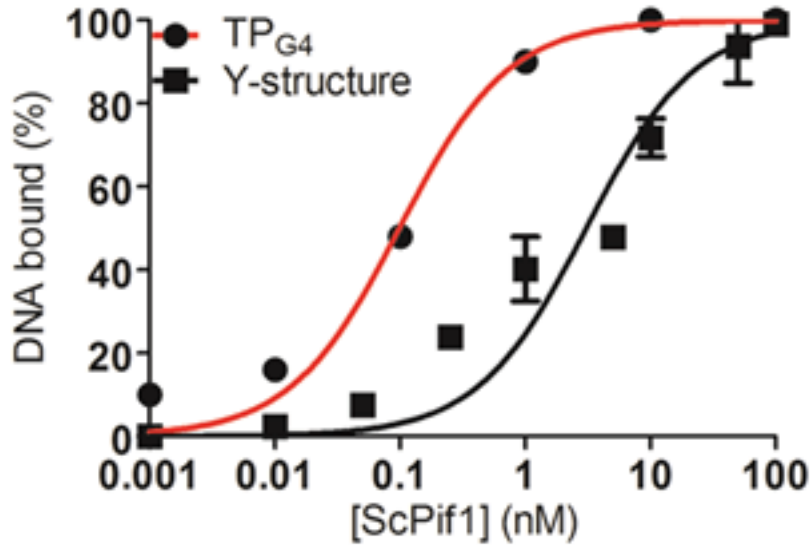


## Supplementary Information

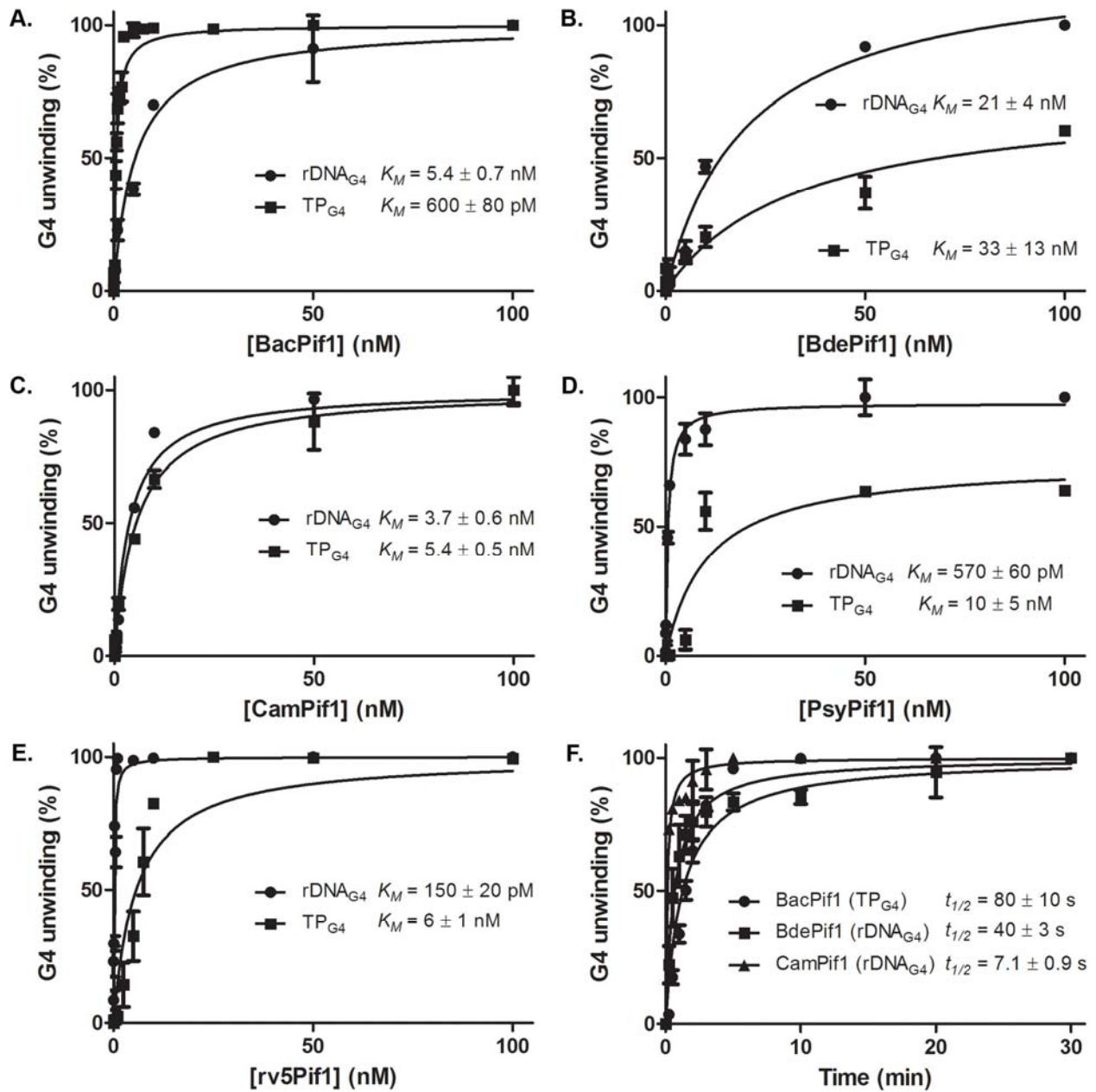
### CGE analysis

In the standard GCR assay, expression of both *CAN1* and *URA3* must be lost to generate clones that will grow on plates containing 5-FOA and canavanine (FOA+Can). After standard GCR events, these genes are not expressed because they are physically lost by *de novo* telomere addition or non-reciprocal translocation or large deletion. However, PCR and Southern analysis demonstrated that telomere addition was not common, and *URA3* and *CAN1* were present at their original locations in many *pif1-m2* + G4 and *pif1-m2 rrm3Δ* + G4 (and some *pif1-m2 rrm3Δ*) clones. To determine if genes were mutated, the genes and ~200 bp of their up- and downstream flanking sequences were cloned and sequenced. Similar analyses of 11 independent *sgs1Δ* + G4 GCR clones showed that *CAN1* and *URA3* were missing (data not shown), suggesting that the *pif1-m2* + G4, *pif1-m2 rrm3Δ* + G4, and *pif1-m2 rrm3Δ* silencing of *CAN1* and *URA3* is enhanced by lack of nuclear Pif1 function and a nearby G4 motif. To investigate this phenomenon in more detail, we tested all of the markers (*URA3*, *CAN1*, and *LEU2*, which marks the G4 motif) in the parental GCR strains and found that the WT, *pif1-m2*, *sgs1Δ*, and *pif1-m2 rrm3Δ* strains all grew on –Ura medium (and those with the G4 insert grew on –Leu medium) but not on media containing FOA, Can, or both (data not shown). Thus, before the GCR assay, all of the markers functioned as expected. Similar plating assays with the post-GCR *pif1-m2* + G4, *pif1-m2 rrm3Δ* + G4, and *pif1-m2 rrm3Δ* clones demonstrated that they grew on FOA, Can, and FOA+Can plates, as well as on –Ura (data not shown), despite the presence of WT *URA3* and *CAN1*. Together, the above data suggest that either transcription or translation of *URA3* is silenced, and this silencing is

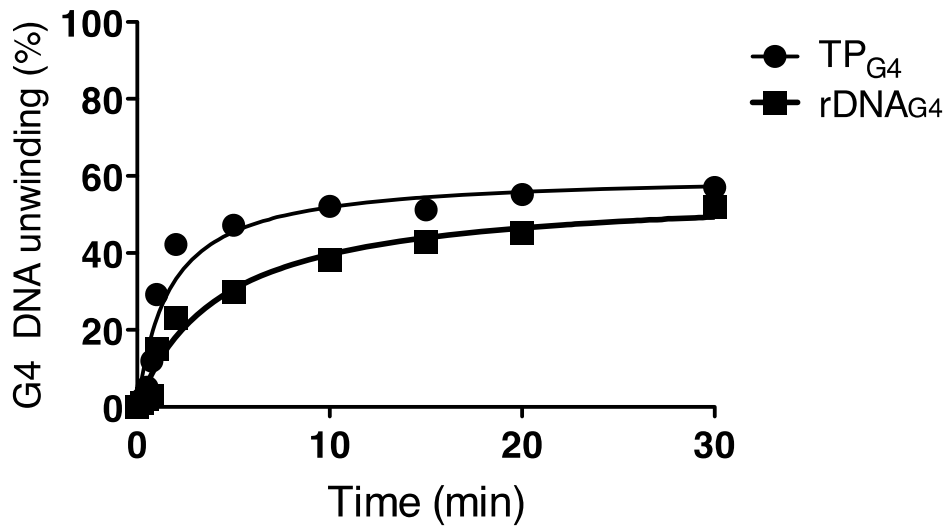
somehow related to lack of nuclear Pif1 and the presence of the G4 insert. As a control for this hypothesis, we characterized the rare GCR events that occur in the *pif1-m2* “no insert” strain that were not due to telomere addition and found that the *CAN1* and *URA3* markers were missing, not silenced.



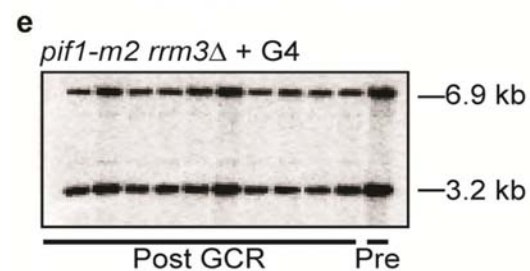
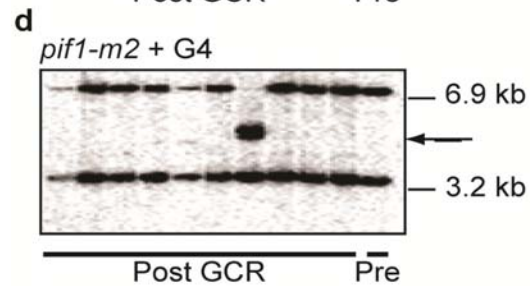
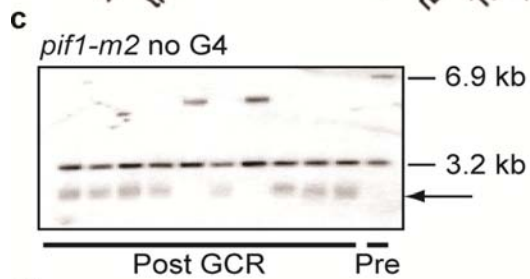
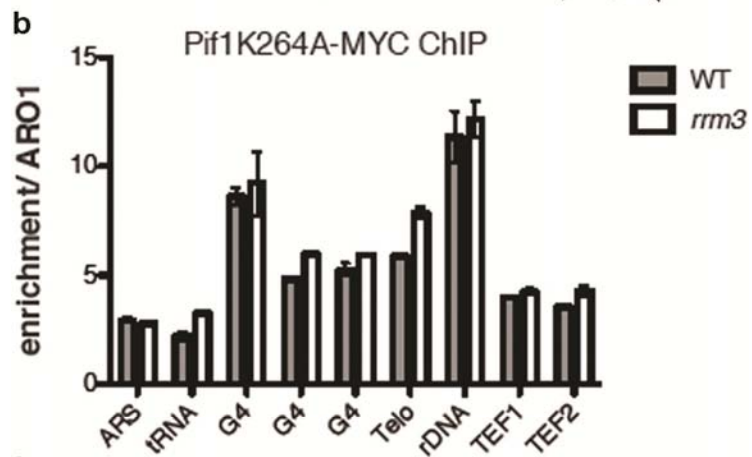
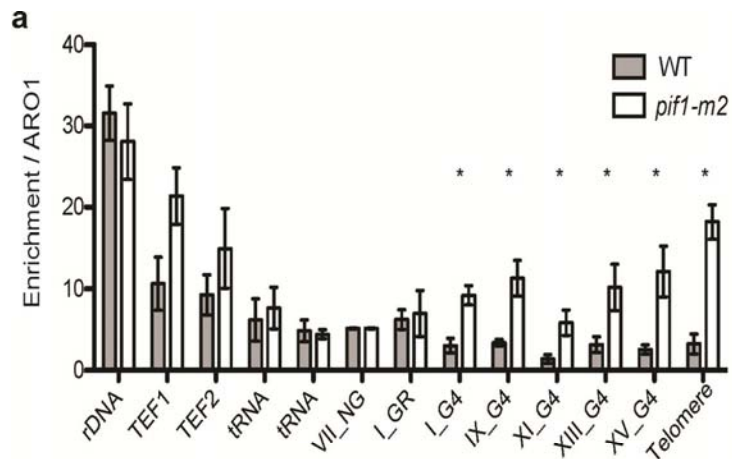
**Supplementary Figure 1.** Binding of the TP<sub>G4</sub> and Y-structure substrates (0.1 nM) as a function of [ScPif1]. Error bars here and in all subsequent figures correspond to one standard deviation of the mean from  $\geq 3$  independent experiments.



**Supplementary Figure 2.** Prokaryotic Pif1 G4 DNA helicase assays. Unwinding of the rDNA<sub>G4</sub> and TP<sub>G4</sub> substrates (0.1 nM) as a function of the concentration of (a) BacPif1, (b) BdePif1, (c) CamPif1, (d) PsyPif1, and (e) rv5Pif1. The apparent  $K_M$ s represent the midpoints of the protein titration curves. (f) Time course analyses of G4 DNA unwinding by the BacPif1, BdePif1, and CamPif1 helicases. The rates of BacPif1, BdePif1, and CamPif1 G4 unwinding are 38, 75, and 420 pM G4/min.



**Supplementary Figure 3.** hWRN G4 DNA helicase assays. Time course of the unwinding of the TP<sub>G4</sub> and rDNA<sub>G4</sub> substrates (100 pM) by 100 nM hWRN at 37°C. There are no error bars because the data are the average of two independent experiments.



**Supplementary Figure 4.** Increased Rrm3 binding to G4 motifs in the absence of ScPif1 and types of GCR events. **(a)** Rrm3-Myc binding to five G4 motifs and eight non-G4 motifs in WT (gray) and *pif1-m2* (white) cells. Binding was normalized to input DNA and *ARO1*. \* indicates a statistically significant difference ( $p < 0.0014$ ) in Rrm3 binding in *pif1-m2* vs. WT cells as calculated by the Student's *t*-test. **(b)** ChIP-qPCR analysis of Pif1-K264A binding in WT (grey bars) and *rrm3Δ* (white bars) cells. Pif1-K264A was C-terminally tagged at its endogenous locus with 13 Myc epitopes as described<sup>19</sup>. Pif1-K264A binding was measured at three candidate G4 motifs, two non-G4 Pif1 binding sites (rDNA; and telomere VI-R, Telo), and four Rrm3-dependent sites (*tRNA*, *ARS*, *TEF1*, and *TEF2*) (Rrm3 dependent sites are places where replication pauses in the absence of Rrm3<sup>18</sup>). Values were normalized to both input and the amount of *ARO1* DNA in the immunoprecipitate and are not significantly different by the Student's *t*-test. **(c-e)** Southern blot analyses of *AlwNI*-digested DNA from independent GCR clones from **(c)** *pif1-m2*, **(d)** *pif1-m2*+G4, and **(e)** *pif1-m2 rrm3Δ*+G4 cells. Blots were digested with *AlwNI* and probed with *CIN8* (primer pair 3 from A). In d, e, and f, 8/10, 1/10, and 0/10 clones display apparent telomere additions ("fuzzy" bands, arrow). See Supplementary Information for an explanation of the banding pattern. The banding pattern for the *pif1-m2 rrm3Δ* clones containing no G4 insert looks similar to f (data not shown), but the silencing phenotype of the cells is not the same. The lack of telomere addition in the *pif1-m2 rrm3Δ* strains with or without a G4 insert is consistent with the previous finding that *rrm3Δ* suppresses *pif1* telomere addition<sup>26</sup>.

Chr X G4 TCTAGA...ATAATGGGTCCTCCAAGCGGTAAAACCTTACATGGG---ATGGTGGGGTCACATGGGTGGTC...GGATCC original sequence

*sgs1* TCTAGA...ATAATGGGTCCTCCAAGCGGTAAAACCTTACATGGG---ATGGTGGGGTCACATGGGTGGTC...GGATCC no change

*pif1-m2* GGATCC...A-----C-T--A-G---T-----...TCTAGA deletion/flipped  
TCTAGA...-----GCGTTGTAAATGTTGGTACCCAAACCA---ATTGTC-----TACAAGTT-TC...GGATCC recombined with *PMA1*  
TCTAGA...A-A-T-----A-G-GG-----G-----T-T----C-C-G---AA...GGATCC deletion  
TCTAGA...AGTTGGGGTTCTGATAACAATCACAAATTCGTCGG---TTGGTTGACCGTTGGTACCGGTG...GGATCC insertion  
TCTAGA...AGTAGGGGTTCCGAAAGATCCGGTCATTCGTCGG---TNGGTTGAACGATGGTACCGGTG...GGATCC insertion  
TCTAGA...GGTAGGAGTC-TCC---C-TA----T-CA-G-----T-----AC----- deletion

*rrm3* TCTAGA...ATAATGGGTCCTCCAAGCGGTAAAACCTTACATGGG---ATGGTGGGGTCACATGGGTGGTC...GGATCC no change  
TCTAGA...GTAATGGACCC-----ATGTGTCTAGG---A-----GTT-----TC...GGATCC deletion/mutation  
TCTAGA...AGTTGGAGTCCCCCAGCGGTAAAAGGGTTCCGA---AGGTCGGGGTCACTTGGGTGGTC...GGATCC mutation/insertion

*pif1-m2 rrm3* GGATCC...GTAT-----CGATAAGCTTGATATCGA---AT-----TC...TCTAGA deletion/flipped?  
TCTAGA...NGTTGTAGTCCTCCCATCAGTAAAAGGGTTCCGA---AGGCNGGGGTCATTTGGGTGGTC...GGATCC insertion  
TCTAGA...AGATGGAGTCCTCCCTTCGGTAAAAGGGTTTGGA---AGGGTGGGGTCACTTGGGTGGTC...GGATCC mutation  
TCTAGA...ATAATGGGTCCTCCAAGCGGTAAAACCTTACATGGG---ATGGTGGGGTCACATGGGTGGTC...GGATCC mutation  
GGATCC...A-----C-T--A-G---T-----...TCTAGA deletion/flipped?

Chr I G4 TCTAGA...GGAATCCCAACAATTATCTCAAAATTCCCCCAATTCTCATCA--GTAACACCCCCCCCGTATT...GGATCC original sequence

*sgs1* TCTAGA...GGAATCCCAACAATTATCTCAAAATTCCCCCAATTCTCATCA--GTAACACCCCCCCCGTATT...GGATCC no change

*pif1-m2* TCTAGA...GTGAGCGGGGATAACAATTTACACAGGAAACAGCTATGACCATGATTACGNCAAGCTCGAAATT...GGATCC mutation/insertion  
TCTAGA...GGTACCCAAAC-----CCAATTGTCTACAAGTTTCTTAA--GCAATACC-----...GGATCC recombined with *PMA1*  
TCTAGA...GGTA---AAGAA-----CCAAATTGTC-----TACT...GGATCC deletions/mutations  
TCTAGA...GGAACCCCAG-----TCCATGGTT-----CAGG...GGATCC deletions/mutations  
TCTAGA...ACCAGTCCAA-----GTTTCTC-----TGTG...GGATCC deletions/mutations  
TCTAGA...ACNAGGCCAT-----GTTTCTC-----TGTG...GGATCC deletions/mutations

*rrm3* TCTAGA...GGTACCCAAAC-----CCAATTGTCTACAAGTTTCTTAA--GCAATACC-----...GGATCC recombined with *PMA1*  
TCTAGA...GGTACCCAAAC-----CCAATTGTCTACAAGTTTCTTAA--GCAATACC-----...GGATCC recombined with *PMA1*

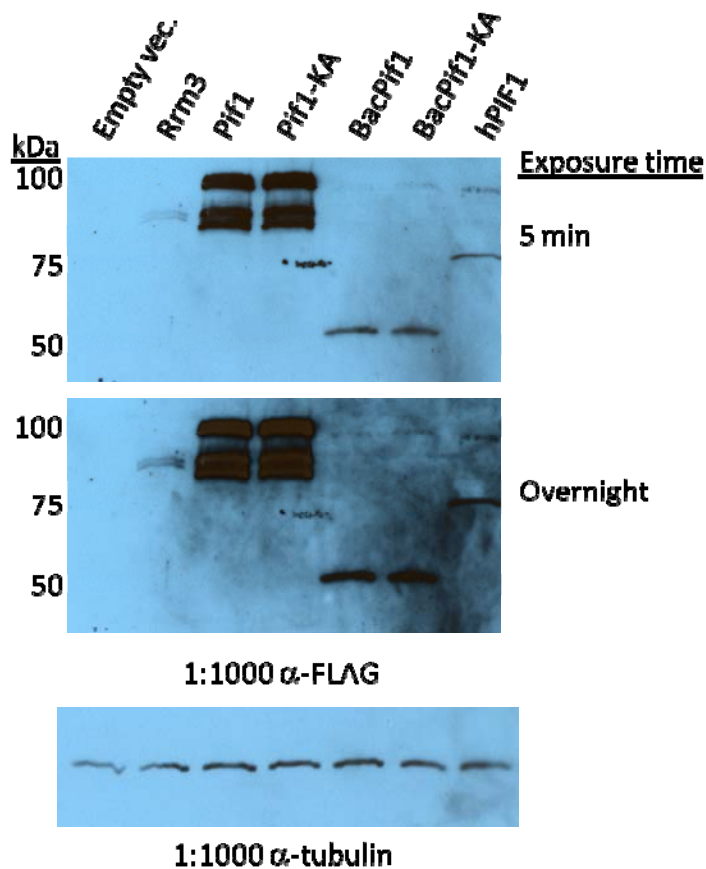
*pif1-m2 rrm3* TCTAGA...GGA---TAACAATTTACACAGGAAACAGCTATGACCATGA--TTA-CGCCAAGCTCGAAATT...GGATCC mutations/deletions  
TCTAGA...GGTTCTTGTGGGNTGGAGTCCCCCTTCGGTAGTAGGGTTCGGAAGGGAGGGGTCATTTCGG...GGATCC insertion  
TCTAGA...GGACTTTTGGGATAATGGGTCTCCATCCGGTAAAACAAACA--TGAAGGGAGGGGCCA----GGATCC mutation  
----- large deletion

**Supplementary Figure 5.** Sequences of G4 inserts in representative GCR clones. ClustalW alignment of the G4 insert sequences after

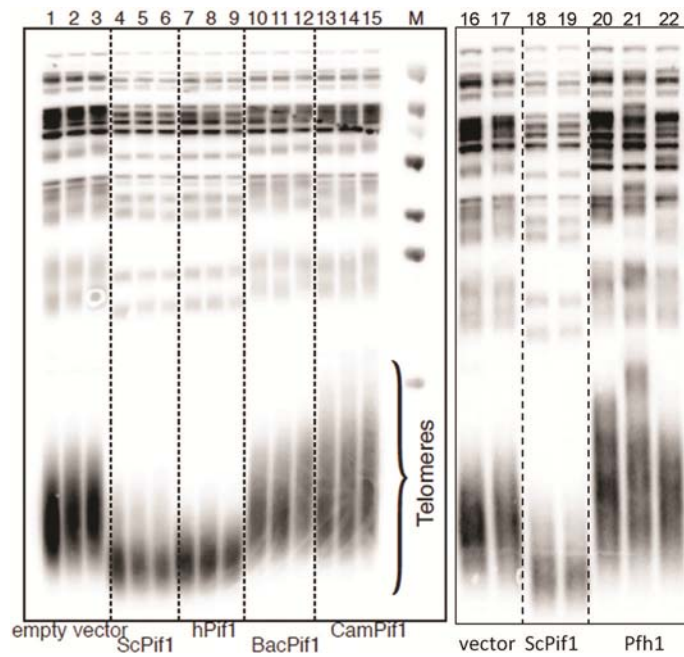
GCR assays in the strains indicated on the left relative to the original Chr I<sub>G4</sub> and Chr X<sub>G4</sub> sequences (underlined on left). The *Xba*I



and *Bam*HI sites used to clone the inserts into pRS415 (Supplementary Table 8) are highlighted in yellow and red, respectively; this ~200 bp region is within the ~1 kb region that was amplified by PCR prior to sequencing. The ellipses represent endogenous flanking sequences that were PCR amplified with the G4 motifs from *S. cerevisiae* genomic DNA, which was omitted to simply presentation of the data. The types of post-GCR alterations noted in the G4 motifs extended into these flanking sequences. The G4 motif begins and ends at the first and last underlined residues, respectively, in the Chr I<sub>G4</sub> and Chr X<sub>G4</sub> sequences. In the original motifs, the underlined residues are predicted to participate in G-G base pairing to form the G4 structure; the Chr I<sub>G4</sub> forms on the complementary strand. In the mutated sequences, the underlined residues are no longer predicted to form G4 structures. The type of event that occurred at the G4 motif (*e.g.*, deletion or mutation) is indicated on the right. In the GCR clones, dashes represent deleted residues. In the original Chr I<sub>G4</sub> and Chr X<sub>G4</sub> sequences, dashes represent sites of insertions in one or more GCR clones.



**Supplementary Figure 6.** Helicase expression in the *pif1-m2 rrm3Δ* + X<sub>G4</sub> strains. The various helicases were C-terminally tagged with 3xFLAG and expressed from a *TRP1*-marked *CEN* vector under the control of the *RRM3* promoter. Whole cell extracts were prepared, proteins were separated on an 8% polyacrylamide gel, transferred to nitrocellulose, and probed with an anti-FLAG antibody. The blot was then stripped and re-probed with an anti-tubulin antibody as a loading control. The expected molecular weights of the recombinant proteins are indicated. Three bands appear in the ScPif1 and ScPif1-KA lanes; the two lower molecular weight bands are likely degradation products from proteolysis of the N-terminus. Expected molecular weights: Rrm3, 84 kDa; Pif1 and Pif1-KA, 100 kDa; BacPif1 and BacPif1-KA, 52 kDa; and hPIF1, 75 kDa.



**Supplementary Figure 7.** Telomere length in *pif1-m2* cells heterologously expressing Pif1 family helicases. Full blot image of Southern blots shown in Figure 5c.

**Table S1.** Helicases tested for G4 DNA binding and unwinding.

Enzyme	Species	Type (polarity)	Protein length	G4 binding	G4 unwinding	Reference(s)
ATRX	Human	DNA (N.D.)	Full length	Yes	N.D. <sup>1</sup>	21
BLM	Human	DNA (3'-5')	Full length	Yes (2.8) <sup>2</sup>	Yes	22-25
ChlR1/DDX11	Human	DNA (5'-3')	Full length	Yes (~2-8)	Yes <sup>3</sup>	26
DHX9/NDH II/RHA	Human	RNA (3'-5')	Full length	N.D.	Yes	27
DnaB	<i>E. coli</i>	DNA (5'-3')	Full length	N.D.	Yes	M. Bochman, unpublished results
Dna2	<i>S. cerevisiae</i>	DNA (5'-3')	Not described	Yes	Yes	28
hDna2	Human	DNA (5'-3')	Not described	Yes (~6)	Yes	28
DNA helicase IV/nucleolin	Human	Both (5'-3')	284-709/710 <sup>4</sup>	Yes ( $\geq 79$ )	N.D. <sup>5</sup>	29
FANCI	Human	DNA (5'-3')	Full length	N.D.	Yes (~1-2)	30
gp41	phage T4	DNA (5'-3')	Full length	N.D.	Yes	M. Bochman, unpublished results

<sup>1</sup> N.D. = not determined.

<sup>2</sup> The numbers in parentheses indicate the  $K_d$  (G4 binding) or  $K_M$  (G4 unwinding) in nM.

<sup>3</sup> Preferentially unwinds two-stranded antiparallel G2' G4 substrate relative to four-stranded parallel G4 substrate.

<sup>4</sup> Indicates the amino acids comprising the truncated polypeptide/total amino acids in the full-length protein.

<sup>5</sup> Hypothesized to induce G4 formation.

LTA <sub>g</sub>	SV40	DNA (3'-5')	Full length	N.D.	Yes	31, 32
Pif1	<i>S. cerevisiae</i>	DNA (5'-3')	Full length <sup>6</sup>	Yes (~0.07-0.14)	Yes (~0.01)	<sup>33</sup> Current manuscript
hPIF1	Human	DNA (5'-3')	Full length <sup>6</sup>	Yes	Yes	34
RecBCD	<i>E. coli</i>	DNA (bi-polar)	Full length	N.D.	No <sup>7</sup>	23
RecQ	<i>E. coli</i>	DNA (3'-5')	Full length	N.D.	Yes	35
RecQ1	Human	DNA (3'-5')	57-649/649	Yes (1.8)	No <sup>8</sup>	24
RHAU/DHX36/ G4R1	Human	RNA	Full length	Yes (low pM to nM range)	Yes	36, 37
RHAU/CG9323	<i>Drosophila melanogaster</i>	RNA	Full length	Yes	Yes	37
SARS cov helicase	coronavirus	DNA	Full length	Yes	Yes	38
Sgs1	<i>S. cerevisiae</i>	DNA (3'-5')	Full length	Yes <sup>9</sup>	Yes <sup>10</sup>	Current manuscript
Sgs1 truncation	<i>S. cerevisiae</i>	DNA (3'-5')	400-1268/1447	Yes (5)	Yes	25

<sup>6</sup> Nuclear isoform.

<sup>7</sup> Only tested on one G4 substrate (TP) but unwound forked and partial duplex DNAs under same reaction conditions.

<sup>8</sup> Inactive on G2' and parallel G4 substrates but active on forked and Holliday junction substrates.

<sup>9</sup> Binding is poor to three tested G4 substrates, precluding the accurate determination of a dissociation constant.

<sup>10</sup> G4 DNA unwinding is poor relative to unwinding of a forked DNA substrate.

Srs2	<i>S. cerevisiae</i>	DNA (3'-5')	1-898/1174	N.D.	Yes	M. Bochman, unpublished results
WRN	Human	DNA (3'-5')	Full length	N.D.	Yes	22, 27, 39

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**Table S2.** Sequences used in DNA binding and helicases assays.

<b>G4 motif</b>	<b><i>S. cerevisiae</i> chromosome</b>	<b>Sequence (5'-3')</b>
Poly(dA)	N/A	AAAAAAAAAAAAAAAAAAAAA
Poly(dC)	N/A	CCCCCCCCCCCCCCCCCC
Poly(dG)	N/A	GGGGGGGGGGGGGGGGGG
Poly(dT)	N/A	TTTTTTTTTTTTTTTTTTTT
G-rich	N/A	CGGCCCGGCGGTCCGCGGTC
Non G-rich	N/A	CAAAGGACCCTTTGTGGATC
rDNA <sub>G4</sub>	XII	GGGTAACGGGGAATAAGGGTTCGATTCCGGAGAGGG
TP <sub>G4</sub>	N/A	GGGGGAGCTGGGGTAGATGGGAATGTGAGGG
Chr IV <sub>G4</sub>	IV	GGGGAGGGGAAGGGGAGGGG
Chr IX <sub>G4</sub>	IX	GGGTACGGTGGGTAATAAGGGAAGGTATCGGG
Chr X <sub>G4</sub>	X	GGGTCCTCCAAGCGGTAAACTTACATGGGATGGTGGGGTCACA
Chr XI <sub>G4</sub>	XI	TGGG
Chr XII <sub>G4</sub>	XII	GGGCACGGGCACTCAATGGACGGGGTATCCACCCAGCTTGGAAA GGGG
Y-structure	N/A	GGGGTGGTGCTTCAGCCTGGGGTAACAAATCAAGTTGGGGCGGT GCATCCACTTGGGCGTCGGG Top = TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTGACGCTGCCGAATTCTG GCTTGCT Bottom = TGAGTGAGCAAGCCAGAATTCGGCAGCGTCTTTTTTTTTTTTTTTTT TTTTTTTTTTTTTTTT

Tested G4 motifs are from the *S. cerevisiae* genome<sup>10,19</sup> except G4-TP. G4-TP is a well-characterized four-stranded parallel G4 DNA substrate derived from a mouse immunoglobulin locus<sup>23</sup>. All G4 substrates contained either a 5' or 3' 10-nt poly(dA) tail (not shown) for ScPif1 or Sgs1 experiments, respectively. The Y-structure substrate was prepared by annealing the top and bottom oligonucleotides listed above and filling in the top strand with  $\alpha^{32}\text{P}$ -dCTP and unlabeled dATP, dGTP, and dTTP using Klenow Fragment (3'→5' exo<sup>-</sup>) (NEB).

**Table S3.** Sequences used in GCR assays.

<b>G4 motif</b>	<b><i>S. cerevisiae</i> chromosome</b>	<b>Sequence (5'-3')</b>
I <sub>G4</sub>	I	CCCAACAATTATCTCAAAATTCCCCAATTCTCATCAGTAACACC CCACCCC
X <sub>G4</sub>	X	GGTGGGTAATAAGGGAAGGTATCGGGATTGGGG
NG	VII	CTAATCTTTCAGCGTTGTAAATGTTGGTACCCAAACCCAATTGTC TACAAGTTTCCTTAGC
GR	I	ATGGTGGTCATCTCAGTAGATGTAGAGGTGAAAGTACCGGTCCA TGGCTCGGT



**Table S4.** Yeast strains

Strain	Genotype	Reference or source
YPH500	<i>MAT<math>\alpha</math> ura3-52 lys2-801 ade2-101 trp1<math>\Delta</math>63 his3<math>\Delta</math>200 leu2<math>\Delta</math>1</i>	<sup>1</sup>
YPH499	<i>MAT<math>\alpha</math> ura3-52 lys2-801 ade2-101 trp1<math>\Delta</math>63 his3<math>\Delta</math>200 leu2<math>\Delta</math>1</i>	<sup>1</sup>
KP359	<i>MAT<math>\alpha</math> ura3-52 lys2-801 ade2-101 trp1<math>\Delta</math>63 his3<math>\Delta</math>200 leu2<math>\Delta</math>1</i> <i>Rrm3-MYC13::TRP1 pif1-m2</i>	This study
KP349	<i>MAT<math>\alpha</math> ura3-52 lys2-801 ade2-101 trp1<math>\Delta</math>63 his3<math>\Delta</math>200 leu2<math>\Delta</math>1</i> <i>Rrm3-MYC13::HIS3</i>	This study
KP448	<i>MAT<math>\alpha</math>/<math>\alpha</math> ura3-52 lys2-801 ade2-101 trp1<math>\Delta</math>63 his3<math>\Delta</math>200 leu2<math>\Delta</math>1</i> <i>PIF1/pif1-m2</i>	This study

**Table S5.** *Saccharomyces cerevisiae* strains used for gross-chromosomal rearrangement assays.

<b>Strain</b>	<b>Gene(s) of interest</b>	<b>Insert</b>	<b>Plasmid</b>
MBY49	WT	No insert	-
KP296	WT	G4-I	-
KP327	WT	G4-X	-
KP298	WT	G-rich	-
KP299	WT	Non-G-rich	-
KP313	<i>pif1-m2</i>	No insert	-
KP323	<i>pif1-m2</i>	G4-I	-
KP322	<i>pif1-m2</i>	G4-X	-
KP330	<i>pif1-m2</i>	G-rich	-
KP329	<i>pif1-m2</i>	Non-G-rich	-
MBY79	<i>rrm3</i> Δ	No insert	-
KP304	<i>rrm3</i> Δ	G4-I	-
KP328	<i>rrm3</i> Δ	G4-X	-
KP332	<i>rrm3</i> Δ	G-rich	-
KP331	<i>rrm3</i> Δ	Non-G-rich	-
MBY132	<i>sgs1</i> Δ	No insert	-
KP314	<i>sgs1</i> Δ	G4-I	-
KP324	<i>sgs1</i> Δ	G4-X	-
KP311	<i>pif1-m2 rrm3</i> Δ	No insert	-
KP325	<i>pif1-m2 rrm3</i> Δ	G4-I	-
KP326	<i>pif1-m2 rrm3</i> Δ	G4-X	-
KP334	<i>pif1-m2 rrm3</i> Δ	G-rich	-

KP333	<i>pif1-m2 rrm3Δ</i>	Non-G-rich	-
KP351	<i>pif1-m2 sgs1Δ</i>	No insert	-
KP352	<i>pif1-m2 sgs1Δ</i>	G4-I	-
KP353	<i>pif1-m2 sgs1Δ</i>	G4-X	-
MBY225	<i>pif1-m2 rrm3Δ</i>	G4-X	pMB13 (empty vector)
MBY222	<i>pif1-m2 rrm3Δ</i>	G4-X	pMB282 (ScPif1)
MBY223	<i>pif1-m2 rrm3Δ</i>	G4-X	pMB283 (ScPif1-KA)
MBY224	<i>pif1-m2 rrm3Δ</i>	G4-X	pMB274 (Rrm3)
MBY194	<i>pif1-m2 rrm3Δ</i>	G4-X	pMB272 (Pfh1)
MBY233	<i>pif1-m2 rrm3Δ</i>	G4-X	pMB292 (hPIF1)
MBY213	<i>pif1-m2 rrm3Δ</i>	G4-X	pMB270 (BacPif1)
MBY217	<i>pif1-m2 rrm3Δ</i>	G4-X	pMB288 (BacPif1-KA)
MBY252	<i>pif1-m2 rrm3Δ</i>	G4-X	pMB267 (BdePif1)
MBY250	<i>pif1-m2 rrm3Δ</i>	G4-X	pMB289 (BifPif1)
MBY209	<i>pif1-m2 rrm3Δ</i>	G4-X	pMB303 (CamPif1)
MBY251	<i>pif1-m2 rrm3Δ</i>	G4-X	pMB304 (V99B1Pif1)
MBY221	<i>pif1-m2 rrm3Δ</i>	G4-X	pMB305 (PsyPif1)

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\*All tested inserts (~200-250 bp) were identified as regions with strong Pif1 binding<sup>19</sup>. G4-I, G4 motif from chromosome I; G4-X, G4 motif from chromosome X; G-rich, G-rich sequence from chromosome I; Non-G-rich, non-G-rich sequence from chromosome VII.

**Table S6.** Oligonucleotides used for cloning.

## A) Oligonucleotides used for gross-chromosomal rearrangement strain construction.

<b>Modification</b>	<b>Oligo</b>	<b>Sequence (5'-3')</b>
<i>hxt13::URA3</i>	MB262	GCACGTAAGGCATAACAATCAAAAAAAGAAAAAAGAAA CAAAAGTTAAACCAAAAAAACAACAGCTGAAGCTTCGT ACGC
	MB277	CACATTATTATGTAAACTATAATATAACAATGTTGCCTATC AAGACAAACATATGCACTCTATGACATAGGCCACTAGTG GATCTG
<i>rrm3::HIS3MX6</i>	MB30	GAACAAGCTCAAAGTCGAGAGATTTGTTCTTATAAGAC ATCCCGCGGATCCCGGGTTAATTAA
	MB31	GAAAAGAAAACCTTCAACTAGAGTATATGCATTTATTCGTT GCAAGGAATTCGAGCTCGTTTAAAC
<i>sgs1::his5<sup>+</sup></i>	MB32	TGTTGTATATATTTAAAAAATCATAACACGTACACACAAGG CGGTACAGCTGAAGCTTCGTACGC
	MB33	GAATGGTGTCTAGTTATAAGTAACACTATTTATTTTTCT ACTCTCGTATCCGGTGATCACCTAGAC
<i>prb1::insert</i>	KP321f	CAAACCTAAGAGTCCAATTAGCTTCATCGCCAATAAAAA AACAAACTAAACCTAATTCTAACA
	KP321r	TTGTAACCTCGAGACGCCTAAGGAAAGAAAAAGAAAAA AAAAGCAGCTGAAATTTTTCTAAA

\*The definitions of G4-I, G4-X, G-rich, and Non-G-rich are the same as in Tables S2 and 3.

## B) Prokaryotic Pif1 cloning primers.

<b>Species (helicase, accession #)</b>	<b>Oligo</b>	<b>Sequence (5'-3')*</b>
<i>Bacteroides</i> sp. 2_1_16 (BacPif1, GG705209)	MB215	CGGGATCCATGGAAGATATGATTTTGACAGA AGAGATGCAAAAAATAATGAATCTC
	MB216	GATCCGCTCGAGATCTAACCGTTTACCATAA TAGTTACCTTCTGATTTGTATGCCC
<i>Bdellovibrio bacteriovorus</i> HD100 (BdePif1, BX842655)	MB211	CTGAAGATCTATGAATTCAGAGTCCAAATGC TATCGTTTTTATATTTGCTTCGC
	MB212	GATCCGCTCGAGAAGACCTTCAAACCTGCTTA TAAAAATGCAAACTTTTGGATCG
<i>Campylobacter jejuni</i> subsp. <i>jejuni</i> NCTC 11168 (CamPif1, AL111168)	MB196	CGGGATCCATGTTTGATAAACTAGAAAAAAT TTTAGCTTATGATAATGTTTTTTAAGTG
	MB198	GATCCGCTCGAGGATTTGTTCTTGTATTTTCG

		AGATCTAAAAAATTATGTTTTTT
<i>Escherichia coli</i> phage rv5 (rv5Pif1, DQ832317)	MB427	<u>CGGGATCC</u> GAAAAAGGGCAGTATGATGTAG GCACAGATGC
	MB428	GATCCGCT <u>CGAGT</u> ACGTCCCCCTCTTCTGCT CTGGTTTC
<i>Psychrobacter</i> sp. PRwf-1 (PsyPif1, CP000713)	MB455	CAGTCG <u>ATTAAT</u> ATGGGATCCAAACAAGCG ACCGCACTGG
	MB457	GATCCGCT <u>CGAGAT</u> CAATAAAGACAAGCGC TAAGCGGATGG

\*Restriction sites used to clone into the *Bam*HI and *Xho*I sites of the expression plasmids are underlined.

**Table S7.** Multiplex PCR primers.

<b>Primer pair</b>	<b>Sequences (5'-&gt;3')</b>	<b>Expected PCR product size (bp)</b>
PCM1 For	CGATGAAGGTTGATTACGAGC	615
PCM1 Internal Rev	GAAGGCTCCTATAAAGAACG	
IG SOM1 Rev	CGAGGTCACGGACACATATACC	
MB634	GACACGAAATTACAAAATGGAATATGTTC ATAGGGTAGACG	500
*Internal CIN8 Rev	GAAAATCGACATAATAAGAGTAGATTTC	414
Internal CIN8 For	GATTTGCGATAGCGTCGCTGCC	
Internal NPR2 Rev2	CTTAGTTTAGAAATTTTGGCAATG	Rev2+Inside=340
Inside Hot Spot For	GAAAAGGATACAAAGGATATGAG	Rev2+Outside=451
Outside Hot Spot Rev	GTTTTCTTCGATTTGAAGGTGTTGG	
IG before CAN1 For	GAGTTTGCTAGATTCATAAAAAGCC	241
IG before CAN1 Rev	CCGATAATGTCTGAGTTAGGTGAG	

\*The PCR product generated by the *CIN8* primer pair was used as the probe for the Southern blots in Fig. 3.

**Table S8.** Plasmids

Name	Feature(s) of interest	Reference or source
pFA6a-Myc13-His3MX6	13xMyc, <i>HIS3</i>	5
pET28b-Pif1-His	<i>Saccharomyces cerevisiae PIF1</i>	40
pFB-MBP-SGS1-His	<i>S. cerevisiae SGS1</i>	7
pUC19+Bd3546 (for)	<i>Bdellovibrio bacteriovorus</i> Pif1	This study*
pMB116	N-terminal 4xStrepII tag, C-terminal 6xHis tag	This study
pMB131 <sup>a</sup>	<i>Bacteroides</i> sp. 2_1_16 Pif1	This study
pMB142 <sup>a</sup>	<i>B. bacteriovorus</i> Pif1	This study
pMB135 <sup>a</sup>	<i>Campylobacter jejuni</i> Pif1	This study
pMB226 <sup>a</sup>	<i>Escherichia coli</i> phage rv5 Pif1	This study
PMB241 <sup>a</sup>	<i>Psychrobacter</i> sp. PRwf-1 Pif1	This study
pUG72	<i>URA3</i>	4
pFA6a-His3MX6	<i>HIS3</i>	5
pUG27	<i>his5</i> <sup>+</sup>	4
pRS415	CEN <i>LEU2</i>	1
KP114 <sup>b</sup>	G4 Chr I	This study
KP118 <sup>b</sup>	G4 Chr X	This study
KP116 <sup>b</sup>	GR Chr I	This study
KP117 <sup>b</sup>	Chr VII	This study
pMB258	<i>CEN1</i> , <i>TRP1</i> , <i>RRM3</i> promoter, C-terminal 3xFLAG	This study
pMB282 <sup>c</sup>	<i>S. cerevisiae PIF1</i>	This study
pMB307 <sup>c</sup>	<i>CEN1</i> , <i>TRP1</i> , <i>PIF1</i> promoter, <i>PIF1</i> , C-terminal 3xFLAG	This study
pMB283 <sup>c</sup>	<i>S. cerevisiae pif1</i> -K264A	This study
pMB274 <sup>c</sup>	<i>S. cerevisiae RRM3</i>	This study
pMB272 <sup>c</sup>	<i>Schizosaccharomyces pombe pfh1</i> <sup>+</sup>	This study
pMB292 <sup>c</sup>	<i>Homo sapiens PIF1</i>	This study
pMB270 <sup>c</sup>	<i>Bacteroides</i> sp. 2_1_16 Pif1	This study
pMB288 <sup>c</sup>	<i>Bacteroides</i> sp. 2_1_16 Pif1-KA	This study
pMB267 <sup>c</sup>	<i>C. jejuni</i> Pif1	This study
pMB289 <sup>c</sup>	<i>P. sp.</i> PRwf-1 Pif1	This study
pMB303 <sup>c</sup>	<i>B. infantis</i> Pif1	This study
pMB304 <sup>c</sup>	<i>Emiliana huxleyi</i> Virus 99B1 Pif1	This study
pMB305 <sup>c</sup>	<i>B. bacteriovorus</i> Pif1	This study
pMB348 <sup>d</sup>	<i>Schizosaccharomyces pombe</i> Pfh1	This study
pMB363 <sup>d</sup>	<i>S. pombe</i> Pfh1-KA	This study

<sup>a</sup> Plasmid backbone = pMB116.

<sup>b</sup> Plasmid backbone = pRS415.

<sup>c</sup> Plasmid backbone = pRS258.

<sup>d</sup> Plasmid backbone = pRS424.

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