

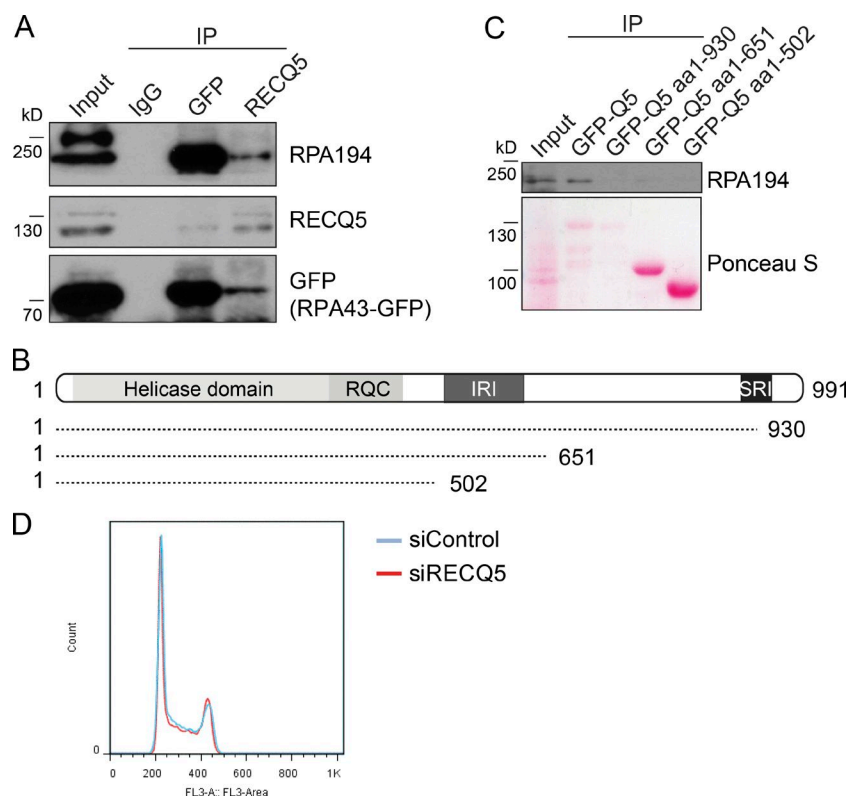
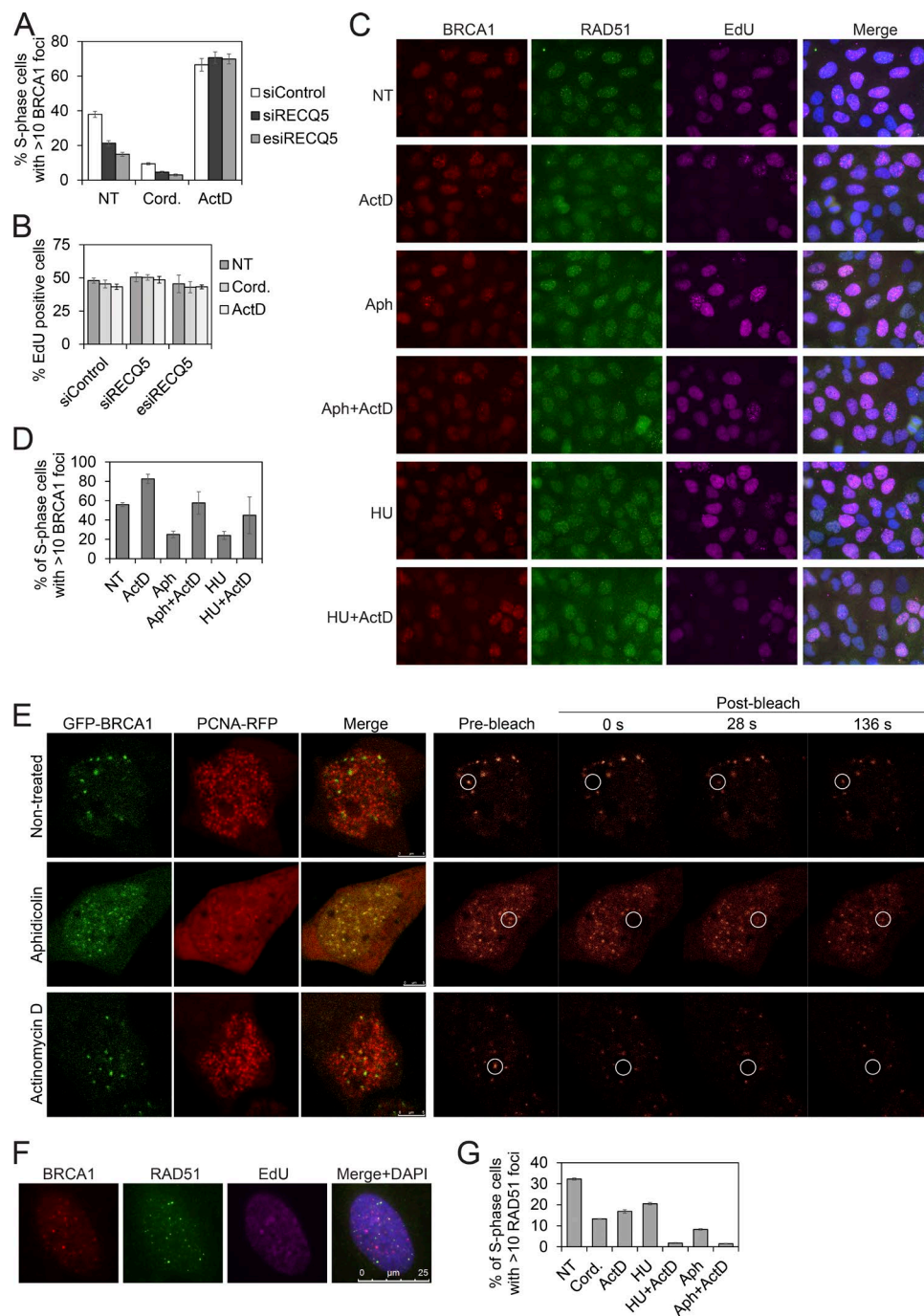
Urban et al., <http://www.jcb.org/cgi/content/full/jcb.201507099/DC1>

Figure S1. **Interaction between RECQ5 and RNAPI.** (A) Reciprocal coimmunoprecipitation of RECQ5 and RNAPI. Ectopically expressed subunit of RNAPI, RPA43-GFP, was pulled down from HEK293 cell extract using GFP-Trap\_A beads. RPA194, the catalytic subunit of RNAPI. (B) Scheme of truncated variants of RECQ5 used for mapping of its RNAPI-interacting domain. Helicase domain, RecQ-C terminal (RQC) domain, IRI domain and SRI domain are depicted. (C) Coimmunoprecipitation of RPA194 with indicated RECQ5-GFP variants expressed in HEK293 cells. GFP-RECQ5 variants are visualized by Ponceau S staining. Note that GFP-RECQ5 aa 1–930 is referred to as GFP-RECQ5ΔCt. (D) Cell cycle distribution of mock- and RECQ5-depleted (siRECQ5 #1) HEK293 cells.



**Figure S2. BRCA1 and RAD51 form foci at sites of interference between replication and transcription.** (A) Quantitative analysis of BRCA1 foci in mock- and RECQ5-depleted U2OS cells represented in Fig. 5 A. Where indicated, cells were treated with cordycepin (Cord.; 50  $\mu$ M) for 2 h or ActD (1  $\mu$ g/ml) for 1 h before fixation. EdU-positive cells containing more than 10 BRCA1 foci were scored as positive. (B) Effect of ActD (1  $\mu$ g/ml) and cordycepin (50  $\mu$ M) on EdU incorporation in mock- and RECQ5-depleted cells. Percentage of EdU-positive cells is plotted. (C) Representative images of U2OS cells stained for BRCA1, RAD51, and EdU upon treatment with replication and/or transcription inhibitors as indicated. Aphidicolin (Aph; 5  $\mu$ M) and hydroxyurea (HU; 2 mM) were added 90 min before fixation, whereas ActD (1  $\mu$ g/ml) was added 1 h before fixation. EdU was added 1 h before addition of replication inhibitors. For ActD-treated cells, EdU was added 1 h before fixation. Nuclei were stained with DAPI. NT, nontreated. (D) Quantitative analysis of BRCA1 foci in EdU-positive nuclei represented in C. Cells containing more than 10 foci were scored as positive. (E) Representative images from a FRAP sequence showing fluorescence recovery of GFP-BRCA1 foci in mock-, Aph-, and ActD-treated HEK293 cells. Cells were transfected with GFP-BRCA1 and PCNA-RFP constructs, and measurements were performed 6–9 h after release of cells from NC block. (F) Partial colocalization of BRCA1 and RAD51 foci in EdU-positive U2OS cells. Nuclei were stained with DAPI. (G) Quantitative analysis of RAD51 foci in EdU-positive nuclei represented in C. Cells containing more than 10 foci were scored as positive. For A, B, D, and G, data are represented as mean  $\pm$  SD.

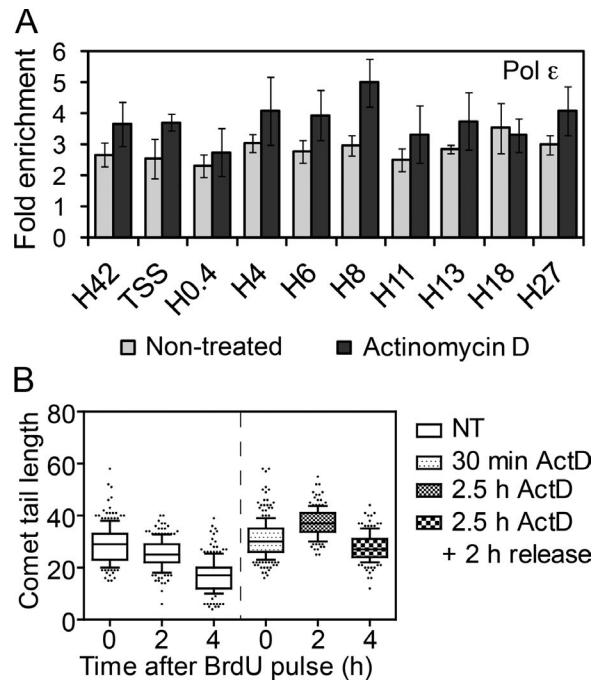


Figure S3. **Halted transcription complexes form a barrier for replication fork progression.** (A) Occupancy of Pol  $\epsilon$  on rDNA repeat unit in nontreated and ActD-treated (1  $\mu\text{g}/\text{ml}$ ) cells. ChIP assay was performed on chromatin used in Fig. 1 B. Data are represented as mean  $\pm$  SD. (B) Statistical analysis of BrdU-positive comet tail lengths measured for mock- or ActD-treated U2OS cells. ActD (1  $\mu\text{g}/\text{ml}$ ) was added 10 min before the BrdU pulse labeling (20 min) and readed after the BrdU wash-off. Whiskers indicate 10th to 90th percentile.

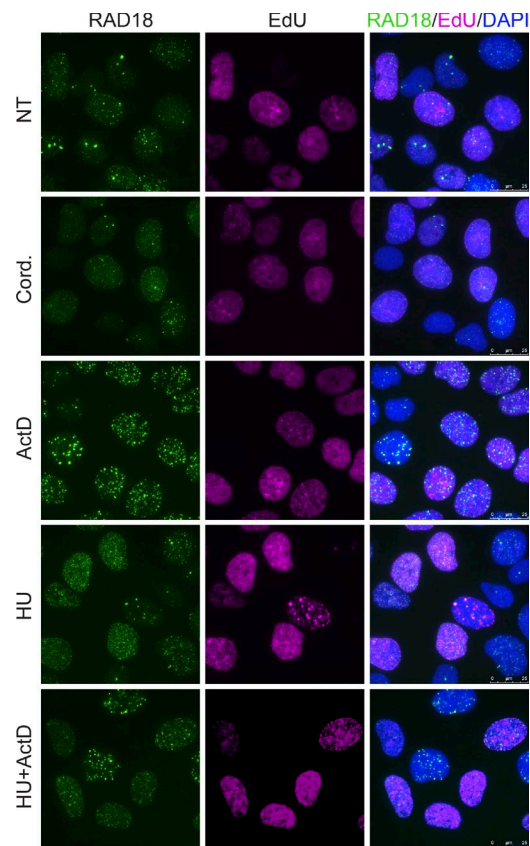


Figure S4. **RAD18 forms foci at sites of interference between replication and transcription.** Representative images of U2OS cells stained for RAD18 and EdU upon treatment with replication and/or transcription inhibitors as indicated. HU (2 mM) was added for 90 min, ActD (1  $\mu$ g/ml) was added for 1 h, cordycepin (Cord.; 50  $\mu$ M) was added for 2 h. EdU was added 1 h before addition of HU or fixation. Nuclei were stained with DAPI. NT, nontreated.

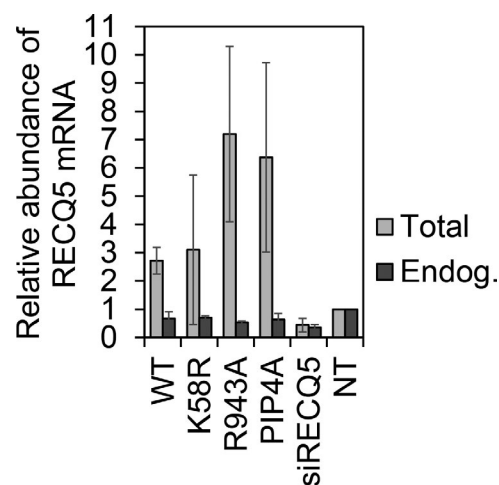


Figure S5. **Relative expression of Flag-tagged RECQ5 variants and endogenous RECQ5 in U2OS T-REx cells.** Relative mRNA abundance of endogenous or total RECQ5 (endogenous plus Flag-tagged wild-type RECQ5, RECQ5-K58R, RECQ5-R934A, or RECQ5-PIP4A) normalized to ALAS1 (5'-aminolevulinate synthase 1). Cells were cultured in the presence of doxycycline (0.4 ng/ml) for 48 h. Parental U2OS T-REx cells were transfected with siControl or siRECQ5. RNA was isolated using TRI reagent (Sigma-Aldrich). cDNA was prepared using the Superscript III reverse transcription (Invitrogen) with 1  $\mu$ g total RNA in the reaction. cDNA was analyzed by qPCR using primers to distinguish endogenous RECQ5 (forward primer match to shRNA silent mutations in Flag-tagged variants) from total RECQ5 (Table S1). Data are represented as mean  $\pm$  SD.

Table S1. Sequences of primers used for quantitative real-time PCR

Amplicon	Forward (5' to 3')	Reverse (5' to 3')
H42	AGAGGGGCTGCGTTTTCGGCC	CGAGACAGATCCGGCTGGCAG
TSS	CCCGGGGAGGTATATCTTT	CCAACCTCTCCGACGACA
H0.4	CAGGCGTTCTCGTCTCCG	CACCACATCGATCGAAGAGC
H4	CGACGACCCATTGGAACGTCT	CTCTCCGGAATCGAACCTGA
H6	CAGCTAGCTGCGAGAATTAATG	CGATTGATCGGCAAGCGAC
H8	AGTCGGGTTGCTTGGGAATGC	CCCTTACGGTACTTGTTGACT
H11	GGACCAGGGGAATCCGAC	CGCTTCATTGAATTTCTTCAC
H13	ACCTGGCGCTAAACCATTCGT	GGACAAACCTTGTGTCGAGG
H18	GTTGACGTACAGGGTGGACTG	GGAAGTTGTCTTCACGCCTGA
H27	CCTTCCACGAGAGTGAGAAGCG	CTCGACCTCCCGAAATCGTACA
H33	ATCTCTTGACCTCGTGACCCG	TTGCGTTTCTCTGGACTGACTTC
Oct-4	GTATTCAGCCAAACGACCATC	CCCACCCTTACCTCCTGAAG
RSP19	GAGGCAGAGGTTGCAGTGAGTC	CTGGTAGAGAACAAGCTCCCAT
RPL22	GTGAACGGAAAAGCTGGGAAC	CTGGGCACTGGGTGCACGCA
ACTG1	GGTGACACAGCATCACTAAGGG	GACAGCACCGTGTTGGCGTA
ACTG1-I	GGTGACACAGTGAGACCCTATCT	GGCGTTCTTTACATATTGTGGAT
RECQL5, total	GTGAACAGCTGGCCATAGAGC	GGACCTTCTCCTCCATCCAGTC
RECQL5, endogenous	CCTTTACAGGAGAGTGCGACC	GGAGAGACTACAATGGTGATGC
ALAS1	CCACTGGAAGAGCTGTGTGATGTG	GCGATGTACCCTCCAACAACCC