**SUPPLEMENTAL MATERIALS**

**Table S1.** Oligonucleotides used in this study.

|  |  |
| --- | --- |
| **Name** | **Sequence (5ʹ to 3ʹ)** |
| MB355 | GAATGGTGTCGTAGTTATAAGTAACACTATTTATTTTTCTACTCTGCATAGGCCACTAGTGGATCTG |
| MB373 | CAGCCAGCTTGGAGTCATTGGCTAGAGG |
| MB374 | GAGCAATGTGCACACCACAATATGTCGTGGTTG |
| MB525 | AAAACAGAACCGTTATACATATTGAGATGGTTAAGGTCGTAGAAAAGAAATGTTCATTTGAGAAGGAAAACGGATCCCCGGGTTAATTAA |
| MB526 | TCCACCAAGTGAATCTACAAGTAGTAGAATAGAGTATTTATATTCGGTTTACAAACTACAAATAGCGTGCGAATTCGAGCTCGTTTAAAC |
| MB527 | GTGAATTGCTCAGAAGAGAAAGGCATACCGTC |
| MB528 | CTGTGCATCAACAAGGTGACAGAATGTTGATG |
| MB765 | ATGGTGACGAAGCCGTCACATAACTTAAG |
| MB768 | AAAGTGCCCAAAAAGAATGCTTGGCGAATGGTGTCGTAGTTATAAGTAACACTATTTATTTTTCTACTCTGAATTCGAGCTCGTTTAAAC |
| MB769 | GGGAGAAATCCAGTGTTAGTCAAAAGGATGAGG |
| MB932 | CCGGAAGTATATCAGGGTATGGAACACG |
| MB1395 | GGCTAGAGGAAAGGAAAAAATACAGATTATTGTTGTATATATTTAAAAAATCATACACGTACACACAAGGCGGTAACGGATCCCCGGGTTAATTAA |
| MB1396 | GTCGACCTGCAGCGTACGAAGCTTCAGCTGTCACTTTCTTCCTCTGTAGTGACCTCGGTA |
| MB1397 | TACCGAGGTCACTACAGAGGAAGAAAGTGACAGCTGAAGCTTCGTACGCTGCAGGTCGAC |

**Table S2.** *hrq1* x single-gene deletion SGA results (see File S2).

**Table S3.** *hrq1-K318A* x single-gene deletion SGA results (see File S2).

**Table S4.** *sgs1* x single-gene deletion SGA results (see File S2).

**Table S5.** *sgs1-K706A* x single-gene deletion SGA results (see File S2).

**Table S6.** *hrq1* *sgs1* x single-gene deletion SGA results (see File S2).

**Table S7.** *hrq1* *sgs1-K706A* x single-gene deletion SGA results (see File S2).

**Table S8.** *hrq1-K318A* *sgs1* x single-gene deletion SGA results (see File S3).

**Table S9.** *hrq1-K318A* *sgs1-K706A* x single-gene deletion SGA results (see File S2).

**Table S10.** *hrq1* x temperature-sensitive SGA results (see File S3).

**Table S11.** *hrq1-K318A* x temperature-sensitive SGA results (see File S3).

**Table S12.** *sgs1* x temperature-sensitive SGA results (see File S3).

**Table S13.** *sgs1-K706A* x temperature-sensitive SGA results (see File S3).

**Table S14.** *hrq1* *sgs1* x temperature-sensitive SGA results (see File S3).

**Table S15.** *hrq1* *sgs1-K706A* x temperature-sensitive SGA results (see File S3).

**Table S16.** *hrq1-K318A* *sgs1* x temperature-sensitive SGA results (see File S3).

**Table S17.** *hrq1-K318A* *sgs1-K706A* x temperature-sensitive SGA results (see File S3).

\**nota bene*: Tables S2-S17 contain lists of significant genetic interactors. For the full SGA data, please see File S4.

**Table S18.** Complete *hrq1* x single-gene deletion SGA results (see File S4).

**Table S19.**Complete *hrq1-K318A* x single-gene deletion SGA results (see File S4).

**Table S20.**Complete *sgs1* x single-gene deletion SGA results (see File S4).

**Table S21.**Complete *sgs1-K706A* x single-gene deletion SGA results (see File S4).

**Table S22.**Complete *hrq1* *sgs1* x single-gene deletion SGA results (see File S4).

**Table S23.**Complete *hrq1* *sgs1-K706A* x single-gene deletion SGA results (see File S4).

**Table S24.**Complete *hrq1-K318A* *sgs1* x single-gene deletion SGA results (see File S4).

**Table S25.**Complete *hrq1-K318A* *sgs1-K706A* x single-gene deletion SGA results (see File S4).

**Table S26.** Complete *hrq1* x temperature-sensitive SGA results (see File S4).

**Table S27.**Complete *hrq1-K318A* x temperature-sensitive SGA results (see File S4).

**Table S28.**Complete *sgs1* x temperature-sensitive SGA results (see File S4).

**Table S29.**Complete *sgs1-K706A* x temperature-sensitive SGA results (see File S4).

**Table S30.**Complete *hrq1* *sgs1* x temperature-sensitive SGA results (see File S4).

**Table S31.**Complete *hrq1* *sgs1-K706A* x temperature-sensitive SGA results (see File S4).

**Table S32.**Complete *hrq1-K318A* *sgs1* x temperature-sensitive SGA results (see File S4).

**Table S33.**Complete *hrq1-K318A* *sgs1-K706A* x temperature-sensitive SGA results (see File S4).



**Figure S1. Confirmation of SGA hits.** The top-five negative and positive genetic interactors from all screens were confirmed by hand. Examples are shown of a strong negative genetic interaction (A), a strong positive genetic interaction (B), and a weaker positive genetic interaction (C, left) that was also confirmed by growth curve analysis (C, right). For the spot dilution assays, overnight cultures of the indicated strains were diluted to OD660 = 1, and serially diluted 10-fold to 10-4 before plating 5 L of each dilution on YPD. For growth curve analysis, overnight cultures were diluted to OD660 = 0.1 into fresh YPD in 96-well plates, and growth was monitored for 24 h in a plate reader that was heated to 30°C and vigorously shaking between measurements. The averages of three independent cultures are plotted; the error bars correspond to the standard deviation.

**Chart, scatter chart

Description automatically generated**

**Figure S2. Full SGA data comparisons between *hrq1* or *sgs1* screens.** All of the SGA scores from the *hrq1* and *hrq1-K318A* screens (A) and the *sgs1* and *sgs1-K706A* screens (B) were compared using scatter plots. Values falling along the blue diagonal were identical between screens. The black dashed lines represent the deviation in the trend of the data from the diagonal, and the R2 values are noted on the plots. Select genetic interactors that greatly deviated from the diagonal are noted.

**Chart, map, scatter chart

Description automatically generated**

**Figure S3. Pairwise comparisons of the full SGA data from all triple mutant screens.** The SGA scores from the indicated (A-F) were compared using scatter plots. Values falling along the blue diagonal were identical between screens. The black dashed lines represent the deviation in the trend of the data from the diagonal, and the R2 values are noted on the plots. Select genetic interactors that greatly deviated from the diagonal are noted.

**Chart, bubble chart

Description automatically generated**

**Figure S4. Comparison of *hrq1* and *sgs1* SGA screens.** Significant positive genetic interactors are compared between previously published data (Costanzo *et al.* 2010; Costanzo *et al.* 2016) in green and our current data in gold for *hrq1* (A) and *sgs1* (C) SGA screens. Similarly, significant negative genetic interactors are compared between the previously published data in red and our current data in blue for *hrq1* (B) and *sgs1* (D) SGA screens.

**References**

Costanzo, M., A. Baryshnikova, J. Bellay, Y. Kim, E. D. Spear *et al.*, 2010 The genetic landscape of a cell. Science 327**:** 425-431.

Costanzo, M., B. VanderSluis, E. N. Koch, A. Baryshnikova, C. Pons *et al.*, 2016 A global genetic interaction network maps a wiring diagram of cellular function. Science 353.