

**Table S3. Primers used in this study**

| Number | Name                                | Template plasmid   | Sequence (5'-3')  |
|--------|-------------------------------------|--|---|
| RE652  | OPI1_S114*_for                      | pMAL-OPI1  | ccaacaagagacagaagctgtaacgggcgattg   |
| RE653  | OPI1_S114*_rev                      |  | caatcgcccggttacagcttctgtctcttgttgg  |
| RE734  | OPI1_R180*_for                      | pMAL-OPI1  | ctttgcacaagcaagatggaaatgcttagacgaccactggagc   |
| RE735  | OPI1_R180*_rev                      |  | gctccagtggtcgtctaaagcatttccatcttgccttgcaag  |
| RE529  | OPI1_mGFP_cTerm<br>Hom_EcoRI_fwd    | pFA6a-mGFP-NatMX6  | gactaGAATTCgagacagattgaggtctttc   |
| RE530  | OPI1_mGFP_cTerm<br>Hom_SpeI_rev     |  | gactaACTAGTcttgcgtggactcctccatg   |
| RE528  | OPI1_mGFP_275up_<br>ATG_HindIII_fwd | pFA6a-mGFP-NatMX6-C-<br>term-homology  | gactaAAGCTTgacgcttacgcagacatctc   |
| RE440  | OPI1_mGFP_BamHI<br>_rev             |  | gactaGGATCCgtccttgctatccacggt   |
| RE548  | OPI1_cfor                           | yeast gDNA   | cctcaatgagagggattaataagagg  |
| RE916  | OPI1_crev                           |  | cgaagcatgatgcggcagt   |
| RE647  | OPI1_mRFP_fwd                       | pFA6a-mRFP-KanMX4  | GGATGGCAATTACGTAAGCCCTCTCAGGACAACGTGGATAGCAAG<br>GACGcctcctccgaggacgtcatc           |
| RE648  | OPI1_mRFP_rev                       |  | CGTATAATATTATTACTGGTGGTAATGCATGAAAGACCTCAATCTG<br>TCTCGatcgatgaattcgagctcgtttaaactg |
| RE649  | OPI1_mRFP_cfw                       | yeast gDNA   | caacttttgcagcagcaactcct   |
| RE646  | mRFP_rev_113bp                      |  | ggtctgggtgcctcgtagg   |
| RE589  | OPI1_Y127A_for                      | pMAL-OPI1 <sup>R180*</sup> / pFA6a-<br>promoter-OPI1-mGFP-<br>NatMX6-C-term-homology | ggaaggacaacttgaaagaggccaagctgaacatgtccatcg  |
| RE590  | OPI1_Y127A_rev                      |  | cgatggacatgttcagcttggcctctttcaagttgtccttcc  |
| RE465  | OPI1_Y127L_for                      | pMAL-OPI1 <sup>R180*</sup> / pFA6a-<br>promoter-OPI1-mGFP-<br>NatMX6-C-term-homology | ggaaggacaacttgaaagagttaaagctgaacatgtccatcgag  |
| RE466  | OPI1_Y127L_rev                      |  | ctcgatggacatgttcagctttaactctttcaagttgtccttcc  |
| RE545  | OPI1_Y127D_for                      | pMAL-OPI1 <sup>R180*</sup> / pFA6a-<br>promoter-OPI1-mGFP-<br>NatMX6-C-term-homology | gaaggacaacttgaaagaggacaagctgaacatgtccat   |
| RE546  | OPI1_Y127D_rev                      |  | atggacatgttcagcttgcctctttcaagttgtccttc  |
| RE776  | OPI1_Y127W_for                      | pMAL-OPI1 <sup>R180*</sup> / pFA6a-<br>promoter-OPI1-mGFP-<br>NatMX6-C-term-homology | gaaggacaacttgaaagagtggaaagctgaacatgtccatcga   |
| RE777  | OPI1_Y127W_rev                      |  | tcgatggacatgttcagcttccactctttcaagttgtccttc  |
| RE577  | OPI1_Y127R_for                      | pMAL-OPI1 <sup>R180*</sup> / pFA6a-<br>promoter-OPI1-mGFP-<br>NatMX6-C-term-homology | ggaaggacaacttgaaagagcgcaagctgaacatgtccatcg  |
| RE578  | OPI1_Y127R_rev                      |  | cgatggacatgttcagcttgcgctctttcaagttgtccttcc  |
| RE579  | OPI1_Y127K_for                      | pMAL-OPI1 <sup>R180*</sup> / pFA6a-<br>promoter-OPI1-mGFP-<br>NatMX6-C-term-homology | gaaggacaacttgaaagagagaagctgaacatgtccatcg  |
| RE580  | OPI1_Y127K_rev                      |  | cgatggacatgttcagcttcttctctttcaagttgtccttc   |

|       |                      |  |  |
|-------|----------------------|--|--|
| RE591 | OPI1_G120W_for       | pMAL-OPI1 <sup>R180*</sup> / pFA6a-promoter-OPI1-mGFP-NatMX6-C-term-homology                       | cgggcgattgccaag <u>t</u> ggaaggacaacttga                                 |
| RE592 | OPI1_G120W_rev       |  | tcaagttgtcctt <u>cc</u> acttggcaatcgccccg                                |
| RE595 | OPI1_ΔNLS_Q5_for     | pFA6a-promoter-OPI1-mGFP-NatMX6-C-term-homology  | caggcgCTGTTCGCGGGCGATTGCC  |
| RE596 | OPI1_ΔNLS_Q5_rev     |  | tgccgcGTTGGTGAAGAACTCATCGTCGTTG  |
| RE581 | OPI1_D203A_for       | pFA6a-promoter-OPI1-mGFP-NatMX6-C-term-homology  | cgacgatgaggagttttt <u>g</u> ctgcctcagagcagg                              |
| RE582 | OPI1_D203A_rev       |  | cctgctctgaggcag <u>ca</u> aaaaactcctcatcgctcg                            |
| RE835 | OPI1_2xAH_Q5_for     | pMAL-OPI1 <sup>R180*</sup>   | ttgaaagagtacaagctgaacatgtccATCGAGTCTAAGAAGAGGC                           |
| RE836 | OPI1_2xAH_Q5_rev     |  | gttgtccttccccttggcaatcgccccGGACATGTTTCAGCTTGATAC                         |
| RE949 | OPI1_3K3R_Q5_for     | pMAL-OPI1 <sup>R180*</sup> / pFA6a-promoter-OPI1-mGFP-NatMX6-C-term-homology                       | caagttgtccctCCCCTTGGCAATCGCCCCG  |
| RE950 | OPI1_3K3R_Q5_rev     |  | agagagtacaggCTGAACATGTCCATCGAGTCTAAGAAGAG                                |
| RE957 | OPI1_2K2R_Q5_2for    | pMAL-OPI1 <sup>3K3R,R180*</sup> / pFA6a-promoter-OPI1 <sup>3K3R</sup> -mGFP-NatMX6-C-term-homology | ccgcgacagcctCTGTCTCTTGGTGGTGAAG  |
| RE958 | OPI1_2K2R_Q5_2rev    |  | gcgattgccaggGGGAGGGACAACCTTGAGAG   |
| RE804 | OPI1_D122K_E126K_for | pMAL-OPI1 <sup>R180*</sup> / pFA6a-promoter-OPI1-mGFP-NatMX6-C-term-homology                       | gcgattgccaaggggaaga <u>aga</u> acttgaaaa <u>agt</u> tacaagctgaaca<br>tgt |
| RE805 | OPI1_D122K_E126K_rev |  | acatgttcagcttgtactttttcaagtt <u>ctt</u> cttccccttggcaat<br>cgc           |

Underlined sequences indicate the sites of point mutation. Capitalized sequences indicate either restriction sites or sequences used for homologous recombination.