This Supplemental materials file contains code used in the analysis and figure construction of “Copy number variation alters local and global mutational tolerance”.   
  
Supplemental code and (where necessary additional data objects required for the analysis) have been provided, separated into thematic folders based on the experiment, analysis, and project:

SCode1\_

**SCode2\_Growth\_rate\_analysis**

Files associated with the calculation of growth rates in YPGal, YPGal + CCCP and Glutamine limited media. Code for the generation of Figures 1B,C; 3D,E; and Supplemental Figures S3, S15B, S26

**SCode3\_RNAseq\_analysis**

Files associated with RNAseq analysis, including the replicate correlation, copy-number correction, DESeq2, and the GSEA. Code for the generation of Figure 4; and Supplemental Figures S4, S15A, S16, S17, S18, S25  
  
**SCode4\_Hermes\_analysis**

Files associated with the analysis of HERMES data, including the conversion from alignments to unique insertion sites, replicate correlation, copy-number correction, and DESeq2. Code for the generation of Figure 2B; 3A,C; and Supplemental Figures S6, S7, S8, S9, S10, S11, S12, S13

**SCode5\_Tn\_to\_GSEA**

Files associated with the GSEA of HERMES data, including DESeq2, heatmap plots, GSE and GSEA GO term compression. Code for the generation of Figure 3B and Supplemental Figures S14

**SCode6\_Compare\_to\_Prev**

Files associated with comparison of gene set expression data between this study and previous studies. Code for the generation of Figure 5 and Supplemental Figures S19, S20, S21, S22, S23, S24

**SCode7\_Miscellaneous\_analysis**

Files not associated with any single or substantial aspect of analysis, including downstream TF analysis, ncRNA enrichment, and zscore calculation. No association with any figures.

**Figure source data and code used in analysis**

**Figure 1A**

Data from: 10.1128/mra.00729-22

**Figure 1B**

Data from: 10.1371/journal.pbio.3000069

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

Analyzed by:

SCode2\_Growth\_rate\_anaylsis/Growth\_rate\_summary.rmd

Section “Figure 1B”

**Figure 1C**

Raw Data:

SCode2\_Growth\_rate\_anaylsis/growth\_curves/

Analyzed by:

SCode2\_Growth\_rate\_anaylsis/Growth\_rate\_summary.rmd

Section “Figure 1C”

**Figure 2B**

Normalized insertions per gene from Supplemental\_Table\_S9

Analyzed by:

SCode4\_Hermes\_analysis/Make\_Fig2B.py

**Figure 3A**

Normalized strain insertion data from: Supplemental\_Table\_S9

Analyzed by:  
 SCode4\_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:

SCode5\_Tn\_to\_GSEA/make\_gse.r

SCode5\_Tn\_to\_GSEA/make\_compressed.py

SCode5\_Tn\_to\_GSEA/make\_insert\_GSEA.py

**Figure 3C**

Normalized strain insertion data from: Supplemental\_Table\_S9

Analyzed by:  
 SCode4\_Hermes\_analysis/Make\_Fig3C.rmd

**Figure 3D**

Raw Data:

SCode2\_Growth\_rate\_anaylsis/growth\_curves/

Analyzed by:

SCode2\_Growth\_rate\_anaylsis/functions.r

SCode2\_Growth\_rate\_anaylsis/Growth\_rate\_summary.rmd

Section “Figure 3D”

**Figure 3E**

Raw Data:

SCode2\_Growth\_rate\_anaylsis/growth\_curves/

Analyzed by:

SCode2\_Growth\_rate\_anaylsis/functions.r

SCode2\_Growth\_rate\_anaylsis/Growth\_rate\_summary.rmd

Section “Figure 3E”

SCode2\_Growth\_rate\_anaylsis/error\_bars/error\_bars\_\*\_mult.py

**Figure 4**

Normalized expression data from: Supplemental\_Table\_S23

Analyzed by:

SCode3\_RNAseq\_analysis/ Make\_Fig4.py

**Figure 5**

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

SCode6\_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

Analyzed by:

SCode2\_Growth\_rate\_anaylsis/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:

SCode3\_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:

SCode4\_Hermes\_analysis/Make\_Supplemental\_Fig\_S6.r

**Supplemental\_Fig\_S7**

Strain insertion data from: Supplemental\_Table\_S8

Analyzed by:

SCode4\_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:

SCode4\_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:

SCode4\_Hermes\_analysis/Make\_ Supplemental\_Fig\_S9.Rmd

**Supplemental\_Fig\_S10**

Normalized strain insertion data from: Supplemental\_Table\_S9

Analyzed by:

SCode4\_Hermes\_analysis/Make\_ Supplemental\_Fig\_S10.Rmd

**Supplemental\_Fig\_S11**

Normalized strain insertion data from: Supplemental\_Table\_S9

Analyzed by:

SCode4\_Hermes\_analysis/Supplemental\_Fig\_S11\_Make\_Insert\_R-squared.r

SCode4\_Hermes\_analysis/Make\_Supplemental\_Fig\_S11.py

SCode4\_Hermes\_analysis/Supplemental\_Fig\_S11\_Enrichment\_of\_outliers.py

**Supplemental\_Fig\_S12**

Normalized strain insertion data from: Supplemental\_Table\_S9

Analyzed by:

SCode4\_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:

SCode4\_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:

SCode5\_Tn\_to\_GSEA/make\_gse.r

SCode5\_Tn\_to\_GSEA/make\_compressed.py

SCode5\_Tn\_to\_GSEA/make\_insert\_GSEA.py

**Supplemental\_Fig\_S15A**

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

SCode3\_RNAseq\_analysis/Make\_Supplemental\_Fig\_S15.rmd

**Supplemental\_Fig\_S15B**

Raw Data:

SCode2\_Growth\_rate\_anaylsis/growth\_curves/

Analyzed by:

SCode2\_Growth\_rate\_anaylsis/functions.r

SCode2\_Growth\_rate\_anaylsis/Growth\_rate\_summary.rmd

Section “Figure 3E”

SCode2\_Growth\_rate\_anaylsis/error\_bars/error\_bars\_\*\_additive.py

**Supplemental\_Fig\_S16**

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

SCode3\_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:

SCode3\_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:

SCode3\_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:

SCode6\_Compare\_to\_Prev/Compare\_to\_Previous\_Studies.rmd

**Supplemental\_Fig\_S25**

Using DESeq2 data from SCode3\_RNAseq\_analysis/Supplemental\_File\_DESeq\_for\_GSEA/

Analyzed by:

SCode3\_RNAseq\_analysis/make\_gse\_for\_rna.r

**Supplemental\_Fig\_S26**

Raw Data:

SCode2\_Growth\_rate\_anaylsis/growth\_curves/

Analyzed by:

SCode2\_Growth\_rate\_anaylsis/functions.r

SCode2\_Growth\_rate\_anaylsis/Growth\_rate\_summary.rmd

Section “Effect of CCCP”