Supplementary Table 1. Antibodies used in this study.

Name	Description	Source/Reference	Catalogue number
RNA Polymerase II 8WG16 antibody	Mouse monoclonal IgG2a antibody recognizing the non-phosphorylated form of RNAPII CTD repeat YSPTSPS (used for ChIP)	Covance	MMS-126R
RNA Polymerase II H5 antibody	Mouse monoclonal IgM antibody recognizing the phosphoserine 2 version of RNAPII CTD repeat YSPTSPS (used for Western blot)	Covance	MMS-129R
RNA Polymerase II CTD repeat YSPTSPS (phospho S2) antibody	Rabbit polyclonal antibody recognizing the phosphoserine 2 version of RNAPII CTD repeat YSPTSPS (used for ChIP)	Abcam	ab5095
RNA polymerase II 7C2 antibody	Mouse monoclonal antibody that recognizes CTD irrespective of its phosphorylation status (used for Western blot)	A kind gift from Prof. Jean Marc Egly	
RECQ5 antibody	Rabbit polyclonal antibody raised against C-terminal fragment of RECQ5 (amino acids 675-991)	Kanagaraj et al (2006)	
GFP antibody	Rabbit polyclonal antibody against GFP (used for ChIP)	Abcam	ab290
β Tubulin (D-10) antibody	Mouse monoclonal antibody raised against a recombinant protein corresponding to amino acids 210-444 of human β-tubulin	Santa Cruz	sc-5274
Sheep anti-mouse IgG HRP	Secondary antibody used for Western blot	GE Healthcare	NA931V
Goat anti-mouse IgM HRP	Secondary antibody used for Western blot	Southern Biotech	1021-05

Supplementary Table 2. Sequences of primers and parameters of amplicons used for quantitative real time PCR analysis.

Amplicon	Sequences of primers	Annealing temperature (°C)	Length of amplicon (bp)	Central bp position relative to TSS
ACTG1				
1	FOR: 5′-ggaaagatcgccatatatggac-3′ REV: 5′-tcaccggcagagaaacgcgac-3′	57	174	-15
2	FOR: 5′-gctgttccaggctctgttcc-3′ REV: 5′-atgctcacacgccacaacatgc-3	57	134	+1055
3	FOR: 5′-gtgacacagcatcactaagg-3′ REV: 5′-acagcaccgtgttggcgt-3	57	134	+1722
4	FOR: 5′-tctgtcagggttggaaagtc-3′ REV: 5′-aaatgcaaaccgcttccaac-3	57	114	+2605
5	FOR: 5′-gtgacacagtgagaccctat-3′ REV: 5′-gatttgtaggcgttctttac-3	57	110	+4837
DHFR				
1	FOR: 5′-ctgcacctgtggaggagga-3′ REV: 5′-tccttgccctgccatgtctc-3′	50	143	+196
2	FOR: 5′-gttctatagtcactgcatcttagtc-3 REV: 5′-tgctaattctggttgttcagtaag-3	50	168	+16967
3	FOR: 5′-gagtatgtttctgtcttagattgg-3 REV: 5′-atgagaacctgctcgctgac-3	50	168	+26533
4	FOR: 5′-ttgtttcagggacagggtctt-3 REV: 5′-ctgtggtgggaagatggct-3	50	109	+46523
CDKN1A	FOR: 5′-ccagctgggctctgcaatt-3′ REV: 5′-ccttccagtccattgactg-3′	54	129	+7907
HSP70-2	FOR: 5′-cgtgctcatctttgacctgg-3′ REV: 5′-ggtggctcaccatgcggttg-3′	54	140	+1042

Note that ACTG1-3 and DHFR-2 amplicons were used for qPCR analysis shown in Figure 5. TSS, transcription start site; FOR, forward primer; REV, reverse primer; bp, base pair.

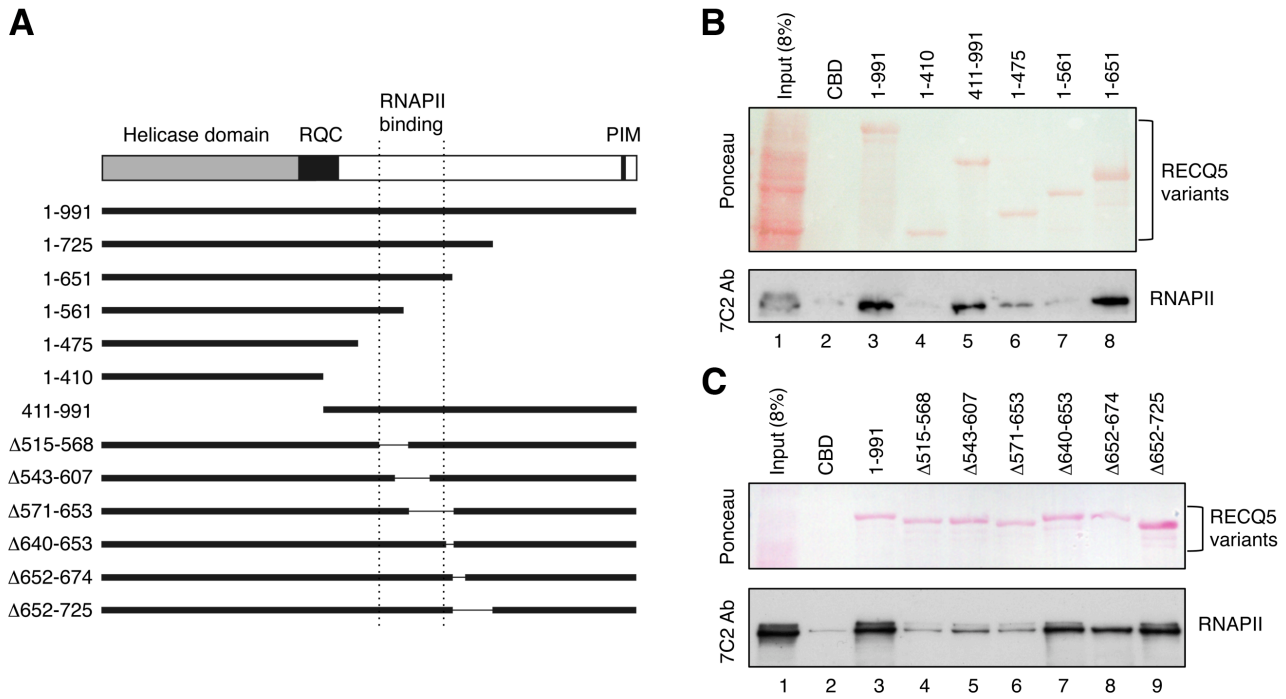

Figure S1. Mapping the RNAPII-interacting domain in RECQ5. **(A)** Domain organization of human RECQ5 and schemes of RECQ5 deletion variants used in this study. RQC, RecQ C-terminal domain, which contains a Zn^{2+}-binding motif that is essential for the helicase activity of RECQ5; PIM, PCNA-interacting motif. The dashed lines indicate the location of the RNAPII-interacting domain. **(B,C)** CBD pull-down assay. The indicated RECQ5 variants were produced in *E. coli* as fusions with the chitin-binding domain (CBD) and bound to chitin beads. Beads were incubated with a 293T cell extract and RNAPII binding was analyzed by Western blotting using the 7C2 antibody that binds to RNAPII CTD repeats irrespective of the phosphorylation status. Blots were also stained with Ponceau S to visualize RECQ5 and its variants. Note that phosphatase inhibitors were not included in these assays so that RNAPII is predominantly in the hypophosphorylated state.

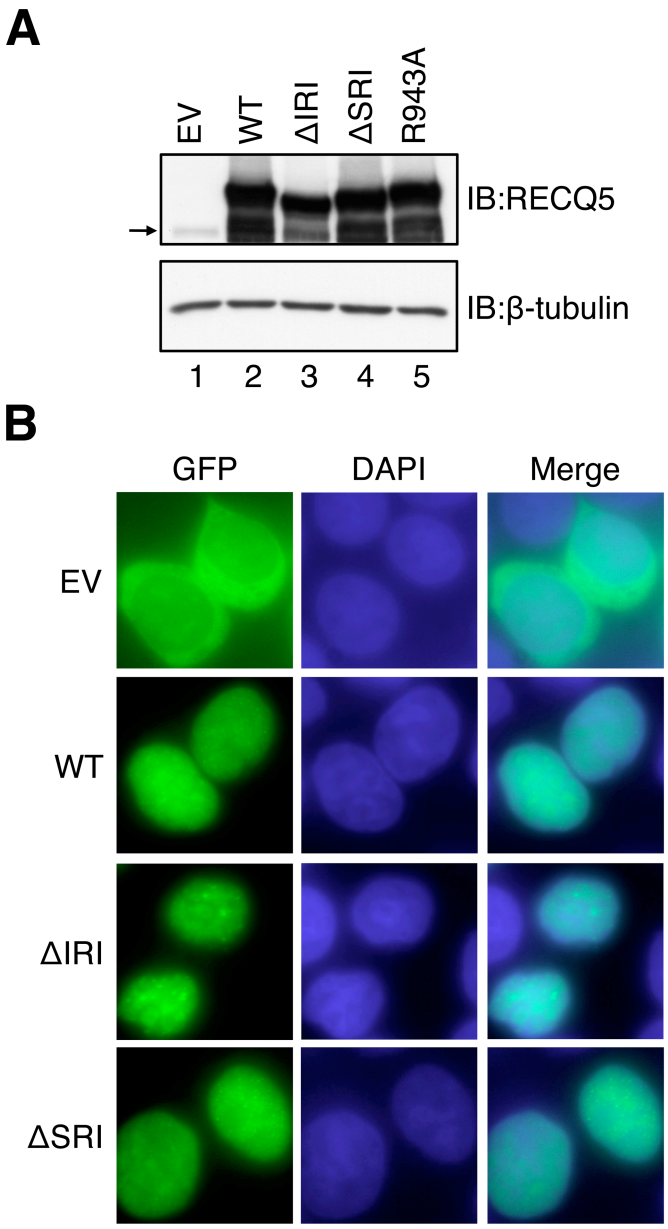

Figure S2. Expression of recombinant GFP-RECQ5 protein and its mutants in HEK293 cells. **(A)** Western blot analysis of extracts of HEK293 cells transiently transfected with constructs expressing GFP-tagged wild-type (WT) RECQ5 or its mutants (as indicated) or empty vector (EV). Transient transfection of expression vectors was performed as described in Materials and Methods. 48 hours post-transfection, whole-cell extracts (20 μg of total protein) were analyzed by Western blotting using antibodies against RECQ5 and β-tubulin (loading control). The arrow indicates the position of endogenous RECQ5 protein. Note that the endogenous RECQ5 in lanes 2-5 is masked by degradation products of recombinant RECQ5 proteins **(B)** Subcellular localization of GFP-RECQ5 and its mutants over-expressed in HEK293 cells. 48 hours post-transfection, cells were fixed with ice-cold methanol at -20 °C for 30 minutes. Fixed cells were washed thrice with PBS and mounted on slides using Vectashield mounting medium containing DAPI (Vector Laboratories, Inc.). Images were captured on an Olympus IX81 fluorescence microscope with a CCD camera (Orca AG, Hamamatsu) using cellR software (Olympus). IB: immunoblot.

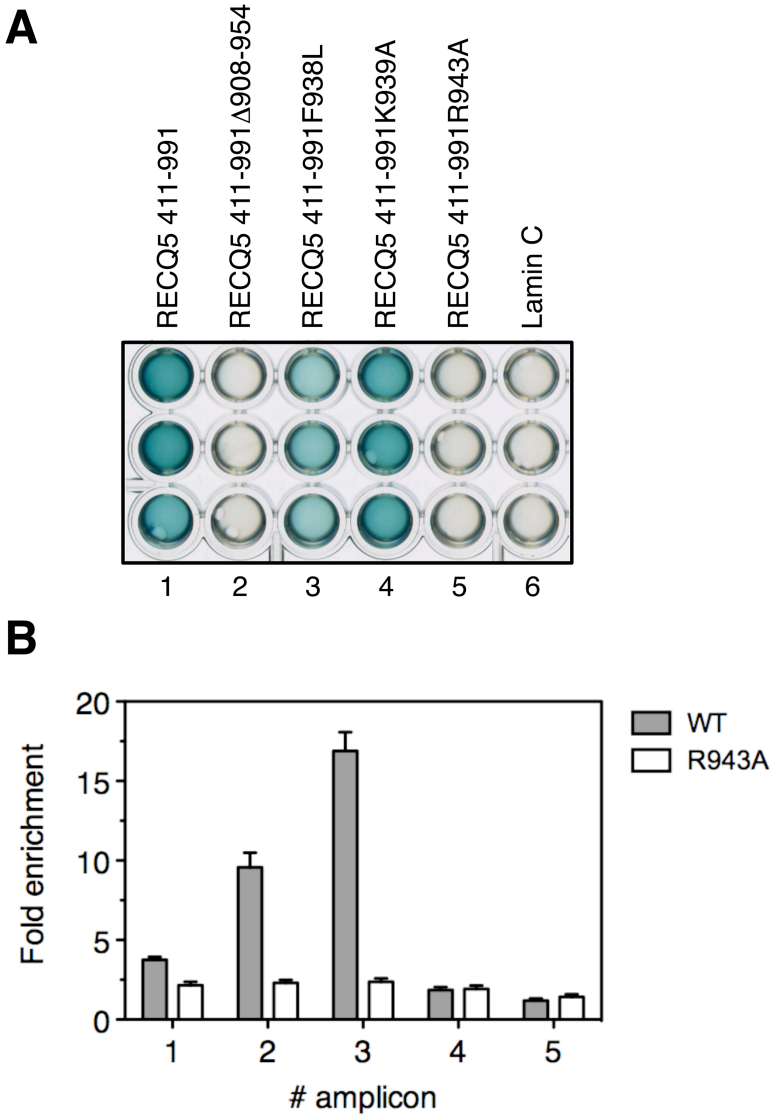

Figure S3. Mutational analysis of the conserved residues in the SRI domain of RECQ5. **(A)** Interaction of RECQ5 mutants with the CTD in the yeast two-hybrid system. Clones carrying the bait (RECQ5) and prey (CTD) plasmids were tested for β-galactosidase activity using the pellet X-gal assay. Blue color indicates a positive interaction. **(B)** Effect of the R943A substitution in the SRI domain of RECQ5 on its association with the ACTG1 gene. HEK293 cells were transfected either with empty vector (mock) or with vectors expressing wild-type (WT) or mutant (R943A) forms of RECQ5 as fusions with GFP. Forty-eight hours post-transfection, chromatin was immunoprecipitated using an anti-GFP antibody and subjected to qPCR analysis with the same amplicons as in Figure 4A (*top panel*). Fold enrichment was calculated as described in the legend of Figure 5.

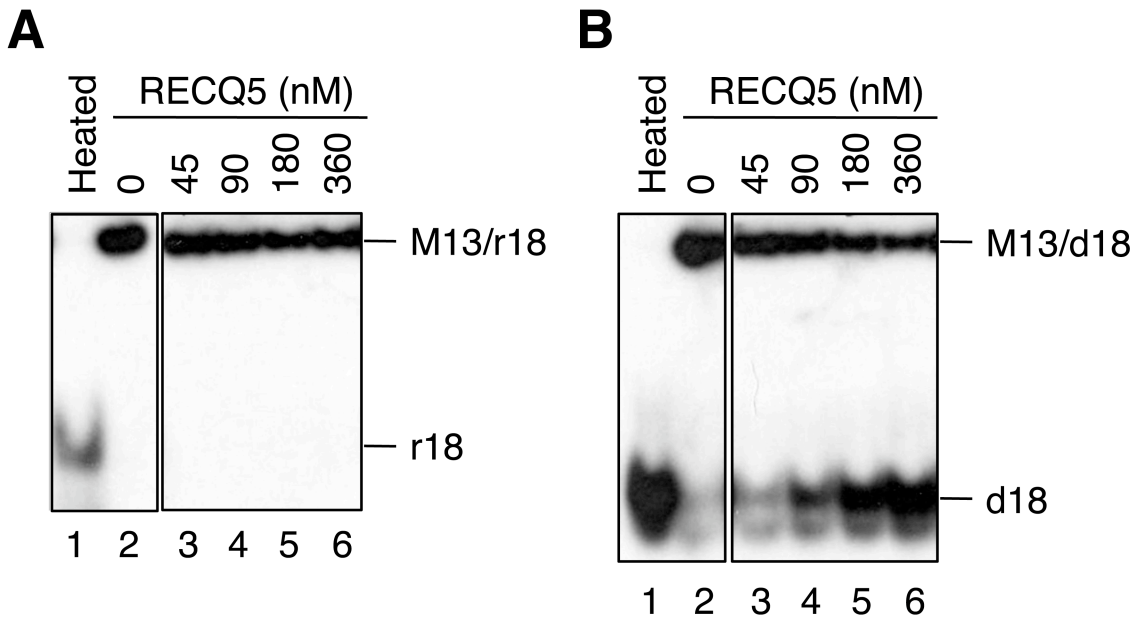

Figure S4. RECQ5 can not unwind RNA:DNA hybrid duplexes. Helicase activity of RECQ5, at indicated concentration, was measured on 1 nM M13mp2/r18 (DNA:RNA; panel A) and 1 nM M13/d18 (DNA:DNA; panel B) partial duplexes, respectively, as described in Materials and Methods. Lane 1, heat-denatured substrate.