**Supplemental information**

# RECQL4 promotes DNA end resection in repair of DNA double-strand breaks

Huiming Lu[1,4], Raghavendra A. Shamanna[1,4], Guido Keijzers [2], Roopesh Anand [3], Lene Juel Rasmussen [2], Petr Cejka [3], Deborah L. Croteau[1], Vilhelm A. Bohr[1,*]

[1]Laboratory of Molecular Gerontology, National Institute on Aging, National Institutes of Health, MD 21224, USA

[2]Center for Healthy Aging, Department of Cellular and Molecular Medicine, University of Copenhagen, Denmark

[3]Institute of Molecular Cancer Research, University of Zurich, Winterthurerstrasse 190,

8057 Zurich, Switzerland

*Corresponding author:

Vilhelm A. Bohr

Email: vbohr@nih.gov

Running title: RECQL4 promotes DNA end resection

Keywords: RECQL4, Rothmund-Thomson Syndrome, DNA resection, homologous recombination, DNA repair, RecQ-like helicase

[4] These authors contributed equally to this work.

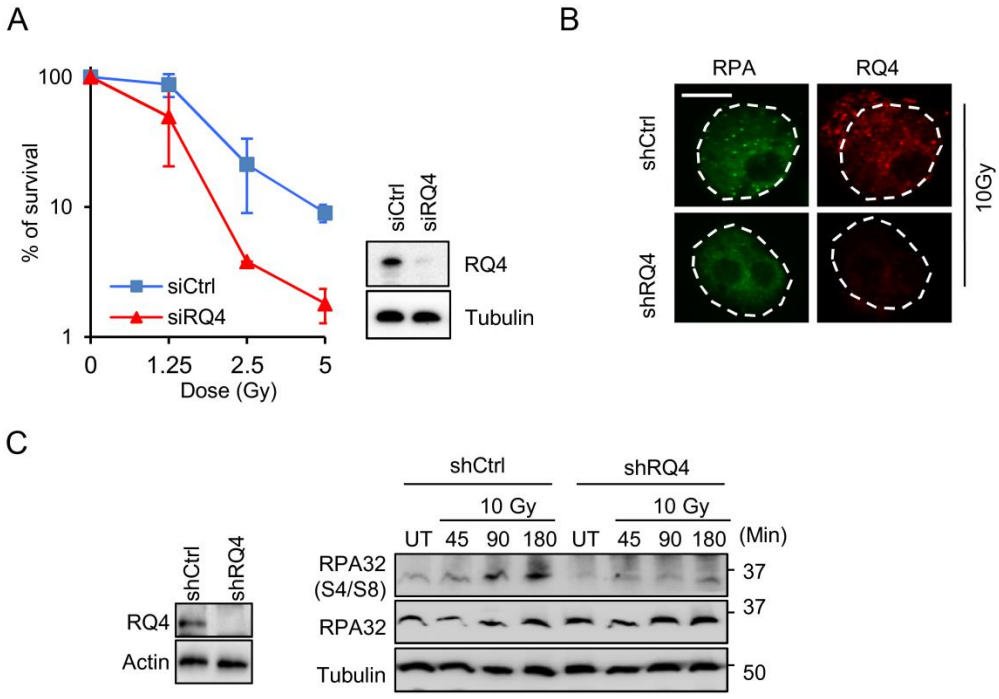


**Figure. S1. RECQL4 is important for cell survival and RPA foci formation after IR stress, related to Fig. 1. A.** Colony formation analysis of HeLa cells treated with RECQL4 siRNA (siRQ4) and control siRNA (siCtrl) after γ radiation. Data are presented as mean±SD from three independent repeats. **B.** RPA foci in RECQL4-depleted (shRQ4) and control HeLa cells after IR. Cells were irradiated for 10 Gy and allowed to recover under normal culture conditions for 1 hr and then fixed for immunostaining with anti-RPA32 antibody. **C.** IR-induced RPA32 phosphorylation on serine 4 and 8 in RECQL4-depleted HEK293T cells and control cells. HEK293T cells expressing control or RECQL4 shRNA were exposed to 10 Gy of IR then allowed to recover for the indicated time, UT, untreated.

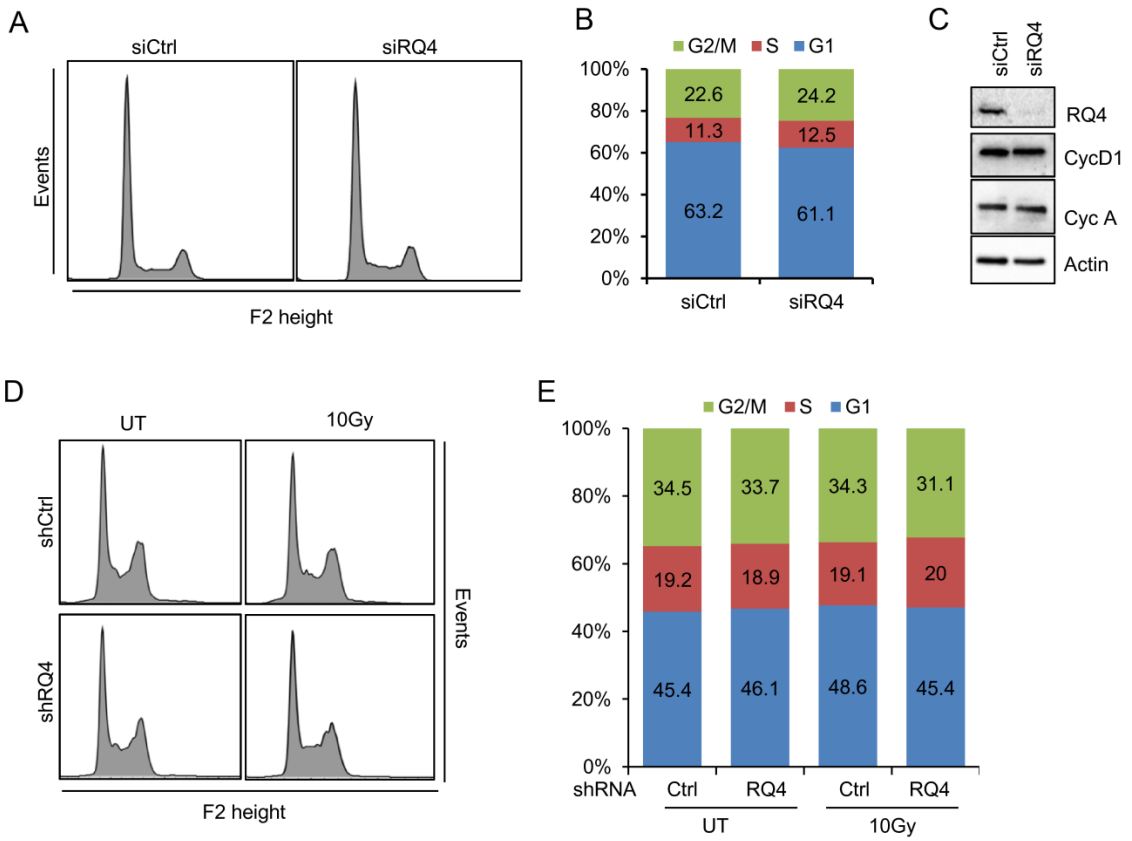


**Figure S2. RECQL4 loss did not alter cell cycle distribution in U2OS and HEK293T cells, related to Fig. 1 and 3. A.** Cell cycle of U2OS cells treated with *RECQL4* siRNA (siRQ4) or control siRNA (siCtrl) was analyzed by FACS after staining with propidium iodide. **B.** Quantification of the proportion of cells in the cell cycle phases using FlowJo. **C.** Expression level of Cyclin A and Cyclin D1 in DR-GFP-U2OS cells after control or RECQL4 siRNA treatment. **D.** Cell cycle analysis of RECQL4-depleted or control HEK293T cells by FACS after staining with propidium iodide. **E.** Quantification of the proportion of cells in the cell cycle phases from Panel D using FlowJo. For IR, cells were fixed 10 min post a 10 Gy IR.

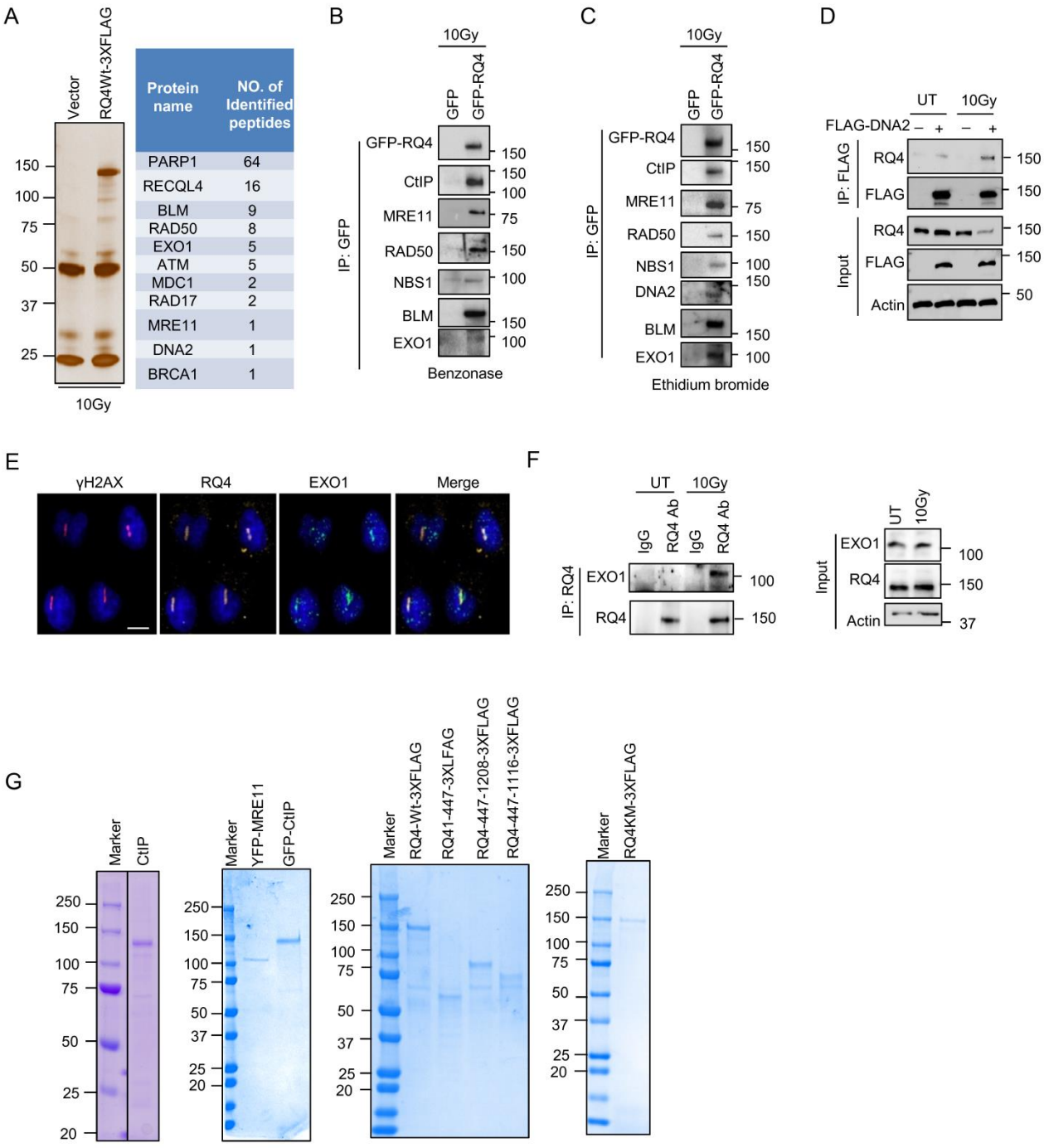


**Figure S3. RECQL4 interacts with DNA end resection proteins after IR, related to Figures 2 to 4. A.** Identification of RECQL4-interacting proteins by mass spectrometry, related to Figures 2-4. RQ4Wt-3XFLAG-was pulled down with its interacting partners in the presence of benzonase from HEK293T cells after IR and visualized by silver

staining. All identified proteins are listed in Supplemental Table 1. The interaction between RECQL4 and DNA end resection proteins were further confirmed using GFP-RECQL4 pull-down from irradiated HEK293T cells after IR in the presence of 20 U/mL benzonase (**B**) or 50 µg/mL ethidium bromide (**C**) in the IP lysis buffer. **D.** RECQL4 interacts with DNA2. RECQL4 was pulled down with FLAG-DNA2 from HEK293T cells in the presence of benzonase after IR. **E.** Co-localization of RECQL4 and EXO1 at laser-induced DSB sites in U2OS cells. **F.** Co-immunoprecipitation of EXO1 by RECQL4 antibody from irradiated U2OS cells in the presence of benzonase. Cells were allowed to recover for 10min after IR or laser treatment before immunoprecipitation or immunostaining. **G.** Coomassie blue staining gels with proteins used for *in vitro* immunoprecipitation. CtIP protein was purified from insect cells. YFP-MRE11 and GFP-CtIP were purified from HEK293T cells. 3XFLAG-tagged RECQL4 and it truncated fragments were purified from HEK293T cells. RQ4KM-3XFLAG was purified from HEK293T cells. The details of purification are described in the extended experimental procedures in the Supplemental materials.

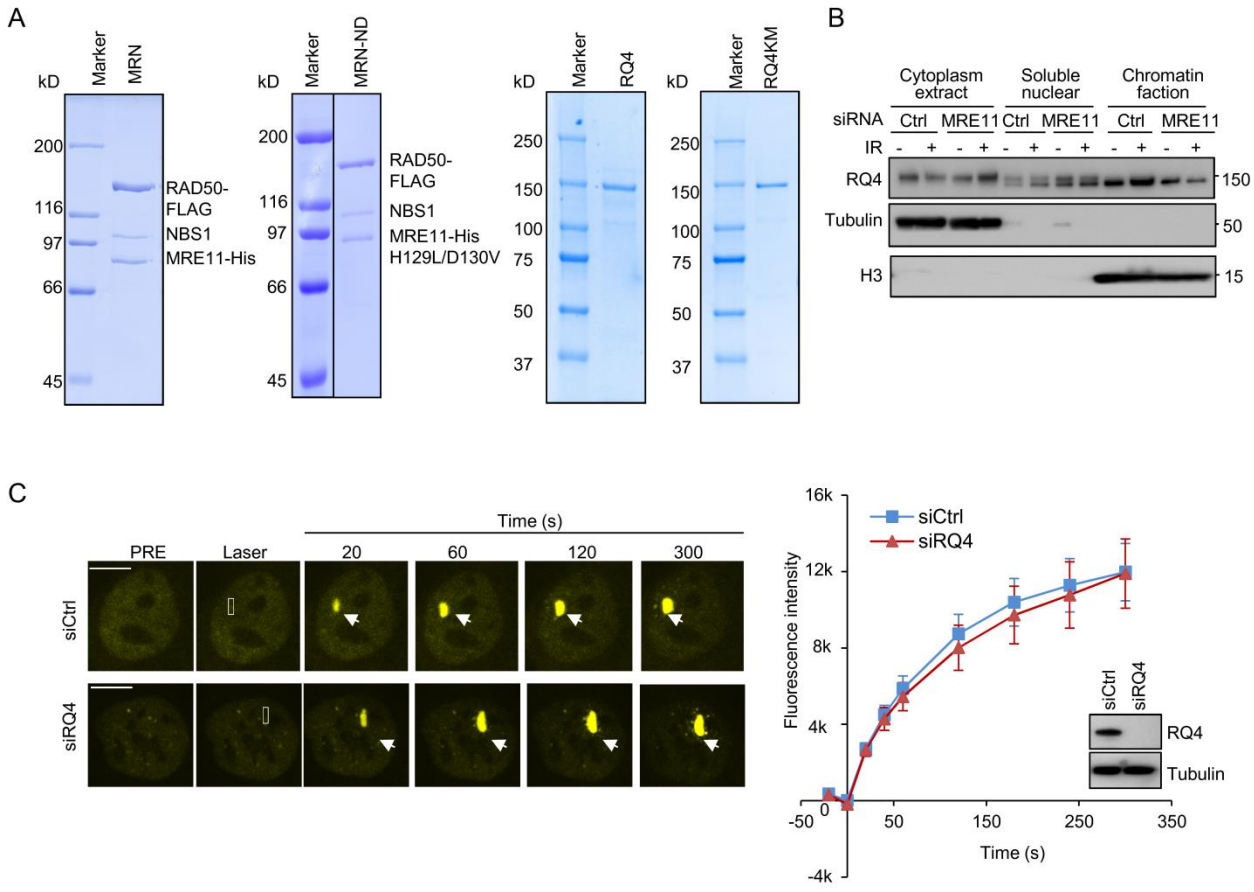


**Figure S4. Knockdown of RECQL4 does not affect recruitment of MRE11 to laser-induced DSBs, related to Figure 2. A.** Coomassie blue staining gel of recombinant MRN, nuclease-dead MRN mutant MRN-ND, wildtype RECQL4 and helicase dead RECQL4 mutant RQ4KM. **B.** Abundance of RECQL4 in subcellular fractions from control and MRE11-depleted U2OS cells. The cells were irradiated for a dose of 10 Gy, and allowed to recover under normal culture conditions for 10 min before subcellular fractionation. **C.** The recruitment of YFP-MRE11 to laser-induced DSBs in control or RECQL4-depleted U2OS cells. Data were presented as mean ± SEM from 25 cells. Bar, 10 μm.

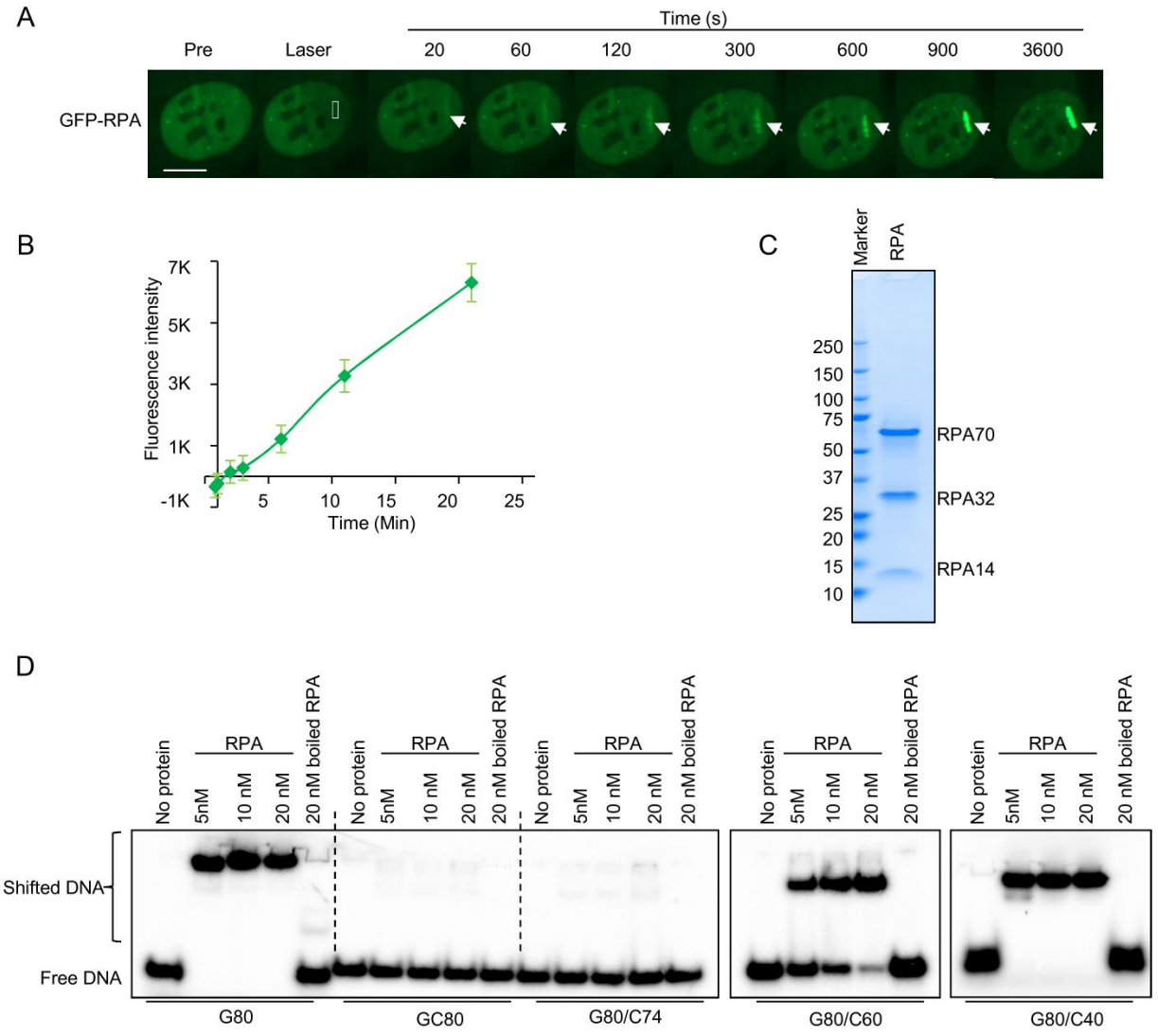


**Figure S5 Recruitment dynamics of GFP-RPA to laser-induced DSBs and DNA binding of RPA to DNA substrates by electrophoretic mobility shift assay, related to Figure 5. A.**Three plasmids expressisng the three GFP-tagged RPA subunits were transfected into U2OS cells. 24hrs after DNA transfection, the cells were summited for measuring rescruitment dynamics of GFP-RPA to laser-induced DSBs. Bar, 10 μm. **B.** Quantification of GFP-RPA fluorescence intensity were presented as mean ± SEM from 12 cells. **C.** Coomassie blue staining gel of recombinant RPA subunits. **D.** DNA binding

activity of RPA to ssDNA (G80), blunt dsDNA (GC80) and 3’ extended dsDNA substrates (G80/C74, G80/C60, G80/C40).

**Table S1** RECQL4-interacting proteins identified by mass spectrometry, related to Figures 2 to 4.

**Table S2** Information of oligo used in this study, related to all figures.

## EXTENDED EXPERIMENTAL PROCEDURE

### Cell culture, knockdown, DNA transfection, γ radiation and survival assay

U2OS, HEK293T and HeLa cell lines were cultured in DMEM medium with 10% fetal bovine serum (Sigma), 1X penicillin/streptomycin (Gibco). All cells were cultured in an atmosphere of 5% $CO_2$ at 37ºC. Lentivirus-mediated shRNA knockdown and siRNA knockdown was performed as previously described (Lu et al., 2014). The sequence of siRNA and shRNA are listed in Supplemental Table 2. Plasmid DNA were introduced into target cells with Polyplus JetPrime® according to manufacturer's instruction, and used for downstream experiment after 24-36 hrs. For γ radiation, γ rays were generated using a cesium-137 source (Gammacell Exactor 40, Best Theratronics). Radiation dose is 10 Gy IR, and post-irradiation recovery time is indicated in Figure legends. For colony survival assay, 3000 RECQL4-depleted U2OS or HeLa cells were seeded into 6 cm dishes 12 hrs before γ radiation treatment. Post irradiation, cells were cultured for 10 days, and then fixed and stained with 2% methylene blue in 5% ethanol. The colonies with over 50 cells were counted. The number of surviving colonies was normalized to the untreated to correct for plating efficiencies. The results are presented as mean from three independent experiments with SEM and P value by Student's *t*-test.

### Laser induced DNA damage and real time recruitment of fluorescence proteins

Laser-induced DSB were generated as previously described (Singh et al., 2010). Recruitment of GFP-tagged RECQL4 and GFP-tagged CtIP were performed as described (Singh et al., 2010). For mirin treatment, U2OS cells expressing GFP-RECQL4 were pre-

incubated with 100 μM mirin for 4 hrs under standard cell culture conditions, and then subjected for laser microirradation. For assessing the recruitment dynamics of GFP-CtIP, GFP-BLM and GFP-EXO1, *RECQL4* siRNA transfected U2OS cells were transfected with one additional plasmid three days after siRNA treatment, and then submitted for laser microirradation one day later. The results are presented as mean ± SEM, and P value were measured with Student's *t*-test.

**HR repair assay**

RECQL4 and other target proteins were knocked down by siRNA in the DR-GFP U2OS cells (a gift from Prof. Xiaofan Wang), and three days after siRNA transfection, the HR repair assay was performed according to the previous studies (Pierce et al., 1999; Wang et al., 2014). The plasmids resistant to siRQ4, pCMVTag4A-RQ4-siR and pCMVTag4A-RQ4KM-siR were generated by PCR with primers RQ4-siR-PF and RQ4-siR-PR (Supplementary Table 2) using pCMVtag4A-RQ4 and pCMVtag4A-RQ4KM as templates (Lu et al., 2014). RECQL4 siRNA were transfected into DR-GFP U2OS cells to deplete the endogenous RECQL4. Two day later, 0.5 μg siRNA-resistant plasmids pCMVTag4A-RQ4-siR or pCMVTag4A-RQ4KM-siR expressing 3XFLAG tagged wildtype RECQL4 and helicase-dead mutant RQ4KM, respectively, were transfected into $5X10^5$ RECQL4 siRNA-transfected DR-GFP U2OS cells. As control, the vector pCMVtag4A was transfected into RECQL4 siRNA-/control siRNA-transfected DR-GFP U2OS cells. 24 hr later, cells were transfected with I-SceI plasmid and then processed for HR assay as

described above. The results are presented as mean ± SEM from three independent experiments with P value determined by Student's *t*-test.

### *In vivo* DNA 5' end resection assay

*In vivo* 5' end resection was measured in AID-DIvA U2OS cells (a gift from Dr. Gaëlle Legube), as previously described (Zhou, Caron et al. 2014). Briefly, RECQL4 and other target proteins were knocked down by siRNA and AsiSI-mediated DSBs were induced by treating the cells with 4-OHT for 4h. Genomic DNA was isolated using Blood & Cell Culture DNA mini kit (Qiagen). Three µg of RNaseH treated genomic DNA was digested overnight with *Bam*HI, *Bsr*GI and *Hin*dIII digestion at 37$^{o}$ C. This assay is based on the assumption that ssDNA generated by resection events surrounding the DSB sites is resistant to digestion by restriction enzymes that by definition require duplex DNA. To quantify the extent of resection, the availability of selected DNA sites were measured by qPCR using 18 ng of restriction enzyme digested or undigested genomic DNA using specific Taqman probes (Zhou, Caron et al. 2014). The ssDNA percent was calculated from the change in Ct values of mock and restriction enzyme digested DNA using the following equation: ssdNA% = $1/(2^{\wedge(\Delta Ct-1)+0.5})*100$ (Zhou, Caron et al. 2014). At least three biological repeats were performed and data are presented as the mean $\pm$ SEM. For the rescue assay, RECQL4 siRNA were transfected into AID-DIvA U2OS cells to deplete the endogenous RECQL4. Two days later, 5 µg pCMVTag4A-RQ4-siR, pCMVTag4A-RQ4KM-siR or control vector was transfected into $2X10^{6}$ RECQL4 siRNA-transfected AID-DIvA

U2OS cells. 45 hr later, cells were treated with 4-OHT and then processed for the DNA end resection assay as described above.

**Western blotting, immunofluorescence microscopy and subcellular protein fractionation**

Western blot and immunofluorescence microscopy were performed as previously described (Lu et al., 2014). Antibodies used in this study are listed below. Subcellular fractions were isolated using a subcellular protein fractionation kit (Thermo Fisher) according to the manufacturer's instructions and the resultant fractions were analyzed with Western blotting. The following antibodies were used in immunofluorescence microscopy and western blotting: Anti-RECQL4 antibody (Santa Cruz, SC-16925), anti-γH2AX (pS139) (Millipore, 05-636), anti-RPA32 antibody (Millipore, NA18), anti-RPA32 (S4/S8) antibody (Bethyl Laboratories, A300-245A) anti-RECQL4 antibody (Lu et al., 2014), anti-EXO1 antibody (GeneTex, GTX109891), anti-CtIP antibody (Active Motif, 61141), anti-Mre11 antibody (Abcam, ab214), anti-FLAG antibody (Sigma, F1864), anti-Actin antibody (Sigma, A2228) , anti-Tubulin antibody (Sigma, T5168), anti-53BP1 antibody (BD Biosciences, 612522), anti-DNA2 antibody (Abgent, AP10182c), anti-NBS1 antibody (BD Biosciences, 611870), anti-Histone H3 antibody (Cell signaling, 9715), anti-BLM antibody (Abcam, 2179), anti-RAD50 antibody (BD Biosciences, 611010) and anti-GFP antibody (Abcam, ab38689). The following secondary antibodies were used in this study: Anti-mouse IgG Antibody (HRP-linked) and Anti-rabbit IgG Antibody (HRP-linked) from Cell Signaling, Rabbit

TrueBlot®: Anti-Rabbit IgG HRP and Mouse TrueBlot® ULTRA: Anti-Mouse Ig HRP from Rockland Immunochemicals Inc.

**Protein purification**

The MRE11-RAD50-NBS1 complex was purified from insect cells as previously described (Cheng et al., 2004). Purification of recombinant RECQL4 and helicase-dead mutant RQ4KM were performed as described (Rossi et al., 2010). Purificaiton of RPA was performed as described (Henricksen et al., 1994). For CtIP purification, pYFPn1 (Clontech) was modified with oligos GK131/GK132 and GK136/GK137 to insert a tag of ATG-9xHis-HA, and human rhinovirus 3C protease sites, respectively. The resulting plasmid was inserted with *CtIP* cDNA, which was derived from HEK293 cells by reverse transcriptase kit (Life technologies) with the primers GK119/GK120. The ATG-9xHis-HA-CtIP-YFP fragment was cut from the plasmid and insert into pFastbac1 for making the Bac-Bac expressing virus as described in manufacturer's protocol (Invitrogen). CtIP-YFP virus was incubated with SF21 insect cells for 72 hrs, and harvested, and then resuspended in 10 ml loading buffer (20 mM Tris-HCl pH 8.0, 500 mM NaCl, 2 mM β-Mercaptoethanol, 5 mM imidazole, 1X Roche EDTA-free protease inhibitor cocktail). Cells were disrupted with a homogenizer and centrifuged at 50,000 g for 1 hr at 4°C. The purification was performed with AKTA purifier 10 with frac 950 (GE-healthcare) at a 4ºC room. The supernatant was collected and loaded onto a Ni-column at 4°C. The column was washed loading buffer and then five volume loading buffer plus 30 mM imidazole. The protein was eluted with Elution buffer (50 mM HEPES-KOH pH 7.5, 300 mM NaCl, 2 mM β-mercaptoethanol, 300 mM imidazole),

then digested with 300 unit PreScission Protease (GE-healthcare) after adjusting buffer to include 1 mM DTT, 2 mM EDTA, 10% glycerol.). Selected fractions were pooled and loaded onto a 1 mL ResourceQ column pre-equilibrated with buffer A (50 mM HEPES-KOH pH 7.5, 100 mM KCl, 10% glycerol, 1 mM DTT). The protein was eluted using a gradient buffer B (50 mM HEPES-KOH pH 7.5, 500 mM KCl, 10% glycerol, 1 mM DTT). The CtIP fractions were identified by SDS-PAGE (Fig. S3G), pooled then snap frozen in liquid nitrogen.

3XFLAG-tagged RECQL4 and truncated fragments were purified from irradiated HEK293T cells. The following primers were used to PCR amplify the truncation fragments of RECQL4: Q4-1-F and Q4-427-R for the N-terminal fragment encoding amino acids (aa) 1-427 of human RECQL4; Q4-427-F and Q4-1116-R for the helicase fragment containing aa 427-1116; Q4-427-F and Q4-1208-R for the helicase plus C-terminal fragment containing aa 427-1208. The resulted plasmids were designated as pCMVtag4A-RQ4-1-427-NLS-3XFLAG, pCMVtag4A-RQ4-427-1116-NLS-3XFLAG and pCMVtag4A-RQ4-427-1208-NLS-3XFLAG. Each of the reverse primers encode the simian virus 40 large tumor antigen nuclear localization sequence (PKKKRKV) to ensure nuclear localization. The Not1 and Xho1 sites in the primers were used to subclone into a modified pCMVtag4A vector that encodes the 3XFLAG epitope following the Xho1 site. Thus each fragment expressed from this vector will contain additional aa that code for a nuclear localization followed by the 3XFLAG sequence. The entire open reading from each construct was sequence verified.

Briefly, pCMVtag4A-RQ4, pCMVtag4A-RQ4KM, pCMVtag4A-RQ4-1-427-NLS-3XFLAG, pCMVtag4A-RQ4-427-1116-NLS-3XFLAG or pCMVtag4A-RQ4-427-1208-NLS-3XFLAG were transfected into HEK293T cells. Twenty-four hours later, the cells were

irradiated with 10 Gy IR, washed with cold PBS, lysed and sonicated in Lysis Buffer 1 containing 50 mM HEPES-KOH 7.5, 500 mM NaCl, 0.5% NP-40, 10% Glycerol, 1X Sigma phosphatase inhibitor cocktail 2 and 3, 1X Sigma protease inhibitor cocktail. After removing debris by centrifuging, the supernatants were incubated with M2 FLAG-magnetic beads overnight at 4 $^{o}$C. The beads were then washed with Washing Buffer 1 (50 mM HEPES-KOH pH7.5, 1 M NaCl, 0.5% NP-40, 10% Glycerol), Washing Buffer 2 (50 mM HEPES-KOH pH7.5, 500 mM NaCl, 0.5% NP-40, 10% Glycerol), and Washing Buffer 3 (50 mM HEPES-KOH pH7.5, 150 mM NaCl, 0.5% NP-40, 10% Glycerol). Finally, the 3XFLAG-tagged proteins were eluted with Washing Buffer 3 containing 250 µg/mL 3XFLAG peptide (Sigma-Aldrich). Coomassie blue staining gels of these proteins are shown in Fig. S3G.

**Immunoprecipitation, pull down assay and protein identification**

Control and $\gamma$-irradiated U2OS cells were incubated for 10 minutes, then washed with cold PBS, resuspended in IP Lysis Buffer 2 containing 40 mM Tris-HCl pH 7.4, 150 mM NaCl, 2 mM $MgCl_2$, 0.2% NP-40, 0.4% Triton 100, 1X protease inhibitor cocktail (Thermo fisher), 1X phosphatase inhibitor cocktail 2 and 3 (Sigma-Aldrich), 20 U/mL benzonase (Novagen), and then sonicated on ice. The cell debris was removed by centrifuging at 20,000 xg for 30 minutes. The supernatant containing 2 mg of protein from treated or untreated cells were incubated with 2 µg of RECQL4 antibody (Lu et al., 2014) or normal Rabbit IgG (Thermo Fisher) overnight. Protein A/G agarose beads (Thermo Fisher) were then added and incubated for an additional 4 hrs at 4 $^{o}$C. The beads were washed with cold Washing buffer 4 (20 mM Tris-HCl, pH7.4, 150 mM NaCl, 0.2% Triton X100) for 5

times. The washed beads were resuspended in 1X SDS sample buffer, boiled, and then subjected to Western Blotting.

For FLAG immunoprecipitation, HEK293T cells were transfected with pCMVtag4A-RQ4 expressing RQ4Wt-3XFLAG (Lu et al., 2014) or vector DNA and treated with 10 Gy IR. After incubation for 10 min, cells were lysed in Lysis Buffer 2 as described above. M2 FLAG-magnetic beads (Sigma-Aldrich) were incubated with lysate to pull down RQ4Wt-3XFLAG and its interacting partners. The beads were washed with Washing Buffer 4 for 5 times and then subjected to Western blotting.

For GFP immunoprecipitation, HEK293T or its derivative cells were transfected with GFP-RECQL4, GFP-CtIP or YFP-MRE11, and 24 hrs later, cells were harvested and lysed in Lysis Buffer 2 as described above. For ethidium bromide treatment, 50μg/mL ethidium bromide was included in Lysis buffer 2. Lysates were incubated with GFP-TRAP beads (ChromoTek) overnight at 4 $^{o}$C. The beads were washed with Washing Buffer 4 for 5 times and subjected for Western blotting.

For *in vitro* immunoprecipitation, purified RECQL4 was incubated with purified MRN complex or CtIP in Binding Buffer 1(20 mM Tris-HCl pH7.4, 100 mM NaCl, 0.2% Triton X100) for 2 hrs at 4 $^{o}$C, and then divided into two parts, one part was incubated with rabbit anti-RECQL4 antibody, and the other with normal rabbit IgG (Life technologies). Four hours later, agarose protein A/G beads were added to capture the antibodies. After washing three times with Binding Buffer, the beads were mixed with 1X SDS sample buffer, boiled and analyzed by Western blotting.

GFP-CtIP or YFP-MRE11 were expressed in HEK293T cells, harvested and sonicated in Lysis Buffer 1. The supernatants were then incubated with GFP-TRAP beads (ChromoTek) and washed with Washing Buffer 1, 2, and 3 in order as described above. Finally, the beads were resuspended in Binding Buffer 2 (20 mM Tris-HCl pH7.4, 150 mM NaCl, 0.1% Triton X100) and mixed with 3XFLAG-tagged RECQL4 wildtype, helicase-dead mutant or the truncated fragments, and incubated at 4 $^{o}$C for 8 hrs. The beads were washed with Binding Buffer 2 for three times, and then subjected to Western blotting.

Silver staining was performed with the Pierce™ Silver Stain for Mass Spectrometry kit (Thermo fisher) according the manufacturer' instruction. For mass spectrometry, the proteins pulled down with RQ4Wt-3XFLAG were isolated by SDS-PAGE and stained with coomassie blue, and then the interested bands were sliced and submitted to Harvard Taplin Mass Spectrometry Facility. The details of mass spectrometry are listed in Supplemental Table 1.

**Nuclease assay**

Nuclease assays were carried out as described (Sartori et al., 2007). Briefly, after a 5 min pre-incubation with 20 nM MRN or nuclease-dead MRN-ND with 20 nM RECQL4 or helicase-dead RECQL4 RQ4KM or bovine serum albumin (New England Biolabs) on ice, reactions were activated by mixing the proteins with reaction buffer, which contains 20 mM MOPS, pH 7.2, 1 mM DTT, 5 mM $MgCl_2$, 5 mM $MnCl_2$, 1 mM ATP, and 50 ng PhiX174 circular ssDNA (New England Biolabs). After 3 hrs at 37 $^{o}$C, reaction was stopped with 1/10 (v/v) stopping buffer (2% SDS, 100 mM EDTA).The DNA separated on a 0.8% 1X TAE

agarose gel, stained with SYBR Gold (Life technologies), visualized with Chemidoc™ XRS+ system (Bio-Rad) and quantified with Bio-Rad Image Lab™ (Version 3.0). Data were presented as mean ± SEM from three repeats.

**Electrophoretic mobility shift assay**

Oligo G80 was labeled with γ-$^{32}$P at 5' end by T4 Polynucleotide Kinase (New England Biolabs), then annealed with unlabeled oligos C80, C74,C60 and C40, resulting in the substrates GC80, G80/C74, G80/C60 and G80/C40, respectively. The DNA substrates were incubated with RPA in the reaction buffer (20 mM HEPES pH7.4, 100 mM NaCl, 1 mM $MgCl_2$, 0.1% Triton X100, 200 ng/mL BSA) at RT for 15 min, and then isolated in 8% native-PAGE (Acrylamide/Bis-Acrylamide: 29:1). Images were captured on a GE Typhoon phosphorimager.

***In vitro* displacement assay**

A biotin-labeled oligonucleotide (G80) was annealed with C80, C74, C60 and C40 and resulted in dsDNA GC80 and 3' tailed dsDNA G80/C74, G80/C60, and GC40, respectively. See Supplemental Table 2 for a list of the oligonucleotides and their respective sequences. The ssDNA G80, duplex DNA GC80 and the three dsDNA with 3' overhang substrates were bound to M280-streptavidin beads (Life Technologies) according to manufacturer's instructions. RECQL4 (200 nM) was incubated with 20 nM DNA substrates in the binding buffer (20 mM HEPES pH7.4, 100 mM NaCl, 1 mM $MgCl_2$, 0.1%

Triton X100, 200 ng/mL BSA) at RT for 15 min, unbound RECQL4 was removed by washing with the binding buffer. The remaining RECQL4 coated DNA was divided into two parts. One part was incubated with 200 nM RPA and the other with 200 ng/mL BSA at RT for 15 min. The supernatants were then collected and RECQL4 displacement was determined by Western blotting.

**Supplemental Reference**


Cheng, W.H., von Kobbe, C., Opresko, P.L., Arthur, L.M., Komatsu, K., Seidman, M.M., Carney, J.P., and Bohr, V.A. (2004). Linkage between Werner syndrome protein and the Mre11 complex via Nbs1. J Biol Chem *279*, 21169-21176.

Kao, G.D., McKenna, W.G., Guenther, M.G., Muschel, R.J., Lazar, M.A., and Yen, T.J. (2003). Histone deacetylase 4 interacts with 53BP1 to mediate the DNA damage response. J Cell Biol *160*, 1017-1027.

Lu, H., Fang, E.F., Sykora, P., Kulikowicz, T., Zhang, Y., Becker, K.G., Croteau, D.L., and Bohr, V.A. (2014). Senescence induced by RECQL4 dysfunction contributes to Rothmund-Thomson syndrome features in mice. Cell Death Dis *5*, e1226.

Machwe, A., Xiao, L., Groden, J., Matson, S.W., and Orren, D.K. (2005). RecQ family members combine strand pairing and unwinding activities to catalyze strand exchange. J Biol Chem *280*, 23397-23407.

Myers, J.S., and Cortez, D. (2006). Rapid activation of ATR by ionizing radiation requires ATM and Mre11. J Biol Chem *281*, 9346-9350.

Peng, G., Dai, H., Zhang, W., Hsieh, H.J., Pan, M.R., Park, Y.Y., Tsai, R.Y., Bedrosian, I., Lee, J.S., Ira, G.*, et al.* (2012). Human nuclease/helicase DNA2 alleviates replication stress by promoting DNA end resection. Cancer Res *72*, 2802-2813.

Pierce, A.J., Johnson, R.D., Thompson, L.H., and Jasin, M. (1999). XRCC3 promotes homology-directed repair of DNA damage in mammalian cells. Genes Dev *13*, 2633-2638.

Rossi, M.L., Ghosh, A.K., Kulikowicz, T., Croteau, D.L., and Bohr, V.A. (2010). Conserved helicase domain of human RecQ4 is required for strand annealing-independent DNA unwinding. DNA Repair (Amst) *9*, 796-804.

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

Sartori, A.A., Lukas, C., Coates, J., Mistrik, M., Fu, S., Bartek, J., Baer, R., Lukas, J., and Jackson, S.P. (2007). Human CtIP promotes DNA end resection. Nature *450*, 509-514.

Singh, D.K., Karmakar, P., Aamann, M., Schurman, S.H., May, A., Croteau, D.L., Burks, L., Plon, S.E., and Bohr, V.A. (2010). The involvement of human RECQL4 in DNA double-strand break repair. Aging Cell *9*, 358-371.

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

Wang, Q., Goldstein, M., Alexander, P., Wakeman, T.P., Sun, T., Feng, J., Lou, Z., Kastan, M.B., and Wang, X.F. (2014). Rad17 recruits the MRE11-RAD50-NBS1 complex to regulate the cellular response to DNA double-strand breaks. EMBO J *33*, 862-877.