## Supplemental Figure Legends:

**Figure S1. A)** Relative frequency trajectories for all conditions and replicates. Each line is an individual double barcode strain. Colors indicate the fitness estimate of each strain. **B,C)** Scatter plots of fitnesses **(B)** and interaction scores **(C)** between each of three biological replicates in each of three experimental conditions. Each point is an individual strain.

**Figure S2.** Scatter plot of the mean and the standard deviation of fitness estimates made in three replicate cultures in YPD (**A**), YPD 37°C (**B**) and YPEG (**C**). Each point is an individual strain. Spearman's *rho* and *P*-value are given on the plots.

**Figure S3.** Scatter plot of iSeq interaction score estimates and s-scores reported by Collins *et. al.* 2007 (**A**) and interaction scores reported in Costanzo *et. al.* 2010 (**B**). Each point is a gene pair. Error bars on our measurements represent the standard deviation across 4-8 strains carrying the corresponding gene deletions. In panel **B**, two scores were published for a subset of gene pairs. Error bars on Costanzo *et. al.* data are the standard deviations reported on the estimates.

**Figure S4.** Spot assays for F0 strains from the 5 double gene deletion genotypes that were whole-genome sequenced. Overnight cultures were inoculated from single colonies and diluted to $1x10^8$ cells/mL. Four additional serial dilutions (1:10) were performed, and 3ul of each dilution was plated to either YPD or the media used to select haploid double deletion strains carrying the iSeq double barcode (1.7g/L Difco yeast nitrogen base without amino acids and ammonium sulfate, 1g/L monosodium glutamic acid, 2% glucose, Nat, G418, –Ura, –Lys, –Leu, –Ade, and –Arg). The number of cells plated (columns), and the whole chromosome duplication events identified in each strain (rows), are labeled on each panel.

## Supplemental Table Titles:

**Table S1.** Published genetic interactions for each of the 36 double gene deletion genotypes used in this study. Quantitative scores are either an SGA score (ε), or an S score (*s*). See references for sources of reported interactions. All published interactions were found in the BioGRID repository.
**Table S2.** (Separate excel file) Fitness and interaction score estimates for each individual strain in each replicate of each experimental condition.
**Table S3.** (Separate excel file) Expanded summary of whole genome sequencing data.
**Table S4.** (Separate excel file) Interaction score estimates called as significant using 95% confidence intervals.
**Table S5.** (Separate excel file) Sequencing counts for each strain, at each time point, in each culture, and each strain's doubling time and fitness estimates from Optical Density growth curve fitness assay.