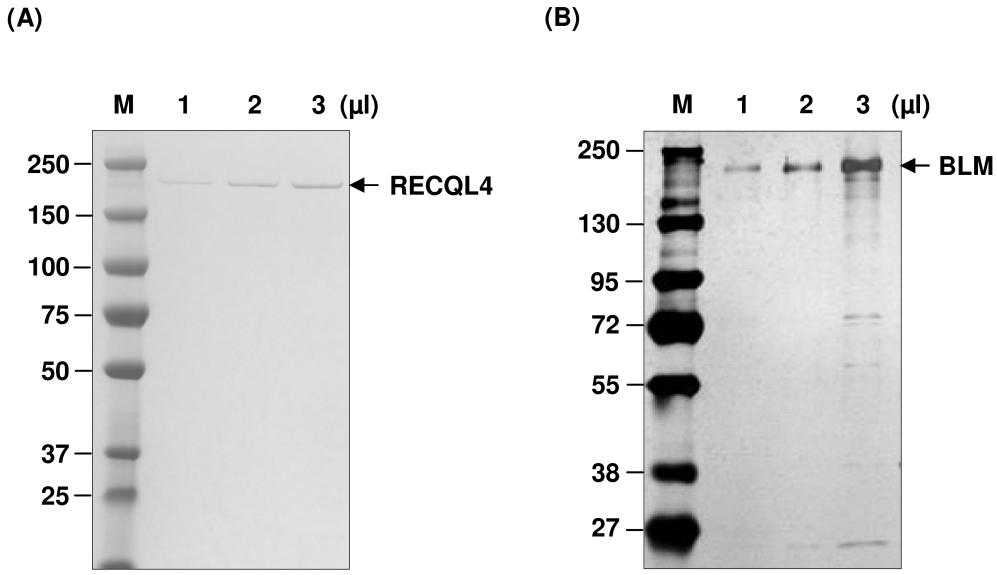
Supplementary Material and Methods 1

Strand exchange assay:

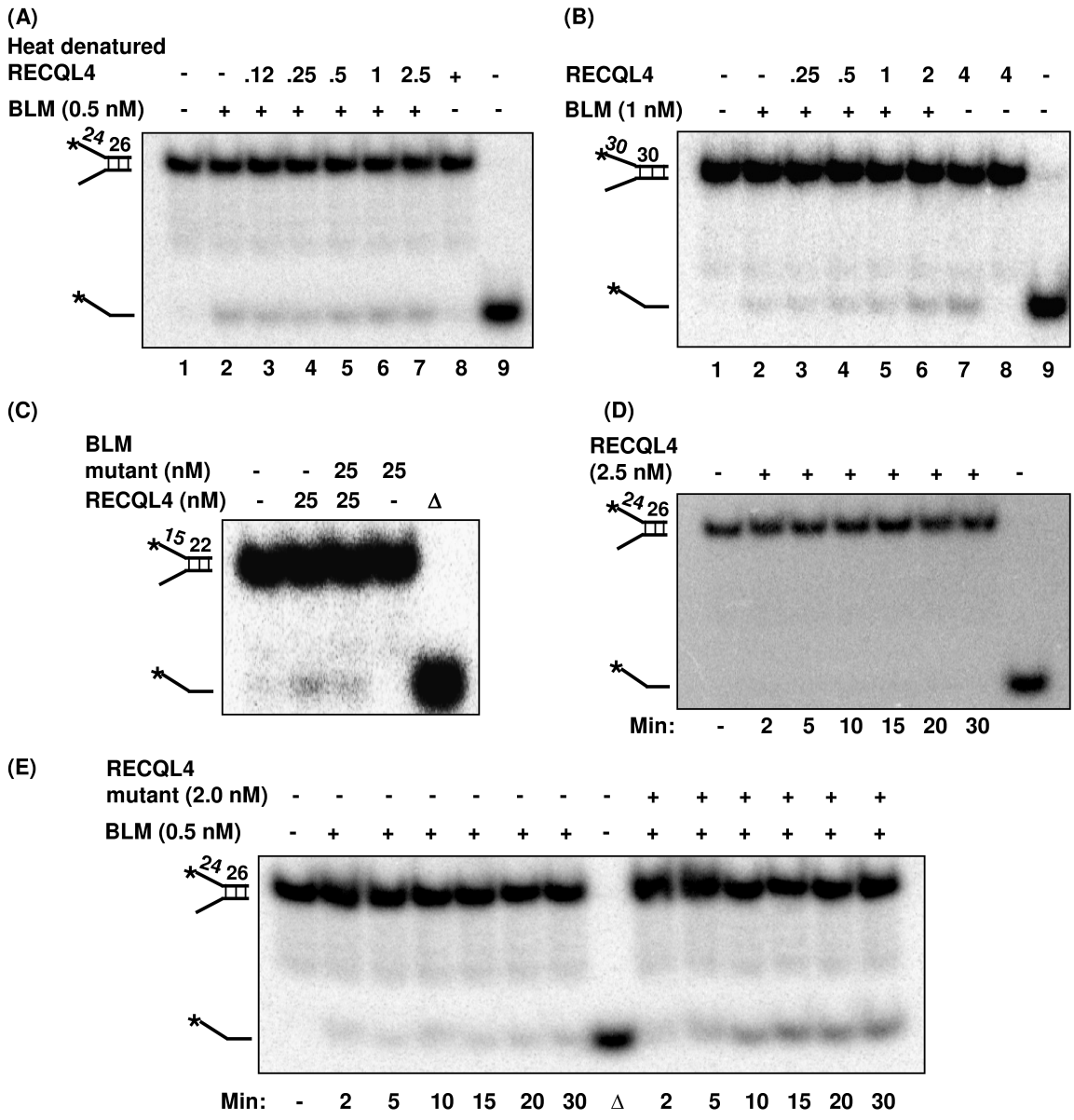
The synthetic stalled replication fork substrate was prepared according to a previously published procedure (35). In brief, partial duplex B was prepared by annealing 5'-radiolabed (γ-^{32}ATP) oligonucleotide as shown (Figure 4D). Then, it was incubated with strand A and BLM in annealing buffer (30 mM Tris pH 7.4, 50 mM KCl, 10 mM $MgCl_2$, 1 mM DTT, 0.1 mg/ml BSA) at 37 °C to get substrate C in a 100 µl reaction volume in two separate vials. After 10 minutes 10 µl aliquots were taken out and mixed with stop buffer. Then 2 mM ATP and RECQL4 were added to it. Again, 10 µl aliquots were taken out at 1, 2, 4, 8 and 16 minutes from each reaction and mixed with stop buffer. The products were separated by 8% non-denaturing PAGE. The radiolabeled DNA was analyzed by phosphorImager and analyzed by ImageQuant software (GE healthcare, Piscataway, NJ, USA).

Supplementary Figure S1



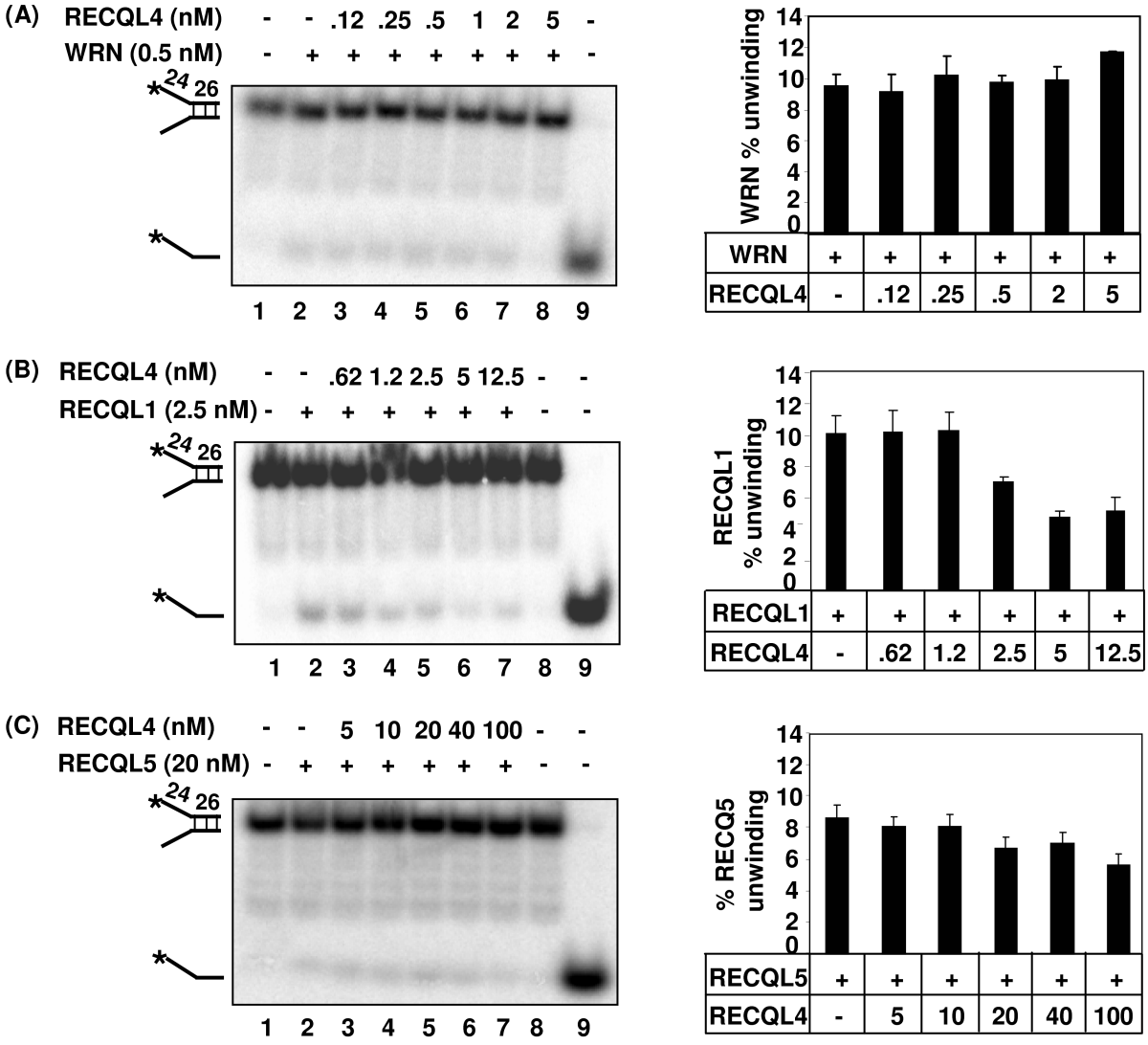
Suppl. Fig S1: (A) Coomassie stained gel of purified His tagged recombinant RECQL4 protein from *E. coli*. (B) Silver stained gel of purified His tagged recombinant BLM protein from *S. cereviceae*. M= Marker.

Supplementary Figure S2



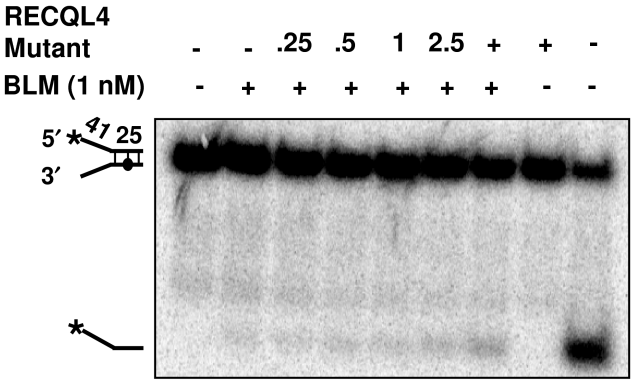
Suppl. Fig. S2: (A) The BLM unwinding activity in presence of increasing concentrations of heat denatured RECQL4. (B) The stimulation of BLM helicase activity in the presence of increasing concentrations of wild type RECQL4 (lanes 3-7) on 30-mer fork DNA duplex substrate. Helicase activities of BLM or RECQL4 alone are shown in lane 2 and 8, respectively. Substrate alone and heat denatured substrate are shown in lanes 1 and 9, respectively. (C) Unwinding activity of RECQL4 on 22-mer DNA duplex substrate in the absence or the presence of helicase-dead mutant BLM protein. (D) Time course kinetics of RECQL4 unwinding activity at different time points as indicated on DNA fork substrate. (E) Time course kinetics of BLM unwinding activity in the absence or the presence of RECQL4 helicase-dead mutant protein on DNA fork substrate.

Supplementary Figure S3



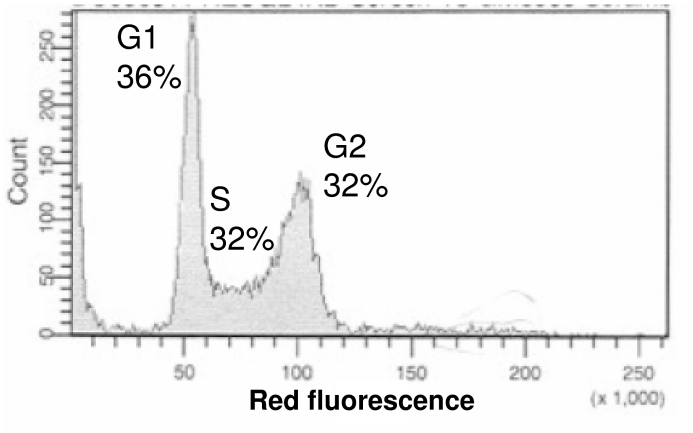
Suppl. Fig. S3. The unwinding activities of different RecQ helicase members (WRN, RECQL1 and RECQL5) as indicated in the absence or the presence of increasing concentrations of RECQL4 are shown in panels (A), (B), and (C) as indicated. The molar ratios of RECQL4 with respect to different RecQ helicases are indicated for each lane. The bar graph is represented along each panels. The experiments were performed in triplicate and the error bars represent the standard deviation (+/-).

Supplementary Figure S4

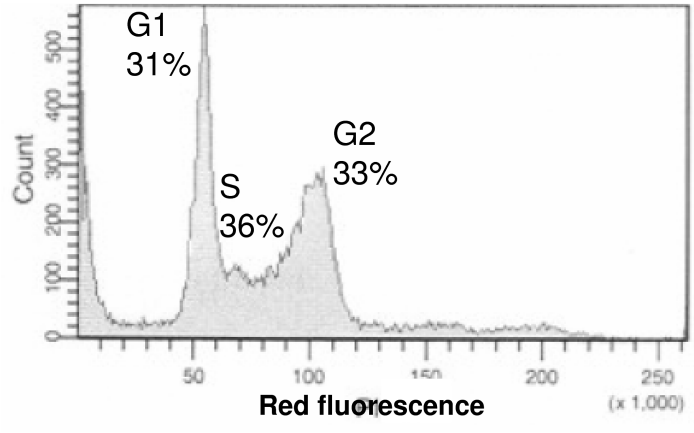


Suppl. Fig. S4. BLM unwinding activity in presence of increasing concentrations of RECQL4 helicase-dead mutant protein on 8-oxoG lesion containing DNA fork substrate.

Supplementary Figure S5



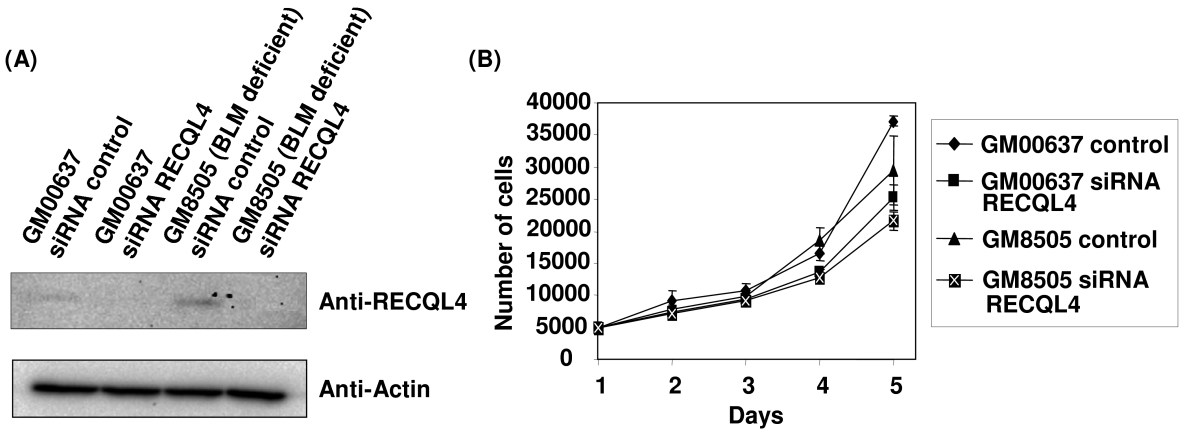
GM 08505 scrambled cells



GM 08505 RECQL4 KD cells

Suppl. Fig. S5: Cell cycle analysis of BLM-deficient cells and BLM- and RECQL4-double deficient cells. Cells were transduced with a lentiviral vector encoding scrambled shRNA or RECQL4 shRNA and harvest 6 days post transduction for analysis. The cells were fixed in acetone: methanol (1:1) and followed by staining with propidium iodide (PI) for FACS analysis.

Supplementary Figure S6



(C) Quantification of SCE frequencies of GM00637 and GM08505 (BLM) cells transfected with either control or the siRNA targeted against RECQL4

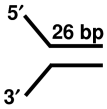
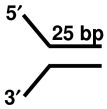
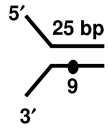
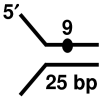
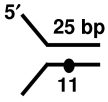
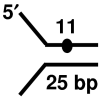
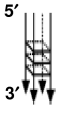
Cell line	Number of SCEs/chromosome										SCEs	Chromosome	SCEs/ Chromosome
	0	1	2	3	4	5	6	7	8	9			
GM637 control	239	59	15								89	313	0.28
GM637 RECQL4 KD	215	46	12	2							76	275	0.27
GM8505 control	156	92	95	39	14	4		1			482	401	1.20
BLM-/- RECQL4 KD	88	44	71	35	13	7	4	1			409	263	1.55

Suppl. Fig S6. (A) Western blot showing RECQL4 silencing in normal fibroblasts (GM00637) and BLM-deficient fibroblasts (GM08505) using siRNA targeted against RECQL4, 48 hrs after transfection. (B) The graph represents the growth assay of various control and RECQL4 knock down cells as indicated. (C) Quantification of frequencies of SCEs/chromosome in different cell lines transfected with either the control or the siRNA targeted against RECQL4 in normal fibroblasts and BLM deficient fibroblasts.

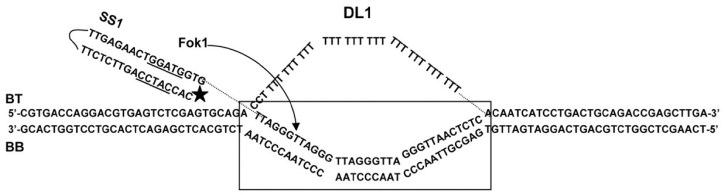
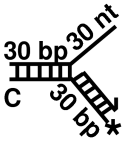
Supplementary Table 1:

Oligonucleotide substrate used in this study

TG, thymine glycol; 8-oxo-G, 8-oxoguanine; T- translocating strand; N- non translocating strand.

DNA substrate	Structure	Sequence	Reference
Fork substrate (26 bp duplex)		5′- GGGACGCGTCGGCCTGGCACGTCGGCCGCTGCGGCCAGGCACCCGATGGC - 3′ 3′- C CCTGCGCAGCCGGACCGTGCAGCCGTTTGTTTGTTTGTTTGTTTGTTT - 5′	
Fork control (25 bp duplex)		TTTTTTTTTTTTTTTTGACGCTGCCGAATTCTGGCCCAAGCGGACATCTTAGCCCACGTTGACCCT TTTTTTTTTTTTTTTTTTGCAGTATCTGCTAATGTAACGATCCTGTAGAATCGGGTGCAACTGGGA	Suhasini et al., 2010
TG-T		TTTTTTTTTTTTTTTTGACGCTGCCGAATTCTGGCCCAAGCGGACATCTTAGCCCACGTTGACCCT TTTTTTTTTTTTTTTTTTGCAGTATCTGCTAATGTAACGATCCTGTAGAATCGGGTGCAACTGGGA g	Suhasini et al., 2010
TG-N		TTTTTTTTTTTTTTTTGACGCTGCCGAATTCTGGCCCAAGCGGACATCTTTgGCCCACGTTGACCCT TTTTTTTTTTTTTTTTTTGCAGTATCTGCTAATGTAACGATCCTGTAGAAA CGGGTGCAACTGGGA	Suhasini et al., 2010
8-oxoG-T		TTTTTTTTTTTTTTTTGACGCTGCCGAATTCTGGCCCAAGCGGACATCTTTGCCCACGTTGACCCT TTTTTTTTTTTTTTTTTTGCAGTATCTGCTAATGTAACGATCCTGTAGAAACGGGTGCAACTGGGA 8oxo	Suhasini et al., 2010
8-oxoG-N		8oxo TTTTTTTTTTTTTTTTGACGCTGCCGAATTCTGGCCCAAGCGGACATCTTAGCCCACGTTGACCCT TTTTTTTTTTTTTTTTTTGCAGTATCTGCTAATGTAACGATCCTGTAGAATCGGGTGCAACTGGGA	Suhasini et al., 2010
G4-quadruplex		5′ - TGGACCAGACCTAGCAGCTATGGGGGAGCTGGGGAAGGTGGGAATGTGA - 3′	Sun et al., 1998
HJ substrate		HJ X12-1: 5′ - GACGCTGCCGAATTCTGGCTTGCTAGGACATCTTTGCCCACGTTGACCCG - 3′ HJ X12-2: 5′ - CGGGTCAACGTGGGCAAAGATGTCCTAGCAATGTAATCGTCTATGACGTC - 3′ HJ X12-3: 5′ - GACGTCATAGACGATTACATTGCTAGGACATGCTGTCTAGAGACTATCGC - 3′ HJ X12-4: 5′ - GCGATAGTCTCTAGACAGCATGTCCTAGCAAGCCAGAATTCGGCAGCGTC - 3′	Karow et al., 2000

Supplementary Table 1 cont. :

DNA substrate	Structure	Sequence	Reference
Telomeric D-loop			Opresko et al., 2004
Stalled replication fork		A: 5′- GAGGTCACTCCAGTGAATTCGAGCTCGCAGCCCCTCTAGGTTACATGACTGAATGATAGT- 3′ B: 5′- ACTATCATTCAGTCATGTAACCTAGTCAATCTGCGAGCTCGAATTCACTGGAGTGACCTC- 3′ C: 5′- ATTGACTAGGTTACATGACTGAATGATAGT- 3′	Kanagaraj et al., 2006