

Supplemental Information

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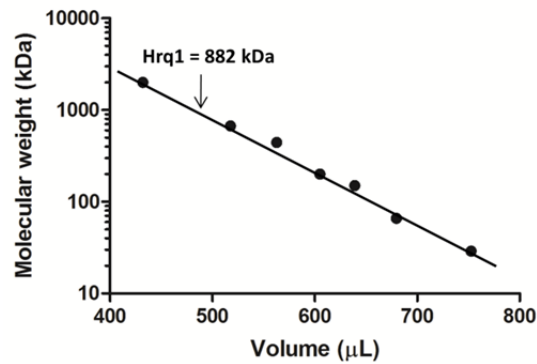


Figure S1: Hrq1 exists as a large oligomer in solution, related to Figure 1. Gel filtration analysis of purified Hrq1. The apparent molecular weight of the slower migrating oligomeric form of Hrq1 from Figure 1H is indicated and is based on a standard curve derived from the peak fractions of blue dextran, thyroglobulin, apoferritin, β -amylase, alcohol dehydrogenase, albumin, and carbonic anhydrase (plotted points from left to right) obtained during column calibration.

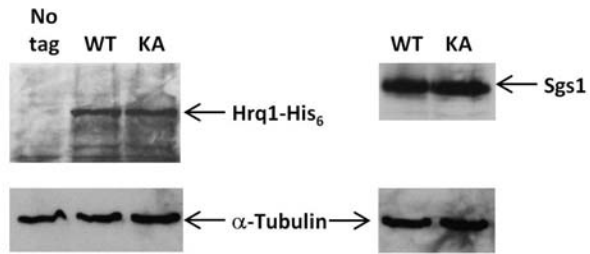


Figure S2: Relative expression levels of Hrql-KA and Sgs1-KA, related to Figures 2-5.

Western blot analysis of Hrql (WT), Hrql-KA (KA) (both left), Sgs1 (WT), and Sgs1-KA (KA) (both right) protein levels. Tubulin levels served as a loading control. All four panels are from the same gel and blotted to the same membrane but were separated for probing with the indicated antibodies. The results shown are representative of three independent experiments.

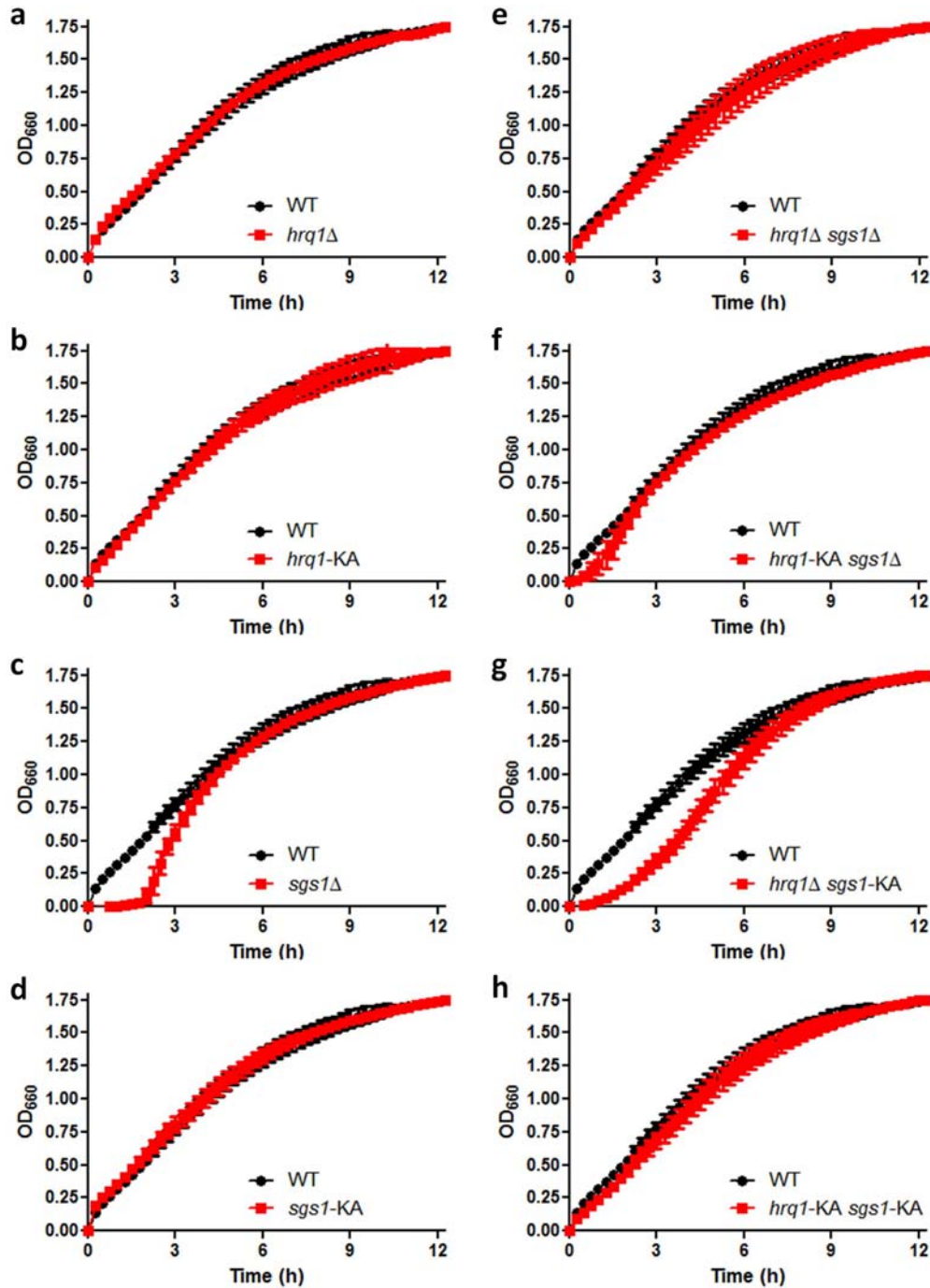


Figure S3: Growth of *hrq1* and *sgs1* mutant cells relative to wild type, related to Figure 2.

Growth curves of the indicated strains. Cells were grown overnight in rich media at 30°C, diluted to OD₆₆₀ = 0.1 in a final volume of 150 μL of rich media in a 96-well plate, and incubated at 30°C in a BioTek EON plate reader with shaking. The OD₆₆₀ was then measured every 15 min

for 12 h. The plotted values are the means of ≥ 3 independent experiments per strain, and the error bars correspond to the standard deviation.

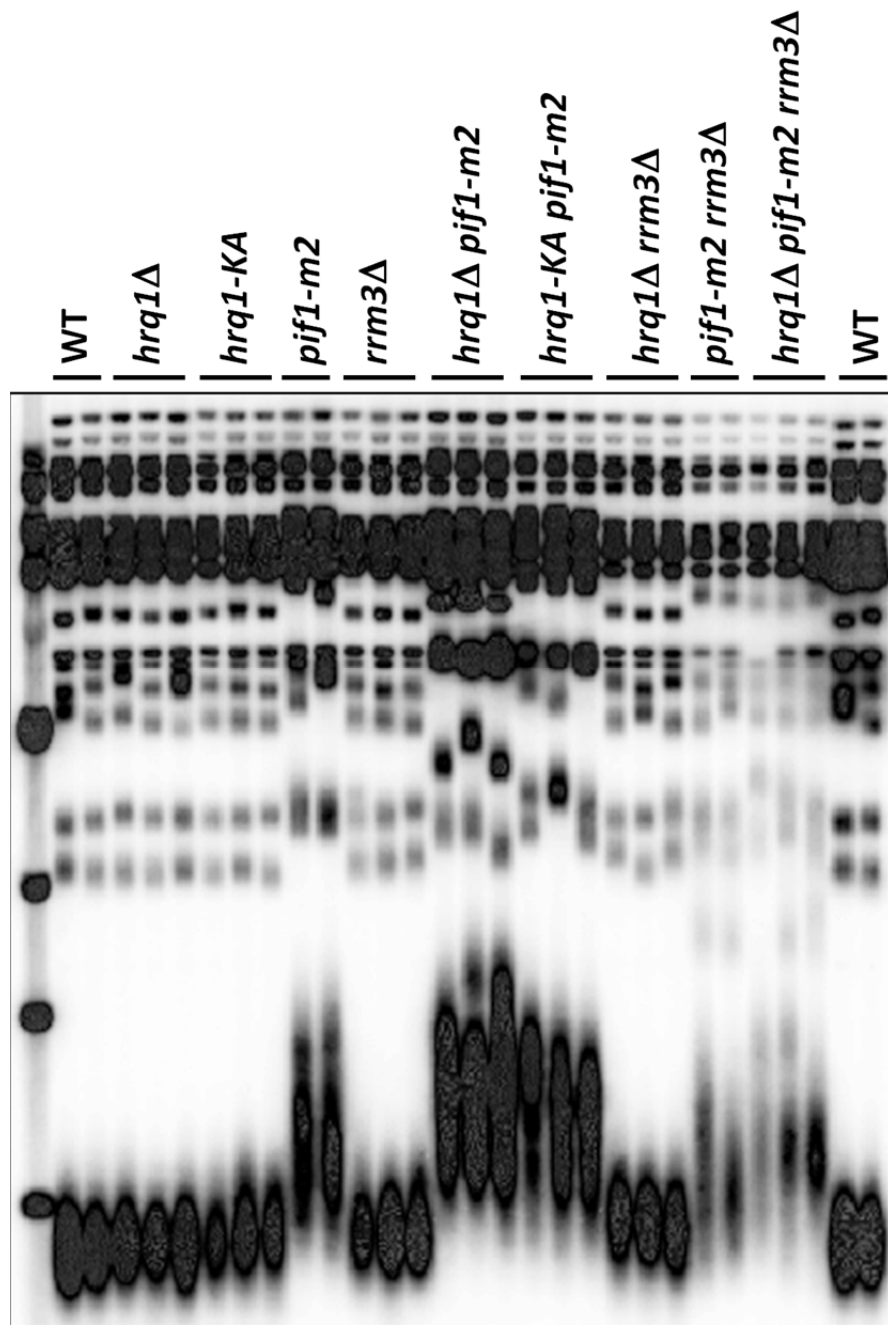


Figure S4: Telomere lengths in *HRQ1*, *PIF1*, and *RRM3* mutant strains, related to Figure 4.

The portions of the left two Southern blots shown in Figure 4A were derived from the full blot shown here.

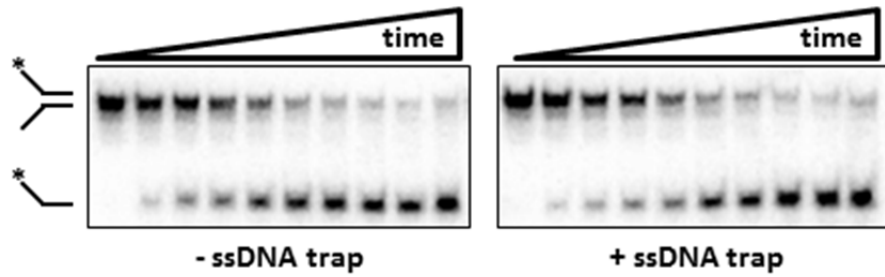


Figure S5: The *in vitro* DNA unwinding activity of Hrql is unaffected by the absence or presence of a ssDNA trap, related to Figures 1 and 5. Identical helicase reactions were performed using 100 nM Hrql as described in the Experimental Procedures, except those in the left panel lacked the ssDNA trap necessary to observe hRecQ4 helicase activity.