Supplementary information

Involvement of the splicing factor SART1 in the BRCA1-dependent homologous recombination repair of DNA double-strand breaks

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Supplementary Methods

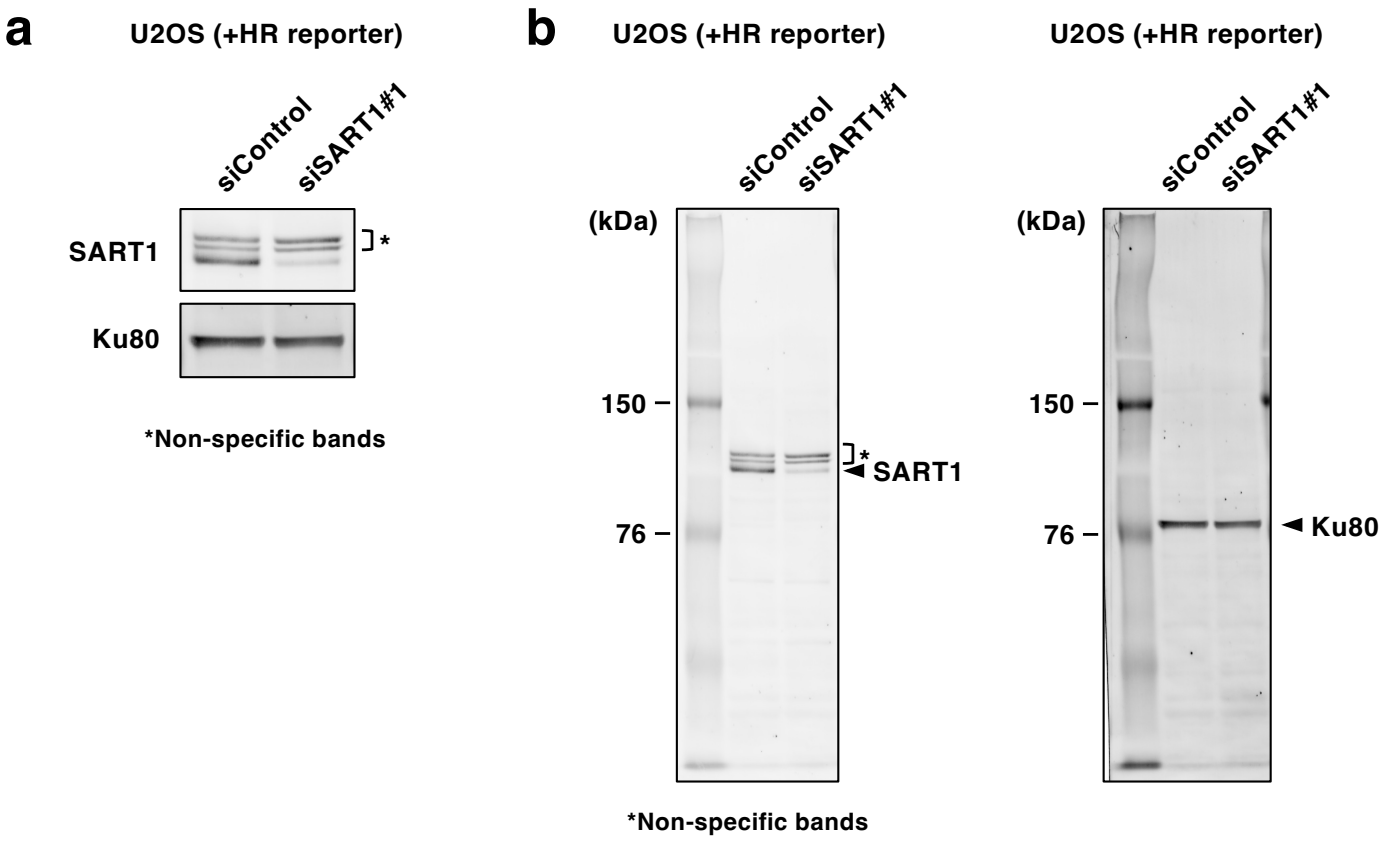
RNA-seq

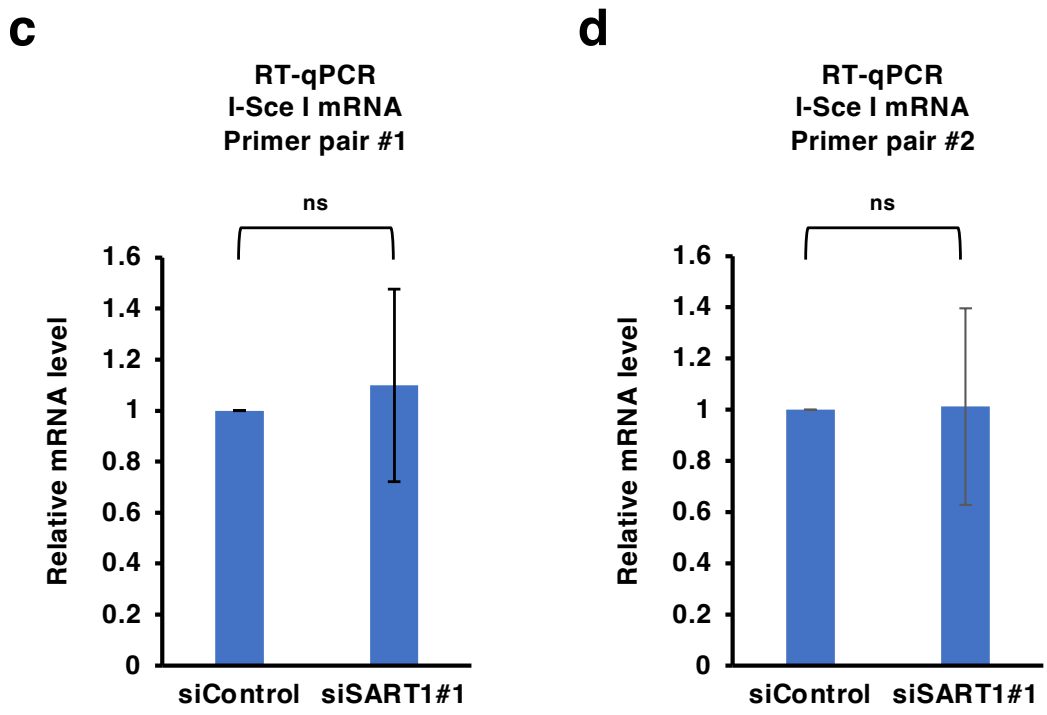
Total RNAs from cultured RPE-hTERT cells was extracted using NucleoSpin RNA (MACHEREY-NAGEL) according to the manufacturer's protocol. Purified RNA quality was evaluated by the RNA integrity number (RIN) using the Agilent RNA6000 Pico Kit and Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). High-quality RNA samples (RNA integrity number (RIN) > 9.8) were used for RNA-seq analysis. One microgram of total RNA was used to prepare sequence libraries using a KAPA mRNA HyperPrep Kit (Kapa Biosystems Inc., Wilmington, MA, USA), following the manufacturer's instructions. The generated libraries and 1% PhiX spike-in were then subjected to paired-end sequencing of 76-bp reads using a NextSeq500 System (Illumina Inc., San Diego, CA, USA) with a NextSeq500 High Output v2.5 Kit (Illumina). Alignment of reads (approximately 43 million reads) to the GRCh38 genome using STAR (version 2.7.6a) was followed by quantification of gene and transcript levels using RSEM (version 1.3.3) based on Ensembl release 100 (Dobin et al., 2013; Li and Dewey, 2011). For the analysis, read counts > 0 were used as thresholds. Count normalization and identification of differentially expressed genes (DEGs) were conducted using the iDEGES/edgeR-edgeR pipeline in the R statistical package TCC (Sun et al., 2013). DEGs were selected based on a q-value of <0.05.

Reagent

An ATM inhibitor (KU55933; Cat. No. 118502) was obtained from Merck Millipore and treated at a final concentration of 10 μM 1 h before laser irradiation.

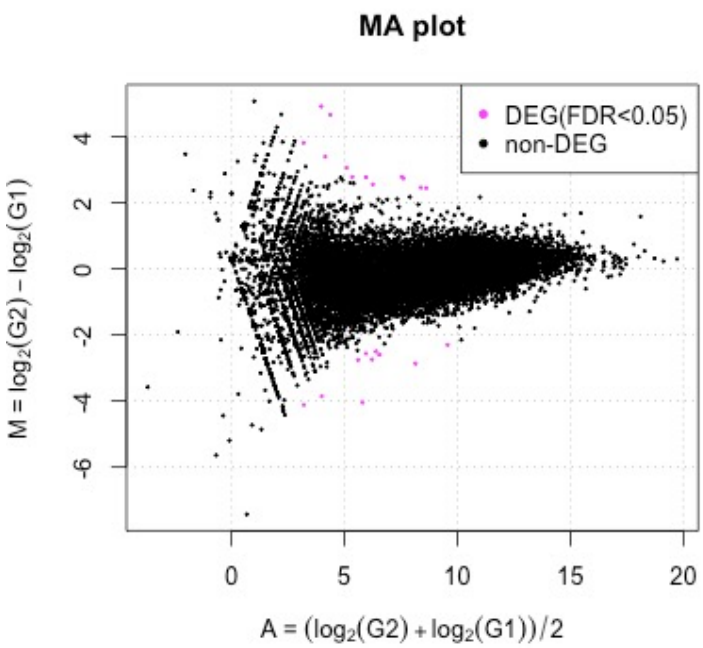
Supplementary Figure S1





Supplementary Figure S2

a

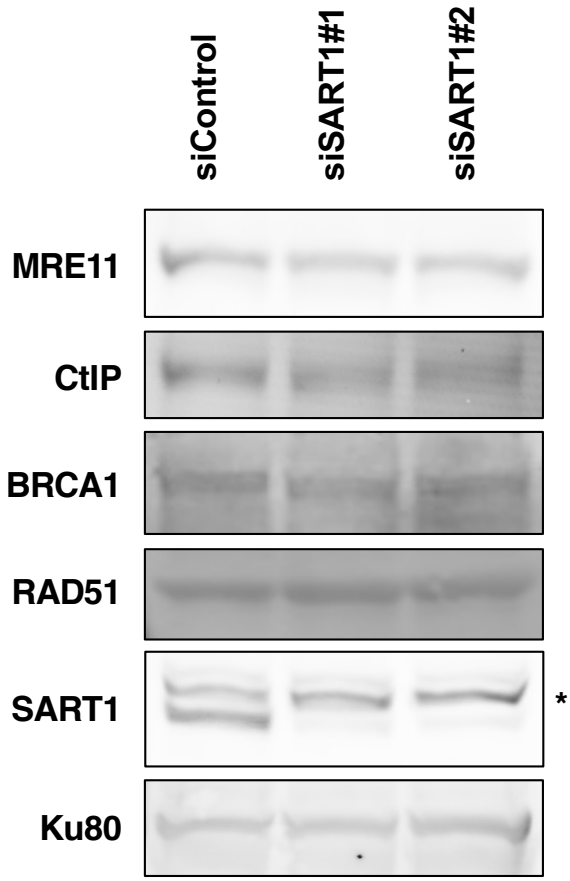


b

FP236241.2
CU634019.2
AC019117.4
CU633906.2
AC009412.1
AL137782.1
CXCL8
AC034102.2
GDNF
SLC37A2
EDN1
NOG
Z95118.2
C5AR1
AC087190.3
SULT1A4
ADAM32
AC116366.3
SART1
GLI1
SDCBP2-AS1
HMGN1P3

Supplementary Figure S3

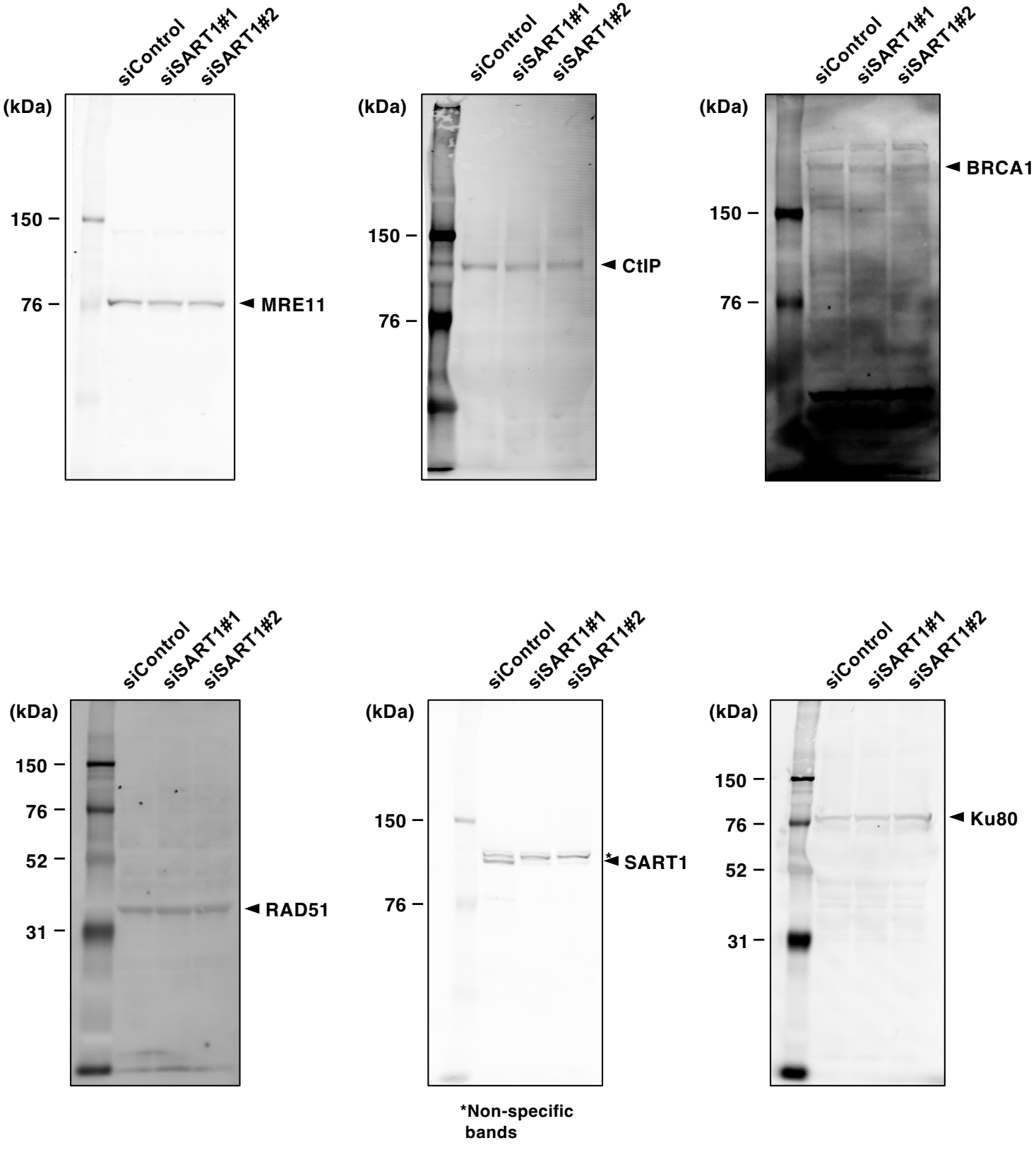
a



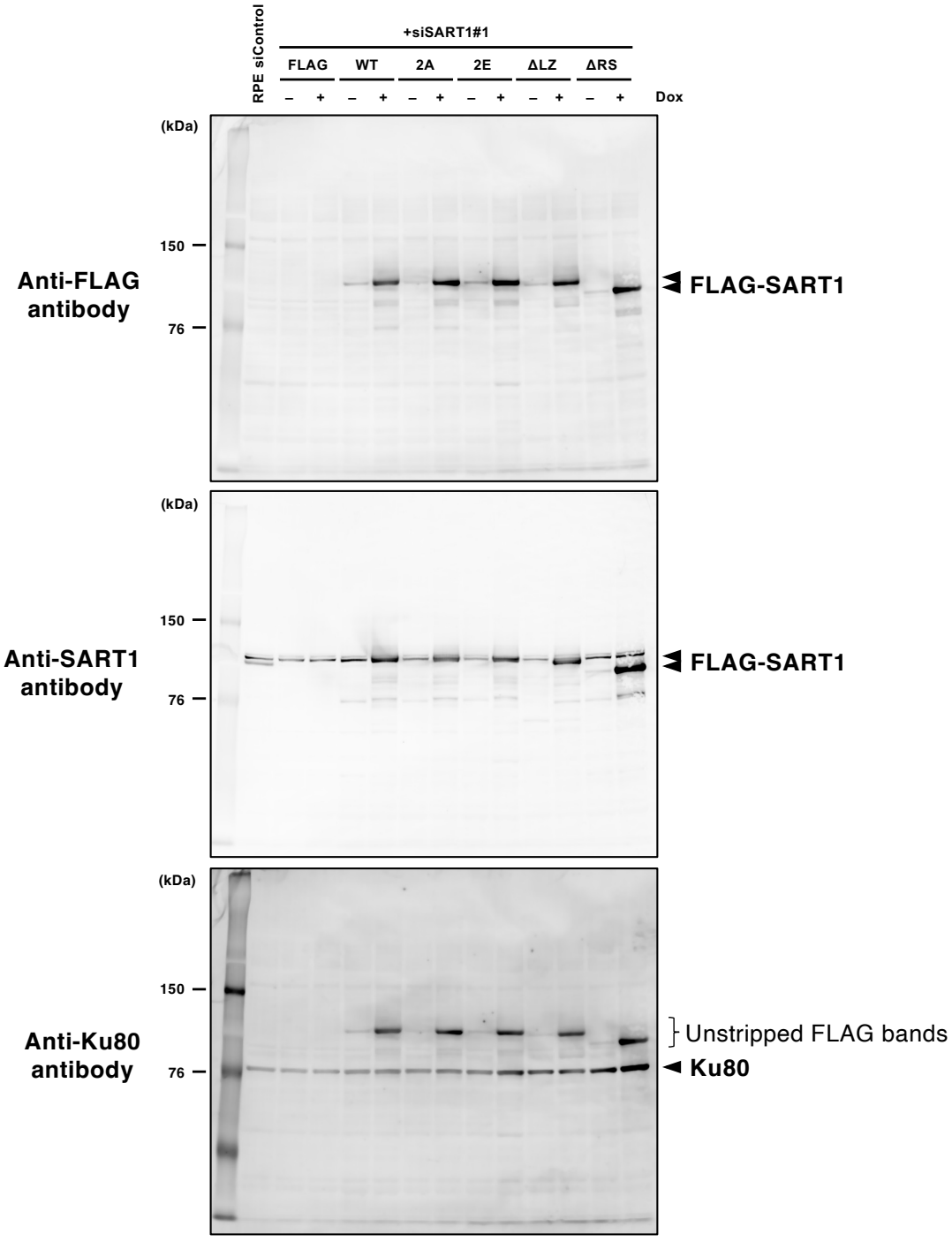
*** Non-specific bands**

Supplementary Figure S3 (continued)

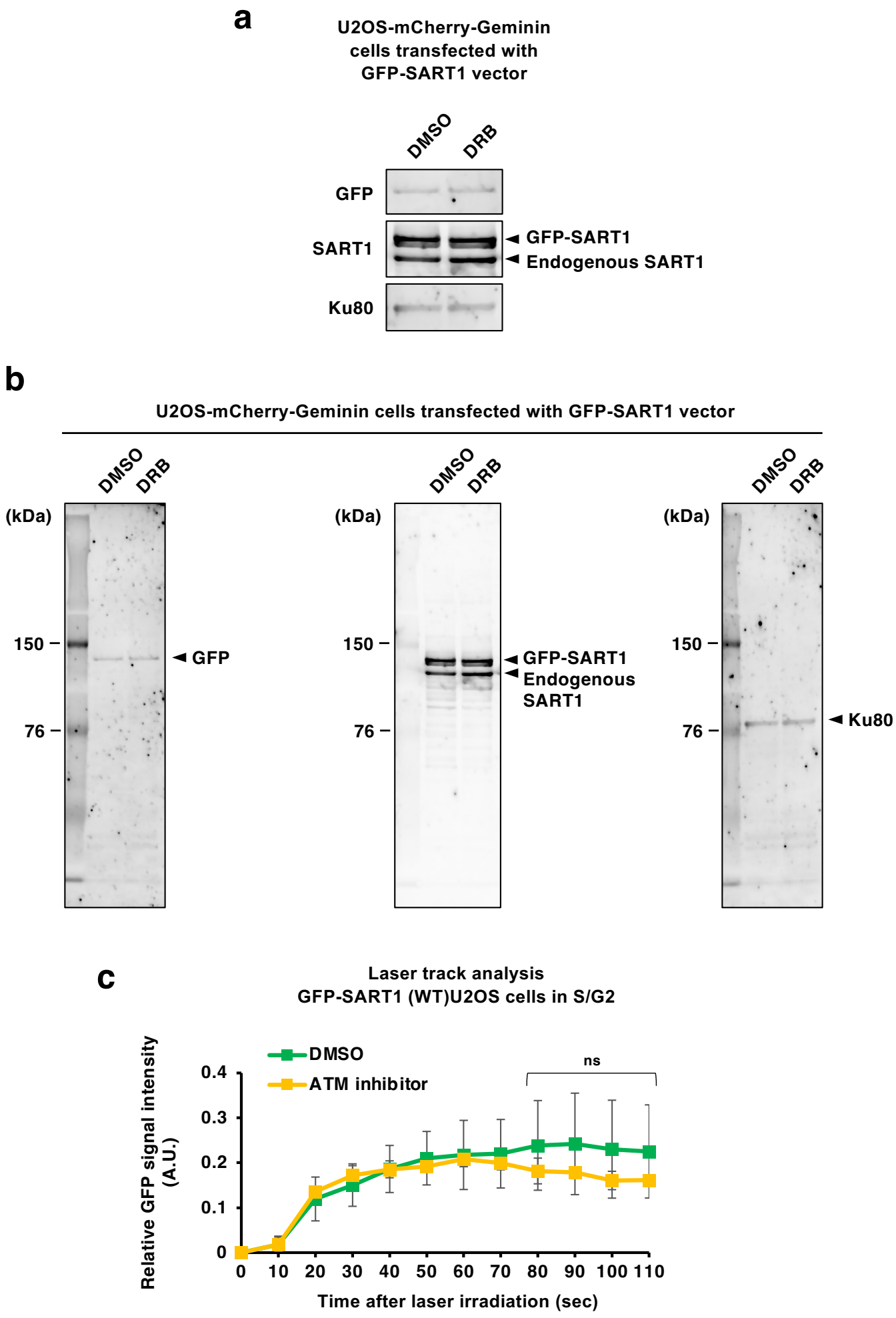
b



Supplementary Figure S4

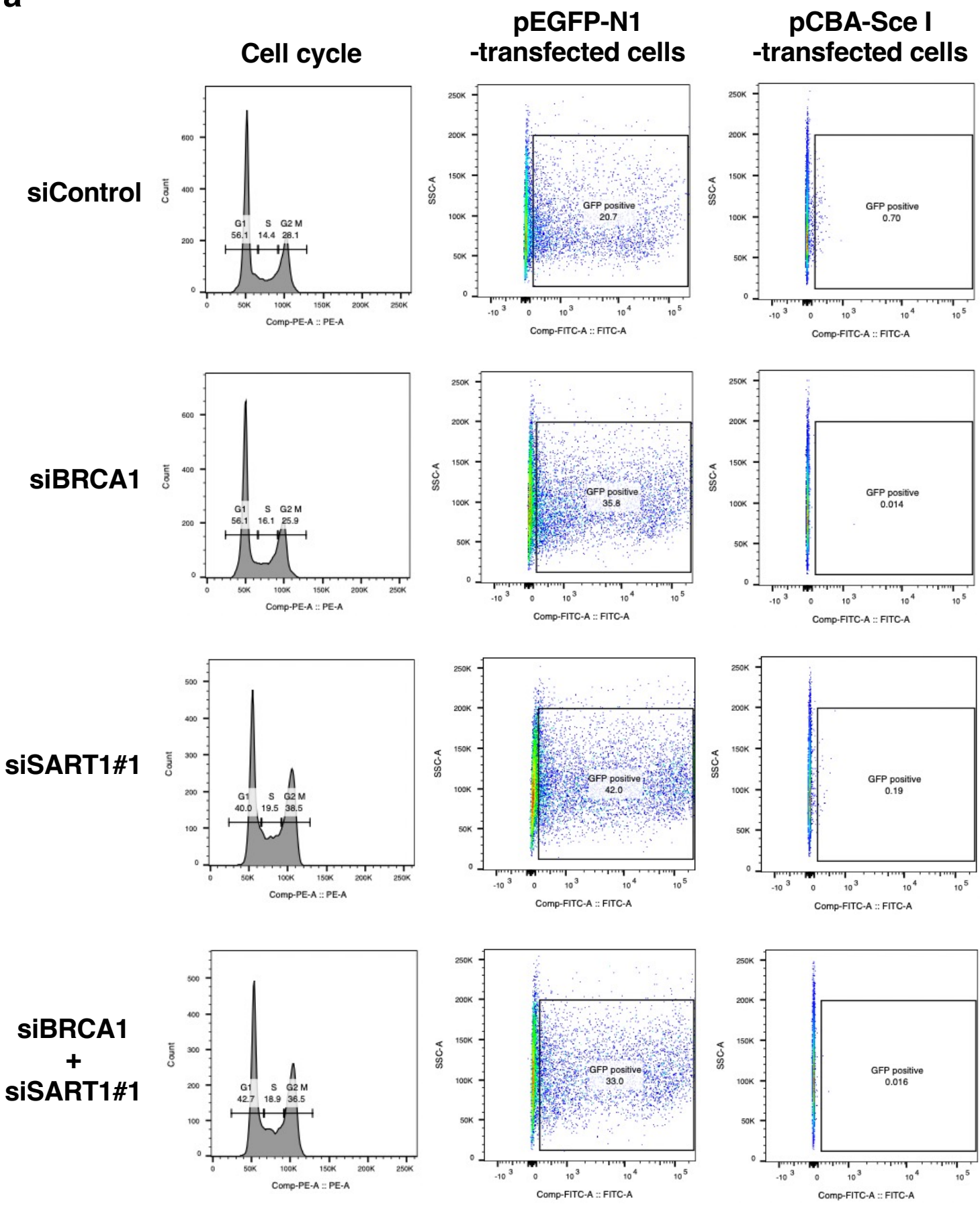


Supplementary Figure S5



Supplementary Figure S6

a



Supplementary Figure S6 (continued)

b

% of each cell cycle phase

	G1	S	G2/M
siControl	56.1	14.4	28.1
siBRCA1	56.1	16.1	25.9
siSART1#1	40.0	19.5	38.5
siBRCA1+ siSART1#1	42.7	18.9	36.5

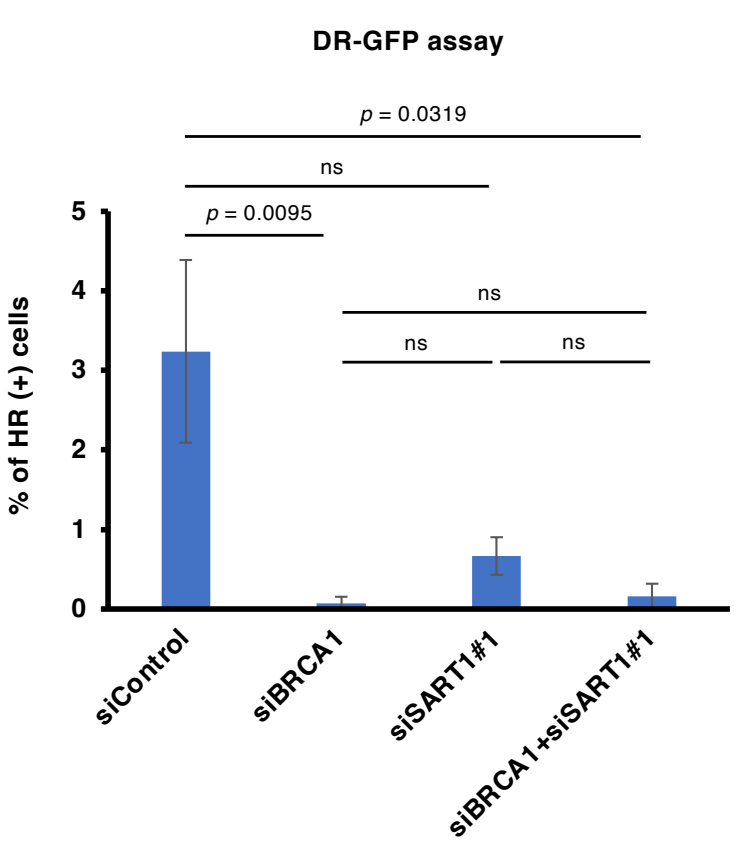
c

HR(+)%

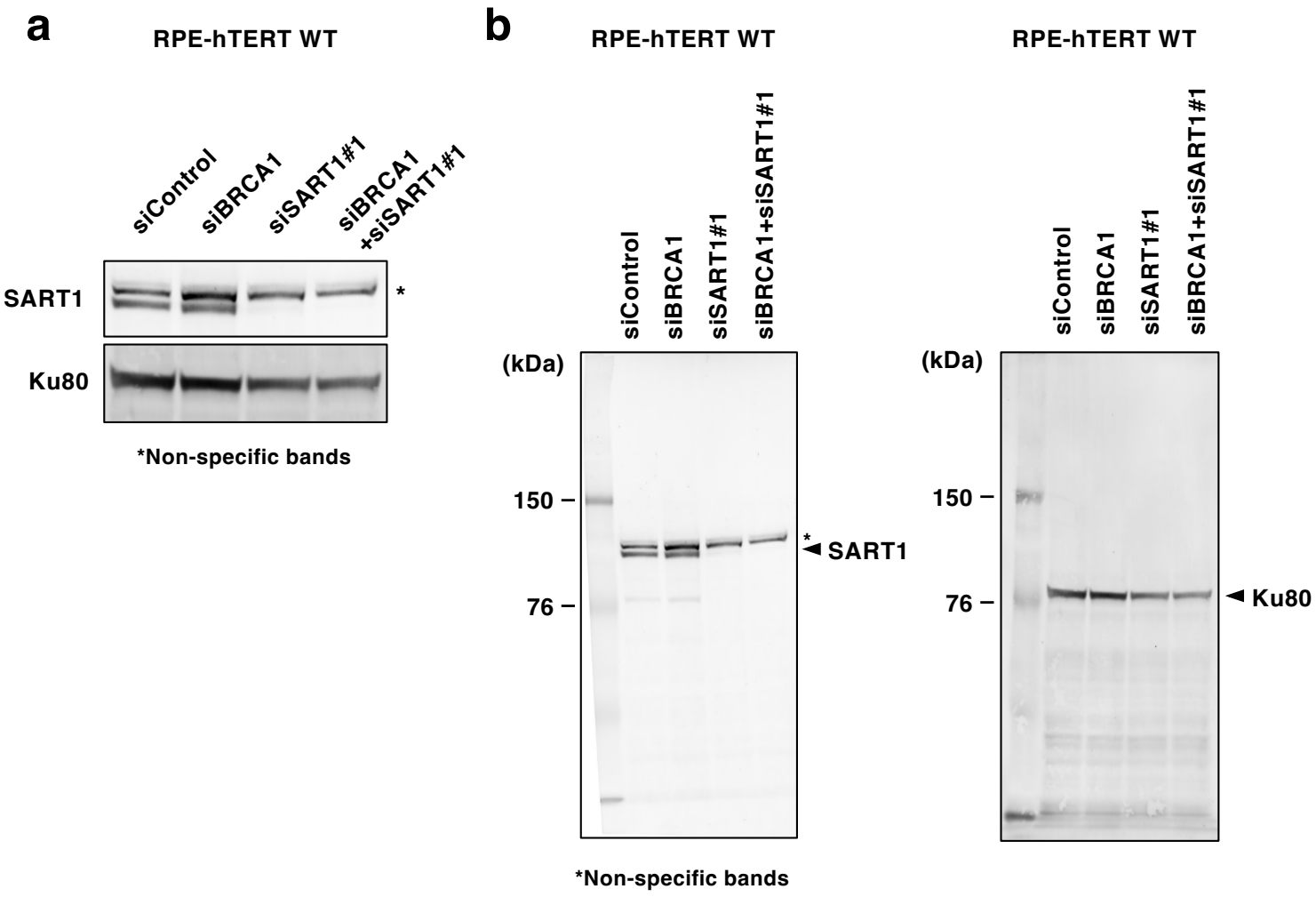
(GFP(+)% of pCBA-Sce I-transfected cells normalized by GFP(+)% of pEGFP-N1-transfected cells)

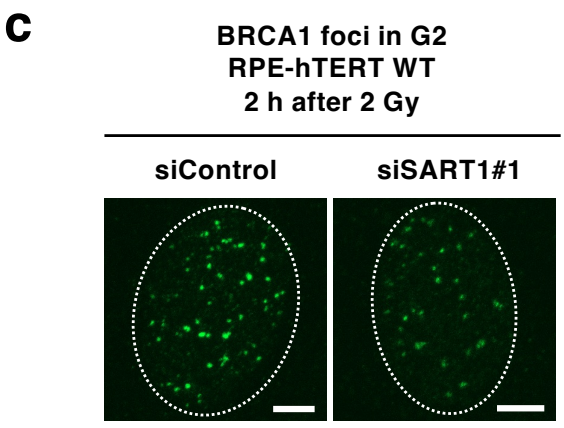
	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Average
siControl	3.38	4.50	1.71	3.37	3.20
siBRCA1	0.04	0.07	0.19	0.00	0.10
siSART1#1	0.45	0.65	1.00	0.56	0.70
siBRCA1+ siSART1#1	0.05	0.27	0.32	0.00	0.21

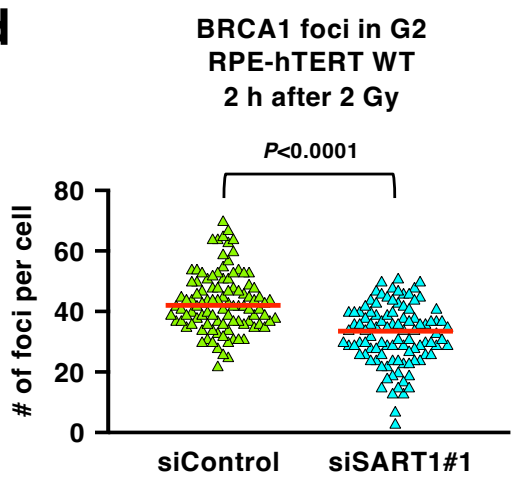
d



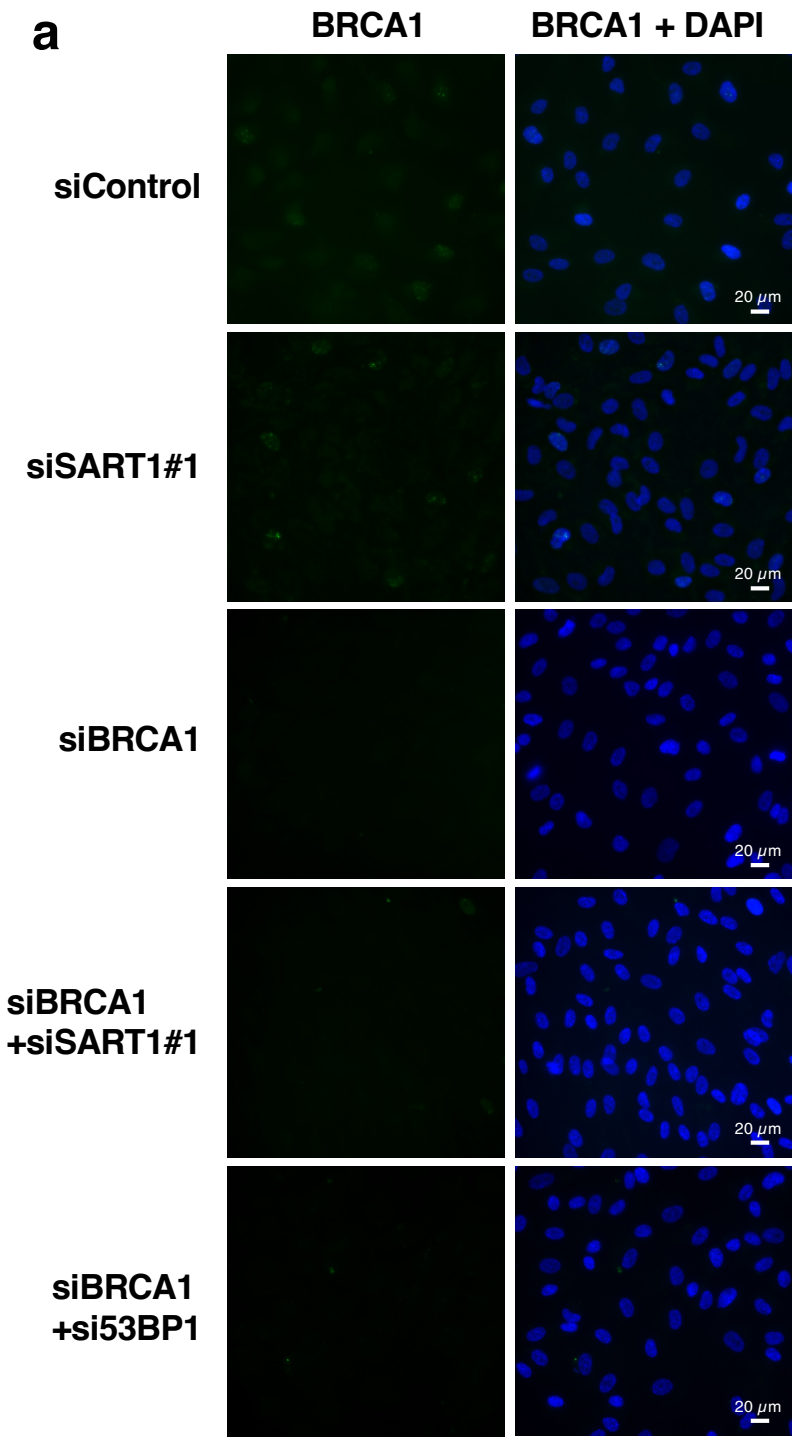
Supplementary Figure S7

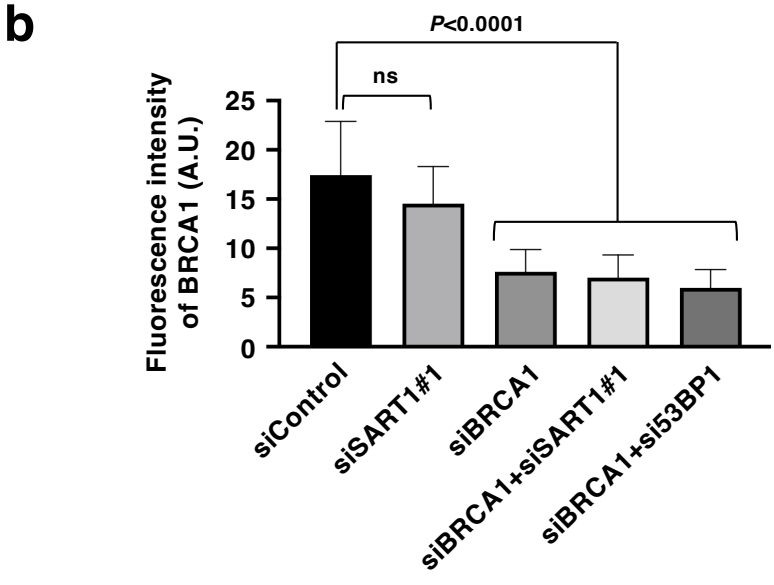




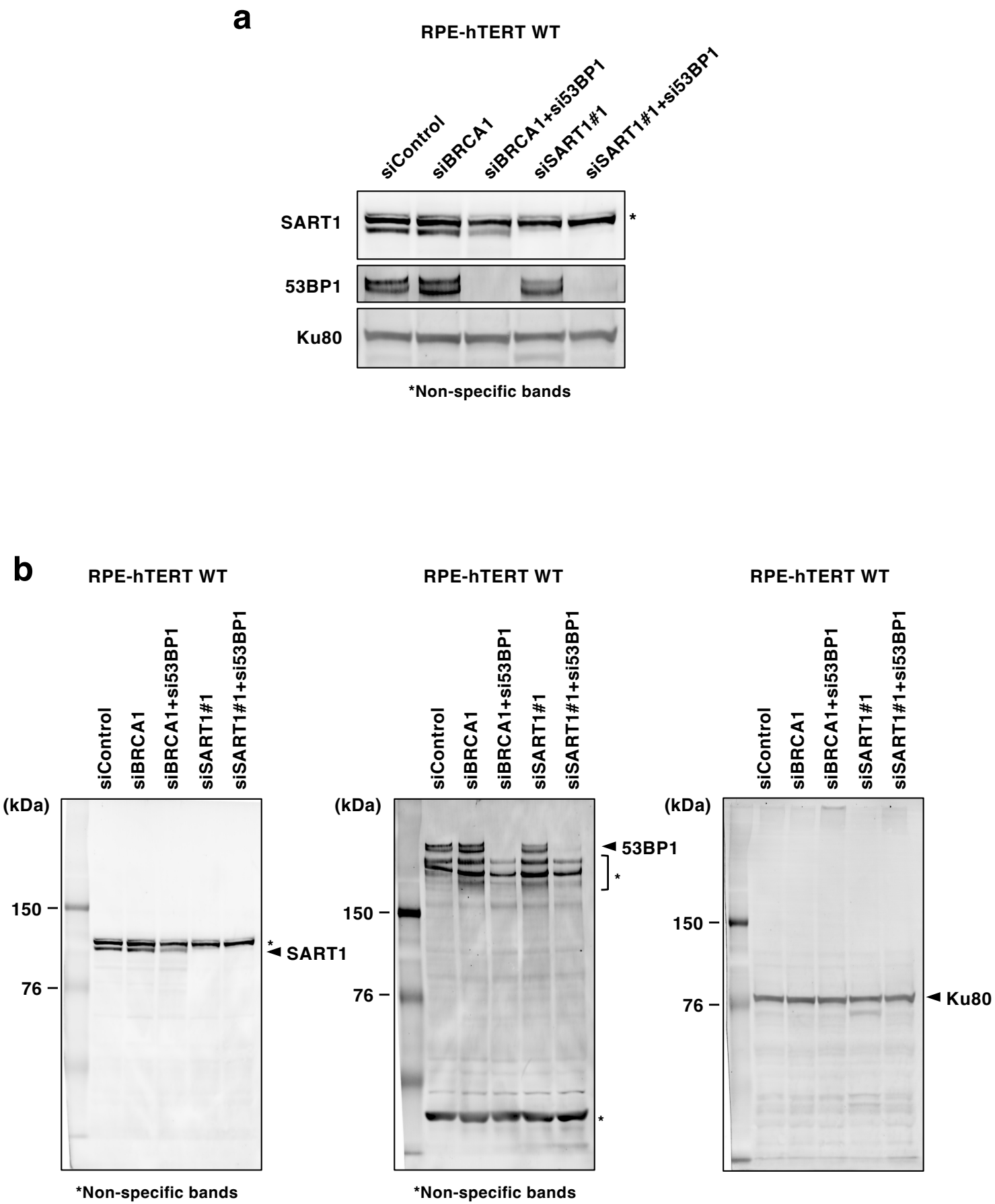


Supplementary Figure S8





Supplementary Figure S9



Supplementary Table S1

Sequence of siRNAs used in the present study

siRNA	Sequence of sense strand of siRNA duplex (5'-3')
siSART1#1	CCGAAUACCUCACGCCUGAdTdT
siSART1#2	GCAAGAGCAUGAACGCGAAdTdT
siBRCA1	GGAACCUGUCUCCACAAAGdTdT
si53BP1	GGACUCCAGUGUUGUCAUUUU

Supplementary Table S2

Antibodies used in the present study

Target	Clone or Cat#	Host	Source	Application (dilution ratio)
RPA2	LS-C38952	Rat	Life Span BioSciences, Inc.	IF (1:500)
RAD51	14B4/GTX70230	Mouse	GeneTex	IF (1:500) WB (1:200)
RAD51	ab133534	Rabbit	Abcam	IF (1:1000)
BRCA1	D-9	Mouse	Santa Cruz Biotech.	IF (1:250)
BRCA1	07-434	Rabbit	Merck	WB (1:200)
53BP1 pT543	3428	Rabbit	Cell Signaling Tech.	IF (1:500)
RIF1	A300-569A	Rabbit	Bethyl Laboratories	IF (1:500)
CENPF	58982	Rabbit	Cell Signaling Tech.	IF (1:1000)
CENPF	610768	Mouse	BD Biosciences	IF (1:1000)
MRE11	31H4/4847	Rabbit	Cell Signaling Tech.	WB (1:200)
CtIP	D76F7/9201	Rabbit	Cell Signaling Tech.	WB (1:200)
SART1	HPA031188	Rabbit	Atlas antibodies	WB (1:200)
Ku80	C48E7/2180	Rabbit	Cell Signaling Tech.	WB (1:200)

IF, Immunofluorescence; WB, Western blotting

Supplementary Figure legends

Figure S1. Confirmation of SART1 knockdown and I-Sce I expression.

(a) Knockdown efficiency of SART1 protein. U2OS cells harboring the HR reporter were transfected with the indicated siRNA. Two days later, cells were lysed and the cell lysates were subjected to western blotting.

(b) Full-length images of western blotting in (a).

(c) and (d) Homogenous expression of I-Sce I in control and SART1 knockdown cells. U2OS cells harboring the HR reporter were transfected with the indicated siRNA. Two days later, total RNA was extracted, reverse transcribed, and subjected to quantitative PCR. The sequence of primers are as follows: Primer pair #1: Forward: 5'-TGCTTACATCCGTTCTCGTG-3', Reverse: 5'-CGTGGTCCATGTATGCTTTG-3', Primer pair #2: Forward: 5'-CCAGCTGATCGAACTGAACA-3', Reverse: 5'-CACGAGAACGGATGTAAGCA-3'.

Figure S2. Transcriptome analysis of SART1 knockdown cells using RNA-seq.

(a) MA plot comparing the transcriptome of SART1 knockdown cells and control cells. Data were obtained using RNA-seq and analyzed as described in the Supplementary Methods.

(b) Genes that were differentially expressed in SART1 knockdown cells compared with control cells.

Figure S3. Effect of SART1 knockdown on protein levels of major HR factors.

(a) RPE-hTERT cells were transfected with indicated siRNAs. Two days later, cells were lysed with 2x Laemli sample buffer (Merck, NJ, USA). The levels of the indicated proteins were examined by western blotting.
(b) Full-length images of western blotting in (a).

Figure S4. Full-length images of western blotting in Fig. 2b. FLAG-SART1-inducible RPE-hTERT cells were transfected with siSART1#1 and were lysed two days later. Dox was add 16-24 h before cell lysis to induce FLAG-SART1 protein. Then, the cell lysates were subjected to western blotting.

Figure S5. Effect of DRB treatment on the expression of transfected GFP-SART1 protein and effect of ATM inhibitor on GFP-SART1 recruitment to the laser track.

(a) Effect of DRB treatment on the expression of transfected GFP-SART1 protein. U2OS-mCherry-Geminin cells were transfected with GFP-SART1 vector. One day later, cells were treated with DRB (100 μM) or DMSO (vehicle control) for 30 min and lysed. Then, the cell lysates were subjected to western blotting.
(b) Full-length images of western blotting in (a).
(c) Effect of ATM inhibitor on GFP-SART1 recruitment to the laser track. U2OS cells expressing mCherry-Geminin (an S/G2 phase marker) were transfected with the wild-type GFP-SART1 vector. One day later, GFP(+)/mCherry(+) cells were irradiated with the 730 nm laser. The

ATM inhibitor (KU55933, 10 μM) and the photosensitizer (Hoechst33342, 10 μg/mL) were added 30 m before irradiation. The intensity of GFP-SART1 in the laser-irradiated regions of S/G2-phase U2OS cells was recorded every 10 sec until 110 sec after irradiation.

Figure S6. DR-GFP assay in BRCA1 and/or SART1 knockdown cells.

(a) U2OS cells harboring the HR reporter were transfected with the indicated siRNA and subjected to the DR-GFP assay. Concomitantly, cell cycle distribution of each sample was monitored. Plots made by FlowJo are shown.
(b) Cell cycle distribution of each sample.
(c) Summary of the results of the DR-GFP assay. GFP(+)% of pCBA-Sce I-transfected cells normalized by GFP(+)% of pEGFP-N1-transfected cells was shown as HR(+)%.
(d) HR(+)% in BRCA1 and/or SART1 knockdown cells. The statistical difference was analyzed using Dunn's multiple comparisons test.

Figure S7. Knockdown efficiency of SART1 and effect of SART1 knockdown on BRCA1 foci in wild-type RPE-hTERT cells.

(a) Knockdown efficiency of SART1 in RPE-hTERT cells. Cells were transfected with indicated siRNA(s) and lysed two days later, followed by western blotting.
(b) Full-length images of western blotting in (a).
(c) Effect of SART1 knockdown on BRCA1 foci in wild-type RPE-hTERT cells. RPE-hTERT WT cells were transfected with indicated siRNA(s). Two days after siRNA transfection, the cells were irradiated with 2 Gy γ-rays and fixed 2 h later. The cells were treated with EdU from 30 min before irradiation until fixation to label S phase cells. The fixed cells were subjected to

BRCA1/CENPF immunofluorescence and EdU detection. The number of BRCA1 foci in the CENPF(+)/EdU(−) G2 phase cells was quantified.

Figure S8. Knockdown efficiency of BRCA1 protein.

(a) Representative images of BRCA1 protein. RPE-hTERT cells were transfected with indicated siRNA(s) and fixed two days later. The fixed cells were subjected to immunofluorescence of BRCA1. The images were obtained using fluorescence microscopy.

(b) Fluorescence intensity of BRCA1 protein in the nucleus. Images of BRCA1 were obtained with the same exposure time and analyzed by Image J. More than 130 cells/sample were analyzed. The statistical difference was analyzed using Dunn's multiple comparisons test.

Figure S9. Knockdown efficiency of SART1 and 53BP1 proteins.

(a) Knockdown efficiency of SART1 and 53BP1 proteins. Cells were transfected with indicated siRNA(s) and lysed two days later, followed by western blotting.

(b) Full-length images of western blotting in (a).