

## Supplementary information

### The essential function of RAD52 protein for viability of BRCA-deficient cells

Kritika Hanamshet and Alexander V. Mazin.

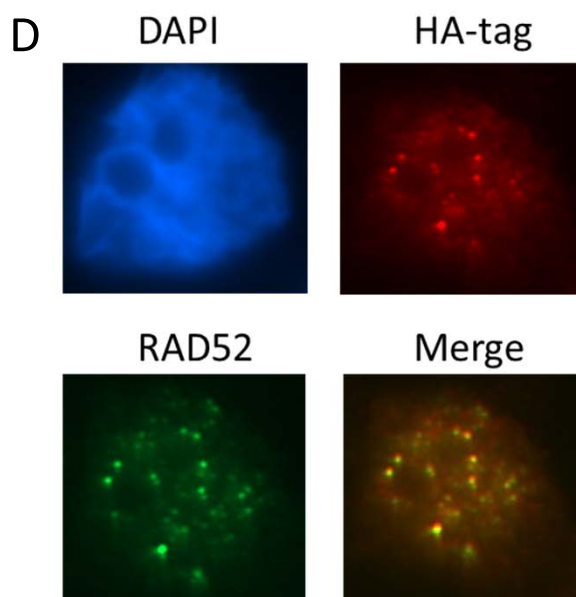
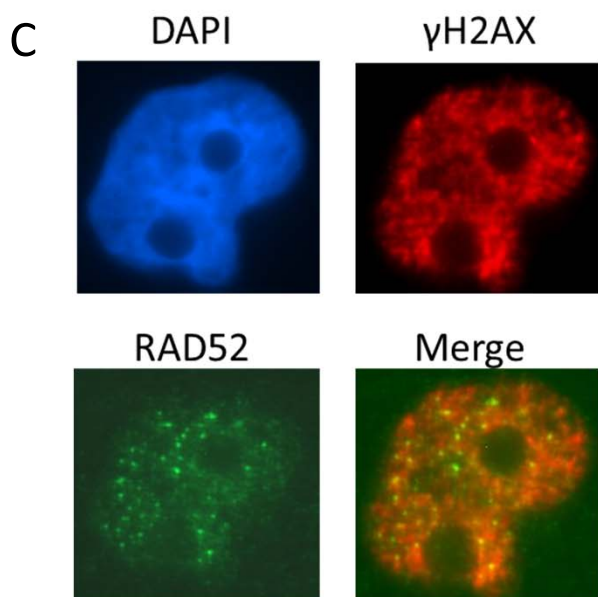
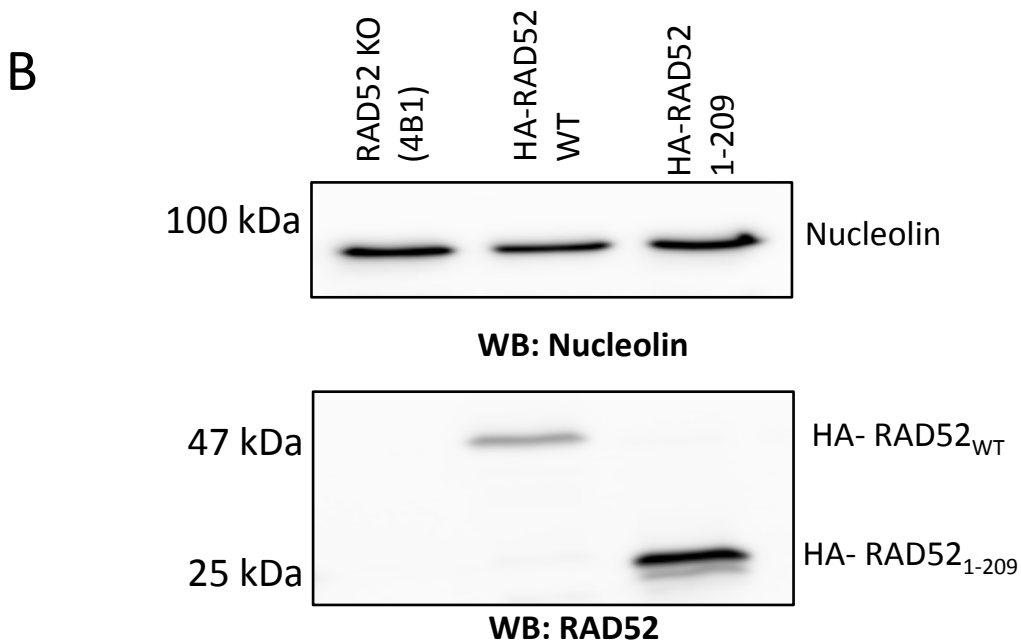
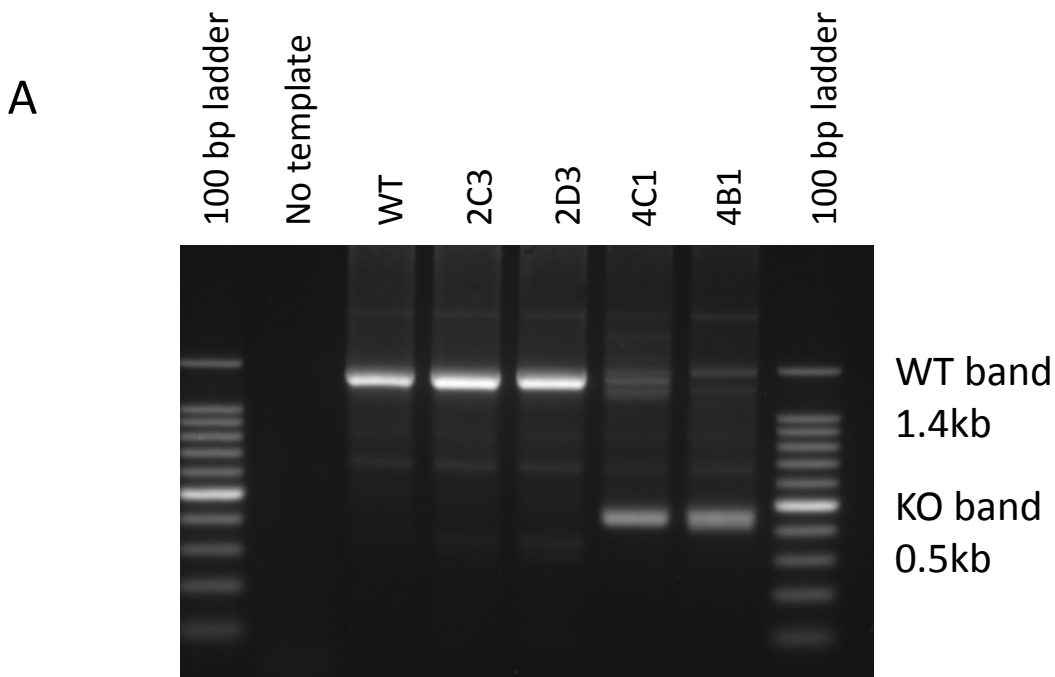
*Department of Biochemistry and Molecular Biology, Drexel University College of Medicine, Philadelphia, PA 19102, USA.*

\*Corresponding Author:  
Alexander Mazin, Ph. D.  
Drexel University College of Medicine  
Department of Biochemistry and Molecular Biology  
245 N 15<sup>th</sup> Street, MS 497, NCB, Room 10103  
Philadelphia, PA 19102-1192  
Tel: 215-762-7195; Fax: 215-762-4452  
E-mail: avm28@drexel.edu

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Figure S1



MDA-MB-436 cells expressing shRAD52 and exogenous HA-RAD52<sub>1-209</sub>

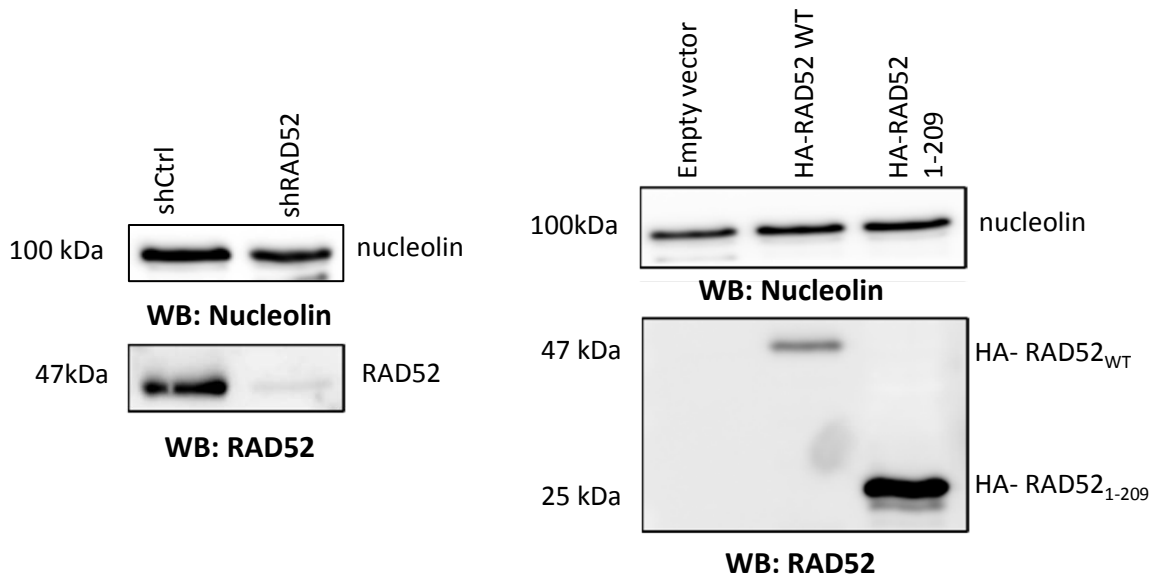
## Figure S1

$RAD52_{1-209}$  forms foci in response cisplatin treatment. (A) Knock-out of *RAD52* by CRISPR-Cas9 in different U2OS cell clones was confirmed by PCR. (B) The expression of exogenous HA- $RAD52_{WT}$  or HA- $RAD52_{1-209}$  proteins in 4B1 U2OS cell clone was confirmed by Western blot. (C) and (D) Representative images of MDA-MB-436 cells stably expressing  $RAD52_{1-209}$  and sh $RAD52$ , stained 6 h after cisplatin (10  $\mu$ M) treatment with DAPI (blue), or probed with fluorescent anti-bodies for  $RAD52$  (green),  $\gamma$ -H2AX (red) in (C), and  $RAD52$  (green), HA-tag (red) in (D).

Figure S2

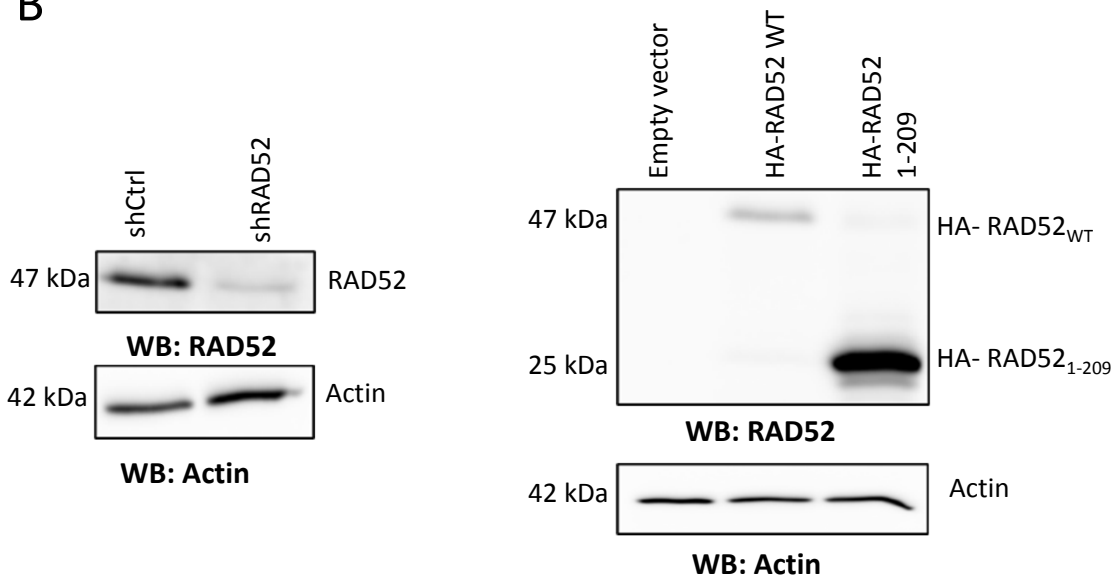
A

MDA-MB-436 cells treated with shRAD52 targeting 3'UTR



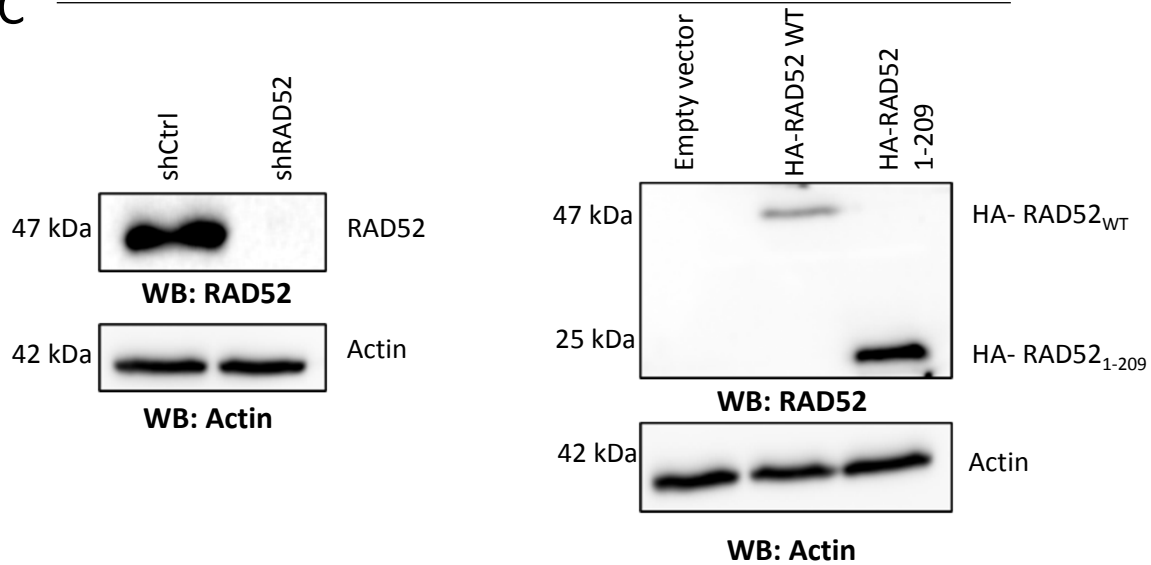
B

HCC1937 cells treated with shRAD52 targeting 3'UTR



C

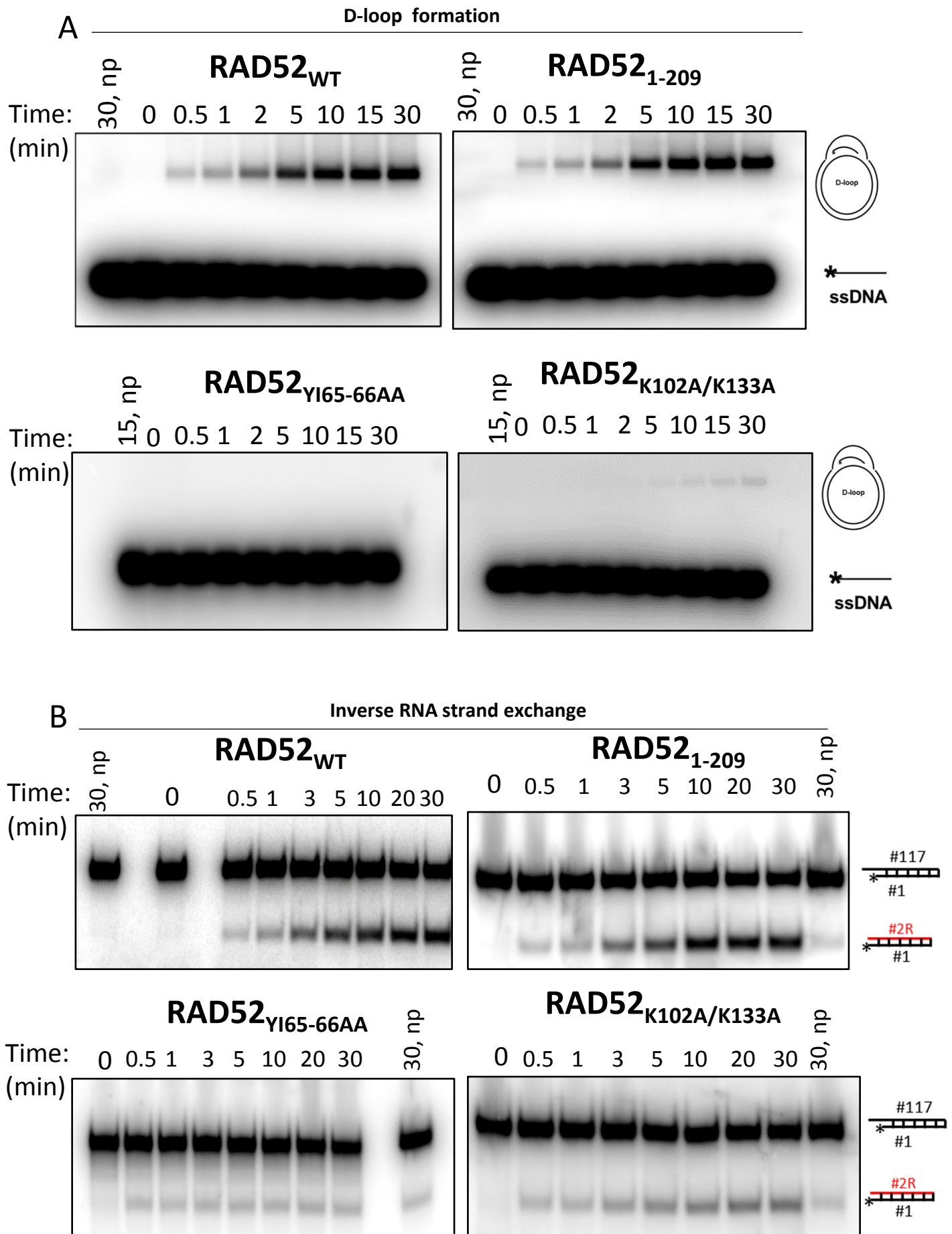
CAPAN1 cells treated with shRAD52 targeting 3'UTR



## Figure S2

Western blot analysis of knock-down of endogenous RAD52. Cells treated with shRAD52 and control shRNA (shCtrl) (left panels) and expression of exogenous HA-RAD52<sub>WT</sub> or HA-RAD52<sub>1-209</sub> in the cells treated with shRAD52 (right panels) in MD-MB-436 (A), HCC1937 (B), and CAPAN1 (C) cells.

Figure S3

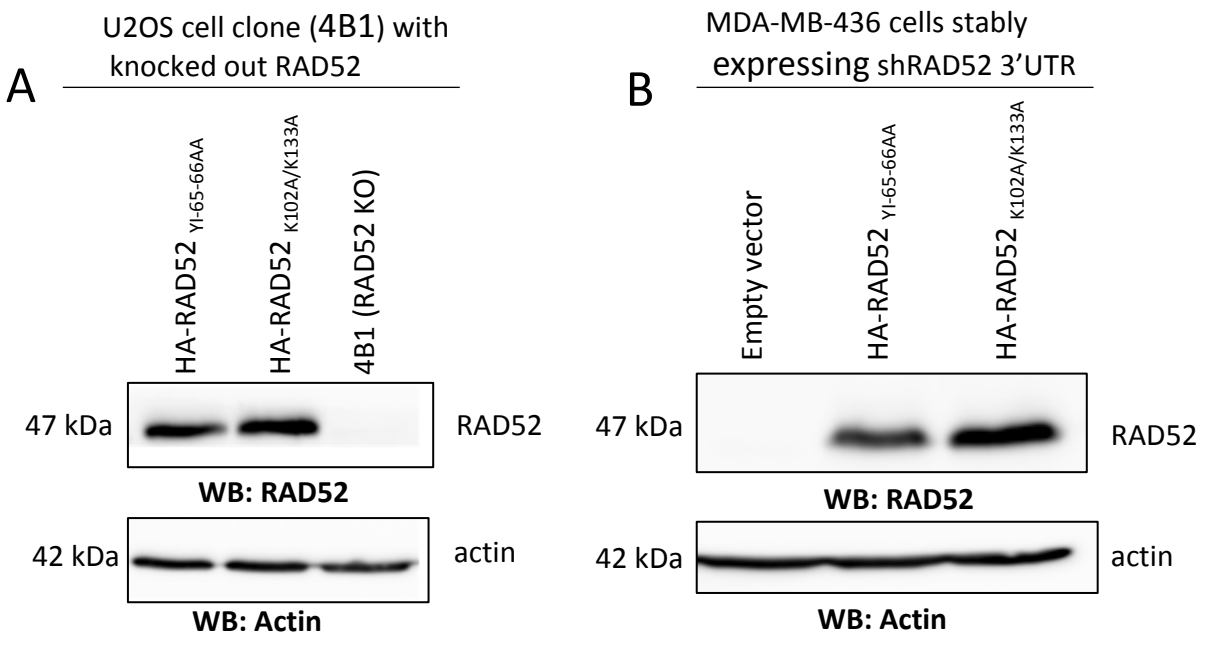


np: no protein

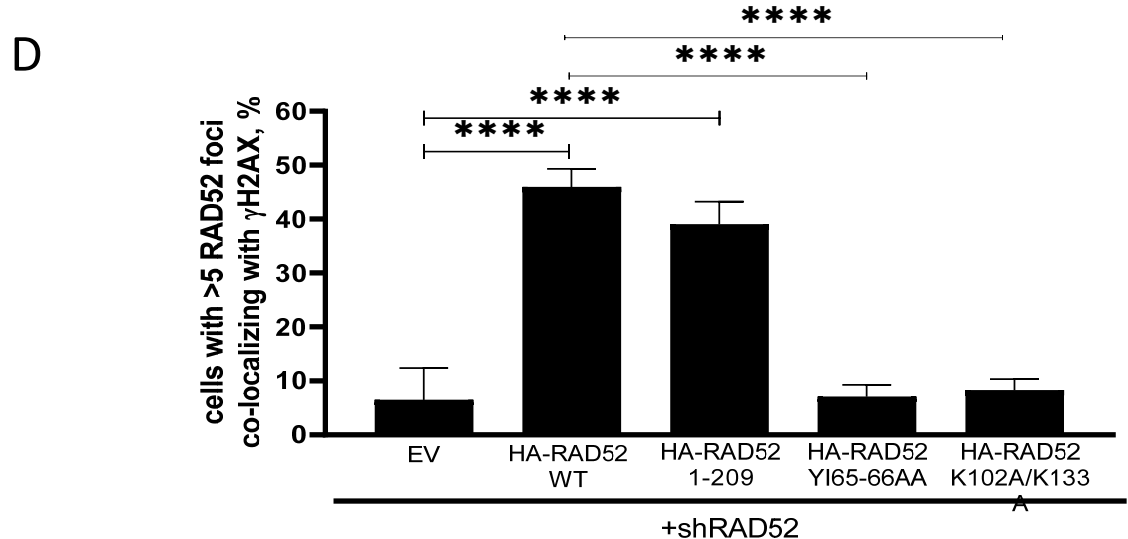
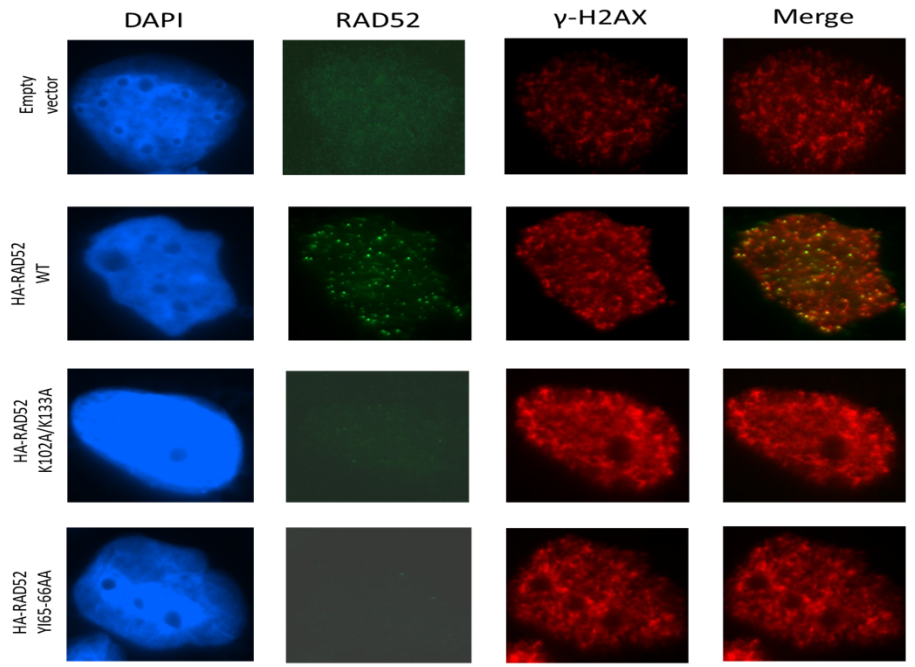
### Figure S3

D-loop formation and inverse RNA strand exchange activity of RAD52<sub>WT</sub> and RAD52 mutants. (A) Representative images of agarose gels containing D-loops formed by for RAD52<sub>WT</sub>, RAD52<sub>1-209</sub>, RAD52<sub>Y165-66AA</sub> or RAD52<sub>K102A/K133A</sub>. The gels were quantified, and the data presented graphically in Figure 3C. (B) Representative images of polyacrylamide gels after inverse RNA strand exchange assay for RAD52<sub>WT</sub>, RAD52<sub>1-209</sub>, RAD52<sub>Y165-66AA</sub> and RAD52<sub>K102A/K133A</sub>. The gels were quantified, and the data presented graphically in Figure 3E. In “np: no protein” control, RAD52 protein was substituted by storage buffer.

Figure S4



**C** MDA-MB-436 cell stably expressing shRAD52 + cisplatin (10  $\mu$ M)

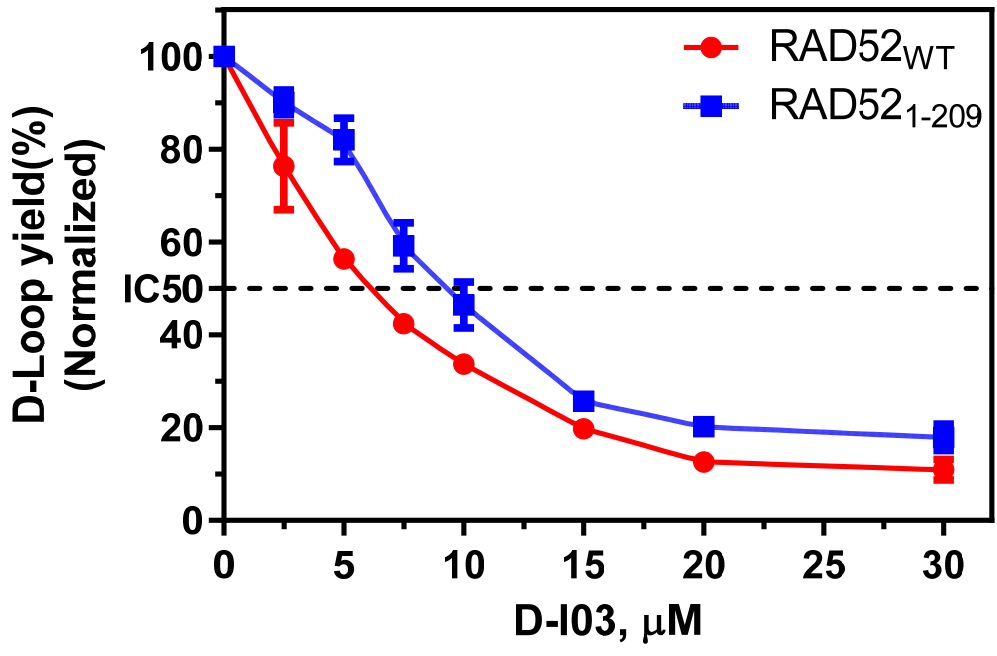




## Figure S4

RAD52<sup>Y165-66AA</sup> and RAD52<sup>K102A/K133A</sup> are incapable to form foci in response cisplatin treatment. (A) Western blot confirmation of knocked out of endogenous RAD52 (KO) and expression of exogenous RAD52<sup>Y165-66AA</sup> and RAD52<sup>K102A/K133A</sup> in U2OS cells. (B) Western blot confirmation of knocked-down endogenous RAD52 by stable expression of shRAD52 and expression of exogenous HA-RAD52<sup>WT</sup> or HA-RAD52<sup>1-209</sup> in MD-MB-436 cells. (C) Representative images of MDA-MB-436 cells stably expressing shRAD52 and RAD52<sup>Y165-66AA</sup> or RAD52<sup>K102A/K133A</sup>, stained 6 h after cisplatin (10  $\mu$ M) treatment with DAPI (blue), fluorescent anti-bodies for RAD52 (green) and  $\gamma$ -H2AX (red). (D) Quantification of RAD52 foci co-localized with  $\gamma$ -H2AX foci in MDA-MB-436 cells treated with shRAD52 and stably expressing indicated exogenous RAD52 protein.

Figure S5



## Figure S5

D-I03 inhibits the D-loop activity of RAD52<sub>WT</sub> and RAD52<sub>1-209</sub> with a similar efficiency.

Table S1: Sequences of the oligonucleotides used in this study.

Oligo name	length	Sequence (5'-3')
160	80	AGCTTTGAGATGCTTGCTTATCAACAGAAGGAGTTACCTCGCGTTGCGTCTATTATGGTACCACACCAATCTTTCCAAGCAACG
1337-BHQ	39	AGCACTGACTACTGTCGTCGATCATCGTGCATCACAGTG-BHQ1
337-F	60	FLU-CACTGTGATGCACGATGATCGACGACAGTAGTCAGTGCTGGGTCAACATCTGTATGCAGG
1	63	ACAGCACCAGATTCAGCAATTAAGCTCTAAGCCATCCGCAAAAATGACCTCTATCAAAAAGGA
117	94	TCCTTTTGATAAGAGGTCATTTTTGCGGATGGCTTAGAGCTTAATTGCTGATCTGGTGCTGTAGGTCAACATGTTGTAAATATGCAGCTAAAG
2R	63	UCCUUUUGAUAAGAGGUCAUUUUUGCGGAUGGCUUAGAGCUUAAUUGCUGAAUCUGGUGCUGU (ribonucleotide)
RAD52 sgRNA L1 up F	25	CACCGCTAGGCTGGAGTCCGACCAG
RAD52 sgRNA L1 up R	25	AAACCTGGTCGGACTCCAGCCTAGC
RAD52 sgRNA R1 down F	25	CACCGACCCACAGCAGACTTTCAGC
RAD52 sgRNA R1 down R	25	AAACGCTGAAAGTCTGCTGTGGGTC
RAD52 check primer Forward	20	AATTCATGTGCCTGGAAAGC
RAD52 check primer Reverse	20	CCCACGTAGAACTTGCCATT
shRAD52 3'UTR Sigma SHCLND-NM_134424.2-1462s21c1	57	CCGGATGAACGTCATTGCGATTTATCTCGAGATAAATCGCAATGACGTTCAATTTTTTG
shControl Sigma SHC202	57	CCGGCAACAAGATGAAGAGCACCAACTCGAGTTGGTGCTCTTCATCTTGTGTTTTT