Supplementary data

Senescence induced by RECQL4 dysfunction contributes to Rothmund-Thomson Syndrome features in mice

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Running title: RECQL4 prevents senescence in human cells and mice

Figure legends

Figure S1 Knockdown of BLM, WRN and RECQL4 induces cellular senescence in primary fibroblast cells. Knockdown of each RecQ helicase by lentivirus-mediated shRNA in human primary fibroblast cells (GM00969, passage 19). (A) knockdown efficiencies were measured by qPCR. (B) Images of SA-β-gal stained cells. (C) Quantitation of SA-β-gal stained cells. Data are from three independent replicates. Student T-test were performed for statistical analysis in this paper.*, $p<0.05$; ***, $p<0.001$.

Figure S2 Knockdown of BLM, WRN or RECQL4-induced cell senescence in telomerase positive cells. The five RecQ helicases were knocked down by lentivirus-mediated shRNA in human primary fibroblast cells BJ and its hTERT-immortalized derivative BJ-5TA. The remaining mRNA of RecQ helicases in BJ **(A)** and BJ-5TA **(C)** were confirmed by RT-PCR. Senescence in RecQ helicases-depleted BJ **(B)** and BJ-5TA **(D)** were measured by SA-β-gal staining. Data are from three independent replicates. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

Figure S3 Apoptosis did not increase in RecQ helicase-deficient human fibroblast cells. RecQ helicases were depleted by shRNA-mediated knockdown in human primary fibroblast cells GM05565 at passage 11. After selection with puromycin, cells were submitted for apoptosis analysis using propidium iodide and Annexin V FACS staining and FACS analysis.

Figure S4 Oxidative stress from high oxygen culture conditions promotes senescence in RecQ helicase-deficient fibroblast cells. RecQ helicase were knocked down in primary human fibroblast cell lines GM00969 at passage 19 and the cells were cultured under low oxygen (3%) or high oxygen (20%) culture conditions for six days. (A) Remaining mRNA of RecQ helicases in GM00969 were measured by qPCR; (B) Quantitation of SA-β-gal stained cells in RecQ helicase-depleted as well as scramble shRNA treated cells. Data are from three independent replicates. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

Figure S5 Increased γH2AX foci in primary fibroblast cells after RecQ helicase loss. RecQ helicases were depleted by lentivirus-mediated shRNA in human primary fibroblast cell line GM05565 at passage 11. The cells in the experiments were six days after transfection of lentivirus. **(A)** The indicated cell lines were immunostained with γH2AX antibody. Bar scale is 20 um. **(B)** Percentage of cells with 5 or more γH2AX foci in the indicated cell lines as in Panel A.

Figure S6 Loss of BLM, WRN or RECQL4 caused similar transcriptome pathway changes. RecQ helicases were knocked down in primary human fibroblast cell line GM05565 at passage 11, and six days after transfection of lentivirus the cells were processed for RNA extraction and microarray analysis. Data are from three independent biological replicates. **(A)** A principal component analysis (PCA) of the unselected average gene expression Z-score from each RecQ helicase knockdown and control samples (n=3). **(B)** Number of up-/ down-regulated pathway in

BLM-, WRN-, or RECQL4-depleted cells. **(C)** Cluster of the top 100 canonical pathways that displayed the greatest Z-score in RecQ helicase-depleted cells compared with controls.

Figure S7 Cluster of the top 100 canonical pathways affected in RecQ helicase depleted fibroblast cells. RecQ helicases were knocked down in primary human fibroblast cell line GM05565 at passage 11, and six days after transfection of lentivirus the cells were processed for RNA extraction and microarray analysis. Cluster of the top 100 canonical pathways that displayed the greatest Z-score from controls. Data are from three independent biological replicates.

Figure S1

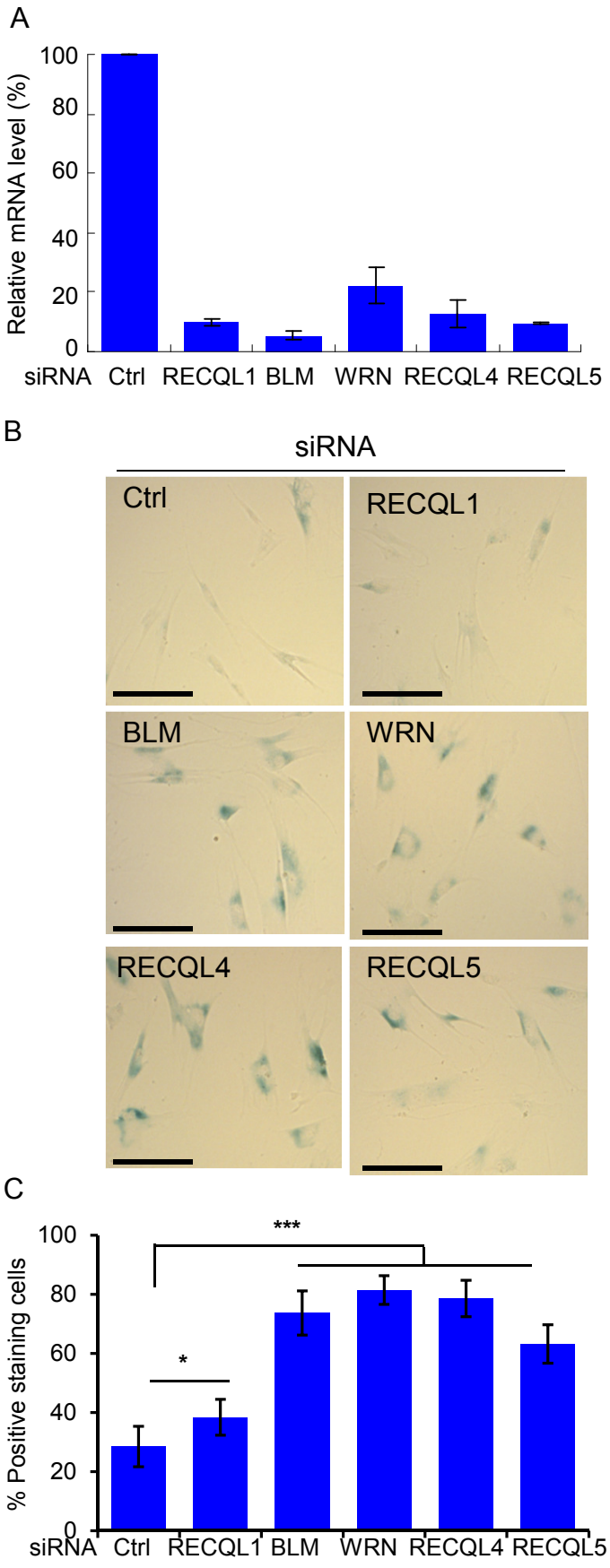

A
Relative mRNA level (%)
100
80
60
40
20
0
siRNA Ctrl RECQL1 BLM WRN RECQL4 RECQL5
B
siRNA
Ctrl
RECQL1
BLM
WRN
RECQL4
RECQL5
C
% Positive staining cells
100
80
60
40
20
0
***
*
siRNA Ctrl RECQL1 BLM WRN RECQL4 RECQL5

Figure S2

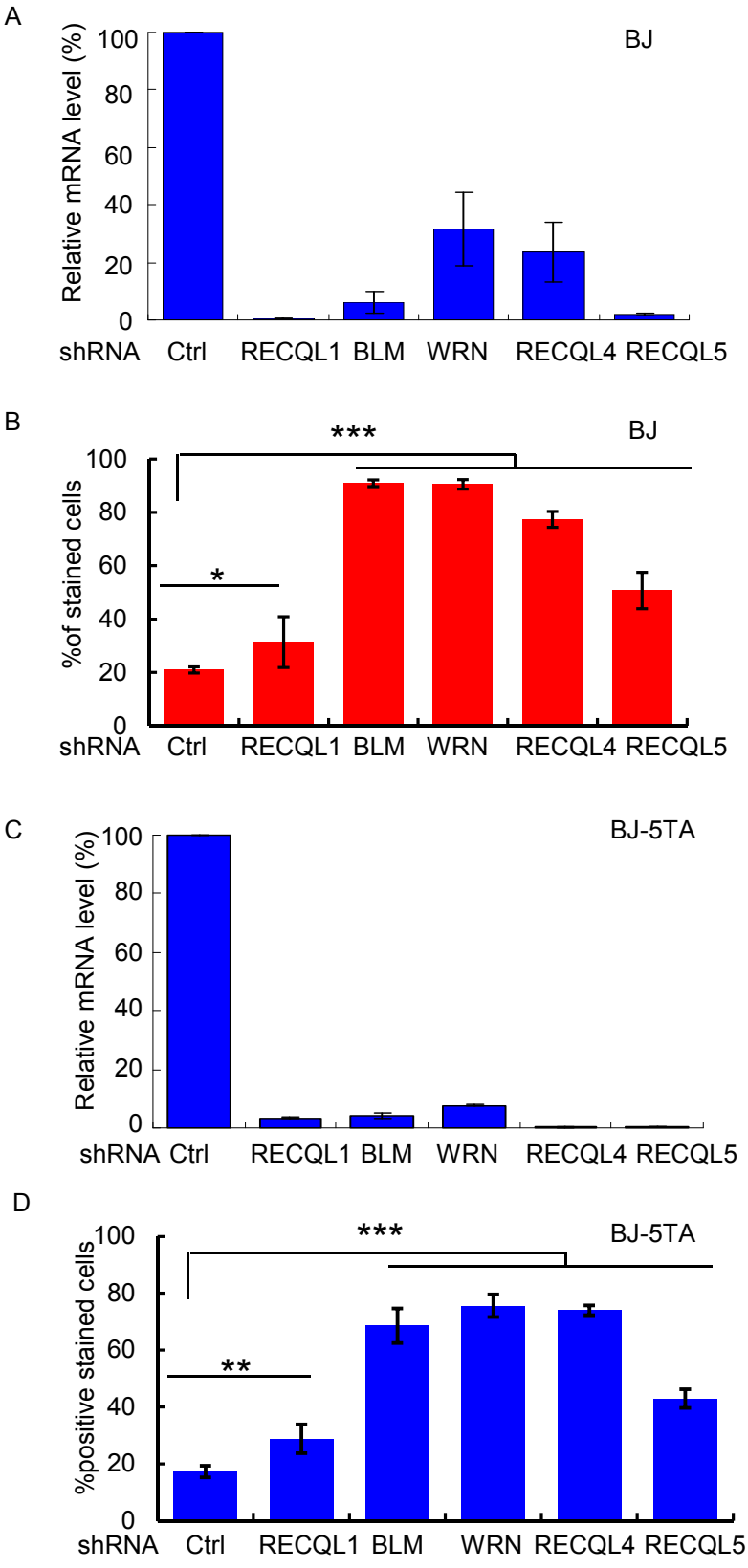

A
BJ
Relative mRNA level (%)
100
80
60
40
20
0
shRNA Ctrl RECQL1 BLM WRN RECQL4 RECQL5
B
BJ
***
*
%of stained cells
100
80
60
40
20
0
shRNA Ctrl RECQL1 BLM WRN RECQL4 RECQL5
C
BJ-5TA
Relative mRNA level (%)
100
80
60
40
20
0
shRNA Ctrl RECQL1 BLM WRN RECQL4 RECQL5
D
BJ-5TA
***
**
%positive stained cells
100
80
60
40
20
0
shRNA Ctrl RECQL1 BLM WRN RECQL4 RECQL5

Figure S3

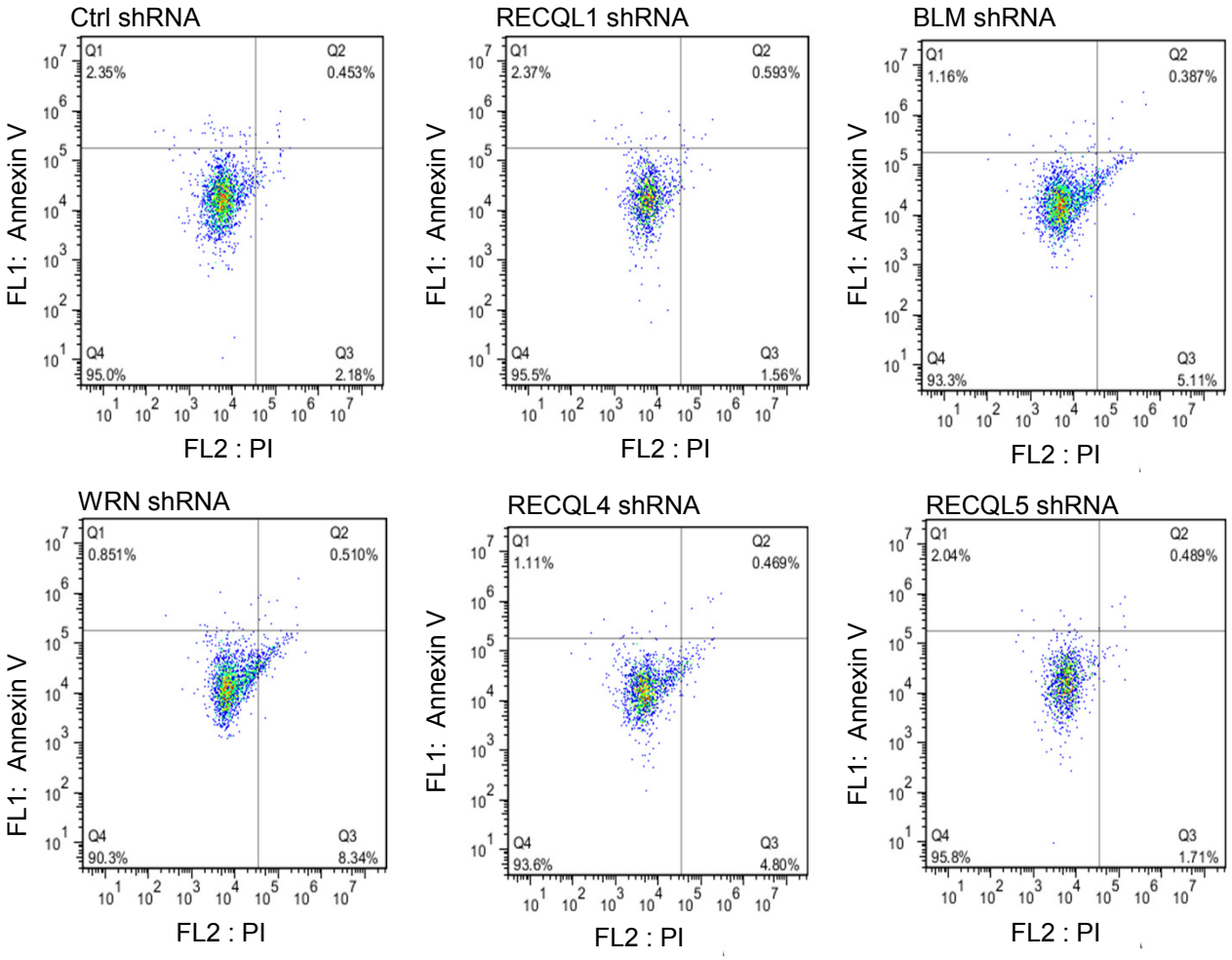

Ctrl shRNA
Q1 2.35%
Q2 0.453%
Q4 95.0%
Q3 2.18%
RECQL1 shRNA
Q1 2.37%
Q2 0.593%
Q4 95.5%
Q3 1.56%
BLM shRNA
Q1 1.16%
Q2 0.387%
Q4 93.3%
Q3 5.11%
WRN shRNA
Q1 0.851%
Q2 0.510%
Q4 90.3%
Q3 8.34%
RECQL4 shRNA
Q1 1.11%
Q2 0.469%
Q4 93.6%
Q3 4.80%
RECQL5 shRNA
Q1 2.04%
Q2 0.489%
Q4 95.8%
Q3 1.71%
FL1: Annexin V
FL2 : PI

Figure S4

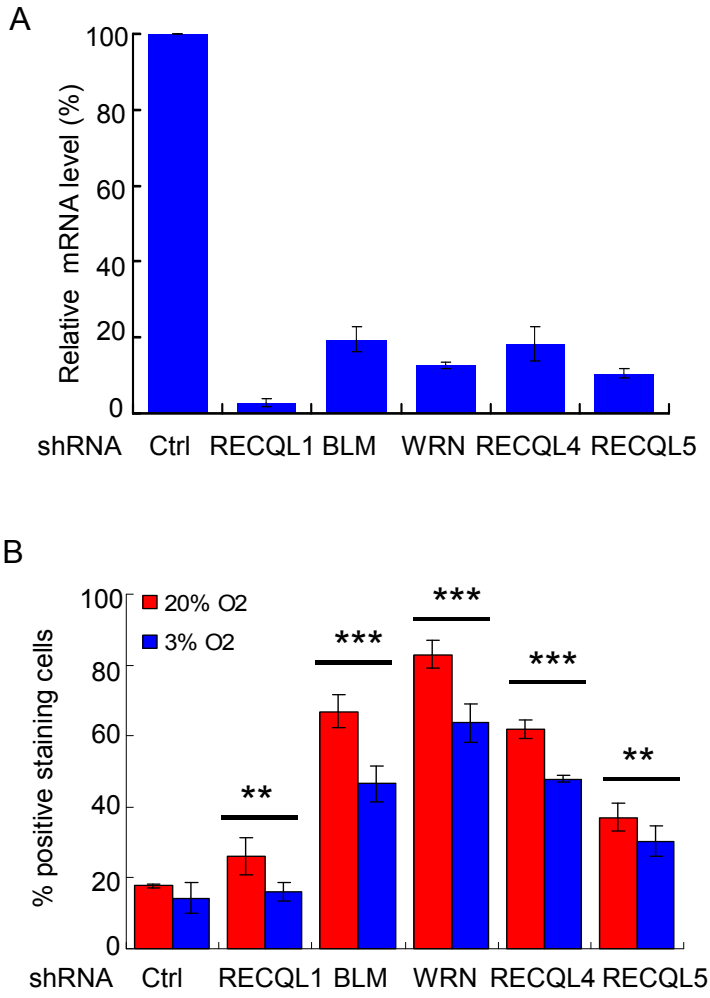
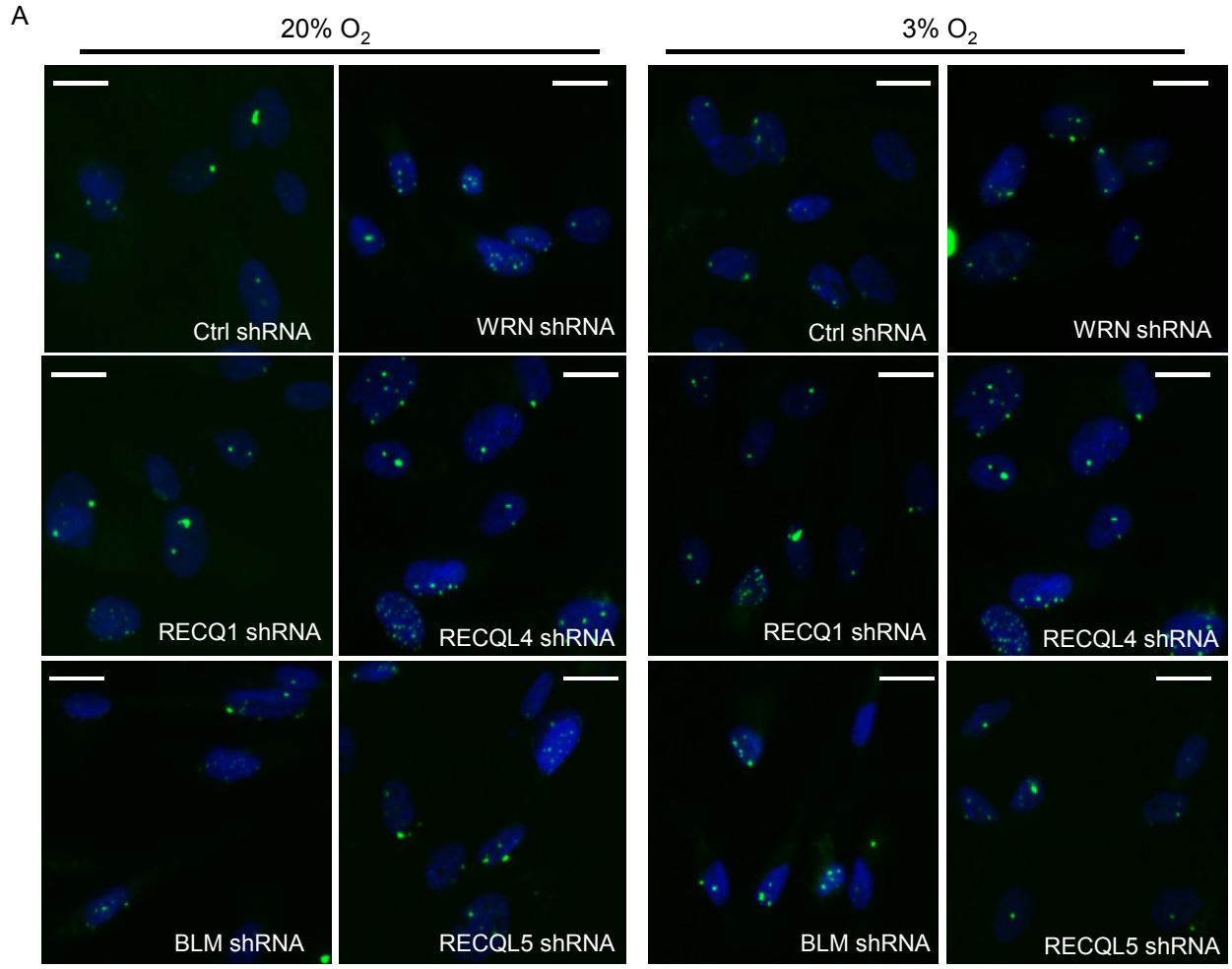

A
Relative mRNA level (%)
100
80
60
40
20
0
shRNA
Ctrl
RECQL1
BLM
WRN
RECQL4
RECQL5
B
% positive staining cells
20% O2
3% O2
***
**
shRNA
Ctrl
RECQL1
BLM
WRN
RECQL4
RECQL5

Figure S5


A
20% O2
3% O2
Ctrl shRNA
WRN shRNA
Ctrl shRNA
WRN shRNA
RECQ1 shRNA
RECQL4 shRNA
RECQ1 shRNA
RECQL4 shRNA
BLM shRNA
RECQL5 shRNA
BLM shRNA
RECQL5 shRNA

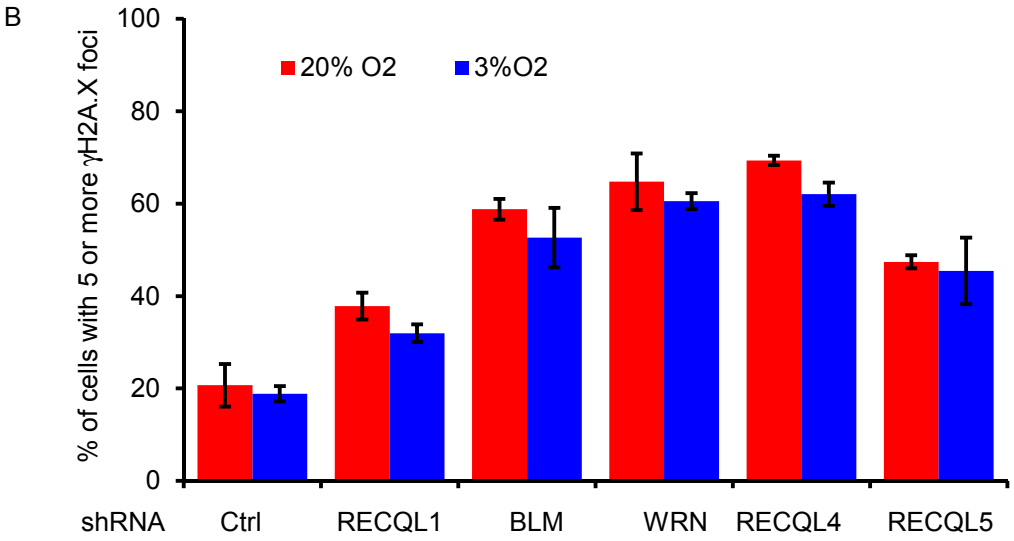
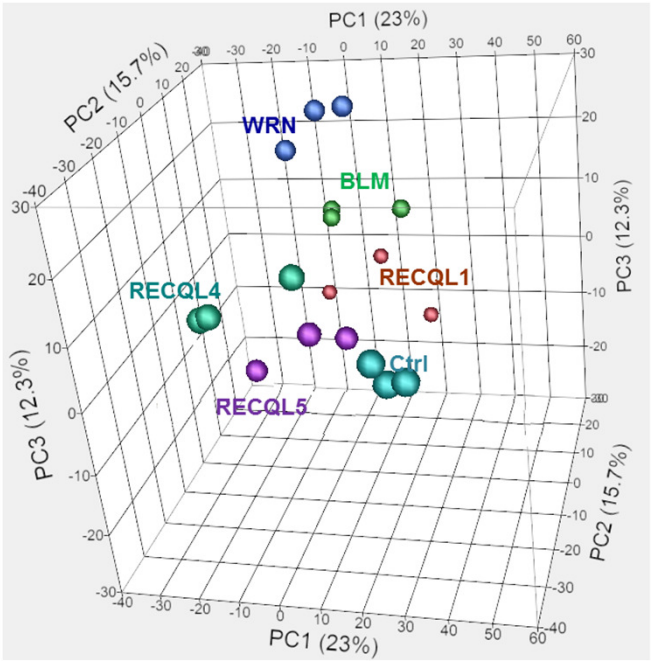

B
% of cells with 5 or more γH2A.X foci
100
80
60
40
20
0
20% O2
3%O2
shRNA
Ctrl
RECQL1
BLM
WRN
RECQL4
RECQL5

Figure S6

A



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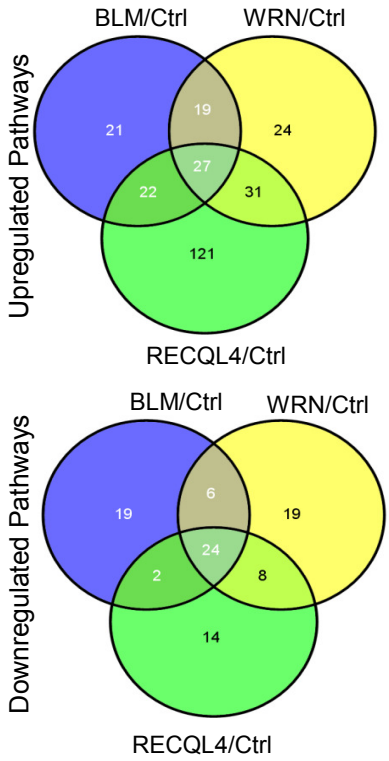

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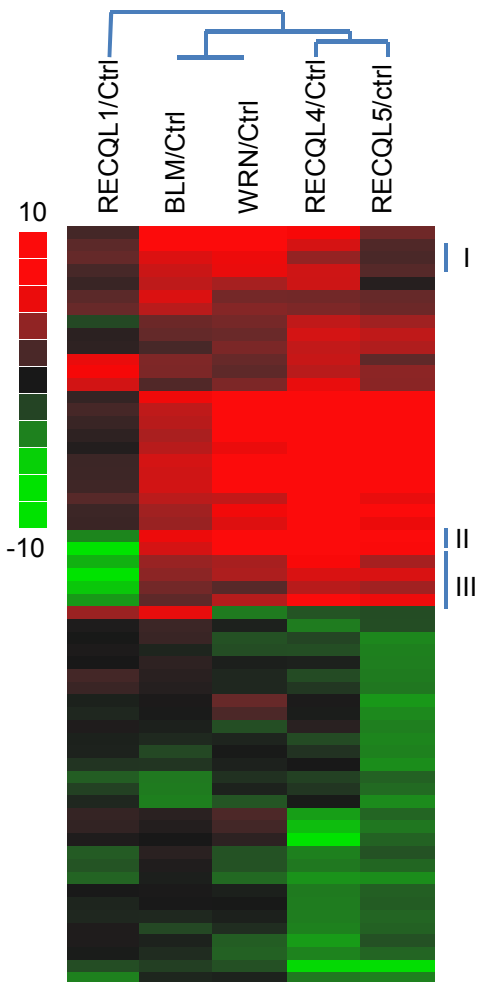

Part	Pathways
I	Packaging of telomere ends
	Telomere maintenance
II	Oxidative phosphorylation
	Electron transport chain
III	Parkinson's Disease
	Diabetes' pathways
	Alzheimer's Disease
	Huntington's Disease

Figure S7

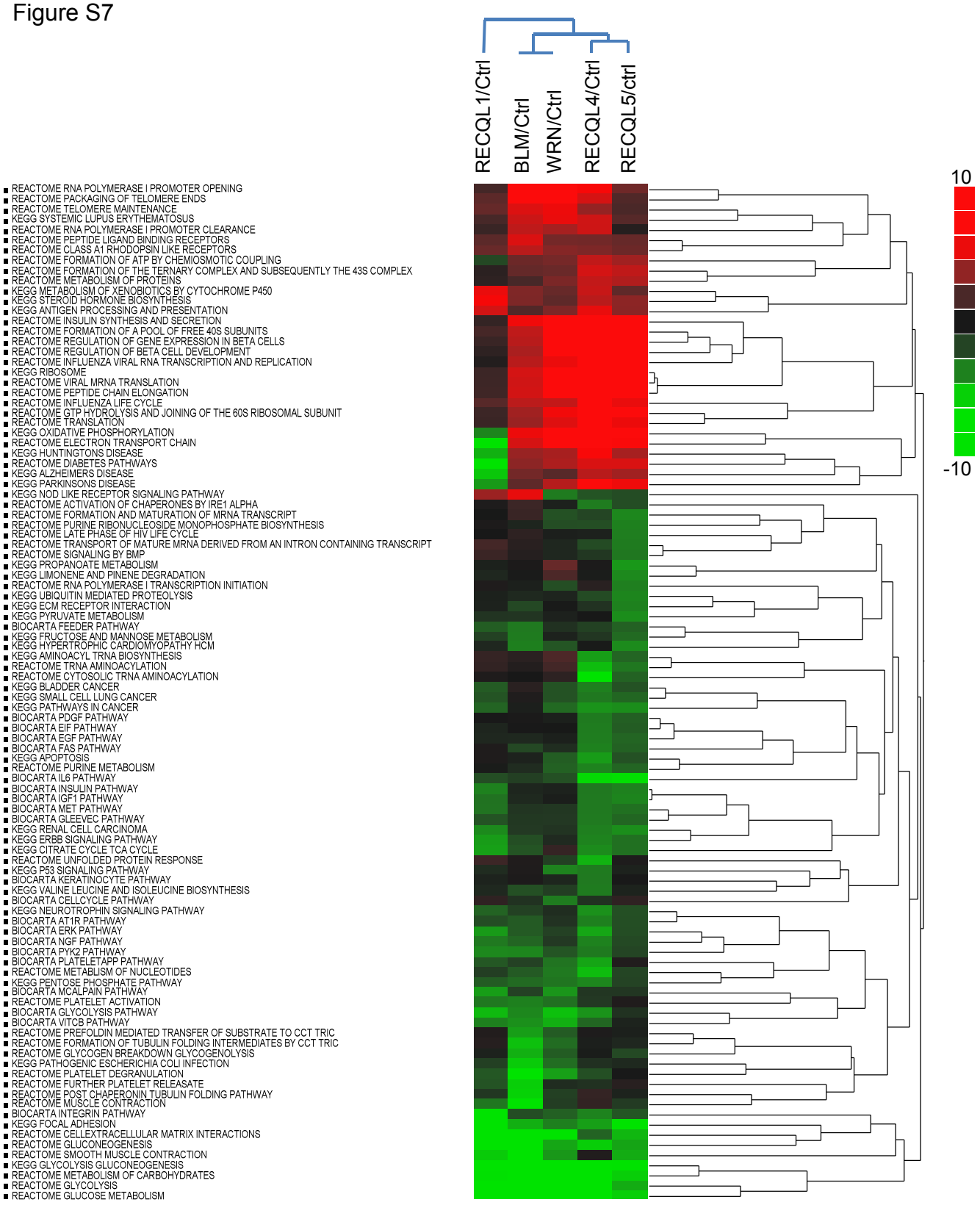
RECQL1/Ctrl
BLM/Ctrl
WRN/Ctrl
RECQL4/Ctrl
RECQL5/ctrl
10
-10
REACTOME RNA POLYMERASE I PROMOTER OPENING
REACTOME PACKAGING OF TELOMERE ENDS
REACTOME TELOMERE MAINTENANCE
KEGG SYSTEMIC LUPUS ERYTHEMATOSUS
REACTOME RNA POLYMERASE I PROMOTER CLEARANCE
REACTOME PEPTIDE LIGAND BINDING RECEPTORS
REACTOME CLASS A1 RHODOPSIN LIKE RECEPTORS
REACTOME FORMATION OF ATP BY CHEMIOSMOTIC COUPLING
REACTOME FORMATION OF THE TERNARY COMPLEX AND SUBSEQUENTLY THE 43S COMPLEX
REACTOME METABOLISM OF PROTEINS
KEGG METABOLISM OF XENOBIOTICS BY CYTOCHROME P450
KEGG STEROID HORMONE BIOSYNTHESIS
KEGG ANTIGEN PROCESSING AND PRESENTATION
REACTOME INSULIN SYNTHESIS AND SECRETION
REACTOME FORMATION OF A POOL OF FREE 40S SUBUNITS
REACTOME REGULATION OF GENE EXPRESSION IN BETA CELLS
REACTOME REGULATION OF BETA CELL DEVELOPMENT
REACTOME INFLUENZA VIRAL RNA TRANSCRIPTION AND REPLICATION
KEGG RIBOSOME
REACTOME VIRAL MRNA TRANSLATION
REACTOME PEPTIDE CHAIN ELONGATION
REACTOME INFLUENZA LIFE CYCLE
REACTOME GTP HYDROLYSIS AND JOINING OF THE 60S RIBOSOMAL SUBUNIT
REACTOME TRANSLATION
KEGG OXIDATIVE PHOSPHORYLATION
REACTOME ELECTRON TRANSPORT CHAIN
KEGG HUNTINGTONS DISEASE
REACTOME DIABETES PATHWAYS
KEGG ALZHEIMERS DISEASE
KEGG PARKINSONS DISEASE
KEGG NOD LIKE RECEPTOR SIGNALING PATHWAY
REACTOME ACTIVATION OF CHAPERONES BY IRE1 ALPHA
REACTOME FORMATION AND MATURATION OF MRNA TRANSCRIPT
REACTOME PURINE RIBONUCLEOSIDE MONOPHOSPHATE BIOSYNTHESIS
REACTOME LATE PHASE OF HIV LIFE CYCLE
REACTOME TRANSPORT OF MATURE MRNA DERIVED FROM AN INTRON CONTAINING TRANSCRIPT
REACTOME SIGNALING BY BMP
KEGG PROPANOATE METABOLISM
KEGG LIMONENE AND PINENE DEGRADATION
REACTOME RNA POLYMERASE I TRANSCRIPTION INITIATION
KEGG UBIQUITIN MEDIATED PROTEOLYSIS
KEGG ECM RECEPTOR INTERACTION
KEGG PYRUVATE METABOLISM
BIOCARTA FEEDER PATHWAY
KEGG FRUCTOSE AND MANNOSE METABOLISM
KEGG HYPERTROPHIC CARDIOMYOPATHY HCM
KEGG AMINOACYL TRNA BIOSYNTHESIS
REACTOME TRNA AMINOACYLATION
REACTOME CYTOSOLIC TRNA AMINOACYLATION
KEGG BLADDER CANCER
KEGG SMALL CELL LUNG CANCER
KEGG PATHWAYS IN CANCER
BIOCARTA PDGF PATHWAY
BIOCARTA EIF PATHWAY
BIOCARTA EGF PATHWAY
BIOCARTA FAS PATHWAY
KEGG APOPTOSIS
REACTOME PURINE METABOLISM
BIOCARTA IL6 PATHWAY
BIOCARTA INSULIN PATHWAY
BIOCARTA IGF1 PATHWAY
BIOCARTA MET PATHWAY
BIOCARTA GLEEVEC PATHWAY
KEGG RENAL CELL CARCINOMA
KEGG ERBB SIGNALING PATHWAY
KEGG CITRATE CYCLE TCA CYCLE
REACTOME UNFOLDED PROTEIN RESPONSE
KEGG P53 SIGNALING PATHWAY
BIOCARTA KERATINOCYTE PATHWAY
KEGG VALINE LEUCINE AND ISOLEUCINE BIOSYNTHESIS
BIOCARTA CELLCYCLE PATHWAY
KEGG NEUROTROPHIN SIGNALING PATHWAY
BIOCARTA AT1R PATHWAY
BIOCARTA ERK PATHWAY
BIOCARTA NGF PATHWAY
BIOCARTA PYK2 PATHWAY
BIOCARTA PLATELETAPP PATHWAY
REACTOME METABLISM OF NUCLEOTIDES
KEGG PENTOSE PHOSPHATE PATHWAY
BIOCARTA MCALPAIN PATHWAY
REACTOME PLATELET ACTIVATION
BIOCARTA GLYCOLYSIS PATHWAY
BIOCARTA VITCB PATHWAY
REACTOME PREFOLDIN MEDIATED TRANSFER OF SUBSTRATE TO CCT TRIC
REACTOME FORMATION OF TUBULIN FOLDING INTERMEDIATES BY CCT TRIC
REACTOME GLYCOGEN BREAKDOWN GLYCOGENOLYSIS
KEGG PATHOGENIC ESCHERICHIA COLI INFECTION
REACTOME PLATELET DEGRANULATION
REACTOME FURTHER PLATELET RELEASATE
REACTOME POST CHAPERONIN TUBULIN FOLDING PATHWAY
REACTOME MUSCLE CONTRACTION
BIOCARTA INTEGRIN PATHWAY
KEGG FOCAL ADHESION
REACTOME CELLEXTRACELLULAR MATRIX INTERACTIONS
REACTOME GLUCONEOGENESIS
REACTOME SMOOTH MUSCLE CONTRACTION
KEGG GLYCOLYSIS GLUCONEOGENESIS
REACTOME METABOLISM OF CARBOHYDRATES
REACTOME GLYCOLYSIS
REACTOME GLUCOSE METABOLISM

Table S1 Number of genes with changed transcriptional level in response to deletion of individual RecQ helicases in human primary fibroblasts

Analysis Set	Up-regulated genes		Down-regulated genes	
	Number	Percentage	Number	Percentage
RECQL1 shRNA/Ctrl shRNA	1478	4.67%	1423	4.50%
BLM shRNA/ Ctrl shRNA	1302	4.11%	1292	4.10%
WRN shRNA/ Ctrl shRNA	1492	4.71%	1500	4.74%
RECQL4 shRNA / Ctrl shRNA	1156	3.66%	1580	5.00%
RECQL5 shRNA / Ctrl shRNA	1166	3.69%	1835	5.80%

Table S2 Information of siRNA, shRNA and primers used in this study.

Name		Sequence	Source	Cat. No	Ref
shRNA targeting RecQ helicases					
	Control shRNA	5'CCTAAGGTTAAGTCGCCCTCGCTCGAGCGAGGGCGACTTAACCTTAGG3'	Addgene	plasmid# 1864	(Sarbassov et al., 2005)
	RECQL1 shRNA	5'CCGGGCACATGCTATTACTATGCAACTCGAGTTGCATAGTAATAGCATGTGCTTTTTG 3'	Sigma-Aldrich	TRCN0000289591	(Sharma and Brosh, 2007)
	BLM shRNA	CCGGCGCTTATGTGATGCTCGGAAACTCGAGTTTCCGAGCATCACATAAGCGTTTTT		TRCN0000004906	(Popuri et al.)
	WRN shRNA	5'CCGGCCTGTAAGATTGCTTTAAGAACTCGAGTTCTTAAAGCAATCTTACAGGTTTTT3'		TRCN0000004899	This study
	RECQL4 shRNA	5'CCGGCCTCGATTCCATTATCATTTACTCGAGTAAATGATAATGGAATCGAGGTTTTTG3'		TRCN0000051169	(Singh et al., 2012)
	RECQL5 shRNA	5'CCGGCCCTAAAGGTACGAGTAAGTTCTCGAGAACTTACTCGTACCTTTAGGGTTTT3'		TRCN0000051415	(Ramamoorthy et al., 2012)
siRNA					
	Control siRNA	ON-TARGETplus Non-targeting siRNA #1	Dharmacon	D-001810-01-05	(Singh et al., 2012)
	siRECQL1	5'GCAAGGAGAUUUACUCGAA3'		Custom design	(Sharma and Brosh, 2007)
	siBLM	5'GGGAAGACAUAAUGCAUAA3'			This study
	siWRN	5'GUGUAUAGUUACGAUGCUAGUGA3'			This study
	siRECQL4	5'CAAUACAGCUUACCGUACA3'			(Singh et al., 2012)
	siRECQL5	5'CCCTAAAGGTACGAGTAAGTT3'			This study
	siRECQL4 UTR-1	5'GCTCCAAAATGCAGAATAA3'			This study
	siRECQL4 UTR-2	5'ACTGAGGACCTGGGCAAAA3'			This study
Primers for RT-PCR					
	RECQL1-RT-PF	5'TGAAGGGTCAAGGGAGGA3'	Custom design		This study
	RECQL1-RT-PR	5'TCCAAATTTGTTTCTAAAATAATCCA3'			

	BLM-RT-PF	5’TTTATCCTGATGCCGACTGG3’		
	BLM-RT-PR	5’ACCCCAGGAGAAACACAGG3’		
	WRN-RT-PF	5’TGCAGCCATTTCTTGTCAAA3’		
	WRN-RT-PR	5’GAAGGACAGTAGATGATTGTTGGA3’		
	RECQL4-RT-PF	5’GGCCGCTACTTTGAGGAAG3’		
	RECQL4-RT-PR	5’TCCTCCCAATCCTGGAGTCT3’		
	RECQL5-RT-PF	5’ACAAAGCATCTGATAAAGCCAC3’		
	RECQL5-RT-PR	5’TCGTCATACCTGCTGAAGTC3’		
	GAPDH-RT-PF	5’GCCACATCGCTCAGACAC3’		
	GAPDH-RT-PR	5’GCCCAATACGACCAAATCC3’		
Primers for constructing plasmids expressing RecQL4 fragments				
	hRQ4-F1-Not1	5'TTAATGGCGGCCGCACCATG GAGC3'		This study
	hRQ4 R447 NLS	5'TATACTCGAGTACCTTTCTCTTCTTTTTTGGGTCCAG GCTGGGCACCTCAG3'		
	hRQ4 F447	5'TTAATGGCGGCCGCACCATGGACCCCACCGTGCTGC CAC3'		
	hRQ4 R860 NLS	5'TATACTCGAGTACCTTTCTCTTCTTTTTTGGCGGCCT GGTGCAGGTGCAG3'		
	hRQ4 F863	5'TTAATGGCGGCCGCACCGAGCAGGAAGGGGCCGTG3 '		
	hRQ4 R1208 NLS	5'TATACTCGAGTACCTTTCTCTTCTTTTTTGGGCGGGC CACCTGCAGGAG3'		
	hRQ4Δ448-860-PF	5'ACTCAGTAAAGCTTTACCTTTCTCTTCTTTTTTGGGT CCAGGCTGGGCACCTCAG 3'		
	hRQ4Δ448-860-PR	5'CAGCCTTAATGGAAGCTTGCCGAGCAGGAAGGGGC CGTG 3'		
	pCMV4A-3XFLAG-fwd	5’GATTATAAAGATCATGACATCGATTACAAGGATGAC GACGAT3’		
	pCMV4A-3XFLAG-rev	5’ACCGTCATGGTCTTTGTAGTCCTCGAGGTCGACGGT ATCGAT3’		
	RecQ4-NotI-Kozak-fwd	5’GAGATTGCGGCCGCACCATGGAGCGGCTGCGGGAC GT3’		
	RecQ4-XhoI-nostop-rev	5’GAGATTCTCGAGGCGGGCCACCTGCAGGAGCT3’		

Reference:

Popuri, V., J. Huang, M. Ramamoorthy, T. Tadokoro, D.L. Croteau, and V.A. Bohr. RECQL5 plays co-operative and complementary roles with WRN syndrome helicase. *Nucleic Acids Res* 41:881-899.

Ramamoorthy, M., T. Tadokoro, I. Rybanska, A.K. Ghosh, R. Wersto, A. May, T. Kulikowicz, P. Sykora, D.L. Croteau, and V.A. Bohr. 2012. RECQL5 cooperates with Topoisomerase II alpha in DNA decatenation and cell cycle progression. *Nucleic Acids Res* 40:1621-1635.

Sarbassov, D.D., D.A. Guertin, S.M. Ali, and D.M. Sabatini. 2005. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science* 307:1098-1101.

Sharma, S., and R.M. Brosh, Jr. 2007. Human RECQ1 is a DNA damage responsive protein required for genotoxic stress resistance and suppression of sister chromatid exchanges. *PLoS One* 2:e1297.

Singh, D.K., V. Popuri, T. Kulikowicz, I. Shevelev, A.K. Ghosh, M. Ramamoorthy, M.L. Rossi, P. Janscak, D.L. Croteau, and V.A. Bohr. 2012. The human RecQ helicases BLM and RECQL4 cooperate to preserve genome stability. *Nucleic Acids Res* 40:6632-6648.