**SUPPLEMENTAL MATERIALS**

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

|  |  |  |
| --- | --- | --- |
| **Name** | **Sequence (5´-3´)** | **Purpose** |
| MB525 | AAAACAGAACCGTTATACATATTGAGATGGTTAAGGTCGTAGAAAAGAAATGTTCATTTGAGAAGGAAAACGGATCCCCGGGTTAATTAA | Deleting *HRQ1* with NatMX |
| MB526 | TCCACCAAGTGAATCTACAAGTAGTAGAATAGAGTATTTATATTCGGTTTACAAACTACAAATAGCGTGCGAATTCGAGCTCGTTTAAAC | Deleting *HRQ1* with NatMX |
| MB527 | GTGAATTGCTCAGAAGAGAAAGGCATACCGTC | PCR-amplifying *hrq1-K318A(NatMX)* and analyzing the *HRQ1* locus  |
| MB528 | CTGTGCATCAACAAGGTGACAGAATGTTGATG | PCR-amplifying *hrq1-K318A(NatMX)* and analyzing the *HRQ1* locus |
| MB932 | CCGGAAGTATATCAGGGTATGGAACACG | Sequencing *hrq1-K318A* |
| MB1028 | TAAGATTATTGACGTTAGAAGAGCTACGAAAGACGATACTCATACAAATGAAATCATTAAAAAAGAGATAGGGGGAGGCGGGGGTGGA | Tagging Hrq1 with 3xFLAG |
| MB1029 | TCCACCAAGTGAATCTACAAGTAGTAGAATAGAGTATTTATATTCGGTTTACAAACTACAAATAGCGTGCGAATTCGAGCTCGTTTAAAC | Tagging Hrq1 with 3xFLAG |

**Table S2.** Synthetic genetic interactions between the single-gene deletion collection and *hrq1* (see file S2).

**Table S3.** Synthetic genetic interactions between the temperature-sensitive allele collection and *hrq1* (see file S2).

**Table S4.** Synthetic genetic interactions between the single-gene deletion collection and *hrq1-K318A* (see file S2).

**Table S5.** Synthetic genetic interactions between the temperature-sensitive allele collection and *hrq1-K318A* (see file S2).

**Table S6.** Hrq1-interacting proteins (see file S3).

**Table S7.** Gene expression levels in *hrq1* and *hrq1-K318A* cells compared to wild-type (see file S4).



**Figure S1. The C-terminal 3xFLAG tag does not interfere with the role of Hrq1 in DNA inter-strand crosslink repair.** The indicated strains were grown overnight in YPD medium at 30°C with aeration, diluted to OD600 = 1 in sterile H2O, and then serially diluted 10-fold to 10-4. Five microliters of these dilutions were then spotted onto YPD agar plates and YPD agar plates supplemented with 50 or 100 g/mL diepoxybutane (DEB). The plates were incubated at 30°C for 2 days before capturing images with a flatbed scanner and scoring growth.