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**Supplemental information**

***BRCA2* deficiency reveals  
that oxidative stress impairs RNaseH1  
function to cripple mitochondrial DNA maintenance**

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## Supplemental Information

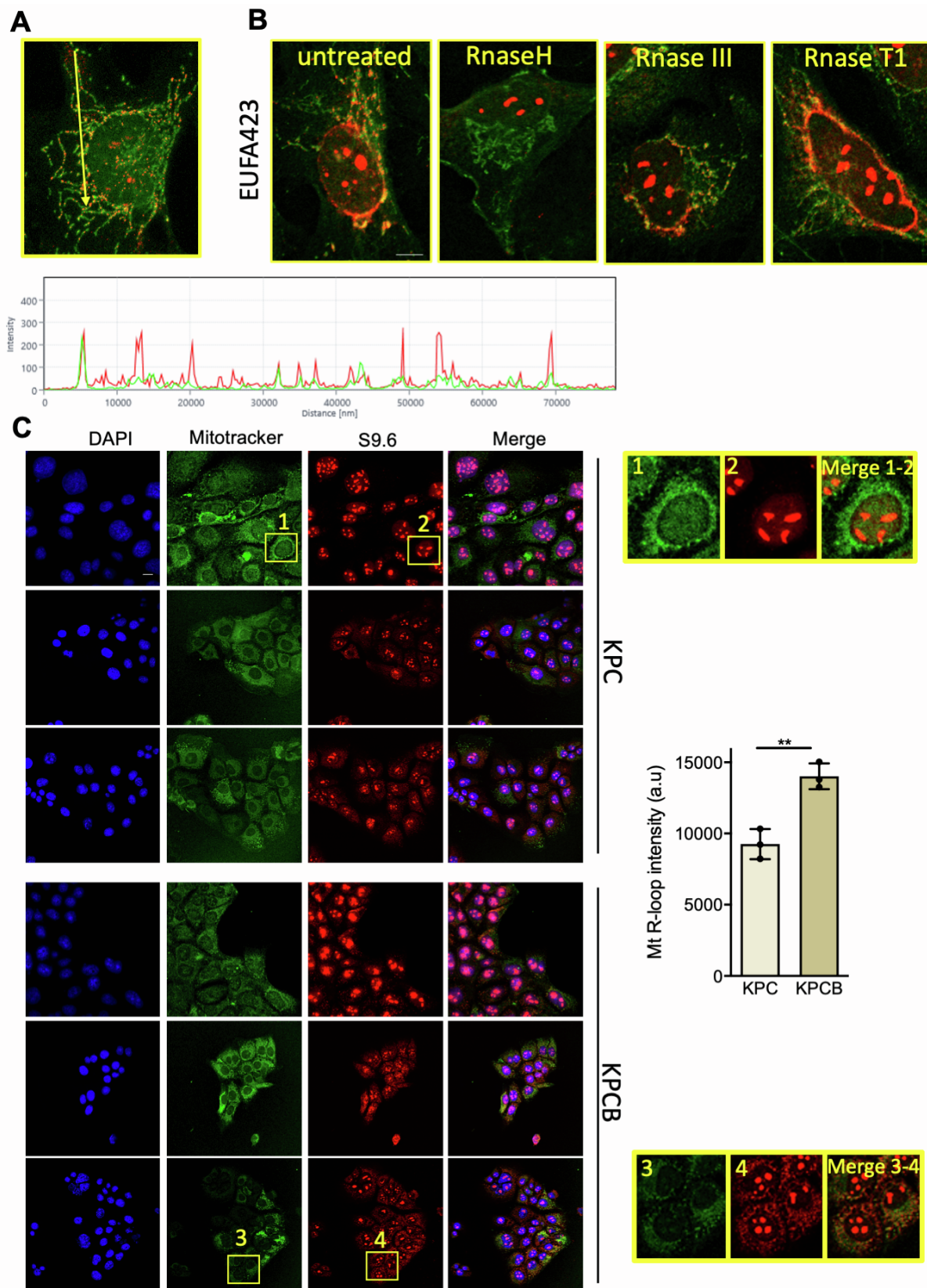
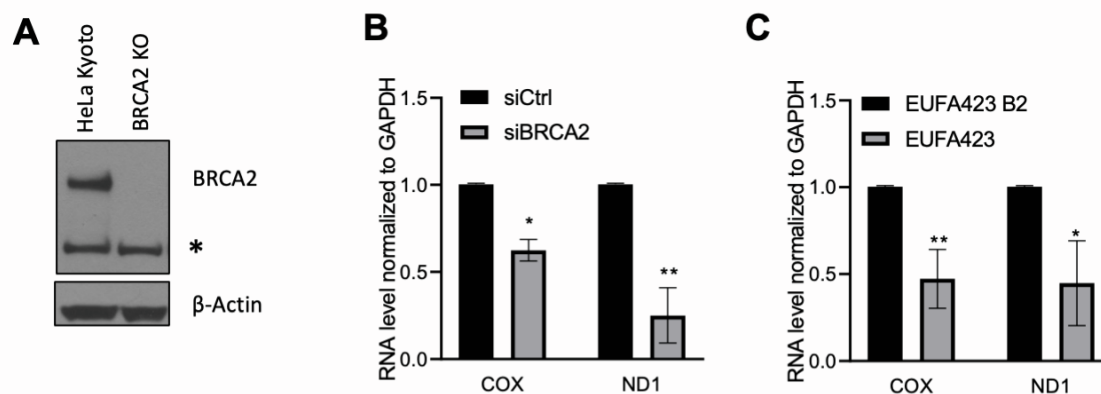


Figure S1, related to Figure 1

**Figure S1, related to Fig. 1. R-loops accumulate in the mitochondria of Brca2-deficient cancers.**

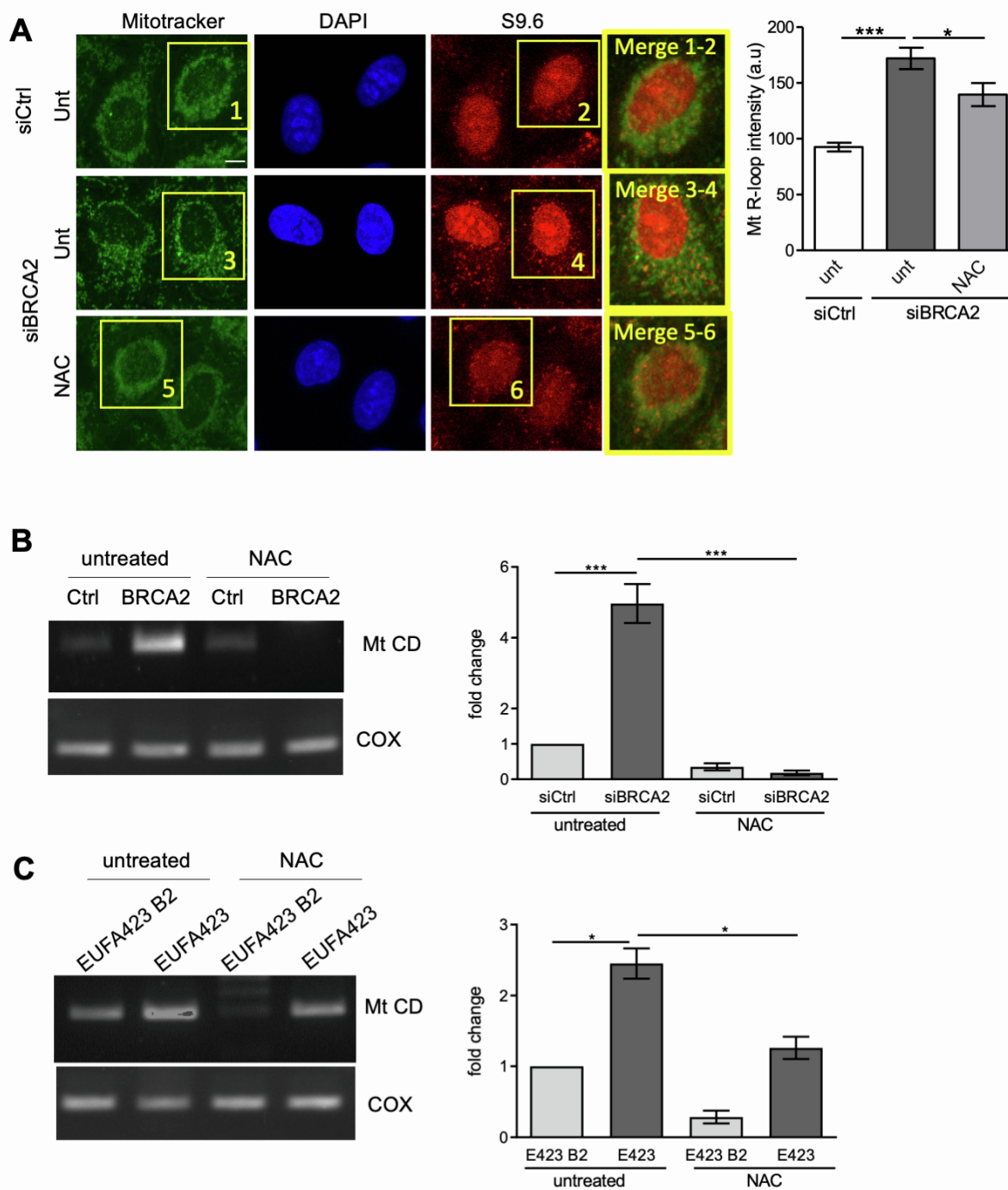
**(A)** Magnification of immunofluorescence detection of R-loops with S9.6 antibody from Fig. 1A with intensity of fluorescence along the arrow indicated in the bottom panel. **(B)** Immunofluorescence detection of R-loops with S9.6 antibody in EUFA423 cells after treatment with the indicated enzymes for 30 minutes at 37°C after fixation. Scale bars 10µm **(C)** Immunofluorescence detection of R-loops with S9.6 antibody in mouse pancreatic ductal adenocarcinoma cell lines from a genetically-engineered autochthonous murine model for Brca2-deficient pancreatic cancer (Skoulidis et al., 2010). KPCB cell lines carry KrasG12D, p53R172H and bi-allelic inactivating mutations in Brca2. KPC cells carry only the Kras and p53 mutations. Magnifications show details of selected cells as indicated. Plot shows the mean  $\pm$  s.d from three different tumours experiments. At least 100 cells were counted for each tumour cell lines. The two-tailed Student's t- test was performed to determine statistical significance between the two groups. \*\*,  $p < 0.01$ . Scale bars 10µm; DAPI (4,6-diamidino-2-phenylindole). Mitotracker stains mitochondria.



**Figure S2, related to Figure 2**

**Figure S2, related to Fig. 2. Transcription of mtDNA-encoded ND1 and COX genes is reduced in BRCA2-deficient cells.**

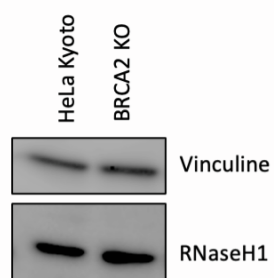
**(A)** Western blot showing BRCA2 expression in HeLa Kyoto wild-type (WT) cells, or in HeLa Kyoto cells engineered by CRISPR/Cas9 to generate BRCA2 nullizygoty (BRCA2-KO). \* denotes a non-specific band detected by BRCA2 antibody **(B-C)** Fold-change in the expression of RNA encoding the ND1 or COX genes in HeLa Kyoto cells transfected with, (si)BRCA2 (A) or (B) in EUFA423 cells compared with EUFA423 B2 controls expressing wild-type BRCA2. Controls in each comparison were assigned a value of 1. Plots show the mean  $\pm$  s.d from three independent experiments. The two-tailed Student's t-test was performed to determine statistical significance between the two groups. \*,  $p < 0.05$ , \*\*,  $p < 0.01$ .



**Figure S3, related to Figure 4**

**Figure S3, related to Figure 4. Mitochondrial genome instability arises from oxidative stress in BRCA2-deficient cells.**

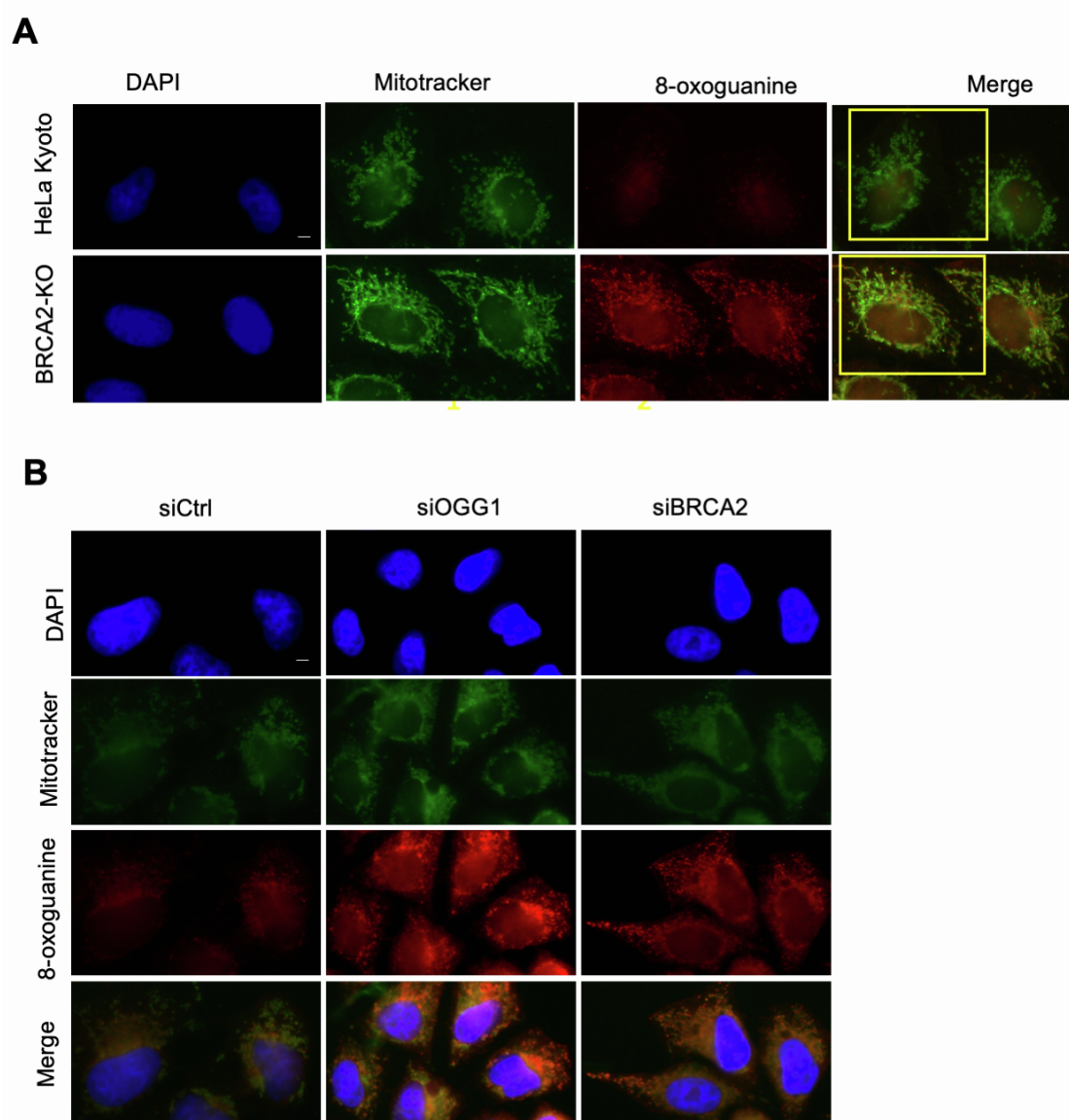
**(A)** Immunofluorescence detection of R-loops with S9.6 antibody in HeLa Kyoto transfected with the indicated siRNA in presence or not of NAC for 24hrs. Magnifications show details of selected cells as indicated. Plots show the relative mean  $\pm$  s.d from three independent experiments. At least 100 cells were counted in each conditions. The two- tailed Student's t-test was performed to determine statistical significance between the two groups. \*,  $p < 0.05$  ; \*\*\* $p < 0,001$ . Scale bars 10 $\mu$ m; DAPI (4,6-diamidino-2-phenylindole), Mitotracker stains mitochondria. **(B-C)** Mitochondrial common deletion measured by nested PCR in HeLa Kyoto transfected with (si)Ctrl (control) or (si)BRCA2, followed by treatment with 2mM NAC for 16hrs (B); or in EUFA423 cells compared with EUFA423-B2 controls expressing wild-type BRCA2 (C). Plots show the relative mean  $\pm$  s.d from three independent experiments measured by densitometry analysis. The two-tailed Student's t-test was performed to determine statistical significance between the two groups. \*,  $p < 0.05$ , \*\*\*,  $p < 0.001$ .



**Figure S4, related to Figure 5**

**Figure S4, related to Figure 5. RNaseH1 level in BRCA2 deficient cells**

Western blot showing RNaseH1 expression in HeLa Kyoto wild-type (WT) cells, or in HeLa Kyoto cells engineered by CRISPR/Cas9 to generate BRCA2 nullizygoty (BRCA2-KO).



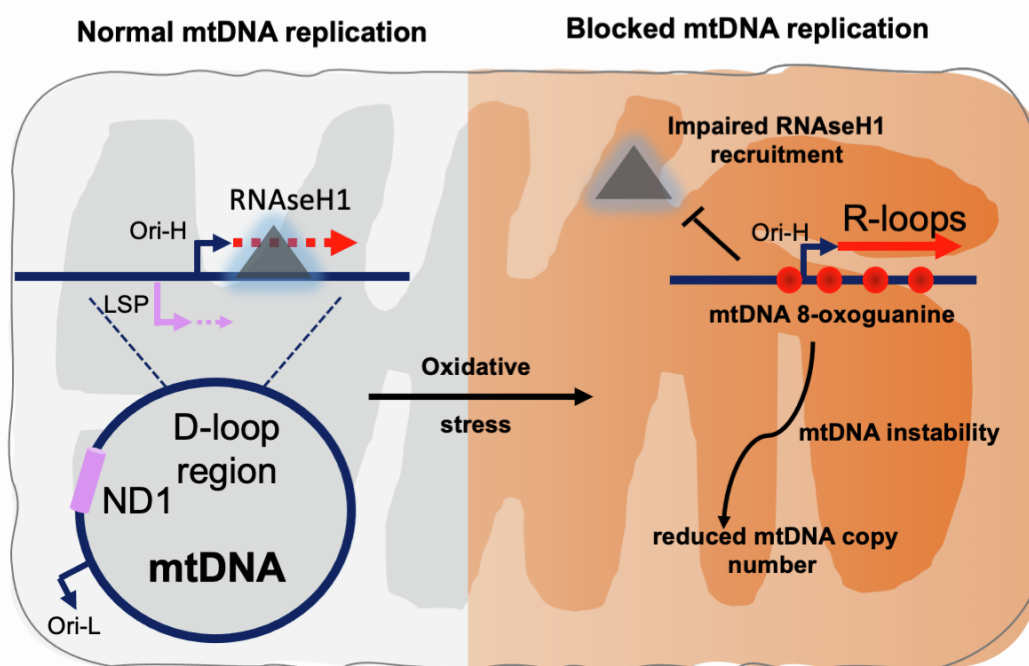
**Figure S5, related to Figure 6**

**Figure S5, related to Figure 6. 8-oxoguanine accumulates in the mitochondria of BRCA2-deficient and OGG1-deficient cells.**

**(A-B)** Immunofluorescence detection of 8-oxoguanine in HeLa Kyoto or in BRCA2 KO cells (A) or in HeLa Kyoto transfected with the indicated siRNAs (B).

Magnifications show details of selected cells as indicated. Scale bars 10 $\mu$ m; DAPI (4,6-diamidino-2-phenylindole), Mitotracker stains mitochondria.





**Figure S6**

**Figure S6, related to Figure 1 to 7. Pathogenic BRCA2 mutations reveal an unrecognized general mechanism wherein oxidative stress restricts mtDNA replication via RNaseH1 impairment.**

Normal mtDNA replication is portrayed in the grey panel. Ori-L and Ori-H indicate mtDNA replication origins; LSP, transcription initiation site for 7S RNA; ND1, NADPH1 gene locus; and the red dashed arrow represents transient RNA formation. Our findings suggest a model wherein oxidative stress (orange panel) causes accumulation of 8-oxoguanine in mtDNA, impairing the recruitment of RNaseH1, an R-loop processing factor essential for mtDNA replication, to the replication-initiating D-loop region. Impaired RNaseH1 recruitment leads to unscheduled R-loop accumulation, depicted by the red arrow blocking mtDNA replication, and reducing mtDNA copy number. BRCA2 deficiency triggers this

mechanism by generating endogenous oxidative stress, as does the depletion of SETX or PRPF8 implicated in neurodegenerative diseases. Similar events occur in wild-type cells exposed to exogenous oxidative stress, speaking to the generality of this mechanism.

## Supplementary Tables

**Supplemental Table S1, related to STAR methods.** Primers used in this study.

Name	sequence 5'-3'
D-loop_Fwd	CTTTCATGGGGAAGCAGATTTG
D-loop_Rev	GCATGGGGAGGGGGTTTTG
ND1_Fwd	TCTCCACCCTTATCACAACA
ND1_Rev	GACTAGTTCGGACTCCCCTT
OriL_Fwd	CCCACAAACACTTAGTTAACAGCT
OriL_Rev	GGCCTCTTTTTACCAGCTCC
Mt-CD1_Fwd	AACCACAGTTTCATGCCCATC
Mt-CD1_Rev	TGTTAGTAAGGGTGGGGAAGC
Mt-CD2_Fwd	ACCCTATAGCACCCCCTCTAC
Mt-CD2_Rev	CTTGTCAGGGAGGTAGCGATG
HPRT_Fwd	TGACACTGGCAAACAATGCA
HPRT_Rev	GGTCCTTTTCACCAGCAAGCT
GAPDH_Fwd	CTCCTGTTTCGACAGTCAGC
GAPDH_Rev	TTCAGGCCGTCCCTAGC

sc-400700: BRCA2 CRISPR/Cas9 KO pool of 3 different gRNA plasmids:	
sc-400700 A:	CTGTCTACCTGACCAATCGA
sc-400700 B:	ATGTAGCACGCATTACATA
sc-400700 C:	CGATTACCTGTGTACCCTTT

**Supplemental Table S2, related to STAR methods.** Antibodies used in this study.

Antibody	Species	Dilution	reference	Supplier
S9.6	Mouse	IF 1/100	N/A	Our laboratory
BRCA2	Mouse	WB 1/500	AB-1 OP95	Merck Millipore
Beta-Actin	Mouse	WB 1/2000	A5441	Sigma
RNAse H1	Rabbit	ChIP 3ug	sc-292711	Santa Cruz

8-oxoguanine	Mouse	IF 1/500	MAB3560	Merck Millipore
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