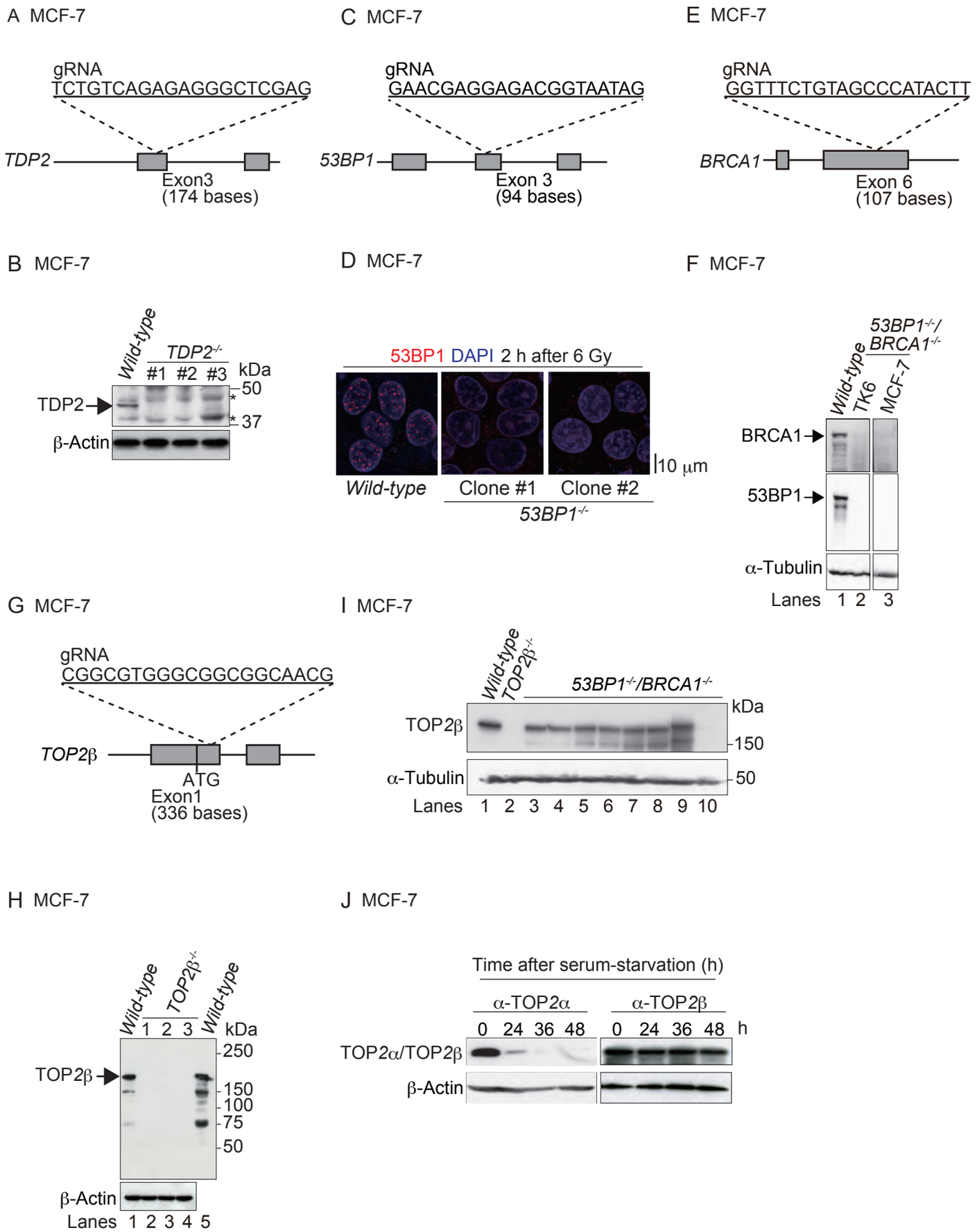
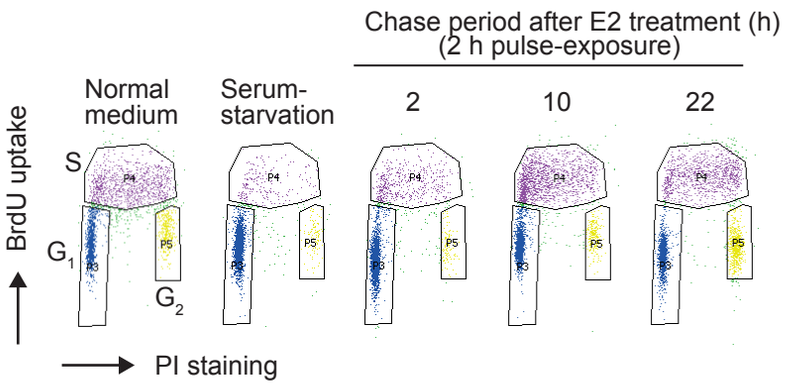
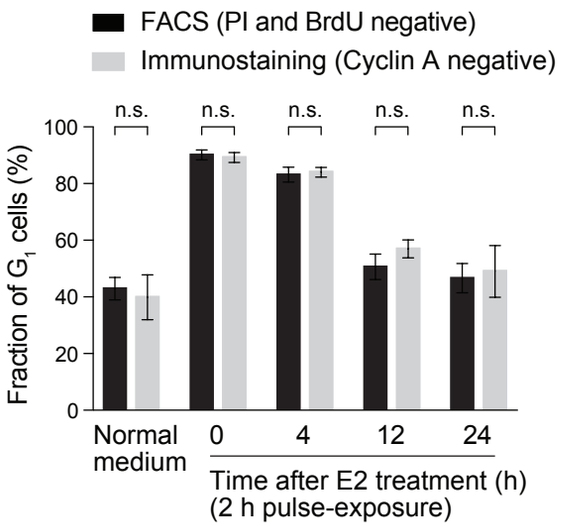

A MCF-7
gRNA
TCTGTCAGAGAGGGCTCGAG
TDP2
Exon3
(174 bases)
C MCF-7
gRNA
GAACGAGGAGACGGTAATAG
53BP1
Exon 3
(94 bases)
E MCF-7
gRNA
GGTTTCTGTAGCCCATACTT
BRCA1
Exon 6
(107 bases)
B MCF-7
Wild-type
TDP2-/-
#1 #2 #3
kDa
TDP2
50
37
β-Actin
D MCF-7
53BP1 DAPI 2 h after 6 Gy
10 μm
Wild-type
Clone #1
Clone #2
53BP1-/-
F MCF-7
53BP1-/-/
BRCA1-/-
Wild-type
TK6
MCF-7
BRCA1
53BP1
α-Tubulin
Lanes 1 2 3
G MCF-7
gRNA
CGGCGTGGGCGGCGGCAACG
TOP2β
ATG
Exon1
(336 bases)
I MCF-7
Wild-type
TOP2β-/-
53BP1-/-/BRCA1-/-
kDa
TOP2β
150
α-Tubulin
50
Lanes 1 2 3 4 5 6 7 8 9 10
H MCF-7
Wild-type
TOP2β-/-
Wild-type
1 2 3
kDa
TOP2β
250
150
100
75
50
β-Actin
Lanes 1 2 3 4 5
J MCF-7
Time after serum-starvation (h)
α-TOP2α
α-TOP2β
0 24 36 48 0 24 36 48 h
TOP2α/TOP2β
β-Actin

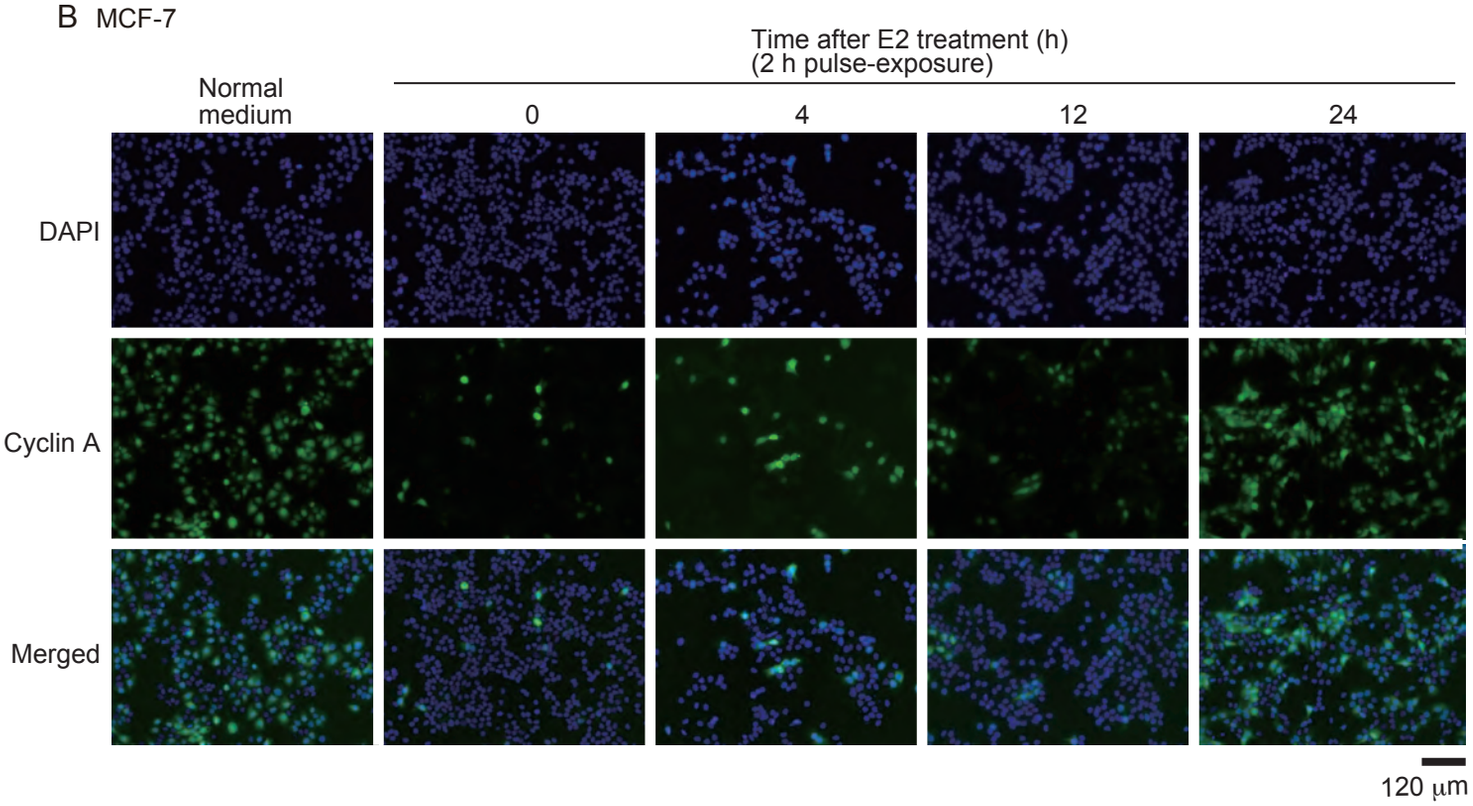
A MCF-7



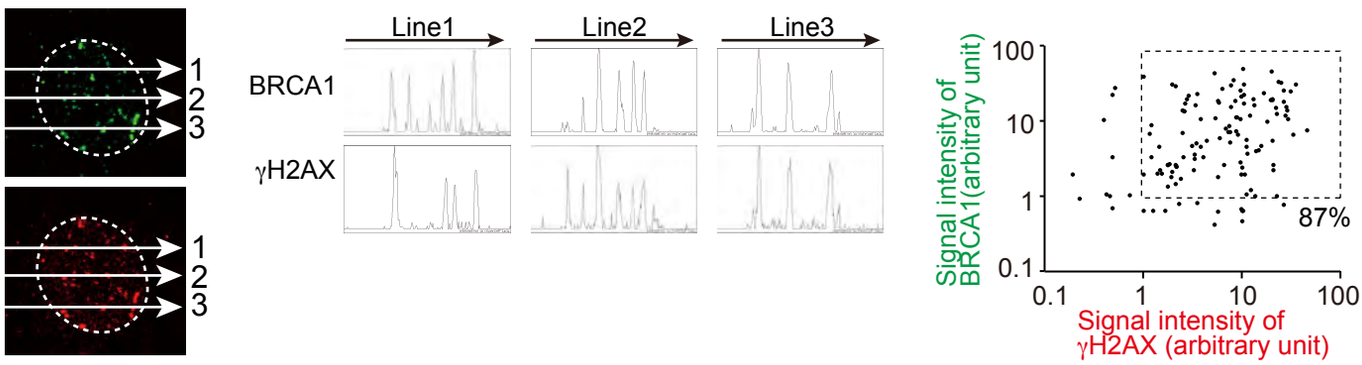
C MCF-7



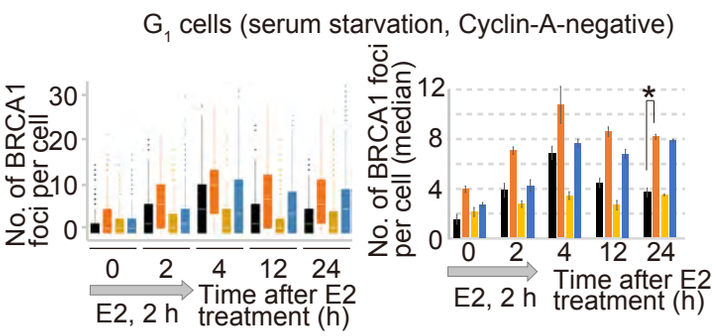
B MCF-7



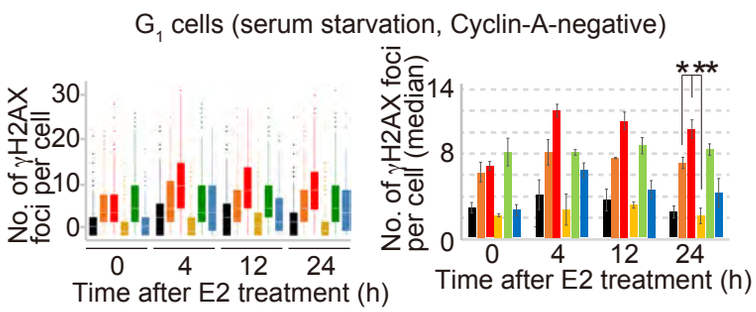
A MCF-7, 0 and 4 h after E2 treatment, γH2AX (green), BRCA1 (red)

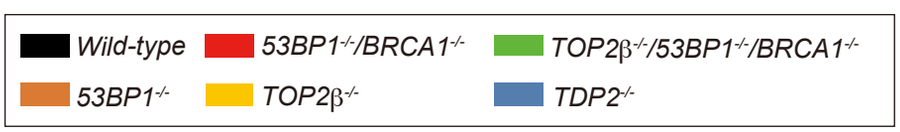


B MCF-7

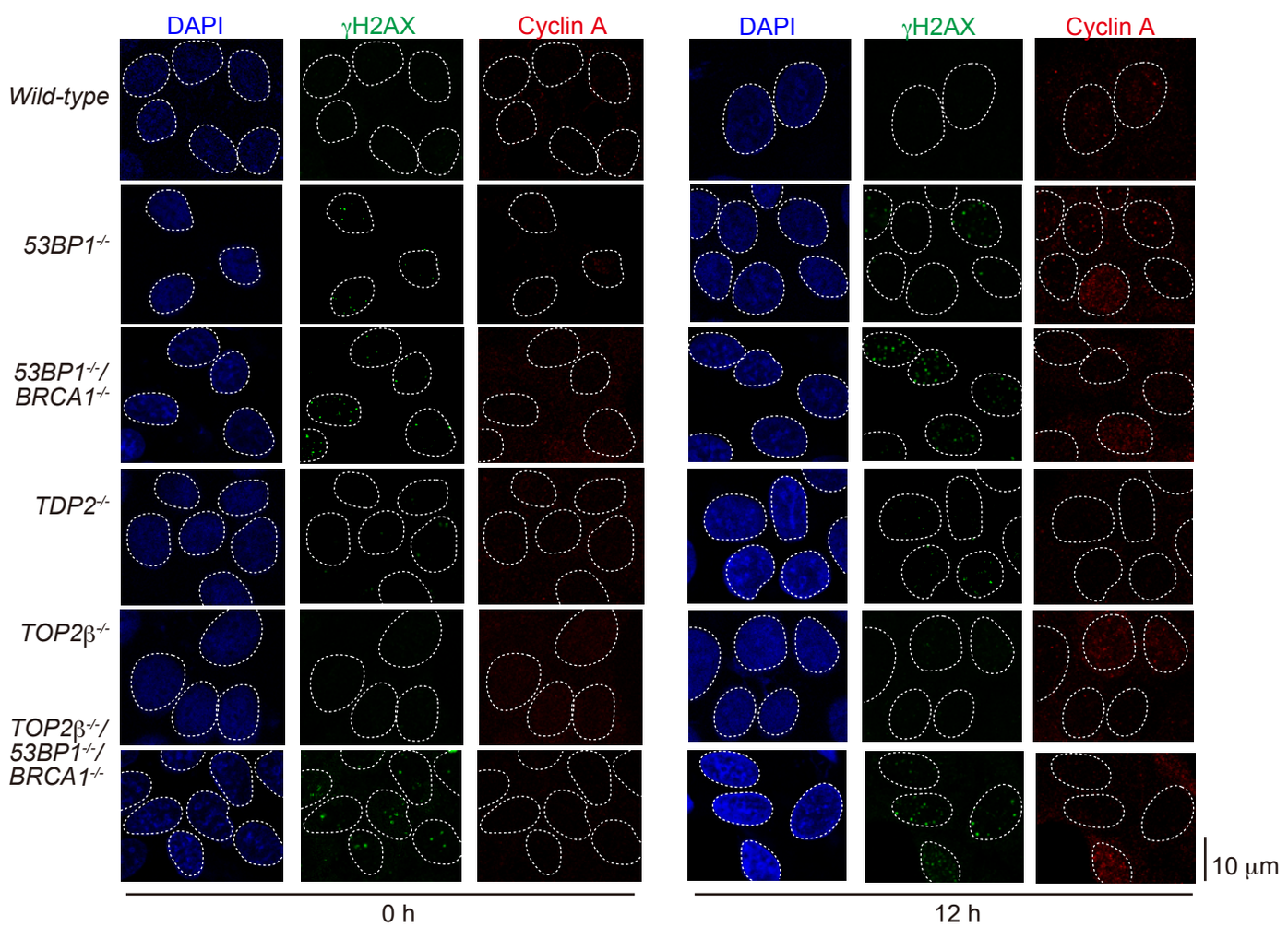


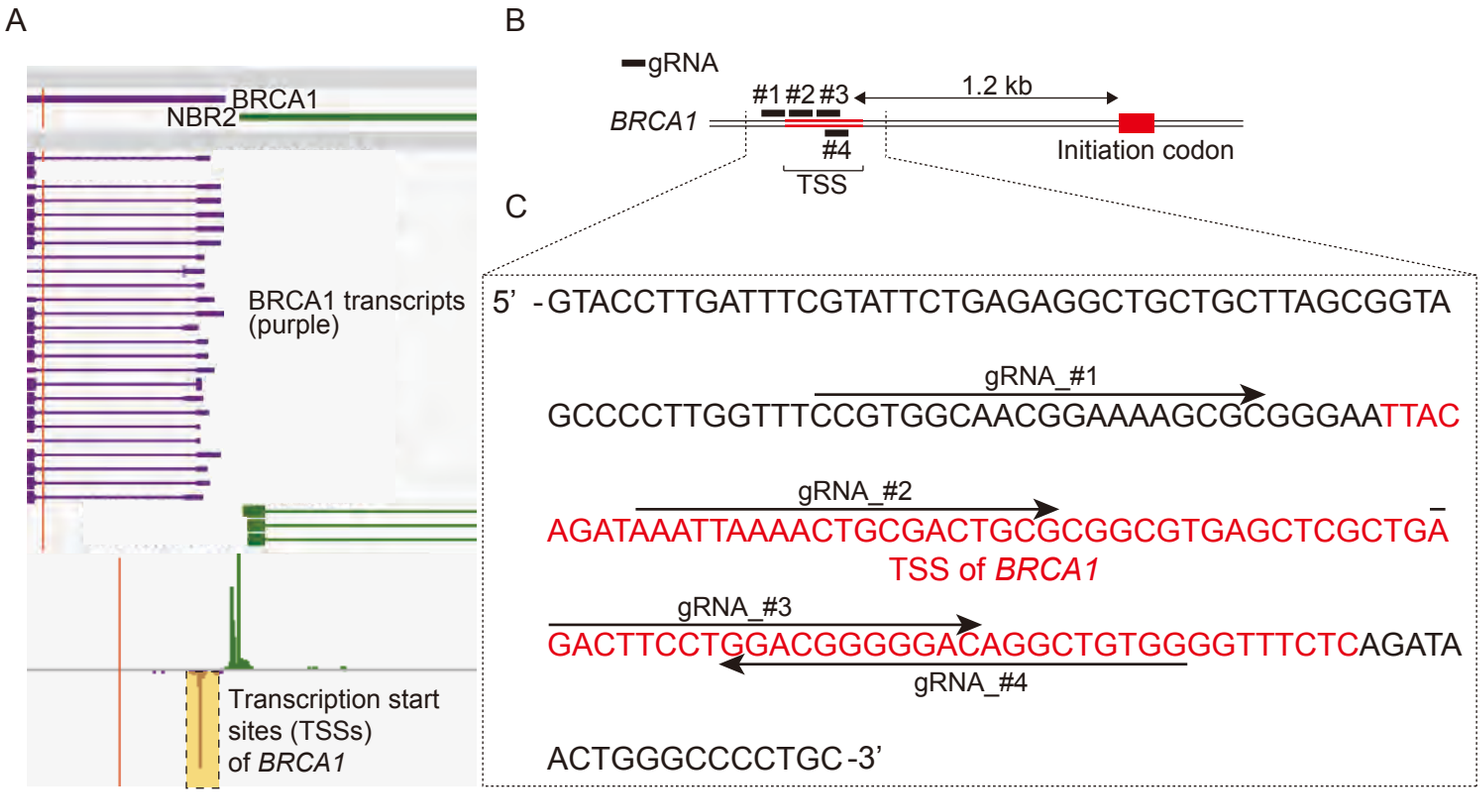
C MCF-7

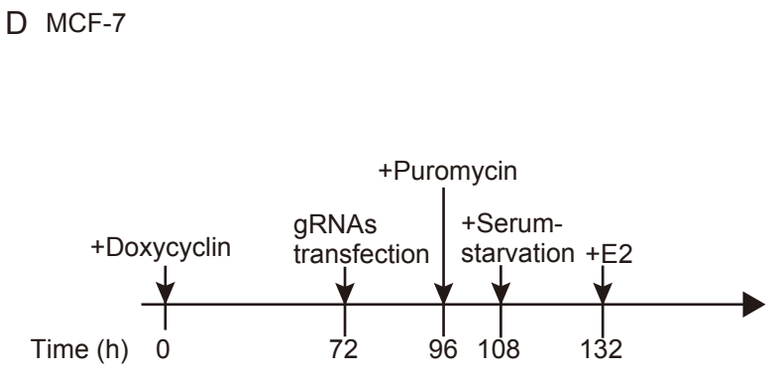


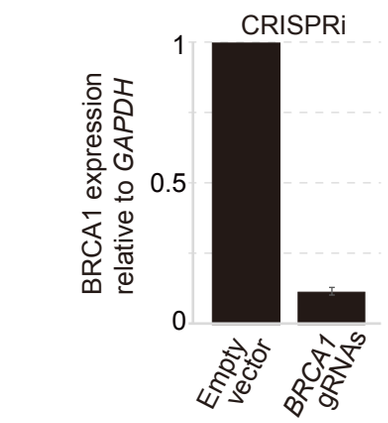


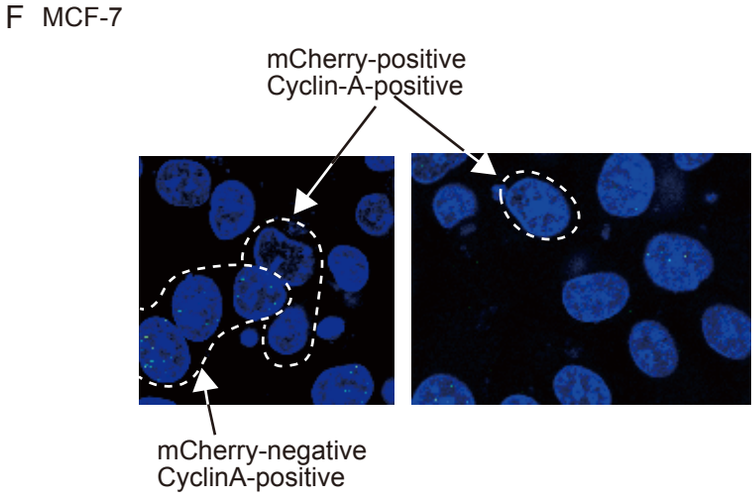
D MCF-7, 0 and 12 h after E2 treatment, DAPI (blue), γH2AX (green), Cyclin A (red)

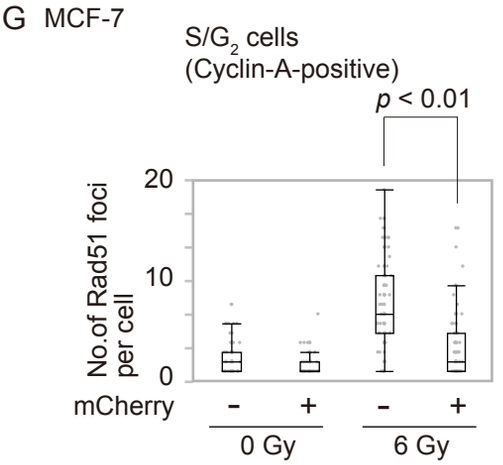



A
BRCA1
NBR2
BRCA1 transcripts (purple)
Transcription start sites (TSSs) of BRCA1
B
gRNA
#1 #2 #3
1.2 kb
BRCA1
#4
TSS
Initiation codon
C
5' -GTACCTTGATTTCGTATTCTGAGAGGCTGCTGCTTAGCGGTA
gRNA_#1
GCCCCTTGGTTTCCGTGGCAACGGAAAAGCGCGGGAATTAC
gRNA_#2
AGATAAATTAAAACTGCGACTGCGCGGCGTGAGCTCGCTGA
TSS of BRCA1
gRNA_#3
GACTTCCTGGACGGGGGACAGGCTGTGGGGTTTCTCAGATA
gRNA_#4
ACTGGGCCCCTGC-3'

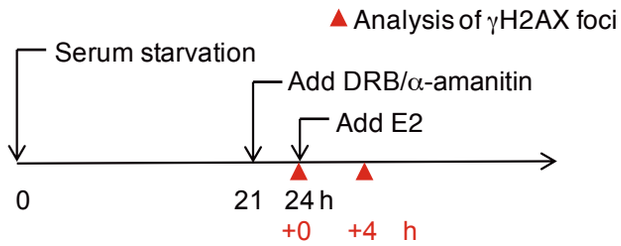

D MCF-7
+Doxycyclin
gRNAs transfection
+Puromycin
+Serum-starvation
+E2
Time (h)
0
72
96
108
132


E MCF-7
CRISPRi
BRCA1 expression relative to GAPDH
1
0.5
0
Empty vector
BRCA1 gRNAs

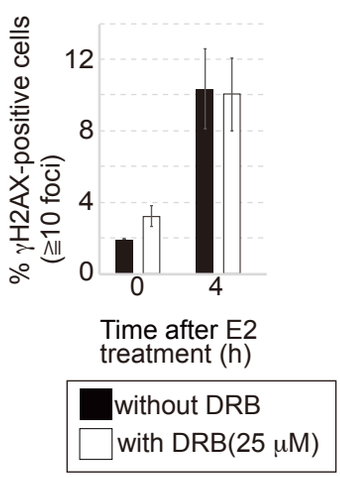

F MCF-7
mCherry-positive Cyclin-A-positive
mCherry-negative CyclinA-positive


G MCF-7
S/G2 cells (Cyclin-A-positive)
p < 0.01
No.of Rad51 foci per cell
20
10
0
mCherry
-
+
-
+
0 Gy
6 Gy

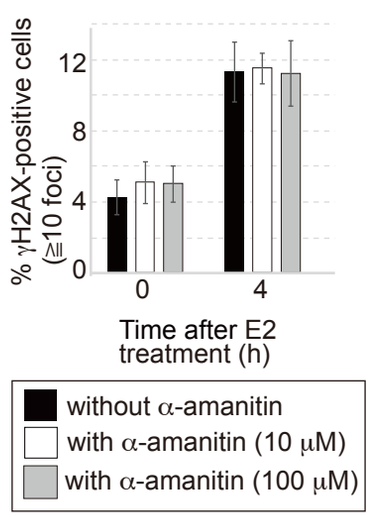
A MCF-7



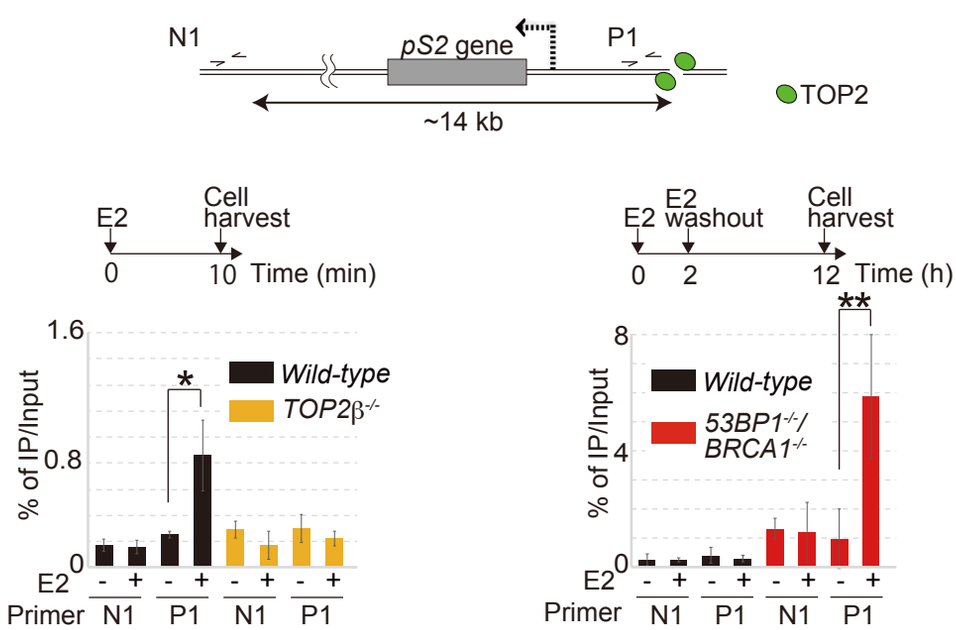
B MCF-7



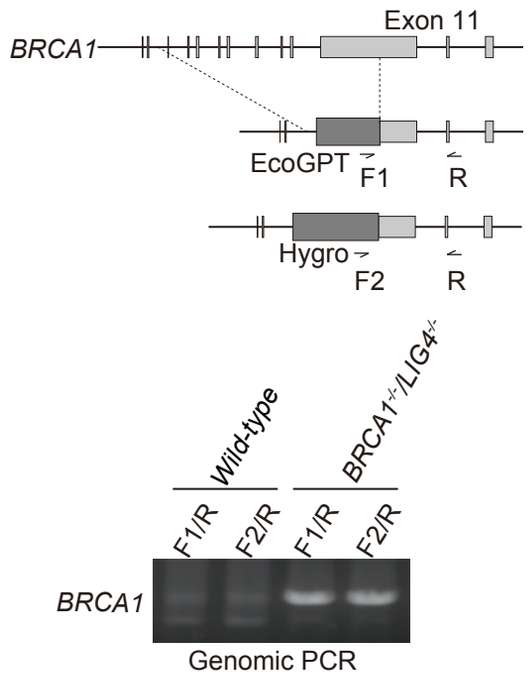
C MCF-7



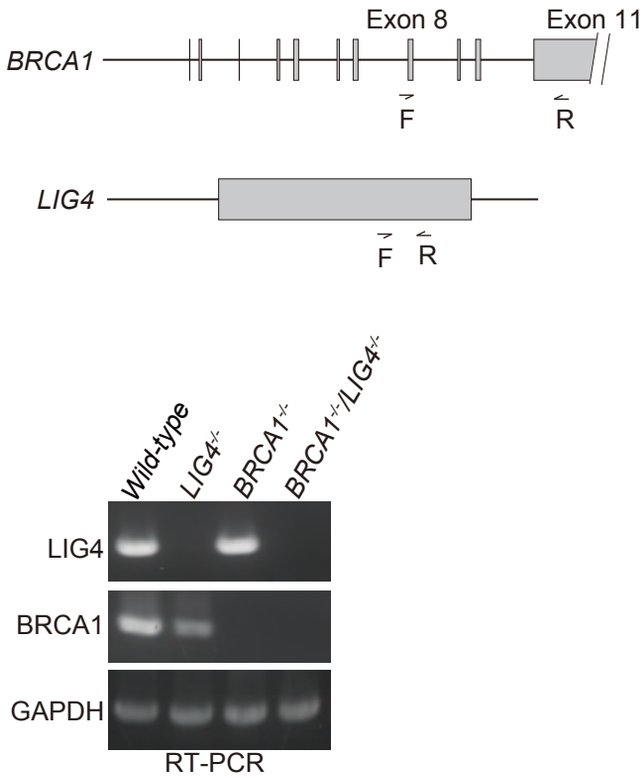
D MCF-7

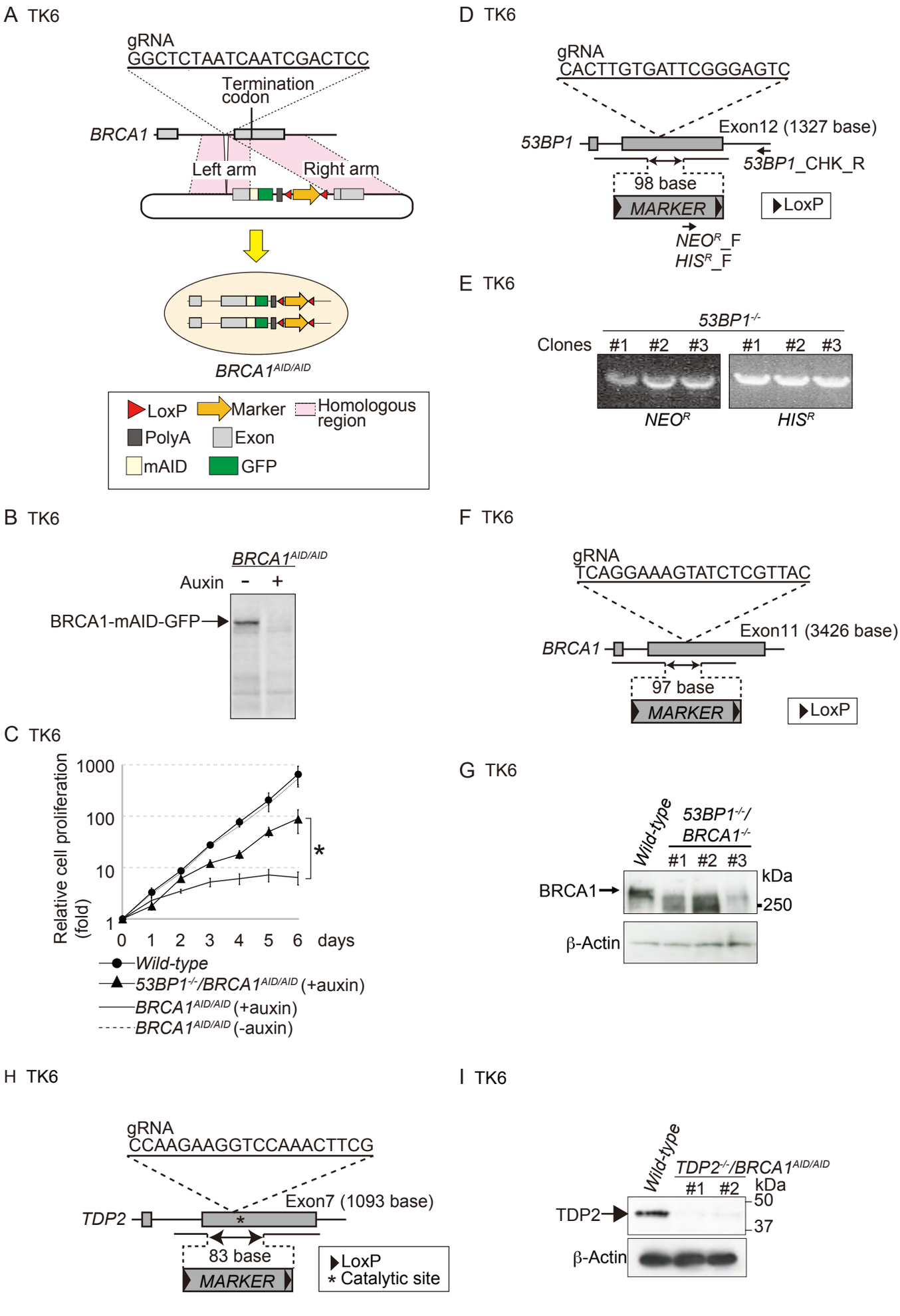


A DT40, genomic PCR



B DT40, RT-PCR




A TK6
gRNA
GGCTCTAATCAATCGACTCC
Termination codon
BRCA1
Left arm
Right arm
BRCA1AID/AID
LoxP
Marker
Homologous region
PolyA
Exon
mAID
GFP
B TK6
BRCA1AID/AID
Auxin - +
BRCA1-mAID-GFP
C TK6
Relative cell proliferation (fold)
1000
100
10
1
0 1 2 3 4 5 6 days
*
Wild-type
53BP1-/-/BRCA1AID/AID (+auxin)
BRCA1AID/AID (+auxin)
BRCA1AID/AID (-auxin)
D TK6
gRNA
CACTTGTGATTCGGGAGTC
53BP1
Exon12 (1327 base)
53BP1_CHK_R
98 base
MARKER
LoxP
NEOR_F
HISR_F
E TK6
53BP1-/-
Clones #1 #2 #3 #1 #2 #3
NEOR
HISR
F TK6
gRNA
TCAGGAAAGTATCTCGTTAC
BRCA1
Exon11 (3426 base)
97 base
MARKER
LoxP
G TK6
Wild-type
53BP1-/-/BRCA1-/-
#1 #2 #3
kDa
BRCA1
250
β-Actin
H TK6
gRNA
CCAAGAAGGTCCAAACTTCG
TDP2
Exon7 (1093 base)
*
83 base
MARKER
LoxP
* Catalytic site
I TK6
Wild-type
TDP2-/-/BRCA1AID/AID
#1 #2
kDa
TDP2
50
37
β-Actin

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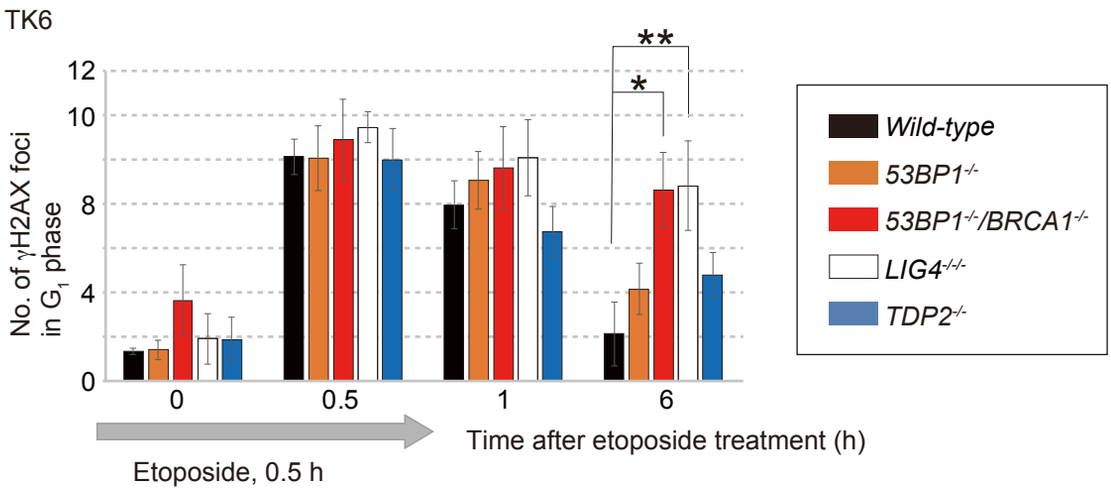
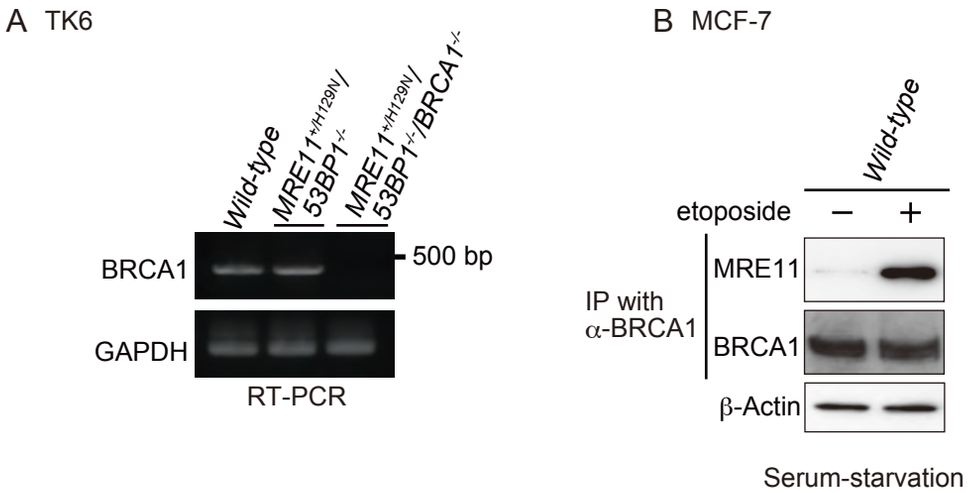
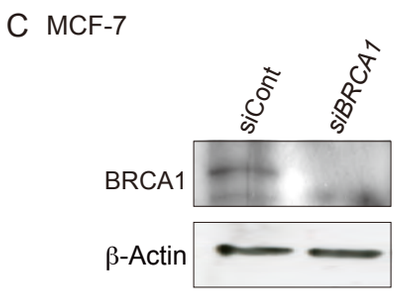
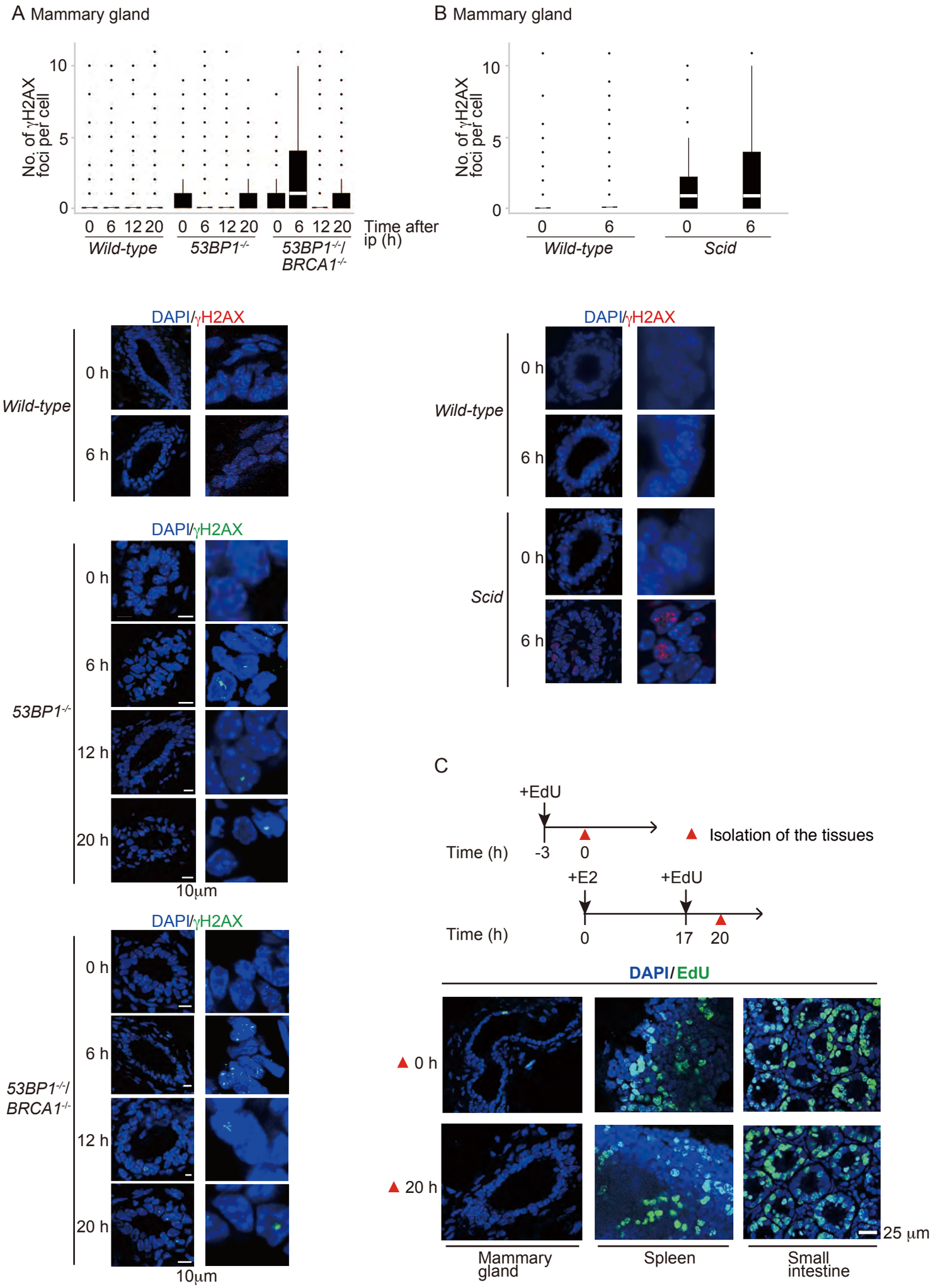



A TK6
Wild-type
MRE11+/H129N/ 53BP1-/-
MRE11+/H129N/ 53BP1-/-/BRCA1-/-
BRCA1
500 bp
GAPDH
RT-PCR
B MCF-7
Wild-type
etoposide
–
+
IP with α-BRCA1
MRE11
BRCA1
β-Actin
Serum-starvation


C MCF-7
siCont
siBRCA1
BRCA1
β-Actin


A Mammary gland
No. of γH2AX foci per cell
10
5
0
0 6 12 20 0 6 12 20 0 6 12 20
Time after ip (h)
Wild-type
53BP1-/-
53BP1-/-/ BRCA1-/-
B Mammary gland
No. of γH2AX foci per cell
10
5
0
0 6 0 6
Wild-type
Scid
DAPI/γH2AX
Wild-type
0 h
6 h
DAPI/γH2AX
Wild-type
0 h
6 h
DAPI/γH2AX
53BP1-/-
0 h
6 h
12 h
20 h
10μm
Scid
0 h
6 h
C
+EdU
Time (h)
-3
0
Isolation of the tissues
+E2
+EdU
Time (h)
0
17
20
DAPI/EdU
0 h
20 h
25 μm
Mammary gland
Spleen
Small intestine
DAPI/γH2AX
53BP1-/-/ BRCA1-/-
0 h
6 h
12 h
20 h
10μm

Supplemental figure legends

Fig. S1. Generation of *TDP2*$^{-/-}$, *53BP1*$^{-/-}$, *53BP1*$^{-/-}$/*BRCA1*$^{-/-}$, and *TOP2β*$^{-/-}$ MCF-7 cells.

(*A*) Schematic diagram of *TDP2* genomic locus, target location, and guide RNA (gRNA) sequence.

(*B*) Western blot of whole-cell extracts prepared from *wild-type* and *TDP2*$^{-/-}$ MCF-7 clones. TDP2 is indicated by arrow, and the asterisks indicate non-specific bands. β-Actin was a loading control.

(*C*) Schematic diagram of *53BP1* genomic locus, target location, and gRNA sequence.

(*D*) Immunostaining of *53BP1* foci in *wild-type* and *53BP1*$^{-/-}$ clones 2 h after ionizing radiation (6 Gy).

(*E*) Schematic diagram of *BRCA1* genomic locus, target location, and gRNA sequence.

(*F*) Western blot analysis of BRCA1 in *53BP1*$^{-/-}$/*BRCA1*$^{-/-}$ MCF-7 clones. Lanes 1 and 2 show *wild-type* MCF-7 and *53BP1*$^{-/-}$/*BRCA1*$^{-/-}$ TK6 cells (*SI Appendix*, Fig. S7*D*-*G*), respectively, representing positive and negative controls. Lanes 3 shows a *53BP1*$^{-/-}$/*BRCA1*$^{-/-}$ MCF-7 clone. α-Tubulin was a loading control.

(*G*) Schematic diagram of *TOP2β* genomic locus, target location, and gRNA sequence. "ATG" indicates the start codon of the *TOP2β* gene.

(*H*) Western blot analysis of TOP2β. Lanes 1 and 5 are *wild-type*. Lanes 2–4 are *TOP2β*$^{-/-}$, generated for this study. β-ACTIN was a loading control.

(*I*) Western blot analysis of TOP2β in *53BP1*$^{-/-}$*/BRCA1*$^{-/-}$ MCF-7 cells. Lanes 1 and 2 are *wild-type* and *TOP2β*$^{-/-}$ control cells, respectively. Lane 10 shows a successful disruption of *TOP2β* in *53BP1*$^{-/-}$*/BRCA1*$^{-/-}$. α-Tubulin was a loading control.

(*J*) Western blot analysis of TOP2α and TOP2β expression in MCF-7 cells at 0, 24, 36, and 48 h after serum-starvation.

Fig. S2. Cell cycle progression following 2 h pulse-exposure of serum-starved MCF-7 cells to E2

(*A*) The cell cycle analysis. The cells were stained with FITC-conjugated anti-BrdU antibody to measure BrdU incorporation into genomic DNA (y-axis, logarithmic scale) and with propidium iodide (PI) to measure the total DNA (x-axis, linear scale). The experimental procedure is the same as in Fig. 1*B* and *C*. The lower-left box identifies

G_1-phase cells, the upper box identifies S-phase cells, and the lower-right box identifies G_2/M-phase cells.

(*B*) The percentage of G_1-phase and S/G_2-phase cells. Error bars were plotted for SD from three independent experiments.

(*C*) Representative images of immunostaining with α-cyclin-A at the same indicated time points as in (*A*).

(*D*) The percentage of cyclin-A-negative cells was calculated from (*C*). Error bars were plotted for SD from three independent experiments. More than 100 cells were analyzed for each experiment.

Fig. S3. E2-induced BRCA1 and γH2AX foci in MCF-7 cells.

(*A)* Densitometric scanning of magnified *TDP2*$^{-/-}$ cells was performed from left to right along the indicated arrows. The resulting data are shown in the dot plot, with γH2AX and BRCA1 foci-signal intensities shown along the x- and y-axes, respectively.

(*B*) The median of the numbers of BRCA1 foci per cell after 2 h transient E2 treatment in individual MCF-7 cells. Error bars are SD of three independent analyses. Error bars

indicate SD calculated from three independent experiments. The asterisk indicates $p <$ 0.005. The *p*-values were calculated by Student's *t*-test.

(*C*) The median of the numbers of γH2AX foci per cell after 2 h transient E2 treatment in individual MCF-7 cells. Data are shown as in (A). Error bars indicate SD calculated from three independent experiments. The single and double asterisks indicate $p < 0.05$ and $p < 0.002$. The *p*-values were calculated by Student's *t*-test.

(*D*) Representative images of E2-induced γH2AX foci. "12 h" indicates cells having been incubated with E2 (10 nM) for 2 h, and then incubated in E2-free medium for an additional 10 h. Blue, green, and red specks indicate DAPI, γH2AX, and cyclin-A signals, respectively. The nuclei are outlined.

Fig. S4. CRISPRi-mediated gene-silencing of *BRCA1* in MCF-7 cells.

(*A*) The transcription start site (TSS) of *BRCA1* is registered in the FANTOM database (http://fantom.gsc.riken.jp/zenbu/). Yellow box includes genomic DNA sequences containing frequently used TSSs of *BRCA1* gene.

(*B*) Four gRNAs used for CRISPRi. We designed them using CRISPRdirect (https://crispr.dbcls.jp/). They are positioned around the TSSs, which are located approximately 1.2 kb upstream of the initiation codon for *BRCA1*.

(*C*) The genomic sequences shown by the yellow box correspond to those shown by the yellow box in (*A*). We input the indicated genomic sequences into CRISPRdirect and obtained the four DNA sequences indicated by the arrows, which correspond to #1 to #4 in (*B*).

(*D*) Experimental method of the CRISPRi-mediated BRCA1 depletion. Doxycyclin was added at time zero to induce the expression of dCas9-KRAB. The expression plasmids containing the gRNAs (*C*) were transiently transfected followed by puromycin selection (2 μg/ml) for 12 h to enrich gRNA-expressing cells. The timing of adding E2 in Fig. 1*D* and 1*E* was at "132" h.

(*E*) BRCA1 expression was analyzed by RT-qPCR of dCas9-KRAB-expressing cells that had been transiently transfected with either an empty vector or expression vector for BRCA1 gRNA. RNA was isolated at 3 d after gRNA transfection (*D*) without E2 treatment.

(*F*) Evaluation of BRCA1 depletion by measuring γ-rays-induced Rad51 foci. Rad51-focus formation was analyzed 2 h after γ–ray exposure (6 Gy) at 3 d after gRNA transfection. In this experiment, we did not conduct the following procedures shown in (*D*): addition of puromycin, serum starvation, or addition of E2. It should be noted that the gRNA-expression plasmid includes both mCherry gene and puromycin-resistance gene. Representative images of Rad51 foci: "mCherry-positive" indicates cells expressing gRNAs and "Cyclin-A-positive" indicates cells in S/G_2 phase.

(*G*) Quantification of Rad51 foci among S/G_2-cells. Cells expressing gRNAs were identified by detecting the expression of mCherry. *p*-value was calculated using Welch's *t*-test.

Fig. S5. E2-induced DSB formation is dependent on both TOP2β and ERs, but is independent of ongoing transcription

(*A*) Schematic diagram of experimental design to examine the effect of RNA polymerase II inhibitors (DRB and α-amanitin) on E2-dependent γH2AX-focus formation. *53BP1*$^{-/-}$*/BRCA1*$^{-/-}$ MCF-7 cells were incubated in serum-free medium for 21

h and then treated with DRB for 3 h. E2 was added at 24 h after incubation in serum-free medium. Red triangles indicate the time points for the analysis of γH2AX foci.

(*B* and *C*) Ongoing transcription is not required for E2-induced DSB formation. γH2AX foci were counted at the indicated times after E2 treatment.

(*D*) ChIP analysis with γH2AX antibody at *pS2* locus in serum-starved *wild-type* and *53BP1*$^{-/-}$*/BRCA1*$^{-/-}$ MCF-7 cells. The top shows the structure of *pS2* gene (gray box), the transcription starting site (leftwards arrow), two sets of primers for quantitative PCR (N1 and P1), and TOP2βcc site (green circles). *Wild-type* and *TOP2*β$^{-/-}$ cells were harvested 10 min after E2 treatment (left graph). *Wild-type* and *53BP1*$^{-/-}$*/BRCA1*$^{-/-}$ cells were incubated with E2 (10 nM) for 2 h ("(+)") or without E2 ("(-)"), followed by incubation in E2-free medium for an additional 10 h (right graph). IP/input percentage was calculated by normalization to non-immunoprecipitated (input) values. Error bars indicate SD calculated from three independent experiments. Single and double asterisks indicate $p < 0.02$ and $p < 0.01$ respectively, calculated by Student's *t*-test.

Fig. S6. Generation of *BRCA1*$^{-/-}$/*LIG4*$^{-/-}$ DT40 cells

(*A*) Schematic diagram of the chicken *BRCA1* genomic locus and results of genomic PCR for the indicated DT40 genotypes. The *BRCA1* allelic genes were disrupted by *EcoGPT* and *HYGRO* markers. The arrows indicate forward and reverse primers for the PCR.

(*B*) RT-PCR for detecting *BRCA1* and *LIG4* transcripts of the indicated DT40 genotypes. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is a control for the loaded RNA. Primers used for this assay are shown by the arrows.

Fig. S7. Generation of *BRCA1*$^{AID/AID}$, *53BP1*$^{-/-}$, *53BP1*$^{-/-}$/*BRCA1*$^{AID/AID}$, *53BP1*$^{-/-}$/*BRCA1*$^{-/-}$, and *TDP2*$^{-/-}$ TK6 cells.

(*A*) Schematic diagram of *BRCA1* genomic locus, gene-targeting construct, and gRNA sequence in TK6. “mAID” indicates the minimized sequence of the Auxin-induced-degron-tag. gRNA binds genomic sequences at the upstream of the exon containing the termination codon. The gRNA sequences were removed from the left arm to avoid digestion of the targeting vector by Cas9.

(*B*) Degradation of BRCA1-mAID-GFP 1 h after exposure of *BRCA1*$^{AID/AID}$ cells to auxin (250 nM). Whole-cell extract was analyzed by western blot with α-BRCA1 antibody.

(*C*) Growth kinetics of the indicated genotypes after auxin treatment from day 0. Cells were continuously exposed to auxin. Error bars indicate SD calculated from three independent experiments.

(*D*) Schematic diagram of *53BP1* genomic locus, gene-targeting construct, and gRNA sequence. "F" and "R" represent forward and reverse primers, respectively.

(*E*) Identification of *53BP1*$^{-/-}$ clones using genomic PCR with *NEO*^{R}_F/*HIS*^{R}_F and *53BP1*_CHK_R primers described in (D).

(*F*) Schematic diagram of *BRCA1* genomic locus, gene-targeting construct, and gRNA sequence.

(*G*) Western blot analysis of BRCA1 to identify *53BP1*$^{-/-}$/*BRCA1*$^{-/-}$ TK6 clones. Arrow indicates BRCA1 protein.

(*H*) Schematic diagram of *TDP2* genomic locus, gene-targeting construct, and gRNA sequence. Targeting vectors were designed to delete the catalytic site (marked by an

asterisk) of the *TDP2* gene. The method for disrupting the *TDP2* gene is the same as that used previously (Hoa *et al*., 2016). *TDP2*$^{-/-}$ TK6 cells used in this study were the same as those used previously (Hoa *et al*., 2016).

(*I*) Western blot analysis of TDP2 to identify *TDP2*$^{-/-}$/*BRCA1*$^{AID/AID}$ TK6 clones. Arrow indicates the band of TDP2. β-Actin is a loading control.

Fig. S8. Repair kinetics of etoposide-induced DSBs.

The indicated TK6 cells were pulse exposed to etoposide from "0" to "0.5 h". The y-axis indicates the average number of γH2AX foci per cell.

Fig. S9. Generation of *MRE11*$^{+/H129N}$/*53BP1*$^{-/-}$/*BRCA1*$^{-/-}$ TK6 cells and siRNA-mediated gene silencing of *BRCA1* in MCF-7 cells.

(*A*) Results of RT-PCR for the indicated TK6 genotypes. Disruption of BRCA1 in *MRE11*$^{+/H129N}$/*53BP1*$^{-/-}$ TK6 cells. Disruption was done as in *SI Appendix*, Fig. S7*F*. The primers, designed at exon 11 of *BRCA1* (Table S2), amplified the 450 base fragment in the intact *BRCA1*.

(*B*) Western blots for detecting MRE11 and BRCA1 in BRCA1 immunoprecipitates from serum-starved MCF-7 cells before (-) and after 2 h treatment (+) with etoposide (10 μM). β-Actin is a loading control.

(*C*) Western blot analysis of *BRCA1*-siRNA-treated MCF-7 cells. β-Actin was used as a loading control.

Fig. S10. E2-induced γH2AX foci in mammary gland tissue of mice.

(*A*) Quantification of γH2AX foci in mammary gland tissues. The boxes indicate the 25–75 percentile and whiskers the 10–90 percentile. Horizontal white line in the box marks the medians. The black dots in each boxplot are outliers, the values of which are either higher than the upper inner fence or lower than the lower inner fence of the boxplot. Representative images of mammary gland tissues stained with DAPI (blue) and α-γH2AX (green or red) in *wild-type*, *53BP1*$^{-/-}$, and *53BP1*$^{-/-}$*/BRCA1*$^{-/-}$ mice at 0, 6, 12, and 20 h after E2 injection. Right images indicate enlarged view of the left.

(*B*) Quantification of γH2AX foci in mammary gland tissues in *wild-type* and *Scid* mice at 0 and 6 h after E2 injection. Representative images of mammary gland tissues stained with DAPI (blue) and α-γH2AX (red) Right images indicate enlarged view of the left.

(*C*) *in vivo* EdU incorporation. Schematic diagrams of experimental design are shown in the upper panels. E2 was intraperitoneally injected into the mice (time 0). After 17 h, mice were given an intraperitoneal injection of EdU at 0.6 mg per 20 g mouse weight. The indicated tissues were isolated 3 h after intraperitoneal injection of EdU. Small intestine and Spleen were analyzed as positive controls.

Table S1. List of Mutant Genotypes used in this study

Genotype	**Name of cell line and species**	**Treat-ment[a]**	**Marker genes**	**Source**	**Identifier**
BRCA1$^{AID/AID}$	Human TK6 (TSCER2)		*neo*^{R}, *puro*^{R}, *his*^{R}		
53BP1$^{-/-}$	Human TK6 (TSCER2)		*neo*^{R}, *his*^{R}		
53BP1$^{-/-}$*/BRCA1*$^{AID/AID}$	Human TK6 (TSCER2)		*neo*^{R}, *puro*^{R}, *his*^{R}	This	N/A

53BP1$^{-/-}$*/BRCA 1*$^{-/-}$	Human TK6 (TSCER2)		*neo*^{R}, *his*^{R}, *hygro*^{R}	study	
MRE11$^{loxP/H129N}$*/ 53BP1*$^{-/-}$*/BRCA 1*$^{-/-}$	Human TK6 (TSCER2)	3/Tam	*puro*^{R}		
TDP2$^{-/-}$*/BRCA1* $^{AID/AID}$	Human TK6 (TSCER2)		*neo*^{R}, *puro*^{R}, *his*^{R}		
53BP1$^{-/-}$	MCF-7		-		
53BP1$^{-/-}$ / *BRCA1*$^{-/-}$	MCF-7		-		
TDP2$^{-/-}$	MCF-7		-		
TOP2β$^{-/-}$	MCF-7		-		
TOP2β$^{-/-}$*/53BP1* $^{-/-}$*/BRCA1*$^{-/-}$	MCF-7		-	This	N/A
MCF-7 conditionally	MCF-7	3/Dox	*neo*^{R}	study	

expressing dCas9-KRAB				This study	N/A
53BP1$^{-/-}$	Chicken DT40		*neo*^{R}, *hygro*^{R}		
53BP1$^{-/-}$*/BRCA 1*$^{-/-}$	Chicken DT40		*hygro*^{R}, *bsr*^{R}, *his*^{R}, *EcoGPT*^{R}		
BRCA1$^{-/-}$*/LIG4*$^{-/-}$	Chicken DT40		*puro*^{R}, *hygro*^{R}, *bsr*^{R}, *EcoGPT*^{R}		
TDP2$^{-/-}$	Human TK6 (TSCER2)		*neo*^{R}, *puro*^{R}	(1)	
MRE11$^{loxP/H129N}$	Human TK6 (TSCER2)	3/Tam	*puro*^{R}, *neo*^{R}, *hygro*^{R}		
Wild-type expressing TIR1	Human TK6 (TSCER2)		*puro*^{R}	(1)	

BRCA1$^{-/-}$	Chicken DT40		*puro*^{R}, *his*^{R}	(2)	
TDP2$^{-/-}$	Chicken DT40		*neo*^{R}, *puro*^{R}	(3)	
LIG4$^{-/-}$	Chicken DT40		*neo*^{R}, *his*^{R}	(4)	

[a] Indicated genotypes were treated with 4-OH tamoxifen (Tam) or doxycycline (Dox) for 3 days. Tam treatment was used to conditionally inactivate *wild-type MRE11* gene for TOP2 trapping experiment. Dox treatment was used to conditionally express dCas9-KRAB for CRISPRi experiment. TSCER2 is a cell line derived from TK6 (5).

Table S2. Quantification of genomic DNA used for Top2 trapping, related to Fig. 3

A Steps for measuring the concentration of genomic DNA.

1, Take 50 μl from 1 ml DNA solution dissolved in buffer B (Step 2 of chromatin fractionation)

2, Proteinase K treatment for > 4 hours

3, Treat twice with phenol chloroform

4, Perform ethanol precipitation and dissolved DNA in 1000 μl TE solution (related to Figure 3E)

5, Measure DNA concentration by spectrophotometer

B

Related to Fig. 3*B*	Etop-oside	#1 (μg/ml)	OD (260nm/280nm)	#2 (μg/ml)	OD (260nm/280nm)	#3 (μg/ml)	OD (260nm/280nm)
Wild-type	-	110.5	1.96	105.4	1.82	105.1	1.88
	+	106.5	1.88	105.2	1.88	99.2	1.89
TDP2$^{-/-}$	-	104.2	1.91	104.4	1.88	102.9	1.92
	+	106.2	1.89	101.3	1.92	99.3	1.83

Related to Fig. 3*C*	Etop-oside	#1 (μg/ml)	OD (260nm/280nm)	#2 (μg/ml)	OD (260nm/280nm)	#3 (μg/ml)	OD (260nm/280nm)
Wild-type	-	109.2	1.90	100.2	1.84	89.4	1.81
	+	106.5	1.89	103.9	1.96	88.4	1.91
53BP1$^{-/-}$	-	104.2	1.99	104.7	1.79	88.8	1.83
	+	106.2	1.84	99.3	1.88	82.4	1.91
BRCA1$^{AID/AID}$	-	99.2	1.92	100.1	1.93	90.2	1.79
	+	98.2	1.88	102.1	1.86	87.4	1.89
53BP1$^{-/-}$/ *BRCA1*$^{AID/AID}$	-	100.9	1.80	99.3	1.91	87.6	1.83
	+	101.7	1.79	98.6	1.85	90.1	1.99
53BP1$^{-/-}$/ *BRCA1*$^{-/-}$	-	100.9	1.80	99.3	1.91	78.1	1.80
	+	101.7	1.79	98.6	1.85	77.9	2.00

Related to Fig. 3*C*	Etop-oside	#1 (μg/ml)	OD (260nm/280nm)	#2 (μg/ml)	OD (260nm/280nm)	#3 (μg/ml)	OD (260nm/280nm)
Wild-type	-	109.2	1.90	100.2	1.84	89.4	1.81
	+	106.5	1.89	103.9	1.96	88.4	1.91
53BP1$^{-/-}$	-	104.2	1.99	104.7	1.79	88.8	1.83
	+	106.2	1.84	99.3	1.88	82.4	1.91
BRCA1$^{AID/AID}$	-	99.2	1.92	100.1	1.93	90.2	1.79
	+	98.2	1.88	102.1	1.86	87.4	1.89
53BP1$^{-/-}$/ *BRCA1*$^{AID/AID}$	-	100.9	1.80	99.3	1.91	87.6	1.83
	+	101.7	1.79	98.6	1.85	90.1	1.99
53BP1$^{-/-}$/ *BRCA1*$^{-/-}$	-	100.9	1.80	99.3	1.91	78.1	1.80
	+	101.7	1.79	98.6	1.85	77.9	2.00

Related to Fig. 3*E*	Etop-oside	#1 (μg/ml)	OD (260nm/280nm)	#2 (μg/ml)	OD (260nm/280nm)	#3 (μg/ml)	OD (260nm/280nm)
Wild-type	-	111.3	1.88	99.2	1.81	101.3	1.79
	+	115.1	1.99	97.4	1.88	100.4	1.81
53BP1$^{-/-}$	-	119.8	1.81	100.4	1.91	110.4	1.86
	+	120.9	1.79	103.3	1.82	109.6	1.99
53BP1$^{-/-}$/ *BRCA1*$^{-/-}$	-	102.1	1.82	99.4	1.82	100.4	1.89
	+	100.8	1.87	99.8	1.94	103.8	1.83
TDP2$^{-/-}$	-	120.3	1.97	100.3	1.97	101.3	1.88
	+	120.8	1.99	101.4	1.88	105.6	1.94

Related to Fig. 4*A*	Etop-oside	#1 (μg/ml)	OD (260nm/280nm)	#2 (μg/ml)	OD (260nm/280nm)	#3 (μg/ml)	OD (260nm/280nm)
MRE11$^{-/H129N}$	+	100.5	1.79	100.2	1.84	100.8	1.87
53BP1$^{-/-}$/ *BRCA1*$^{-/-}$	+	103.2	1.91	98.3	1.92	98.5	1.80
MRE11$^{-/H129N}$ *53BP1*$^{-/-}$/ *BRCA1*$^{-/-}$	+	99.5	1.88	99.9	1.96	101.7	1.94

Table S3. List of reagents and resources used in this study

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rat monoclonal α-Cytokeraitn-8/18	Developmental Studies Hybridoma Bank	Cat# TROMA-I, RRID:AB_531826
Rabbit monoclonal α-γH2AX	Cell Signaling Technology	Cat# 9718, RRID:AB_2118009
Goat polyclonal α-rabbit AlexaFluor 546	ThermoFisher	Cat# 11035, RRID: AB_2534093
Goat polyclonal α-rat AlexaFluor 488	Cell Signaling Technology	Cat# 4416S, RRID:AB_10693769
Goat polyclonal α-mouse AlexaFluor 488	ThermoFisher	Cat# A-11029, RRID:AB_2534088
Goat polyclonal α-rabbit AlexaFluor 488	ThermoFisher	Cat# A11034, RRID:AB_2576217
Goat polyclonal α-rabbit AlexaFluor 594	ThermoFisher	Cat# A11037, RRID:AB_2534095
Donkey polyclonal α-mouse HRP	Santa Cruz	Cat# sc-2314, RRID:AB_641170
Donkey polyclonal α-rabbit HRP	Santa Cruz	Cat# sc-2313, RRID:AB_641181
Rabbit polyclonal α-p53 Binding Protein 1	Calbiochem	Cat# PC712, RRID:AB_10685366
Mouse monoclonal α-β-Tubulin	SIGMA	Cat# T6074, RRID:AB_477582

Rabbit polyclonal α-TDP2 Antibody	Bethyl	Cat# A302-737A, RRID:AB_10631698
Rabbit polyclonal α-CyclinA (clone C19)	Santa Cruz	Cat# sc-596, RRID:AB_631330
Mouse monoclonal α-TOP2α	(Tsutsui et al., 2001)	N/A
Mouse monoclonal α-TOP2β	(Tsutsui et al., 2001)	N/A
Mouse monoclonal α-γH2AX (clone JBW301)	Millipore	Cat# 05-636, RRID:AB_309864
Mouse monoclonal α-MRE11 (clone 12D7)	Gene Tex	Cat# GTX70212, RRID:AB_372398
Mouse monoclonal α-BRCA1 (clone D9)	Santa Cruz	Cat# sc-6954, RRID:AB_626761
Mouse monoclonal α-β-actin	Sigma	Cat# A5411
Rabbit polyclonal α-RAD51	Bio Academia	Cat# 70-001, RRID:AB_2177110
Chicken polyclonal α-mCherry	Abcam	Cat# ab205402
Mouse monoclonal α-CRISPR-Cas9	Abcam	Cat# ab205402 RRID:AB_2692325
Goat polyclonal α-chicken AlexaFluor 647	Abcam	Cat# ab150171
FITC-conjugated α-BrdU	Becton Dickinson	Cat# 347583
Chemicals, Peptides, and Recombinant Proteins		
Charcoal/Dextran treated FBS	Hyclone Laboratories	Cat#SH30068.03
Giemsa's Stain Solution	Nacalai Tesque	Cat# 377114-35
Albumin, Bovine, F-V, pH5.2	Nacalai Tesque	Cat# 01863-48
Skim Milk for immunoassay	Nacalai Tesque	Cat# 31149-75
5-Bromo-2'-deoxyuridine	SIGMA	Cat# B5002
MG132	Wako	Cat# 135-18453
17β-Estradiol solution	Sigma	Cat# E-060

Optical cutting temperature (OCT) compound	Sakura Finetek Japan	Cat# 4583
Paraformaldehyde	Wako	Cat# 16320145
Dynabeads Protein G Immunoprecipitation	ThermoFischer	Cat# 10003D
Protease inhibitor cocktail, Complete	SIGMA	Cat# 11697498001
Lipofectamine 3000 Transfection Kit	ThermoFischer	Cat# L3000008
FuGENE HD Transfection Reagent	Promega	Cat# E2312
Doxycycline Hydrochloride	MP Biomedicals, Inc.	Cat# 195044
(Z)-4-Hydroxytamoxifen	Sigma	Cat# H7904
Fulvestrant	Sigma	Cat# I4409
Dynabeads Protein A for Immunoprecipitation	ThermoFischer	Cat# 10002D
Fluoro-KEEPER Antifade Reagent	Nacalai Tesque	Cat# 12745-74
THUNDERBIRD SYBR® qPCR Mix	TOYOBO	Cat# QPS-201Flu
KaryoMAX COLCEMID Solution	ThermoFischer	Cat# 15210-040
Lovastatin	Enzo Life Sciences	Cat# BML-G226-0010
3'-Indoleacetic Acid	Nacalai Tesque	Cat# 19119-61
Etoposide	Trevigen	Cat# 4886-400-01
5,6-Dichlorobenzimidazole 1-β-D-ribofuranoside	Sigma	Cat# D1916
α-Amanitin	Sigma	Cat# A2263
2.5 g/l-Tripsin/1 mmol/l-EDTA Solution	Nacalai Tesque	Cat# 35554-64
Critical Commercial Assays		
Click-iT RNA Alexa Fluor® 488 Imaging Kit	ThermoFischer	Cat# C10329
GeneArt Seamless Cloning Enzyme Mix	ThermoFischer	Cat# A14606
Experimental Models: Cell Lines		
Chicken: DT40 *WT*	(Buerstedde et al., 1991)	N/A
Human: MCF-7 *WT*	ATCC	Cat# HTB-22
Human: TK6 (TSCER2) *WT*	A gift from Dr. Masamitsu Honma (5)	N/A

Human: Lenti-X^{TM} 293T	TAKARA	Cat# 632180
Experimental Models: Organisms/Strains		
The mutant genotypes of TK6, MCF7 and DT40 are listed in Table S1	This work	
Mice: C57BL/6J	SHIMIZU Laboratory Supplies	N/A
Mice: C57BL/6J *53BP1*$^{-/-}$*/BRCA1*$^{+/+}$	(6)	N/A
Mice: C57BL/6J *53BP1*$^{-/-}$*/BRCA1*$^{-/-}$	(7)	N/A
Mice: CB17/*lcr-Prkdc*^{scid}/CrlCrlj	SHIMIZU Laboratory Supplies, CLEA Japan, Inc. (Bosma et al., 1983)	N/A
Mice: C.B-17/*Icr*	CLEA Japan, Inc. (Bosma et al., 1983)	N/A
Oligonucleotides		
The primers are listed in Table S2	This work	
Recombinant DNA		
Plasmid: px330-U6-Chimeric_BB-CBh-hSpCas9	Addgene	Cat# 42230
Plasmid: *DT-ApA/MARKER*^{R}	CDB, RIKEN, Kobe	N/A
Plasmid: pSpCas9(BB)-2A-Puro(pX459)	(8)	Cat# 48139
Plasmid: pHAGE TRE dCas9-KRAB	(9)	Cat# 50917
Plasmid: pgRNA-humanized	(10)	Cat# 44248
Plasmid: pMD2G	Addgene	Cat# 12259
Plasmid: psPAX2	Addgene	Cat# 1226
Plasmid: *pBS-mAID-GFP-NEO*	A gift from Dr. Masato Kanemaki	N/A
Plasmid: *pBS-mAID-GFP-HIS*	A gift from Dr. Masato Kanemaki	N/A
Other		
Control siRNA	GE Healthcare	Cat# L-001810-03-05

BRCA1 siRNA	GE Healthcare	Cat# L-003461-00-0005

Table S4. List of primers used in this study.

Reagent or resource	Source	Identifier
Oligonucleotides (gRNA sequence)		
MCF-7, *TDP2* (for gene disruption), 5'-TCTGTCAGAGAGGGCTCGAG -3'	Eurofin	N/A
MCF-7, *53BP1* (for gene disruption), 5'-GAACGAGGAGACGGTAATAG -3'	Eurofin	N/A
MCF-7, *BRCA1* (for gene disruption), 5'-GGTTTCTGTAGCCCATACTT -3'	Eurofin	N/A
MCF-7, *TOP2β* (for gene disruption), 5'-CGGCGTGGGCGGCGGCAACG -3'	Eurofin	N/A
TK6, *TDP2* (for gene disruption), 5'-CCAAGAAGGTCCAAACTTCG-3'	Eurofin	N/A
TK6, *BRCA1* (for mAID tagging), 5'-GGCTCTAATCAATCGACTCC -3'	Eurofin	N/A
TK6, *53BP1* (for gene disruption), 5'-CACTTGTGATTCGGGGAGTC -3'	Eurofin	N/A
TK6, *BRCA1* (for gene disruption), 5'-TCAGGAAAGTATCTCGTTAC -3'	Eurofin	N/A
CRISPRi-mediated gene silencing of BRCA1 gRNA #1: 5'-CCGTGGCAACGGAAAAGCGC -3'	Eurofin	N/A
CRISPRi-mediated gene silencing of BRCA1 gRNA #2: 5'-AAATTAAAACTGCGACTGCG -3'	Eurofin	N/A
CRISPRi-mediated gene silencing of BRCA1 gRNA #3: 5'-AGACTTCCTGGACGGGGGAC -3'	Eurofin	N/A
CRISPRi-mediated gene silencing of BRCA1 gRNA #4: 5'-CCACAGCCTGTCCCCCGTCC -3'	Eurofin	N/A
Oligonucleotides (the construction of targeting vectors)		
TK6, *BRCA1* (for mAID tagging), Forward primer of left arm, 5'- AGGGCGAATTGGAGCTCCCC CAGATTGAAGTTCATGTTAATACAG -3'	Eurofin	N/A
TK6, *BRCA1* (for mAID tagging), Reverse primer of left	Eurofin	N/A

arm, 5'- TTGGCGCCTGCACCGGATCC GTAGTGGCTGTGGGGGATCTGGGGT -3'		
TK6, *BRCA1* (for mAID tagging), Forward primer of right arm, 5'- TAGTCCAGGAGAATGAATTGACACT	Eurofin	N/A
TK6, *BRCA1* (for mAID tagging), Forward primer of right arm, 5'- CTCACTGTCACCCAGGCTGGAGTGC	Eurofin	N/A
TK6, *53BP1* (for gene disruption), Forward primer of left arm, 5'- GCGAATTGGGTACCGGGCCTAATTCTTGTATTTTTG GTAGAGA -3'	Eurofin	N/A
TK6, *53BP1* (for gene disruption), Reverse primer of left arm, 5'- CTGGGCTCGAGGGGGGGCCTTCTCTGCCCTTGCAA CCAGTGG -3'	Eurofin	N/A
TK6, *53BP1* (for gene disruption), Forward primer of right arm, 5'- TGGGAAGCTTGTCGACTTAAGTTAGATCAGGAGGA AGCTATG -3'	Eurofin	N/A
TK6, *53BP1* (for gene disruption), Reverse primer of right arm, 5'- CACTAGTAGGCGCGCCTTAAATACACAAAAATTAGC CAGATAGTGGCAT -3'	Eurofin	N/A
TK6, *BRCA1* (for gene disruption), Forward primer of left arm, 5'- GCGAATTGGGTACCGGGCCGCCAGTGATCCTCATGA GGCTTTAA -3'	Eurofin	N/A
TK6, *BRCA1* (for gene disruption), Reverse primer of left arm, 5'- CTGGGCTCGAGGGGGGGCCCTCTCTACAGATCTTT CAGTTTGCA -3'	Eurofin	N/A
TK6, *BRCA1* (for gene disruption), Forward primer of right arm, 5'-	Eurofin	N/A

TGGGAAGCTTGTCGACTTAAAATAAATGTGTGAGT CAGTGTGCAG -3’		
TK6, *BRCA1* (for gene disruption), Reverse primer of right arm, 5’- CACTAGTAGGCGCGCCTTAAATGCATGACTACTTCC CATAGGCTGTTC -3’	Eurofin	N/A
Oligonucleotides (genotyping, RT-PCR)		
NEO^{R}_F (for genotyping PCR), 5’-AACCTGCGTGCAATCCATCTTGTTCAATGG -3’	Eurofin	N/A
HIS^{R}_F (for genotyping PCR), 5’-TTTATCAAATTTAGCGCTGTATTCACGCAG -3’	Eurofin	N/A
*53BP1*_CHK_R (for genotyping PCR), 5’- GGAGTTCGAGACTAGCCTGGCTAACATGGT -3’	Eurofin	N/A
BRCA1, forward primer of RT-qPCR: 5’- ACAGACAAGTAAAAGACATGACAGCGATAC-3’	Eurofin	N/A
BRCA1, reverse primer of RT-qPCR: 5’- AAATGACTGGCGCTTTGAAACCTTGAATGT-3’	Eurofin	N/A
GAPDH, forward primer of RT-qPCR: 5’- TGGCCAAGGTCATCCATGACAACTT-3’	Eurofin	N/A
GAPDH, reverse primer of RT-qPCR: 5’- GCGCCAGTAGAGGCAGGGATGATGT -3’	Eurofin	N/A
EcoGPT, forward primer of genomic PCR (F1 in Fig. S6*A*): 5’- ACTATGTTGTTGATATCCCGCAAGATACC -3’	Eurofin	N/A
HYGRO, forward primer of genomic PCR (F2 in Fig. S6*A*): 5’- GTCTGGACCGATGGCTGTGTAGAAGTAC-3’	Eurofin	N/A
Chicken, *BRCA1*, Forward primer of RT-PCR: 5’- GGCATTGAAAAAGGTGTACTTATAGAGCTT -3’	Eurofin	N/A
Chicken, *BRCA1*, Reverse primer of RT-PCR: 5’- TCCACATCCATTTCTTCTGCATTCTTCAAG -3’	Eurofin	N/A
Chicken, *LIG4*, Forward primer of RT-PCR: 5’- GTACACTTCCATAGCTCTGCATGAAGCTAC -3’	Eurofin	N/A
Chicken, *LIG4*, Reverse primer of RT-PCR: 5’-	Eurofin	N/A

TGATCGGAATTCTTGAAGACGAAAGGGCCT -3’		
Oligonucleotides (chromatin immunoprecipitation)		
Chromatin immunoprecipitation assay P1-forward primer: 5’- CTTCTGCAGTGAGTACAGTATTTACCCTGG -3’	Eurofin	N/A
Chromatin immunoprecipitation assay P1-reverse primer: 5’- TTGCTGATAGACAGAGACGACATGTGGTGA -3’	Eurofin	N/A
Chromatin immunoprecipitation assay N1-forward primer: 5’-CATCTCGTGTAATGGTTTCCCAACTCTGTG -3’	Eurofin	N/A
Chromatin immunoprecipitation assay N1-reverse primer: 5’-CCACCTGACCCATGCAAAAGAGACCACTTC -3’	Eurofin	N/A

SI Materials and Methods

All reagents and resources used in this study are listed in *SI Appendix*, Table S3.

Treatment of MCF-7 cells with estrogens

For G_1/G_0 arrest by serum-starvation in MCF-7, the cells were incubated in phenol-red-free DMEM (Cat# 21063029, GIBCO, US) containing 10% FBS for 2 days. The cells were then incubated in serum-free medium for 24 h and E2 (10 nM) were then added. More than 95% of the cells were arrested in the G_0/G_1 phases by the serum-starvation prior to the addition of E2.

For the chromosome analysis in Fig. 1*E*, following 24 h incubation with the medium containing 1% charcoal-filtrated FBS (Cat#SH30068.03, Hyclone, US) for 24 h, the cells were further incubated in the medium containing 1% charcoal-filtrated serum with and without E2 (10 nM) for 36 h.

General method for gene-editing in TK6 cells

We designed gene-targeting constructs based on the manual provided by GeneArt Seamless Cloning Enzyme Mix (ThermoFischer, US) (1). *SI Appendix*, Table S1 shows the list of mutants and the antibiotic resistance markers (*MARKER*^{R}) used to generate the mutants in this study. To generate gene-targeting constructs, we inserted left and right arms (~1 kb each) of genomic sequences into the *ApaI* and the *AflII* sites of the *DT-ApA*/*MARKER*^{R} *vector*. To this end, we assembled the left and right arms together with *DT-ApA*/*MARKER*^{R} having been digested with *ApaI* and *AflII*, using GeneArt Seamless Cloning Enzyme Mix (ThermoFischer, US). Primer information about the left and right arms (~1 kb each) is described in *SI Appendix*, Table S4. To generate the left arm, we needed to add the upstream and downstream sequences derived from the *ApaI* site *to* the 5' and 3' ends, respectively, of *th*e PCR-amplified left arm. For this purpose, we added "5'-GCGAATTGGGTACCGGGCC" to 5' of the upstream primer and added "5'-CTGGGCTCGAGGGGGGGCC" to 5' of the downstream primer. We added the upstream and downstream sequences from the *AflII* site *to the 5' and 3' ends, respectively, of* the PCR-amplified right arm. We added "5'-TGGGAAGCTTGTCGACTTAA" to 5' of the upstream primer of the

PCR-amplified right arm and added ”5’-CACTAGTAGGCGCGCCTTAA” to 5’ of the downstream primer of the PCR-amplified right arm. The *DT-ApA*/*MARKER*^{R} was provided by the Laboratory for Animal Resources and Genetic Engineering, Center for Developmental Biology, RIKEN Kobe (http://www.clst.riken.jp/arg/cassette.html).

The gRNAs were inserted into the *BbsI* site of pX330 vector (Cat# 42230, Addgene, US), which expresses gRNA and Cas9 from the U6 and chicken □-actin promoters, respectively. The two resulting targeting vectors containing different antibiotic markers were transfected with pX330-gRNA into 6 million TK6 cells. The transfected pX330 expressed the Cas9-gRNA complex, induced DSBs at the specific locus of the genomic DNA and thereby facilitated HDR between the genomic locus and the targeting vectors.

Generation of gene-targeting constructs to create *BRCA1*$^{AID/AID}$, *53BP1*$^{-/-}$, and *BRCA1*$^{-/-}$ TK6 cells

To construct the targeting vectors for the *BRCA1*$^{AID/AID}$ cells (*SI Appendix*, Fig. S7*A*), the left and right arms were amplified using the primers shown in *SI Appendix*, Table S4.

To avoid digestion of the left arm by Cas9, the sequence corresponding to gRNA (5'-GGAGTCGATTAGAGCC) in the left arm was removed (*SI Appendix*, Fig. S7*A*). The left and right arms were assembled with *pBS-mAID-GFP-loxP-NEOMYCIN^R (NEO^R)* digested with *EcoNI* and *SmaI* using GeneArt Seamless Cloning Enzyme Mix (ThermoFischer, US). The left and right arms were assembled with *pBS-mAID-GFP-loxP-HISTIDINOL^R* (*HIS^R*) digested with *EcoNI* and *BamHI*. The gRNA was inserted into the *BbsI* site of pX330 vector (Cat# 42230, Addgene, US). The two resulting targeting vectors containing *NEOMYCIN* and *HISTIDINOL* antibiotics markers and pX330-gRNA vector were transfected into *wild-type* expressing the *TIR1* gene (11).

To generate the targeting vectors for the *53BP1$^{-/-}$* cells (*SI Appendix*, Fig. S7*D*), the left and right arms were amplified using "Forward primer of right"/ "Reverse primer of right" and "Forward primer of left"/"Reverse primer of left", which sequences are shown in *SI Appendix*, Table S4. The arms were assembled with *DT-ApA*/*NEO^R and DT-ApA*/*HIS^R vectors by* GeneArt Seamless Cloning Enzyme Mix (ThermoFischer, US) as described above.

To generate the targeting vectors for the *BRCA1*$^{-/-}$ cells (*SI Appendix*, Fig. S7*F*), the left and right arms were amplified using "Forward primer of right"/ "Reverse primer of right" and "Forward primer of left"/"Reverse primer of left", which sequences are shown in *SI Appendix*, Table S4. The arms were assembled with *DT-ApA*/*HYGRO*^{R} *and DT-ApA*/*HIS*^{R} *vectors by* GeneArt Seamless Cloning Enzyme Mix (ThermoFischer, US) as described above.

CRISPR/Cas9-mediated gene-editing in human MCF-7 cells

The gRNAs were inserted into the *BbsI* site of pX459 (Cat# 48139, Addgene, US). pX459 expresses gRNA under the control of the U6 promoter, and Cas9 under the chicken □-actin promoter. The sequences of gRNAs for *TDP2*, *53BP1*, *BRCA1*, and *TOP2*□ are shown in *SI Appendix*, Table S4. pX459-gRNA was transfected into MCF-7 cells, which were seeded on 6 cm dish containing ~60% confluency, with Fugene HD (Promega, US) according to the manufacture protocol. At 24 h after the transfection, puromycin was added to a final concentration of 2 μg/ml. The MCF-7 cells were further incubated for 48 h with the puromycin-containing medium. After removing puromycin,

we incubated the cells for approximately two weeks to isolate the clones. The gene-disruption events were confirmed by western blotting analysis.

Generation of *BRCA1*$^{-/-}$/*LIG4*$^{-/-}$ DT40 cells

10^7 *LIG4*$^{-/-}$ cells were suspended in 0.5 ml PBS containing 20 μg of linearized plasmid for each transfection and electroporated with a Gene Pulser apparatus (BioRad, Hercules, US) at 550 V and 25 μF. The targeting vectors are the same as used in the previous study (2). Following electroporation, cells were transferred into 20 ml fresh medium and incubated for 24 h. Cells were then resuspended in 80 ml medium containing the appropriate drugs and divided into four 96-well plates. After 7-10 days, drug-resistant colonies were transferred to 24-well plates. Drug-resistant colonies were selected in 96-well microtiter plates with medium containing either 30 μg/ml mycophenolic acid (EcoGPT) (Cat#M5255, Calbiochem, US) or 2.5 mg/ml hygromycin.

Administration of estrogens into mice

We injected E2 (300 μg/kg) into *53BP1*$^{-/-}$, *53BP1*$^{-/-}$*/BRCA1*$^{-/-}$, and *Scid* (CB17/*lcr-Prkdc*^{scid}/CrlCrlj) (SHIMIZU Laboratory Supplies, Japan) mice by intraperitoneal injection (IP injection). The same volume of an E2 solvent (PBS) was injected into the control mice.

Preparation of thin-slice specimen from isolated mammary glands

Mammary gland tissue was harvested from 6–8 week-old-female mice. The isolated mammary tissue was briefly washed with cold PBS, then fixed with paraformaldehyde (4%, Cat# 163-20145, Wako, Japan) for 15 min at 4° C, then washed 3 times with PBS. The tissue samples were incubated with 30% sucrose in PBS for 30 min, then embedded with optimal cutting temperature (OCT) compound (Cat# 4583, Sakura, Japan) into Cryomolds (Cat# 4565, Sakura, Japan). The mammary gland tissue was then frozen in liquid nitrogen and kept at -80° C until use. Prior to use, the frozen blocks of tissue were placed on a cryostat at -55° C and sectioned into 10 μm slices. Slides were heated at 55° C to dry.

Immunostaining of mouse tissues

Tissue samples were incubated with paraformaldehyde (4%) for 10 min, then washed three times with PBS containing Tween-20 (0.05%, PBS-T). The slides were incubated with blocking solution (5%, goat serum in PBS-T) for 1 h (or overnight), and subsequently washed once with PBS-T. Slides were then incubated with both α-Cytokeratin-8/18 (1/10, rat monoclonal, University of Iowa, US) and α-γH2AX antibody (1/500, 20E3, rabbit monoclonal, Cell Signaling Technologies, US) for 1 h (or overnight) at 4° C. After washing with PBS-T, slides were incubated with both α-rabbit (Alexa Fluor 546) and α-rat (Alexa Fluor 488) secondary antibodies (Molecular probe, US). γH2AX and Cytokeratin-8/18 signals were detected using BZ-700 (KEYENCE, Japan) and LSM880 (Zeiss, Germany). Z-stack section images consisting of 10 stacks (1μm intervals) from more than 190 nuclei per sample were taken using a 63× oil objective. Images in Fig. 5*A*, *SI Appendix*, S10*A*, and S10*B* represent maximum projection of Z-stacks.

For *in vivo* EdU incorporation in *SI Appendix*, Fig. S10*C*, we intraperitoneally injected

EdU (30 mg/kg) 17 h after IP injection of E2. After 3 h, mammary gland, small intestine, and spleen tissues were isolated. The incorporated EdU was visualized using Click-iT Plus EdU Alexa Flour 488 imaging kit (Cat#C10329, ThermoFisher, US).

Immunostaining of cells

MCF-7 cells were fixed with methanol for 20 min and permeabilized with TritonX-100 (0.5%) in PBS. After incubation in blocking solution (5%, BSA), cells were incubated with the following antibodies for 1 h: α-BRCA1 (1/500, D-9, mouse monoclonal, Santa Cruz, US), α-γH2AX (1/1000, JBW301, mouse monoclonal, Millipore), α-γH2AX (1/500, 20E3, rabbit monoclonal, CST, US). To detect MRE11 foci, we permeabilized MCF-7 cells with TritonX-100 (0.5%) for 10 min after 30 min exposure to etoposide. We then fixed the cells with formaldehyde (4%) for 15 min. After incubation in blocking solution (5%, BSA), the cells were incubated with α-MRE11 (1/500, 12D7, mouse monoclonal, GeneTex, US) for 1 h.

TK6 cells were spun onto a slide using Cytospin (Shandon, Pittsburgh, US), followed by fixation with formaldehyde (4%) for 10 min and permeabilization with Triton X-100

(0.5%) for 10 min. The antibodies used for the TK6 cells were the same as for MCF-7. The densitometric analysis in *SI Appendix*, Fig. S1*A* was performed using ImageJ software as described on the NIH website (https://imagej.nih.gov/ij/docs/menus/analyze.html#gels).

Cell cycle analysis

Cells were labeled for 10 min with 20 mM 5-bromo-2'-deoxyuridine (BrdU; SIGMA, US). Harvested cells were fixed for 2 h with 70% ethanol at 4 degrees and successively incubated as follows: (1) in 2 M HCl and 0.5% Triton X-100 for 30 min at room temperature; (2) in FITC-conjugated anti-BrdU antibody (Becton Dickinson, US) for 1 h at room temperature; and (3) in 5 mg/mL propidium iodide (PI) in PBS. Cells were washed with PBS containing 2% FBS and 0.1% sodium azide between each incubation. Subsequent flowcytometric analysis was performed with a BD LSRFortessa (Becton Dickinson, US). Fluorescence data were displayed as dot plots using FACSDIVA software.

Measurement of TOP2 trapped by genomic DNA

The protocol for this assay was described previously by Hoa et al. 2016. In Fig. 3*G*, whole-cell extract was prepared from 50 million cells, followed by genomic fragmentation by sonication (UR-21P, Tomy Seiko, Japan). Protein G-magnetic beads (Dynabeads, ThermoFischer, US) incubated with α-TOP2β (a gift from Dr. Tsutsui) were used for immunoprecipitation. The IP fraction was eluted by TE containing SDS (1%) and then ultra-centrifuged to separate the TOP2βccs from free TOP2β. The TOP2βccs that migrated into the CsCl gradient during ultra-centrifuging were detected by slot blot.

siRNA-mediated gene silencing

MCF-7 cells were transfected with siRNA (25 pmol) using Lipofectamine 3000 (ThermoFischer, US) and then incubated for 24 h. Cells were seeded at 60% confluency on glass coverslips in 12-well plates and incubated for an additional 24 h prior to analysis.

CRISPRi-mediated gene silencing

Lenti-X^{TM} 293T cells (TAKARA, Japan) were maintained in DMEM (Gibco, Japan) supplemented with 10% fetal bovine serum (Gibco, US). The pMD2G and pSPAX2 (virus packaging plasmids) and dCas9-KRAB-coding plasmids (Cat# 50917, Addgene, US) were transfected into LentiX-293T cells using Fugene HD (Promega, US) in Opti-MEM (Gibco, US) according to the manufacturer's instructions. Viruses were harvested at 48 h after transfection. To generate MCF-7 cells conditionally expressing dCas9-KRAB under the control of the tetracycline-responsive promoter, MCF-7 cells infected with the virus were selected by G418 (Nacalai, Japan). Clones expressing dCas9-KRAB in response to doxycycline were selected by immunostaining with α-Cas9 antibody (Cat# 191468, Abcam, US) 3 days after doxycycline treatment. For the BRCA1 depletion (CRISPRi) in Fig. 1*D* and 1*E*, 4 BRCA1-gRNAs (#1, CCGTGGCAACGGAAAAGCGC, #2, AAATTAAAACTGCGACTGCG, #3, AGACTTCCTGGACGGGGGAC #4, CCACAGCCTGTCCCCCGTCC) were cloned into pgRNA-humanized, respectively (Cat# 44248, Addgene, US) (*SI Appendix*, Fig S4). A schematic representation of the CRISPRi experiment is shown in *SI Appendix*, Fig.

S4*D*. MCF-7 cells were incubated with doxycycline for 3 days to express dCas9-KRAB, after which 4 gRNAs were transfected using Fugene HD (Promega, US). Cells were incubated in serum-free medium containing puromycin for 24 h after gRNA transfection. Cells were analyzed at 60 h after gRNA transfection. We confirmed that gRNAs targeting the TSS of BRCA1 can efficiently suppress Rad51-focus formation induced by γ-rays (*SI Appendix*, Fig. S4*F* and S4*G*).

EU incorporation

Cells were labeled with EU (1 mM) for 1 h and fixed with paraformaldehyde (4%) for 10 min at room temperature. Fixed cells were washed twice with 1X PBS. After permeabilization withTriton X-100/1X PBS (0.5%) for 20 min at room temperature, the Click-iT reaction cocktail (Click-iT RNA Alexa Fluor 488 Imaging Kit, ThermoFisher, Cat# C10329) was applied to samples for 30 min at room temperature, protected from light. After washing twice in 1X PBS, samples were mounted with Vectashield containing DAPI and imaged using a BZ-700 (KEYENCE, Japan) using a 63X objective lens.

Chromosome aberration analysis in mitotic chromosome spreads

Cells were treated with colcemid (0.1 μg/ml, ThermoFischer) for 3 h. Cells were suspended in potassium chloride (75 mM) for 15 min, washed with Carnoy's solution (a 3:1 mixture of methanol and acetic acid), dropped on slides, and stained with a Giemsa solution (5%) for 10 min.

Immunoprecipitation and Western blot analysis

For immunoprecipitation experiment (*SI Appendix*, Fig S9*C*), whole cell extracts (WCEs) were prepared from serum-starved cells (5 x 10^6). WCEs were incubated wit α-BRCA1 antibody pretreated with Dynabeads protein G (Cat# 10003D, ThermoFicher, US). After 3 h incubation, the beads were washed five times with HEPES buffer (pH 7.5) containing KCL (300mM), Tween20 (0.05%), glycerol (10%), protease inhibitor cocktail (Cat# 11697498001, SIGMA, US) and then boiled with 100 μl SDS sample buffer (Tris–HCl (25mM, pH6.5), SDS (1%), β-mercaptoethanol (0.24 mM), bromophenol blue (0.1%), glycerol (5%)).

For Western blotting analysis, cells (1×10^6) were lysed in 100 µl SDS sample buffer. Whole-cell extracts were separated by electrophoresis, transferred onto polyvinylidene difluoride membranes and blocked in 5% skimmed milk dissolved in Tween-20 (0.1%) in TBS. Membranes were incubated with primary antibodies overnight at 4° C followed by washing in Tween-20 (0.1%) in TBS. Membranes were incubated with appropriate HRP-linked secondary antibodies at room temperature for 1 h and washed thrice prior to signal detection. Membranes were developed by chemiluminescence using ECL reagent.

Chromatin immunoprecipitation (ChIP)

ChIP was performed as described previously, with some modifications (12). Briefly, samples were sonicated to generate DNA fragments (<500 bp). The α-γH2AX antibody (1/250, JBW301, Millipore, US) was incubated with Dynabeads Protein A for 3 h at 4° C. Sheared chromatin was centrifuged at 15,000 rpm for 15 min at 4° C. After centrifugation, supernatants were incubated with antibody-protein A conjugates for 3 h at 4° C. The conjugated beads were washed thoroughly with IP buffer-140, IP

buffer-500, LiCl/detergent, and TE. Real-time PCR was carried out using THUNDERBIRD SYBR qPCR Mix. Primer sequences were listed in *SI Appendix*, Table S4.

Quantification and Statistical Analysis

For all statistical analyses with a *p*-value, unpaired Student's *t*-test or Welch's *t*-test was used. Error bars represent standard deviation (SD), as indicated in the legends.

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