Figure 1A

Previously published: 10.1128/mra.00729-22

Data object = “gene\_median\_relative\_depth\_DNA-Table 1.csv”

Figure 1B

Previously published: 10.1371/journal.pbio.3000069

“Fitness\_Final\_Lauer2018.csv” from: journal.pbio.3000069.s009

Analyzed by:

SF2\_Growth\_rate\_anaylsis/SF2\_Growth\_rate\_summary.rmd

Section “Figure 1B”

Figure 1C

Raw Data:

SF2\_Growth\_rate\_anaylsis/growth\_curves/

Analyzed by:

SF2\_Growth\_rate\_anaylsis/SF2\_Growth\_rate\_summary.rmd

Section “Figure 1C”

Generates file:

rel\_growth\_YPGal.csv

Figure 2B

Normalized insertions per gene from Supplemental\_Table\_S9

Analyzed by:

SF4\_Hermes\_analysis/Make\_Fig2B.py

Figure 3A

Normalized strain insertion data from: Supplemental\_Table\_S9

Analyzed by:  
 SF4\_Hermes\_analysis/Make\_Fig3A.rmd

Figure 3B

Normalized strain insertion data from: Supplemental\_Table\_S9

**All data is located in SF5\_Tn\_to\_GSEA/**

Analyzed by:

SF5\_Tn\_to\_GSEA/make\_gse.r

SF5\_Tn\_to\_GSEA/make\_compressed.py

SF5\_Tn\_to\_GSEA/make\_insert\_GSEA.py

Figure 3C

Normalized strain insertion data from: Supplemental\_Table\_S9

Analyzed by:  
 SF4\_Hermes\_analysis/Make\_Fig3C.rmd

Figure 3D

Raw Data:

SF2\_Growth\_rate\_anaylsis/growth\_curves/

Analyzed by:

SF2\_Growth\_rate\_anaylsis/functions.r

SF2\_Growth\_rate\_anaylsis/SF2\_Growth\_rate\_summary.rmd

Section “Figure 3D”

Figure 3E

Raw Data:

SF2\_Growth\_rate\_anaylsis/growth\_curves/

Analyzed by:

SF2\_Growth\_rate\_anaylsis/functions.r

SF2\_Growth\_rate\_anaylsis/SF2\_Growth\_rate\_summary.rmd

Section “Figure 3E”

SF2\_Growth\_rate\_anaylsis/error\_bars/error\_bars\_\*\_mult.py

Figure 4

Normalized expression data from: Supplemental\_Table\_S23

Analyzed by:

SF3\_RNAseq\_analysis/ Make\_Fig4.py

Figure 5

All previously published data is located in SF6\_Compare\_to\_Prev**/**  
 Analyzed by:

SF6\_Compare\_to\_Prev/Compare\_to\_Previous\_Studies.rmd

**Supplemental\_Fig\_S1**

Based on previously published data: 10.1128/mra.00729-22

**Supplemental\_Fig\_S2**

Based on previously published data: 10.1128/mra.00729-22

**Supplemental\_Fig\_S3**

Copy-number sizes from Supplemental\_Table\_S4

Relative fitness in glutamine limited media from:

“Fitness\_Final\_Lauer2018.csv” from: journal.pbio.3000069.s009

Relative growth rate (YPGal) from

rel\_growth\_YPGal.csv from

SF2\_Growth\_rate\_anaylsis/SF2\_Growth\_rate\_summary.rmd

**Supplemental\_Fig\_S4**

Copy-number sizes from Supplemental\_Table\_S4

TPM data from Supplemental\_Table\_S28

DESeq2 data from Supplemental\_Table\_S23, Supplemental\_Table\_S24

Analyzed by:

SF3\_RNAseq\_analysis/Make\_Supplemental\_Core\_Figure.py

**Supplemental\_Fig\_S6**

CDS lengths for each gene derived from SGD peptide sequences.  
Insertion medians calculated from Supplemental\_Table\_9

Analyzed by:

SF4\_Hermes\_analysis/Make\_Supplemental\_Fig\_S6.r

**Supplemental\_Fig\_S7**

Strain insertion data from: Supplemental\_Table\_S8

Analyzed by:

SF4\_Hermes\_analysis/Make\_Supplemental\_Fig\_S7.py

**Supplemental\_Fig\_S8**

Essential ORFs from Winzeler 1999

Normalized strain insertion data from: Supplemental\_Table\_S9

Analyzed by:

SF4\_Hermes\_analysis/Make\_Supplemental\_Fig\_S8.py

**Supplemental\_Fig\_S9**

Essential ORFs from Winzeler 1999

Quartile essential ORFs from Costanzo et al 2021

Strain insertion data from: Supplemental\_Table\_S8

Analyzed by:

SF4\_Hermes\_analysis/Make\_ Supplemental\_Fig\_S9.Rmd

**Supplemental\_Fig\_S10**

Normalized strain insertion data from: Supplemental\_Table\_S9

Analyzed by:

SF4\_Hermes\_analysis/Make\_ Supplemental\_Fig\_S10.Rmd

**Supplemental\_Fig\_S11**

Normalized strain insertion data from: Supplemental\_Table\_S9

Analyzed by:

SF4\_Hermes\_analysis/Supplemental\_Fig\_S11\_Make\_Insert\_R-squared.r

SF4\_Hermes\_analysis/Make\_Supplemental\_Fig\_S11.py

SF4\_Hermes\_analysis/Supplemental\_Fig\_S11\_Enrichment\_of\_outliers.py

**Supplemental\_Fig\_S12**

Normalized strain insertion data from: Supplemental\_Table\_S9

Analyzed by:

SF4\_Hermes\_analysis/Make\_Supplemental\_Fig\_S12.py

**Supplemental\_Fig\_S13**

Copy number per gene from Supplemental\_Table\_S6

Normalized strain insertion data from: Supplemental\_Table\_S9

Analyzed by:

SF4\_Hermes\_analysis/Make\_Supplemental\_Fig\_S13.r

**Supplemental\_Fig\_S14**

Normalized strain insertion data from: Supplemental\_Table\_S9

**All data is located in SF5\_Tn\_to\_GSEA/**

Analyzed by:

SF5\_Tn\_to\_GSEA/make\_gse.r

SF5\_Tn\_to\_GSEA/make\_compressed.py

SF5\_Tn\_to\_GSEA/make\_insert\_GSEA.py

**Supplemental\_Fig\_S15A**

RNA read abundance from: Supplemental\_Table\_S21  
 Analyzed by:

SF3\_RNAseq\_analysis/Make\_Supplemental\_Fig\_S15.rmd

**Supplemental\_Fig\_S15B**

Raw Data:

SF2\_Growth\_rate\_anaylsis/growth\_curves/

Analyzed by:

SF2\_Growth\_rate\_anaylsis/functions.r

SF2\_Growth\_rate\_anaylsis/SF2\_Growth\_rate\_summary.rmd

Section “Figure 3E”

SF2\_Growth\_rate\_anaylsis/error\_bars/error\_bars\_\*\_additive.py

**Supplemental\_Fig\_S16**

RNA read abundance from: Supplemental\_Table\_S21  
 Analyzed by:

SF3\_RNAseq\_analysis/Make\_Supplemental\_Fig\_S16.rmd

**Supplemental\_Fig\_S17**

RNA read abundance from: Supplemental\_Table\_S21

Normalized strain insertion data from: Supplemental\_Table\_S9  
 Analyzed by:

SF3\_RNAseq\_analysis/Make\_Supplemental\_Fig\_S17.r

**Supplemental\_Fig\_S18**

TPM normalized RNA from Supplemental\_Table\_S22

Copy number per gene from Supplemental\_Table\_S6  
 Analyzed by:

SF3\_RNAseq\_analysis/Make\_Supplemental\_Fig\_S18.r

**Supplemental\_Fig\_S19, Supplemental\_Fig\_S20, Supplemental\_Fig\_S21, Supplemental\_Fig\_S22, Supplemental\_Fig\_S23, Supplemental\_Fig\_S24**

All previously published data is located in SF6\_Compare\_to\_Prev **/**  
 Analyzed by:

SF6\_Compare\_to\_Prev/Compare\_to\_Previous\_Studies.rmd

**Supplemental\_Fig\_S25**

Using DESeq2 data from SF3\_RNAseq\_analysis/Supplemental\_File\_DESeq\_for\_GSEA/

Analyzed by:

SF3\_RNAseq\_analysis/make\_gse\_for\_rna.r

**Supplemental\_Fig\_S26**

Raw Data:

SF2\_Growth\_rate\_anaylsis/growth\_curves/

Analyzed by:

SF2\_Growth\_rate\_anaylsis/functions.r

SF2\_Growth\_rate\_anaylsis/SF2\_Growth\_rate\_summary.rmd

Section “Effect of CCCP”