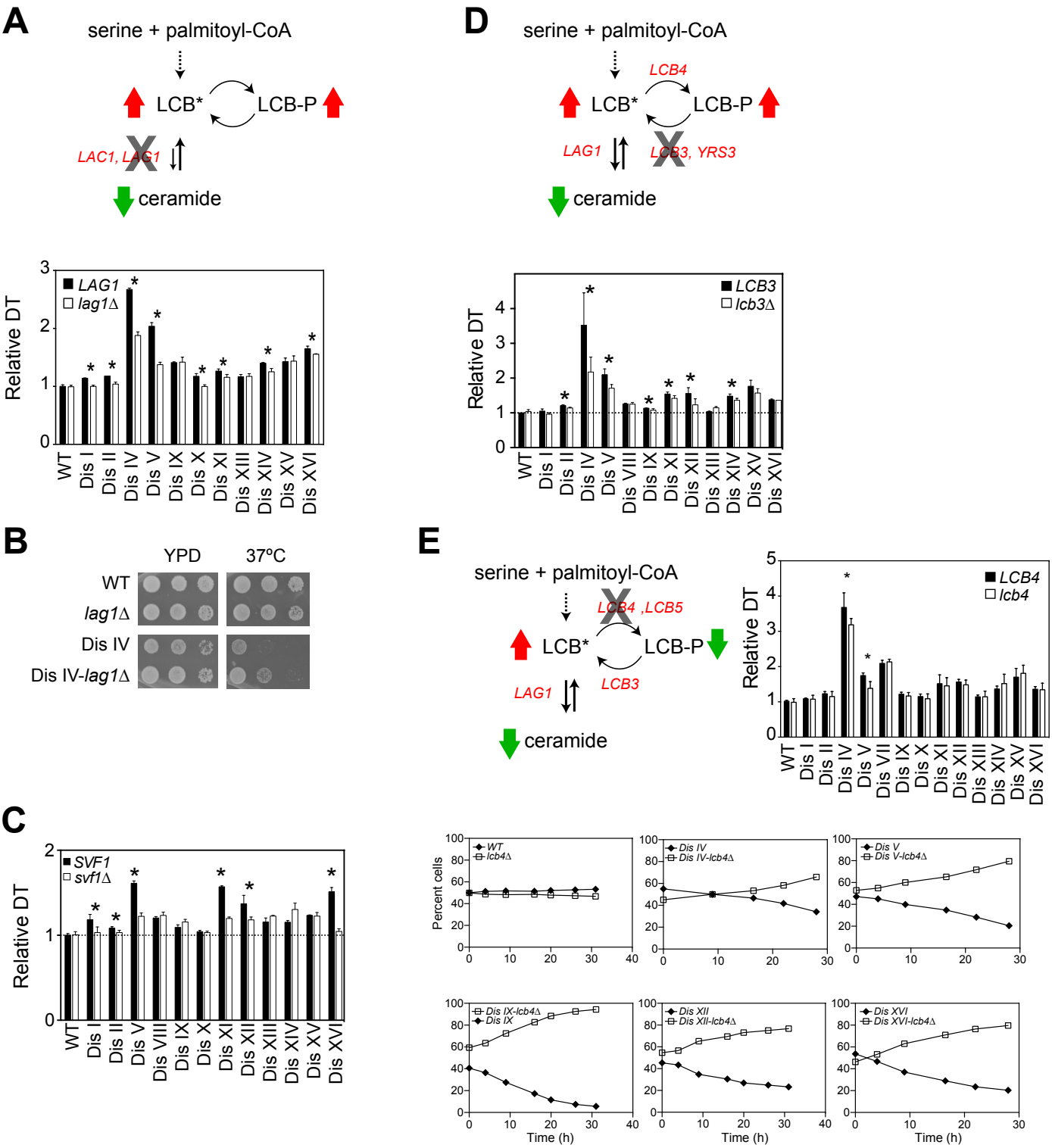


Figure S4. Reduced Ceramide Synthesis Improves the Fitness of Aneuploid Cells. Related to Figure 4.

(A) Doubling times of disomes (black bars) and disomes harboring *lag1*Δ (open bars) relative to WT. Error bars represent +/- SD of 3 biological replicates. Cells were grown at 30ºC in synthetic medium. * represent p-value < 0.05, paired Student's t-test. Strains harboring *LAG1* are marked with *CaURA3* to match the deletions: E192 (WT), E193 (Dis I), E194 (Dis II), E195 (Dis IV), E196 (Dis V), E198 (Dis IX), E199 (Dis X), E200 (Dis XI), E201 (Dis XII), E202 (Dis XIII), E203 (Dis XIV), E204 (Dis XV), and E205 (Dis XVI). Strains harboring the *lag1*Δ: E413 (*lag1*Δ), E414 (Dis I-*lag1*Δ), E415 (Dis II-*lag1*Δ), E416 (Dis IV-*lag1*Δ), E417 (Dis V-*lag1*Δ), E419 (Dis IX-*lag1*Δ), E420 (Dis X-*lag1*Δ), E421 (Dis XI-*lag1*Δ), E908 (Dis XII-*lag1*Δ), E423 (Dis XIII-*lag1*Δ), E424 (Dis XIV-*lag1*Δ), E425 (Dis XV-*lag1*Δ), and E426 (Dis XVI-*lag1*Δ).

(B) Proliferative capability of E192 (WT), E413 (*lag1*Δ), E195 (Dis IV) and E416 (Dis IV-*lag1*Δ) at 37ºC.

(C) Doubling times of disomes (black bars) and disomes harboring *svf1*Δ (open bars) relative to wild type cells. Error bars represent +/- SD of 3 biological replicates. Cells were grown at 30ºC in synthetic medium. * represent p-value < 0.05, paired Student's t-test. Strains harboring *SVF1* are marked with *CaURA3* to match the deletions: E192 (WT), E193 (Dis I), E194 (Dis II), E196 (Dis V), E197 (Dis VIII), E198 (Dis IX), E199 (Dis X), E200 (Dis XI), E201 (Dis XII), E202 (Dis XIII), E203 (Dis XIV), E204 (Dis XV), E205 (Dis XVI). Strains harboring *svf1*Δ: E290 (*svf1*Δ), E291 (Dis I-*svf1*Δ), E292 (Dis II-*svf1*Δ), E293 (Dis V-*svf1*Δ), E294 (Dis VIII-*svf1*Δ), E295 (Dis IX-*svf1*Δ), E296 (Dis X-*svf1*Δ), E297 (Dis XI-*svf1*Δ), E298 (Dis XII-*svf1*Δ), E299 (Dis XIII-*svf1*Δ), E300 (Dis XIV-*svf1*Δ), E301 (Dis XV-*svf1*Δ), and E580 (Dis XVI-*svf1*Δ).

(D) Doubling times of disomes (black bars) and disomes harboring *lcb3*Δ (open bars) relative to WT. Error bars represent +/- SD of 3 biological replicates. Cells were grown at 30ºC in synthetic medium. * represent p-value < 0.05, paired Student's t-test. Strains harboring *LCB3* are: E192 (WT), E193 (Dis I), E194 (Dis II), E195 (Dis IV), E196 (Dis V), E197 (Dis VIII), E198 (Dis IX), E199 (Dis X), E200 (Dis XI), E201 (Dis XII), E202 (Dis XIII), E203 (Dis XIV), E204 (Dis XV), and E205 (Dis XVI). Strains harboring *lcb3*Δ: E445 (WT-*lcb3*Δ), E446 (Dis I-*lcb3*Δ), E447 (Dis II-*lcb3*Δ), E448 (Dis IV-*lcb3*Δ), E449 (Dis V-*lcb3*Δ), E450 (Dis VIII-*lcb3*Δ), E451 (Dis IX-*lcb3*Δ), E452 (Dis X-*LCB3/lcb3*Δ), E453 (Dis XI-*lcb3*Δ), E454 (Dis XII-*lcb3*Δ), E455 (Dis XIII-*lcb3*Δ), E456 (Dis XIV-*lcb3*Δ), E457 (Dis XV-*lcb3*Δ), and E458 (Dis XVI-*lcb3*Δ).

(E) Doubling times of disomes (black bars) and disomes harboring *lcb4*Δ (open bars) relative to WT. Error bars represent +/- SD of 3 biological replicates. Cells were grown at 30ºC in synthetic medium. * represent p-value < 0.05, paired Student's t-test. Strains harboring *LCB4* are: E192

(WT), E193 (Dis I), E194 (Dis II), E195 (Dis IV), E196 (Dis V), E197 (Dis VIII), E198 (Dis IX), E199 (Dis X), E200 (Dis XI), E201 (Dis XII), E202 (Dis XIII), E203 (Dis XIV), E204 (Dis XV), and E205 (Dis XVI). Strains harboring *lcb4*Δ: E460 (*lcb4*Δ), E461 (Dis I-*lcb4*Δ), E462 (Dis II-*lcb4*Δ), E463 (Dis IV-*lcb4*Δ), E464 (Dis V-*lcb4*Δ), E474 (Dis VIII-*lcb4*Δ), E466 (Dis IX-*lcb4*Δ), E497 (Dis X-*lcb4*Δ), E468 (Dis XI-*lcb4*Δ), E469 (Dis XII-*lcb4*Δ), E470 (Dis XIII-*lcb4*Δ), E471 (Dis XIV-*lcb4*Δ), and E473 (Dis XVI-*lcb4*Δ). Bottom: The percentage of cells in co-cultures of strains expressing GFP (closed triangles) and strains harboring *lcb4*Δ (open squares) was determined at the indicated times. The following strains were used (from top left to bottom right): E276 (WT-*PGK1-GFP*) and E460 (*lcb4*Δ), E279 (Dis IV-*PGK1-GFP*) and E463 (Dis IV-*lcb4*Δ), E280 (Dis V-*PGK1-GFP*) and E464 (Dis V-*lcb4*Δ), E282 (Dis IX-*PGK1-GFP*) and E466 (Dis IX-*lcb4*Δ), E284 (Dis XII-*PGK1-GFP*) and E469 (Dis XII-*lcb4*Δ), E289 (Dis XVI-*PGK1-GFP*) and E473 (Dis XVI-*lcb4*Δ).

Figure S5 - Related to Figure 5

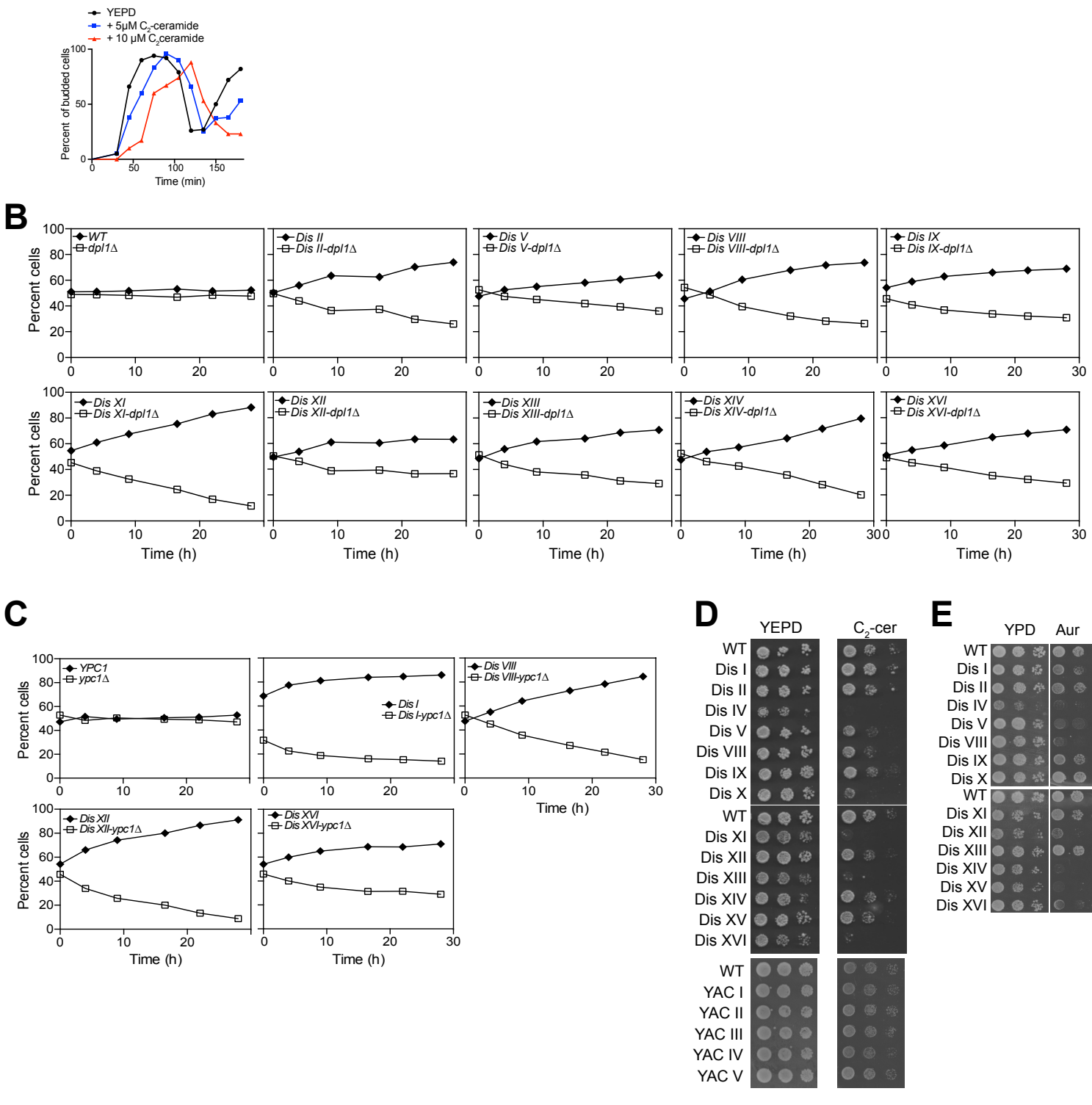


Figure S5. Effects of Shifting the Balance between Ceramide and Long-chain Bases in Aneuploid Strains. Related to Figure 5.

(A) Wild type cells (E113) were arrested in G1 with α-factor pheromone and released from the block as described in (Torres et al., 2007). Samples were taken at indicated times to determine the percentage of budded cells in YEPD medium, or in the presence of increasing concentrations of C_2-ceramide.

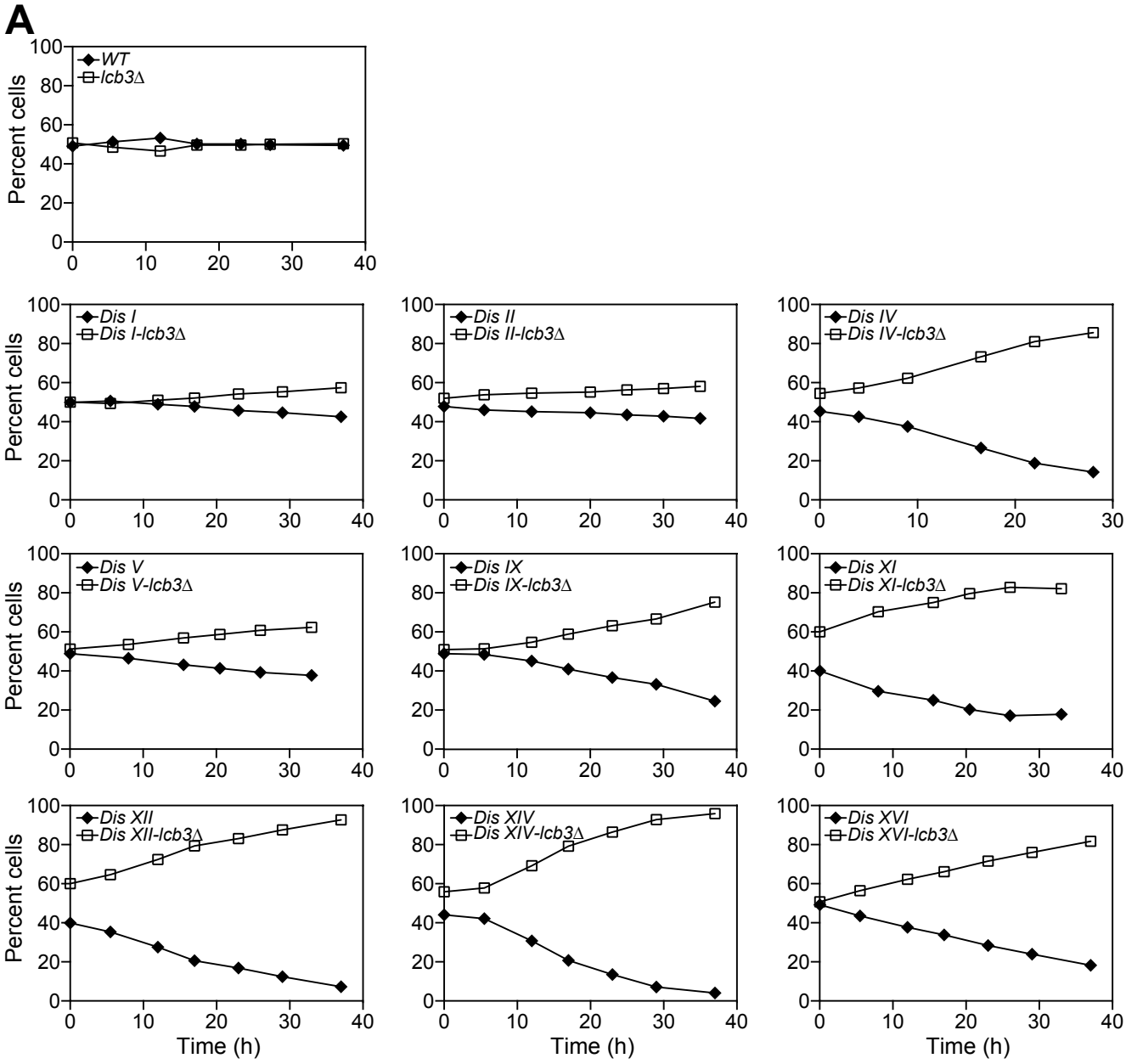
(B) The percentage of cells in co-cultures of strains expressing GFP (closed triangles) and strains harboring *dpl1*Δ (open squares) was determined at the indicated times. Competitions were performed in synthetic medium at 30ºC. Strains used for competitions (from top left to bottom right). E276 (WT-*PGK1-GFP*) and E610 (*dpl1*Δ), E278 (Dis II-*PGK1-GFP*) and E597 (Dis II-*dpl1*Δ), E280 (Dis V-*PGK1-GFP*) and E599 (Dis V-*dpl1*Δ), E281 (Dis VIII-*PGK1-GFP*) and E600 (Dis VIII-*dpl1*Δ), E282 (Dis IX-*PGK1-GFP*) and E601 (Dis IX-*dpl1*Δ), E283 (Dis XI-*PGK1-GFP*) and E603 (Dis XI-*dpl1*Δ), E284 (Dis XII-*PGK1-GFP*) and E604 (Dis XII-*dpl1*Δ), E486 (Dis XIII-*PGK1-GFP*) and E605 (Dis XIII-*dpl1*Δ), E286 (Dis XIV-*PGK1-GFP*) and E606 (Dis XIV-*dpl1*Δ), E289 (Dis XVI-*PGK1-GFP*) and E608 (Dis XVI-*dpl1*Δ).

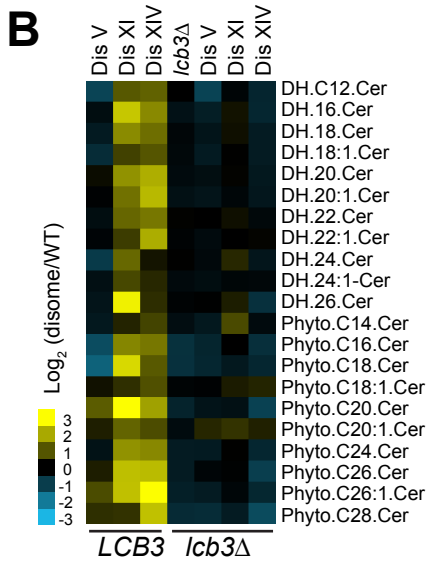
(C) The percentage of cells in co-cultures of strains expressing GFP (closed triangles) and strains harboring *ypc1*Δ (open squares) was determined at the indicated times. Competitions were performed in synthetic medium at 30ºC. The following strains were used (from top left to bottom right): E276 (WT-*PGK1-GFP*) and E619 (*ypc1*Δ), E277 (Dis I-*PGK1-GFP*) and E620 (Dis I-*ypc1*Δ), E2813 (Dis VIII-*PGK1-GFP*) and E624 (Dis VIII-*ypc1*Δ), E284 (Dis XII-*PGK1-GFP*) and E628 (Dis XII-*ypc1*Δ), E288 (Dis XVI-*PGK1-GFP*) and E632 (Dis XVI-*ypc1*Δ).

(D) Proliferative capability of WT and disomes in the presence of C_2-ceramide (10 μM). Top plate: E113 (WT), E88 (Dis I), E89 (Dis II), E91 (Dis IV), E92 (Dis V), E93 (Dis VIII), E94 (Dis IX), and E96 (Dis X); middle plate: E113 (WT), E97 (Dis XI), E98 (Dis XII), E100 (Dis XIII), E101 (Dis XIV), E102 (Dis XV), and E103 (Dis XVI); bottom plate: E113 (WT), E507 (YAC I), E508 (YAC II), E509 (YAC III), E510 (YAC IV), and E511 (YAC V).

(E) Proliferative capability of wild type cells and disomes in the presence of Aureobasidin A (20 ng/ml). Top plate: E113 (WT), E88 (Dis I), E89 (Dis II), E91 (Dis IV), E92 (Dis V), E93 (Dis VIII), E94 (Dis IX), and E96 (Dis X); bottom plate: E113 (WT), E97 (Dis XI), E98 (Dis XII), E100 (Dis XIII), E101 (Dis XIV), E102 (Dis XV), and E103 (Dis XVI).

Figure S6 - Related to Figure 6





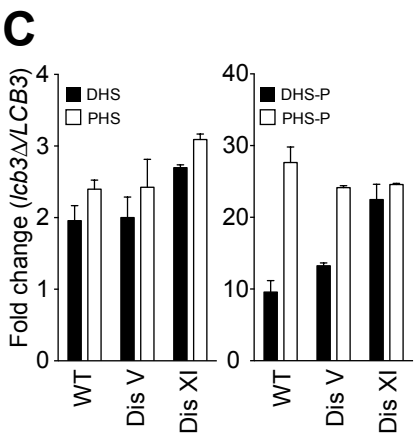
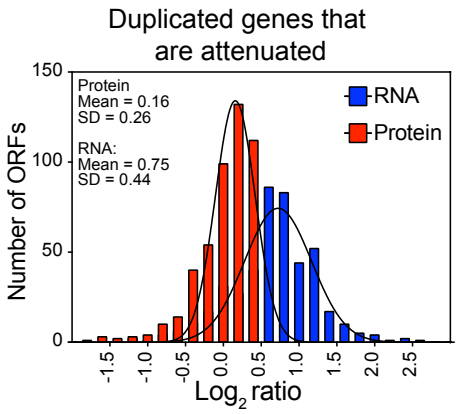


Figure S6. Loss of *LCB3* improves the fitness of aneuploid cells. Related to Figure 6.
(A-B) The percentage of cells in co-cultures of strains expressing GFP (closed triangles) and strains harboring a *lcb3*Δ (open squares) was determined at the indicated times. Competitions were performed in synthetic medium at 30ºC. Competitions were performed between these strains: (A) E276 (WT-*PGK1-GFP*) and E445 (*lcb3*Δ). (B) From top left to bottom right: E277 (Dis I-*PGK1-GFP*) and E446 (Dis I-*lcb3*Δ), E278 (Dis II-*PGK1-GFP*) and E447 (Dis II-*lcb3*Δ), E279 (Dis IV-*PGK1-GFP*) and E448 (Dis IV-*lcb3*Δ), E280 (Dis V-*PGK1-GFP*) and E449 (Dis V-*lcb3*Δ), E282 (Dis IX-*PGK1-GFP*) and E451 (Dis IX-*lcb3*Δ), E283 (Dis XI-*PGK1-GFP*) and E453 (Dis XI-*lcb3*Δ), E284 (Dis XII-*PGK1-GFP*) and E454 (Dis XII-*lcb3*Δ), E487 (Dis XIV-*PGK1-GFP*) and E456 (Dis XIV-*lcb3*Δ), E289 (Dis XVI-*PGK1-GFP*) and E458 (Dis XVI-*lcb3*Δ).
(C) LC/LC-MS analysis of ceramides in the disomes and disomes harboring *lcb3*Δ compared to WT. Columns represent experiments. Strains used from left to right; E192 (WT), E196 (Dis V), E200 (Dis XI), E203 (Dis XIV), E445 (*lcb3*Δ), E449 (Dis V-*lcb3*Δ), (Dis XI-*lcb3*Δ), E456 (Dis XIV-*lcb3*Δ). The average of 3 biological replicates are shown.
(D) Fold change of LCBs (PHS and DHS) and LCB-Ps (PHS-P and DHS-P) in WT, disome V and disome XI cells upon loss of *LCB3*. The average of 3 biological replicates is shown. Error bars represent +/- SD, n = 3. * represent p-value < 0.05, paired Student's t-test. Strains used: ; E192 (WT), E196 (Dis V), E200 (Dis XI), E445 (*lcb3*Δ), E449 (Dis V-*lcb3*Δ), (Dis XI-*lcb3*Δ).

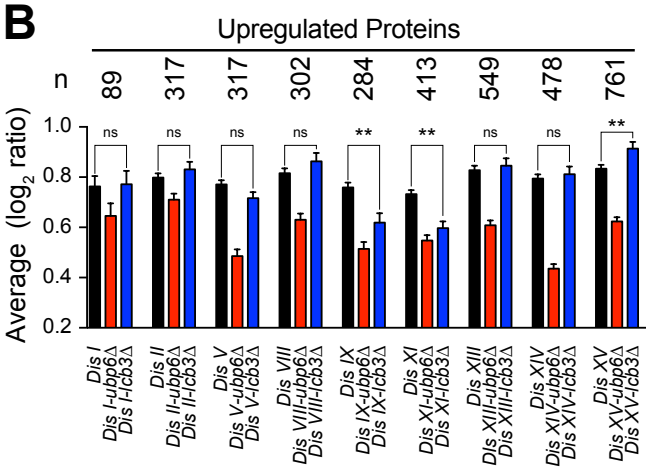
Figure S7 - Related to Figure 7

A



GO TERM	Macromolecular complex	Ribosomal subunit
Number	309 out of 477	72 out of 477
Cluster	64.80%	15.10%
Background	1030 out of 2387	114 out of 2387
P-value	6.01E-24	1.59E-22

B



C

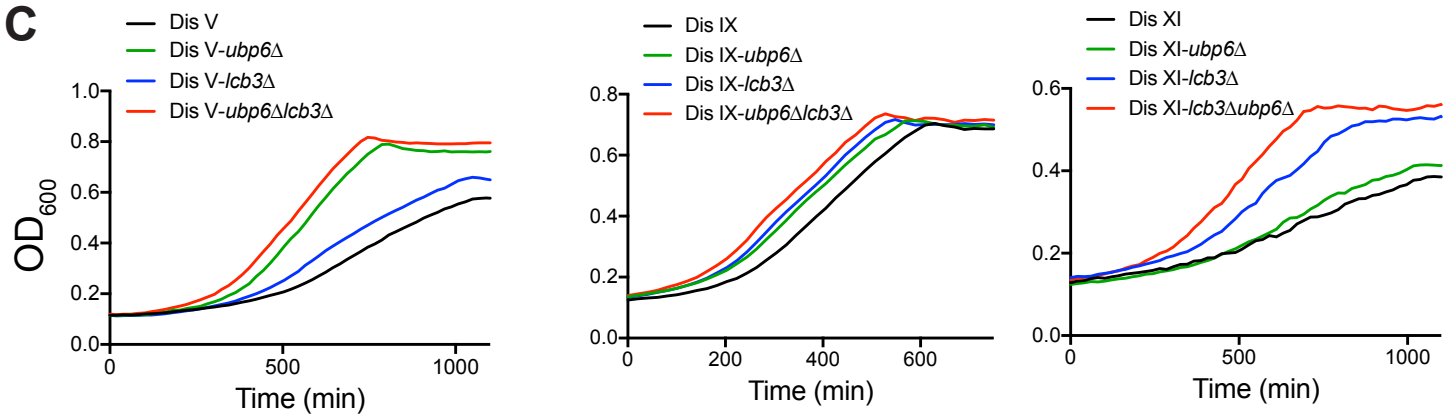


Figure S7. Loss of *LCB3* and *UBP6* enhances the fitness of aneuploid cells. Related to Figure 7.

(A) Histograms of the $\log_2$ ratios of the relative mRNA (blue) and protein levels (red) of the 477 attenuated proteins from disomic cells grown in YEPD medium compared to wild type cells. Fits to normal distribution are shown (black lines).

(B) Average relative levels of the most upregulated proteins, $\log_2$ ratios > 0.4, in the disomes (black), disomes-*ubp6*Δ (red) and disomes-*lcb3*Δ (blue).

(C) Growth curves of disomes V, disome IX, and disome XI harboring no deletions, *ubp6*Δ, *lcb3*Δ, or the double *lcb3*Δ and *ubp6*Δ.