SUPPLEMENTARY MATERIALS AND METHODS

RIP Library

For library preparation of the cDNA converted from RNA isolated from BRCT RIP, poly(A) was added to the end of the RNAs using *E. coli* poly(A) polymerase (NEB) in the presence of RNase inhibitor. After poly(A) addition, beads were washed and treated with proteinase-K to release RNA from the beads. RNA was purified by acid/phenol/chloroform/isoamyl alcohol and converted to dsDNA by reverse transcription (RT) using superscript II reverse transcriptase in the presence of oligo-dT. cDNA was amplified by PCR using oligo-dT and TSO, as shown in Fig. 3C, followed by TA cloning (Thermo Fisher Scientific). TA cloning vectors containing the isolated cDNA were transformed into *E.coli* DH5alpha. Plasmids from 40 positive colonies were isolated and sequenced.

SUPPLEMENTARY FIGURE LEGENDS

Fig. S1. Nucleolar localization of BRCA1.

Representative field images showing immunofluorescent staining of BRCA1 (green, left) and Nucleolin (red, middle) in U2OS cells.

Fig. S2. Nucleolar localization of the ectopically expressed BRCA1 FL and BRCT.

Representative field images showing immunofluorescent staining of FLAG (top, green) and Nucleolin (red, middle) in UWB1 cells transfected with vector, FLAG-BRCA1, FLAG-BRCA1 BRCT WT or FLAG-BRCA1 BRCT S1655A mutant.

Fig. S3. BRCA1 suppresses R-loops at rDNA.

(A) Representative images showing immunofluorescent staining of R-loops (green) using S9.6 antibody in HEK293T (top) and UWB1 cells (bottom).

(B) Representative images showing immunofluorescent staining of R-loops (green) using S9.6 antibody in HCC1937 cells expressing FL BRCA1 or empty vector.

(C) Representative images showing dot blot analysis of the increasing amount of DNA isolated from UWB1 cells with or without BRCA1 expression and with or without RNase H treatment. Membranes were probed with S9.6 antibody (left) and dsDNA antibody (right).

(D) Quantification of the S9.6 dot blot analysis shown in (C) using Image J.

(E) Representative images showing dot blot analysis of the increasing amount of DNA isolated from HCC1937 cells with or without BRCA1 expression and with or without RNase H treatment. Membranes were probed with S9.6 antibody.

(F) Quantification of the S9.6 dot blot analysis shown in (E) using Image J.

Fig. S4. Binding specificity of BRCA1 BRCT.

(A) Percentage of the indicated RNA species co-purified with BRCA1 BRCT fragment by RIP from HEK293T cells transfected with FLAG-BRCA1 BRCT.

(B) Representative image of the electrophoretic mobility shift assay of GST-BRCA1 BRCT WT binding to ^{32}P-end labeled ssDNA containing non-rDNA sequence (left) and rDNA sequence (right).

(C) The percentage of ssDNA bound by GST-BRCA1 BRCT in (B) was quantified by Image J.

Fig. S5. Effect of DNA damage and as-rRNA on R-loops.

(A) Representative image showing northern blot analysis of the RNA samples prepared from UWB1 cells with or without ectopic BRCA1 BRCT expression using a mixture of oligo probes complementary to detect as-5.8S, as-18S and as-28S sequences.

(B-C) DRIP analysis using S9.6 antibody to measure RNase H–sensitive R-loop levels at 5.8S rDNA loci (B) and ACTB EX3 region (C) normalized to the input DNA in UWB1 cells with or without UV irradiation and with or without as-5.8S rRNA overexpression. Y-axis represents fold difference relative to UWB1 cells without UV irradiation or as-5.8S rRNA overexpression.

(D) ChIP analysis of γH2AX levels at 5.8S rDNA loci normalized to input DNA from UWB1 cells with or without UV irradiation and with or without as-5.8S rRNA overexpression. Y-axis represents fold difference relative to UWB1 cells without UV irradiation or as-5.8S rRNA overexpression.

Fig. S6. Effect of BRCA1 on rRNA processing and protein synthesis.

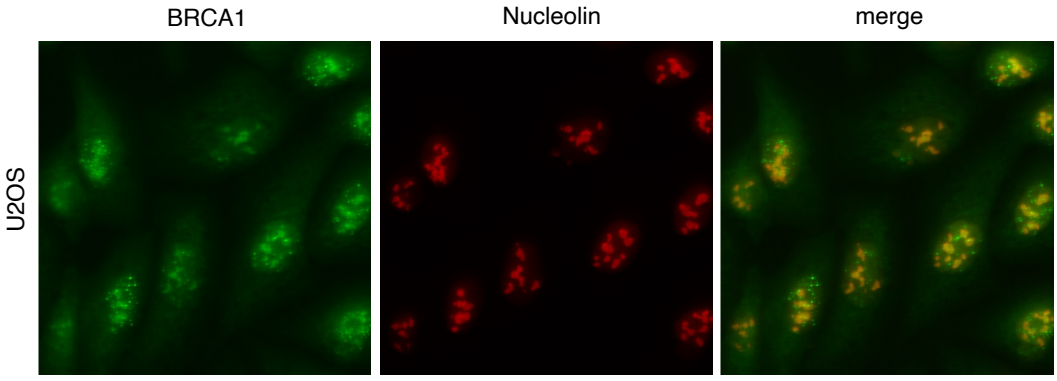
(A) Representative image showing northern blot analysis of the RNA samples prepared from HCC1937 and MDA-436 cells with or without ectopic expression of BRCA1 using an oligo probe complementary to 5.8S sequence.

(B) Representative images of Ponceau staining (left) and western blot analysis (right) using anti-puromycin antibody to detect proteins with puromycin incorporation in UWB1 cells with or without ectopic expression of BRCA1 and with or without treatment with puromycin.

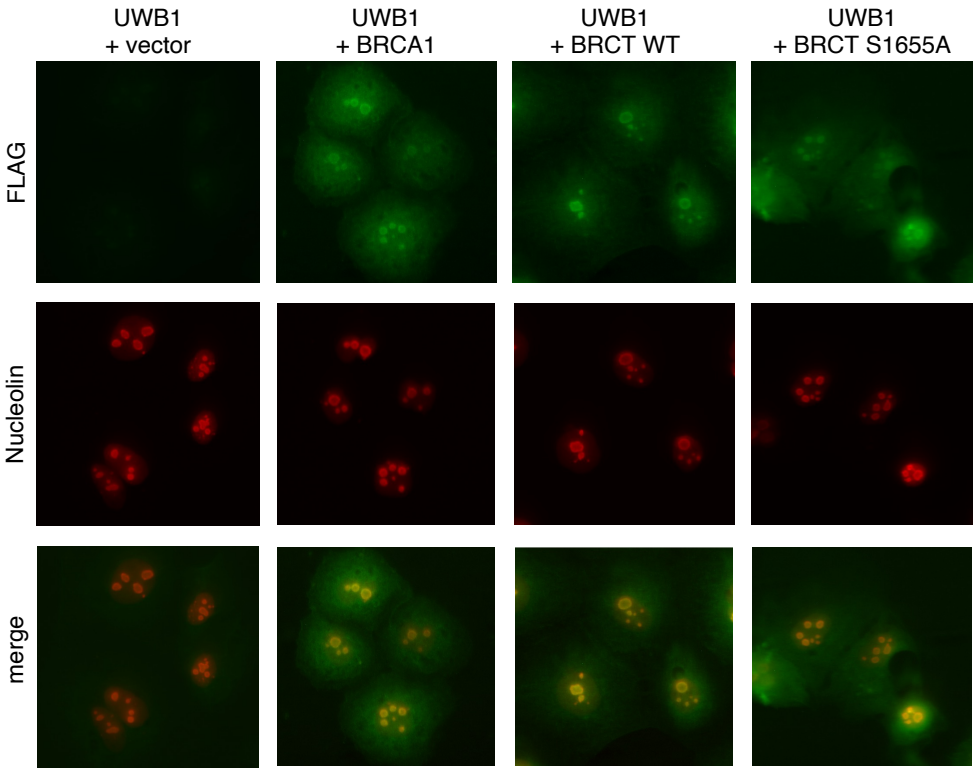
(C) Western blot analysis of BRCA2 using an anti-BRCA2 antibody in whole cell extracts prepared from HEK293T cells transfected with control or BRCA2 - specific siRNAs. β-actin is used as a loading control.

(D) Representative image showing northern blot analysis of the RNA samples prepared from HEK293T cells with or without transfection with BRCA2 siRNA using an oligo probe complementary to 5.8S sequence.

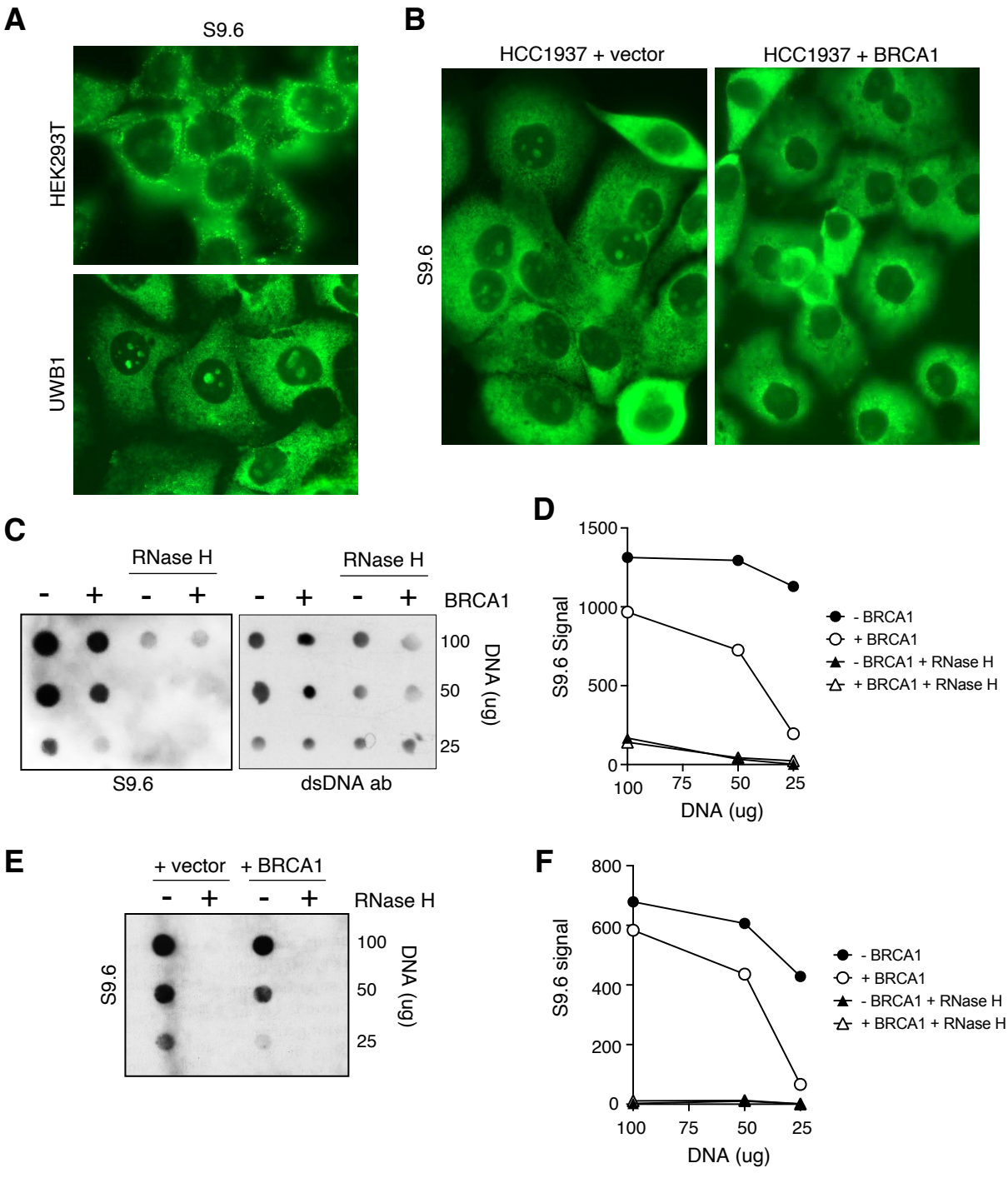
(E) Representative images of Ponceau staining (left) and western blot analysis (right) using anti-puromycin antibody to detect proteins with puromycin incorporation in HEK293T cells transfected with control or BRCA2 siRNA and with or without treatment with puromycin.



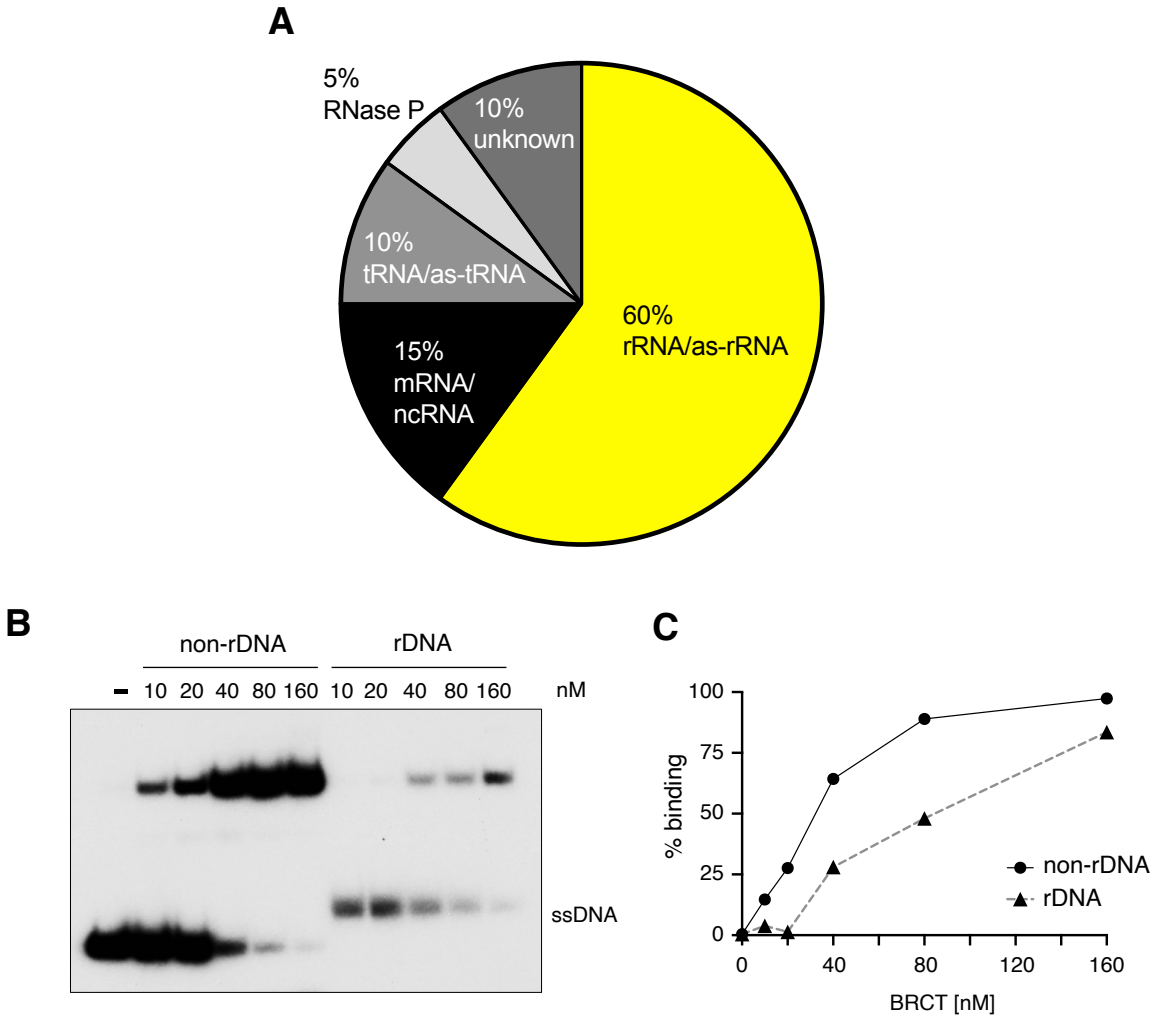
Chang et al, Supplementary Fig. 1



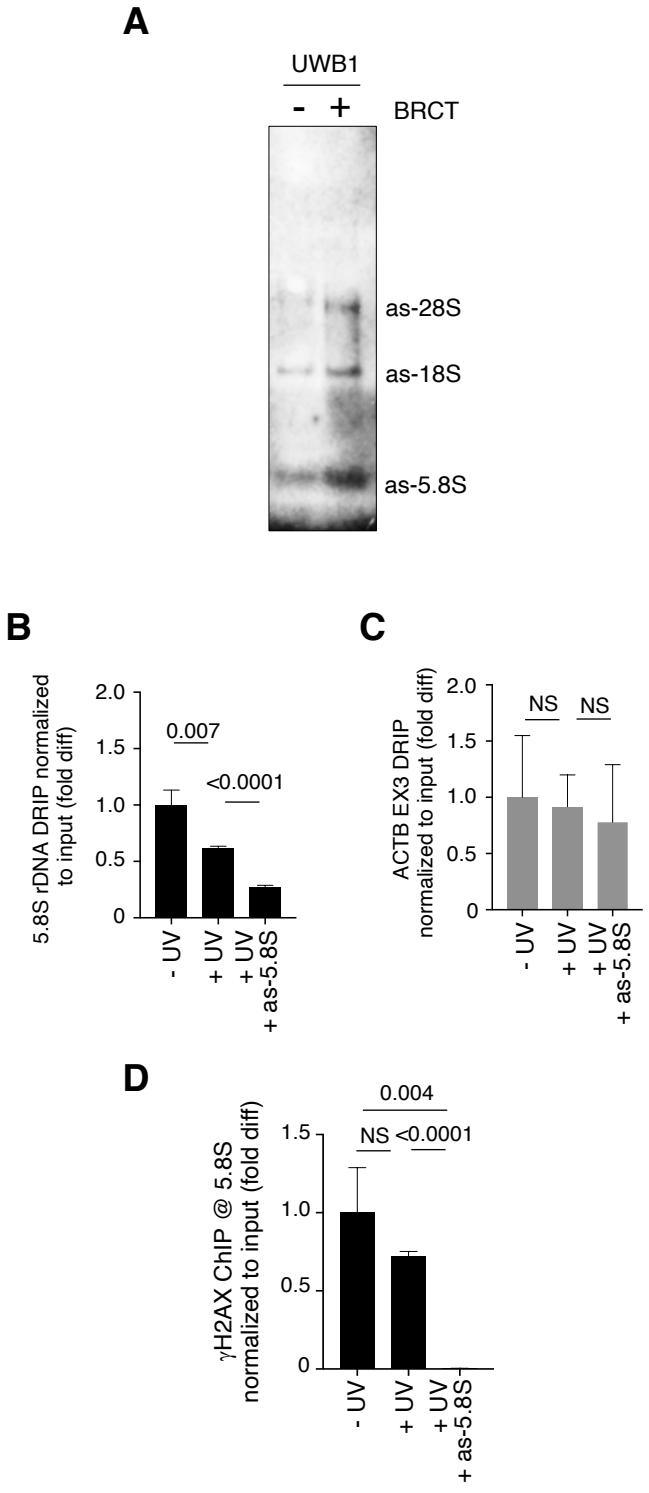
Chang et al, Supplementary Fig. 2



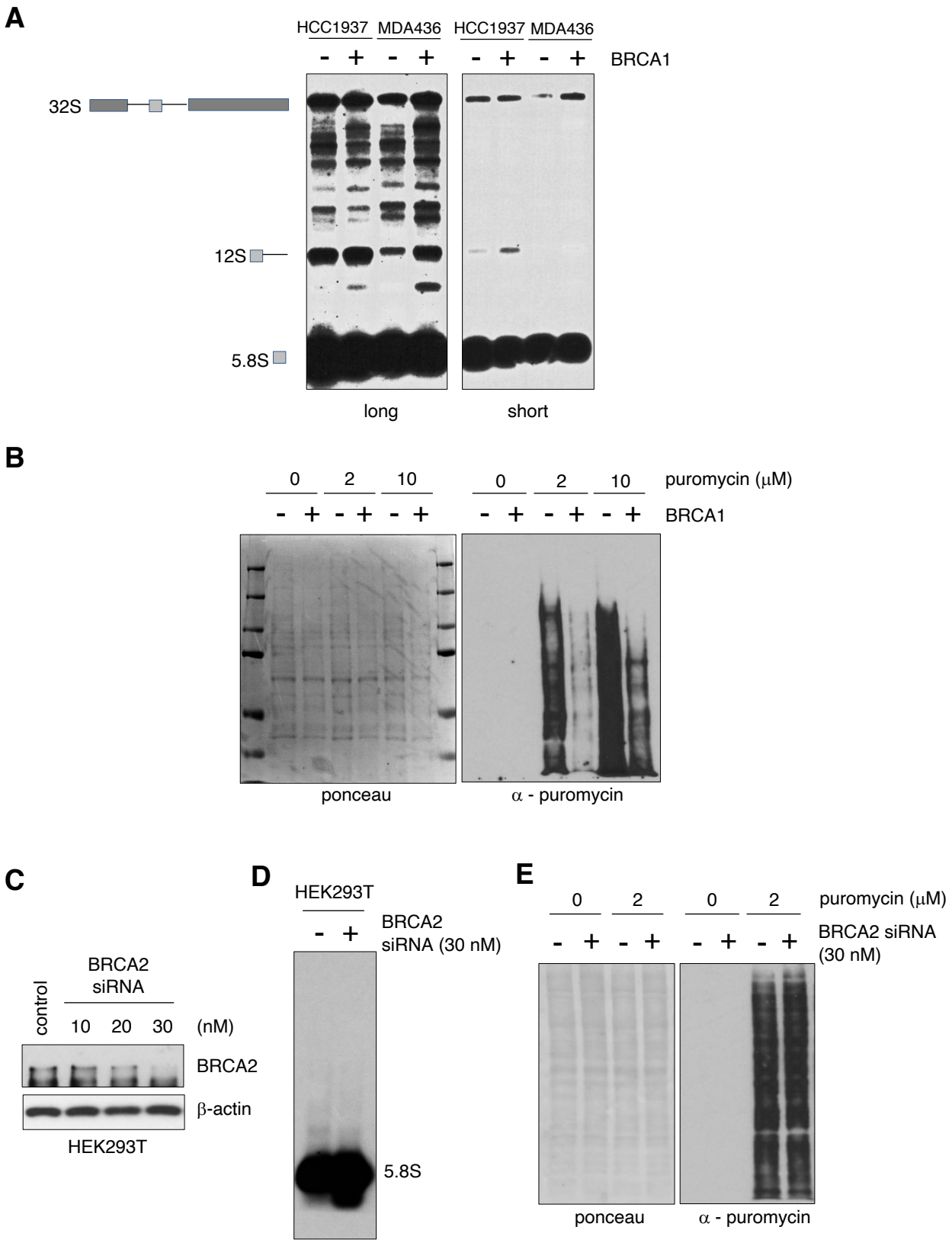
Chang et al, Supplementary Fig. 3



Chang et al, Supplementary Fig. 4



Chang et al, Supplementary Fig. 5



Chang et al, Supplementary Fig. 6