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Supplementary Information for

**The environmental stress response causes ribosome loss in aneuploid yeast cells**

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**This PDF file includes:**

Figures S1 to S6
Tables S1 to S2

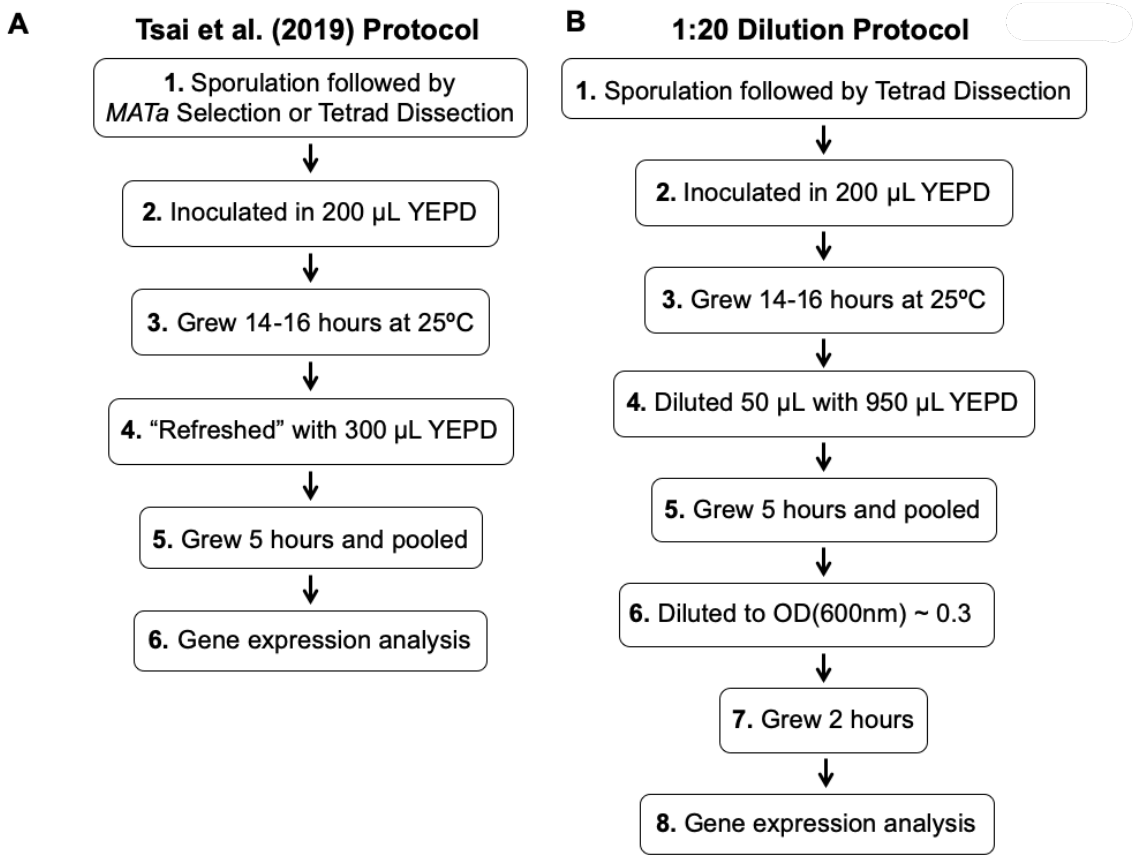



**Figure S1. Generation of heterogeneous aneuploid populations.**
**(*A*)** Protocol developed by Tsai et al. (2019; 9) to generate aneuploid cell populations. Cells harboring random aneuploidies were generated by sporulation of *pRS315-STE2pr-spHIS5* S288C triploids (A40878) and subsequent tetrad dissection or *MATa* selection through histidine prototrophy. Individual colonies were grown for 14-16 hours in 200 µL of YEPD in a 96 deep-well plate. 300 µL of YEPD were then added to cultures. The cultures were grown for 5 additional hours, pooled, and analyzed.
**(*B*)** Protocol to avoid growth of cell populations into stationary phase (1:20 dilution protocol). Cells harboring random aneuploidies were generated by sporulation of *pRS315-STE2pr-spHIS5* S288C triploids (A40878) and subsequent tetrad dissection. Individual colonies were grown for 14-16 hours in 200 µL of YEPD in a 96 deep-well plate. Cultures were then diluted 1:20 in YEPD, grown for another 5 hours, then pooled, and diluted to approximately OD(600nm) = 0.3. The pooled aneuploid populations were grown for an additional 2 hours, and samples were taken.

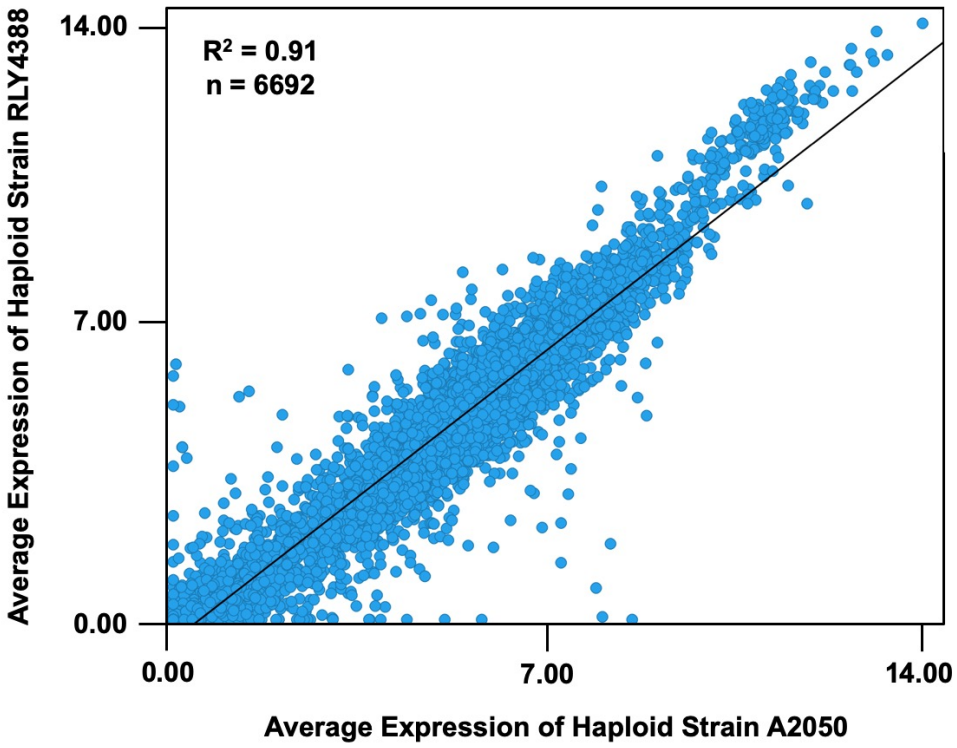



**Figure S2. Correlation between average gene expression of haploid strain RLY4388 and average gene expression of haploid strain A2050.**
RNA-Seq data from strain RLY4388 (Tsai et al. (2019; 9)) and from exponentially growing haploid strain A2050 were processed using the Expectation Maximization (RSEM) method. Transcript per million (TPM) values were calculated and $\log_2$ transformed with a +1 offset to avoid negative expression values. Two techincal replicates from haploid strain RLY4388 and three technical replicates from haploid strain A2050 were averaged, and average gene expression between the two strains was compared (Pearson, $R^2 = 0.91$, $P < 0.001$).

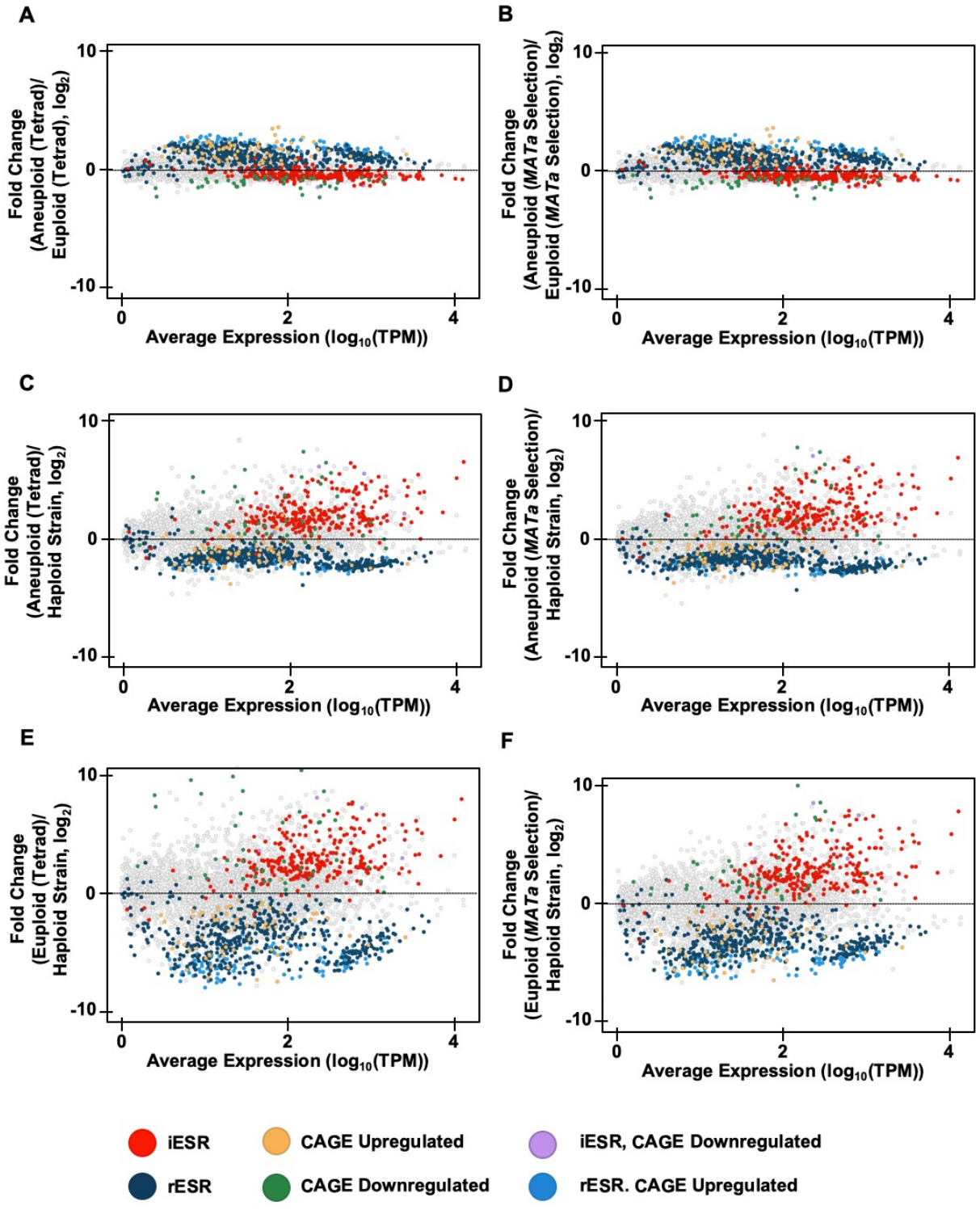
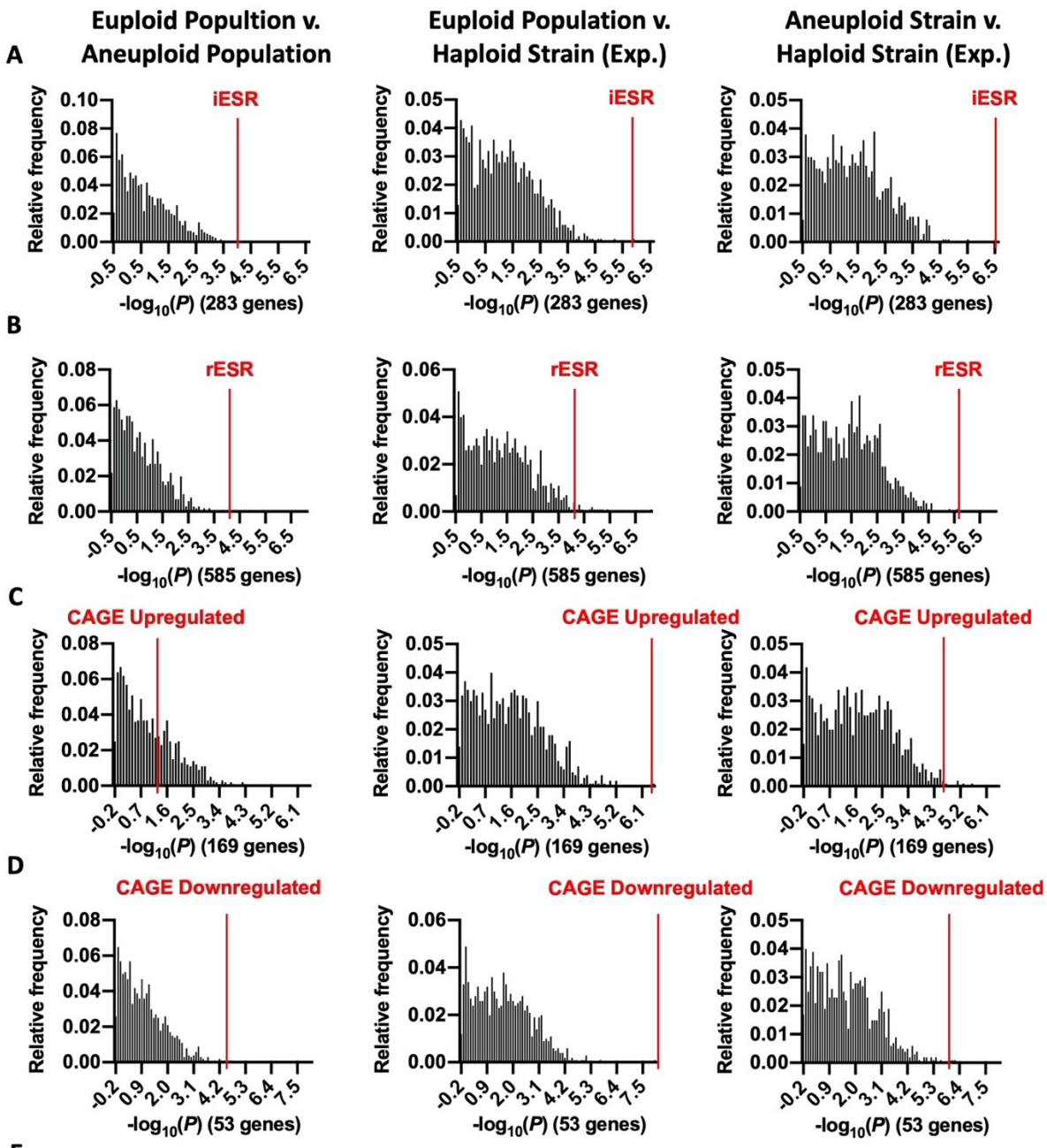

A
B
C
D
E
F
Fold Change (Aneuploid (Tetrad)/ Euploid (Tetrad), log2)
Fold Change (Aneuploid (MATa Selection)/ Euploid (MATa Selection), log2)
Fold Change (Aneuploid (Tetrad)/ Haploid Strain, log2)
Fold Change (Aneuploid (MATa Selection)/ Haploid Strain, log2)
Fold Change (Euploid (Tetrad)/ Haploid Strain, log2)
Fold Change (Euploid (MATa Selection)/ Haploid Strain, log2)
10
0
-10
0
2
4
Average Expression (log10(TPM))
iESR
rESR
CAGE Upregulated
CAGE Downregulated
iESR, CAGE Downregulated
rESR. CAGE Upregulated

**Figure S3. Comparison of aneuploid and euploid gene expression patterns from Tsai et al. (2019; 9) to haploid strain RLY4388.**
RNA-Seq data from Tsai et al. (2019; 9) were processed using the Expectation Maximization (RSEM) method. Transcript per million (TPM) values were calculated and $\log_2$ transformed. Expression data from aneuploid cell populations generated by tetrad dissection were pooled to create "aneuploid populations (Tetrad)". Expression data from aneuploid populations obtained from *MATa* selection were pooled to create "aneuploid populations (*MATa* Selection)". The x axis shows $\log_{10}$(average basal expression), and the y axis shows differential expression between euploid or aneuploid populations (Tetrad and *MATa* Selection) and two separate euploid controls: euploid cell populations and haploid strain RLY4388 (Tsai et al. (2019; 9), accession number: GSE107997). Colors specified refer to iESR, rESR, CAGE upregulated, CAGE downregulated, and those iESR genes downregulated in the CAGE signature and rESR genes upregulated in the CAGE signature. Differential expression graphs are shown for aneuploid cell populations (Tetrad) compared to euploid cell population (Tetrad) **(*A*)**, aneuploid cell populations (*MATa* Selection) compared to euploid cell population (*MATa* Selection) **(*B*)**, aneuploid cell populations (Tetrad) compared to haploid strain RLY4388 **(*C*)**, aneuploid cell populations (*MATa* Selection) compared to haploid strain RLY4388 **(*D*)**, euploid cell population (Tetrad) compared to haploid strain RLY4388 **(*E*)**, and euploid cell population (*MATa* Selection) compared to haploid strain RLY4388 **(*F*)**.




E

| | Euploid Population v. Aneuploid Population | Euploid Population v. Haploid Strain (Exp.) | Aneuploid Population v. Haploid Strain (Exp.) |
|---|---|---|---|
| iESR | = 0.003 | < 0.001 | < 0.001 |
| rESR | = 0.004 | = 0.014 | < 0.001 |
| CAGE Upregulated | = 0.354 | < 0.001 | = 0.006 |
| CAGE Downregulated | = 0.004 | < 0.001 | = 0.002 |

**Figure S4. ssGSEA bootstrapping of ANOVA tests between randomized gene sets.**
To further validate the significance of the differences in gene expression between aneuploid and euploid cell populations shown in Figure 2*B*-*E*, a bootstrapping analysis was performed on four separate groups of gene sets of the same sizes of the iESR gene set (283 genes), rESR gene set (585 genes), CAGE Upregulated gene set (169 genes), and CAGE Downregulated gene set (53 genes). 1000 random gene sets of each size were generated, and ssGSEA projection values for the exponentially growing haploid strain A2050 and the euploid and aneuploid populations (1:20 Dilution Protocol) were calculated. For each randomly generated gene set, the significance of the differences between the euploid population and the aneuploid population (Euploid Population v. Aneuploid Population), the euploid population and the exponentially growing haploid strain A2050 (Euploid Population v. Haploid Strain (Exp.)), and the aneuploid population and the exponentially growing haploid strain A2050 (Aneuploid Population v. Haploid Strain (Exp.)) were calculated as *P* values. *P* values from the randomly generated gene sets were then compared to the *P* value of the corresponding gene set and samples used. For all comparisons where a significant difference was observed, the experimentally obtained *P* value was significantly different from the *P* values from the randomly generated gene sets.
**(*A*-*D*)** 1000 gene sets of four different sizes were randomly generated, and ssGSEA projection values were calculated for the exponentially growing haploid strain A2050 and euploid and aneuploid populations (1:20 Dilution Protocol). With a one-way two-tailed ANOVA test with multiple comparisons and Bonferroni correction (*P* value multiplied by 3), $-\log_{10}(P)$ values were calculated for 1000 gene sets of sizes 283 genes (iESR) **(*A*)**, 585 genes (rESR) **(*B*)**, 169 genes (CAGE Upregulated) **(*C*)**, and 53 genes (CAGE Downregulated) **(*D*)** comparing differences between Euploid Population v. Aneuploid Population, Euploid Population v. Haploid Strain (Exp.), and Aneuploid Population v. Haploid Strain (Exp.). Vertical red lines represent transformed *P* values generated by each comparison for the indicated gene set (iESR, rESR, CAGE Upregulated, and CAGE Downregulated).
**(*E*)** *P* values generated by the bootstrapping analysis for each gene set and comparison. The bootstrapping *P* values were not multiple-test corrected.

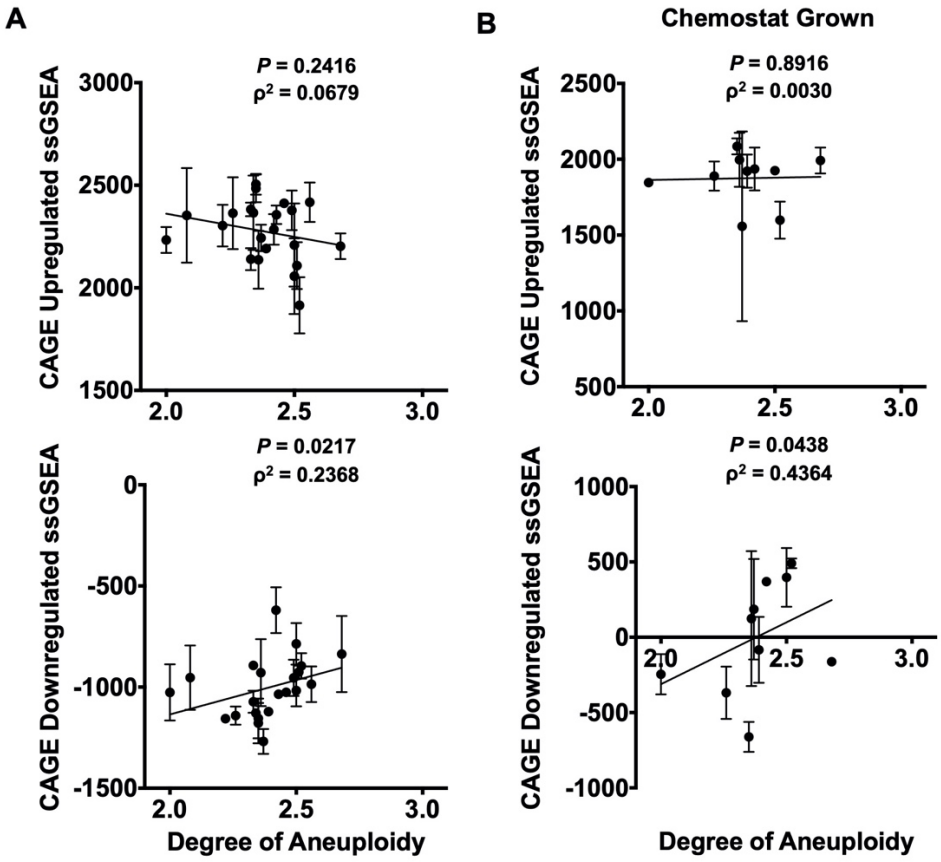



**Figure S5. Correlation between growth rate and the CAGE gene expression signature in complex aneuploid strains**.
Transcriptomes of the complex aneuploid strains were analyzed by RNA-Seq, and ssGSEA projection values were calculated for CAGE upregulated and CAGE downregulated genes.
**(*A*)** Correlation between CAGE upregulated ssGSEA projections and degree of aneuploidy (Spearman, $\rho^2 = 0.0679$, $P = 0.2416$) and CAGE downregulated ssGSEA projections and degree of aneuploidy (Spearman, $\rho^2 = 0.2368$, $P = 0.0217$) in complex aneuploid strains grown in YEPD.
**(*B*)** Correlations between CAGE upregulated ssGSEA projections and degree of aneuploidy (Spearman, $\rho^2 = 0.0030$, $P = 0.8916$) and CAGE downregulated ssGSEA projections and degree of aneuploidy (Spearman, $\rho^2 = 0.4364$, $P = 0.0438$) grown in a phosphate-limited chemostat.
Error bars represent standard deviation from the mean of experimental replicates.

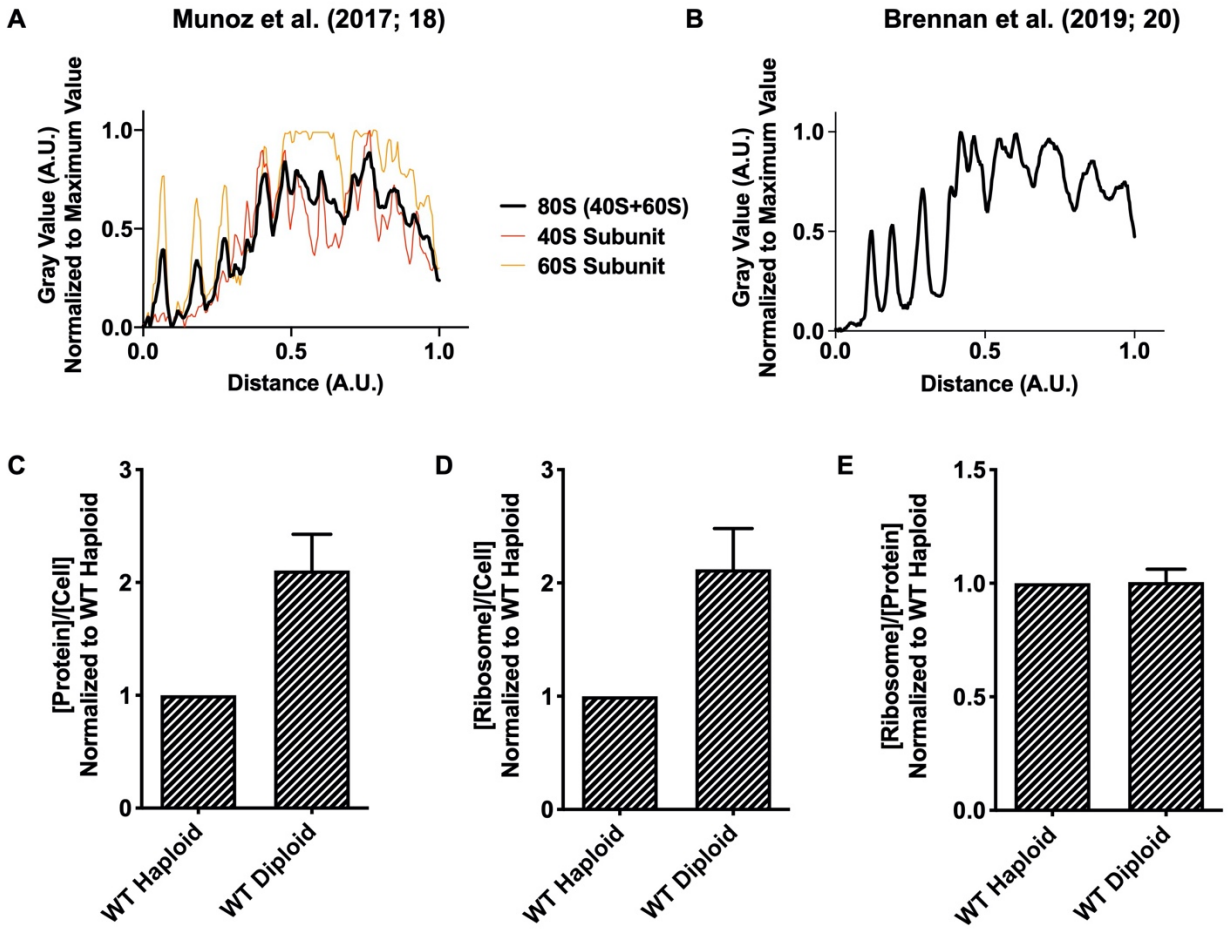



**Figure S6. Ribosome purification method**

**(*A*)** Quantification of Coomassie-stained gels of 40S and 60S ribosomal subunits purified by sucrose cushion in Munoz et al. (2017; 18). A line was drawn in the middle of the lane of the Coomassie-stained gels. Gray values, defined as pixel intensities, were then determined for the gel lanes containing 40S and 60S ribosomal subunits and normalized to the maximum gray value. To infer the banding pattern of 80S ribosomes, averaged the traces obtained from 40S and 60S particles (40S+60S) were averaged prior to normalization.

**(*B*)** Coomassie-stained gel electrophoresis quantification of ribosomes purified by sucrose cushion in Brennan et al. (2019; 20). Gray values were determined as in (*A*) for the lane containing purified ribosomes and normalized to the maximum gray value.

**(*C*-*E*)** Ribosomes were purified from wild-type haploid (A2587) and wild-type diploid (A33821) cultures to determine [Protein]/[Cell] **(*C*)**, [Ribosome]/[Cell] **(*D*)**, and [Ribosome]/[Protein] **(*E*)**. All values were normalized to the wild-type haploid strain A2587. Error bars represent standard deviation from the mean of experimental replicates.

**Table S1. Euploid strains.**
Description of the strain names, genotypes, and source used in this paper.

| Strain Name | Genotype | Source |
|---|---|---|
| A2587 | W303, *MATa ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, psi+* | Kim Nasmyth |
| A33821 | W303, *MATa/α ade2-1, leu2-3, ura3, trp1-1, his3-11,15, can1-100, GAL, psi+* | Kim Nasmyth |
| A2050 | S288C, *MATa ura3-52 his3Δ leu2-3 112 trp1Δ* | Frank Luca |
| A41265 | BY4741, *MATa his3Δ leu2Δ met15Δ ura3Δ slt2Δ::KanMX* | BY4741 Deletion Collection |
| A40877 | BY4743, *MATa/α his3Δ/his3Δ leu2Δ/leu2Δ met15Δ/MET15 ura3Δ/ura3Δ lys2Δ/LYS2 pRS315-STE2pr-spHIS5* | Tsai et al. (2019; 9) RLY9593 |
| A40878 | WT Triploid, *MATa/a/α his3Δ/his3Δ/his3Δ leu2Δ/leu2Δ/leu2Δ met15Δ/met15Δ/MET15 ura3Δ/ura3Δ/ura3Δ lys2Δ/LYS2/LYS2 pRS315-STE2pr-spHIS5* | Tsai et al. (2019; 9) RLY9596 |

**Table S2. Complex aneuploid strains.**
Description of the strain names, aliases, karyotypes and mean doubling times of complex aneuploid strains generated by Pavelka et al. (2010; 12).

| Strain Name | Alias | Chromosome copy number | | | | | | | | | | | | | | | | Degree of Aneuploidy (Normalized to Haploid) | Mean Doubling Time (Minutes) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | I | II | III | IV | V | VI | VII | VIII | IX | X | XI | XII | XIII | XIV | XV | XVI | | |
| RLY4737 | U2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2.00 | 106.2 |
| RLY4888 | A1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 2 | 2 | 2 | 2.08 | 111.2 |
| RLY4946 | A18 | 2 | 2 | 2 | 2 | 3 | 2 | 3 | 2 | 3 | 3 | 3 | 2 | 2 | 2 | 2 | 3 | 2.37 | 134.25 |
| RLY4947 | A19 | 3 | 3 | 2 | 2 | 2 | 2 | 3 | 2 | 3 | 3 | 2 | 3 | 3 | 3 | 2 | 2 | 2.51 | 149.3 |
| RLY4948 | A20 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 2 | 3 | 2 | 2 | 2 | 3 | 3 | 3 | 2 | 2.36 | 139.35 |
| RLY4949 | A21 | 2 | 3 | 2 | 3 | 2 | 2 | 3 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 3 | 2 | 2.52 | 169.85 |
| RLY4950 | A22 | 2 | 3 | 2 | 3 | 2 | 2 | 3 | 3 | 2 | 3 | 3 | 2 | 2 | 3 | 3 | 3 | 2.68 | 158.95 |
| RLY4951 | A23 | 3 | 3 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 2 | 3 | 3 | 3 | 3 | 3 | 2 | 2.50 | 140.3 |
| RLY4952 | A24 | 2 | 3 | 2 | 2 | 2 | 2 | 3 | 2 | 3 | 2 | 2 | 2 | 2 | 3 | 2 | 2 | 2.26 | 126.65 |
| RLY4953 | A25 | 2 | 3 | 2 | 2 | 2 | 2 | 3 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 2 | 2 | 2.22 | 125.05 |
| RLY4954 | A26 | 2 | 3 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 2 | 3 | 3 | 2 | 2 | 2.33 | 130.5 |
| RLY4955 | A27 | 2 | 3 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 2 | 3 | 3 | 2 | 2 | 2.33 | 131.1 |
| RLY4956 | A28 | 2 | 3 | 2 | 2 | 2 | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 3 | 2 | 2 | 2 | 2.46 | 133.7 |
| RLY4957 | A29 | 2 | 3 | 2 | 2 | 2 | 3 | 3 | 2 | 3 | 3 | 2 | 2 | 3 | 2 | 2 | 3 | 2.43 | 127.5 |
| RLY4958 | A30 | 3 | 3 | 2 | 2 | 2 | 3 | 3 | 3 | 3 | 2 | 3 | 2 | 3 | 3 | 2 | 3 | 2.56 | 140.45 |
| RLY4959 | A31 | 2 | 3 | 2 | 2 | 2 | 3 | 3 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 3 | 3 | 2.49 | 148.1 |
| RLY4960 | A32 | 3 | 2 | 2 | 3 | 2 | 2 | 2 | 3 | 3 | 3 | 3 | 2 | 2 | 2 | 2 | 2 | 2.35 | 134.8 |
| RLY4961 | A33 | 2 | 3 | 2 | 2 | 2 | 3 | 3 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 3 | 3 | 2.49 | 144.35 |
| RLY4962 | A34 | 3 | 2 | 2 | 3 | 2 | 2 | 2 | 3 | 3 | 3 | 3 | 2 | 2 | 2 | 2 | 2 | 2.35 | 129.15 |
| RLY4963 | A35 | 3 | 2 | 2 | 2 | 3 | 2 | 3 | 2 | 3 | 2 | 3 | 3 | 2 | 2 | 2 | 2 | 2.34 | 124 |
| RLY4964 | A36 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 2 | 3 | 3 | 2 | 3 | 2 | 3 | 3 | 2.50 | 141.6 |
| RLY4965 | A37 | 2 | 3 | 2 | 2 | 2 | 2 | 3 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 3 | 3 | 2.39 | 136.75 |
| RLY4966 | A38 | 2 | 3 | 2 | 3 | 2 | 2 | 2 | 3 | 3 | 2 | 3 | 2 | 2 | 2 | 3 | 2 | 2.42 | 142.15 |