Cell Reports

Supplemental Information

Poly(ADP-Ribose) Mediates the BRCA2-Dependent Early DNA Damage Response

Feng Zhang, Jiazhong Shi, Chunjing Bian, and Xiaochun Yu

Figure S1

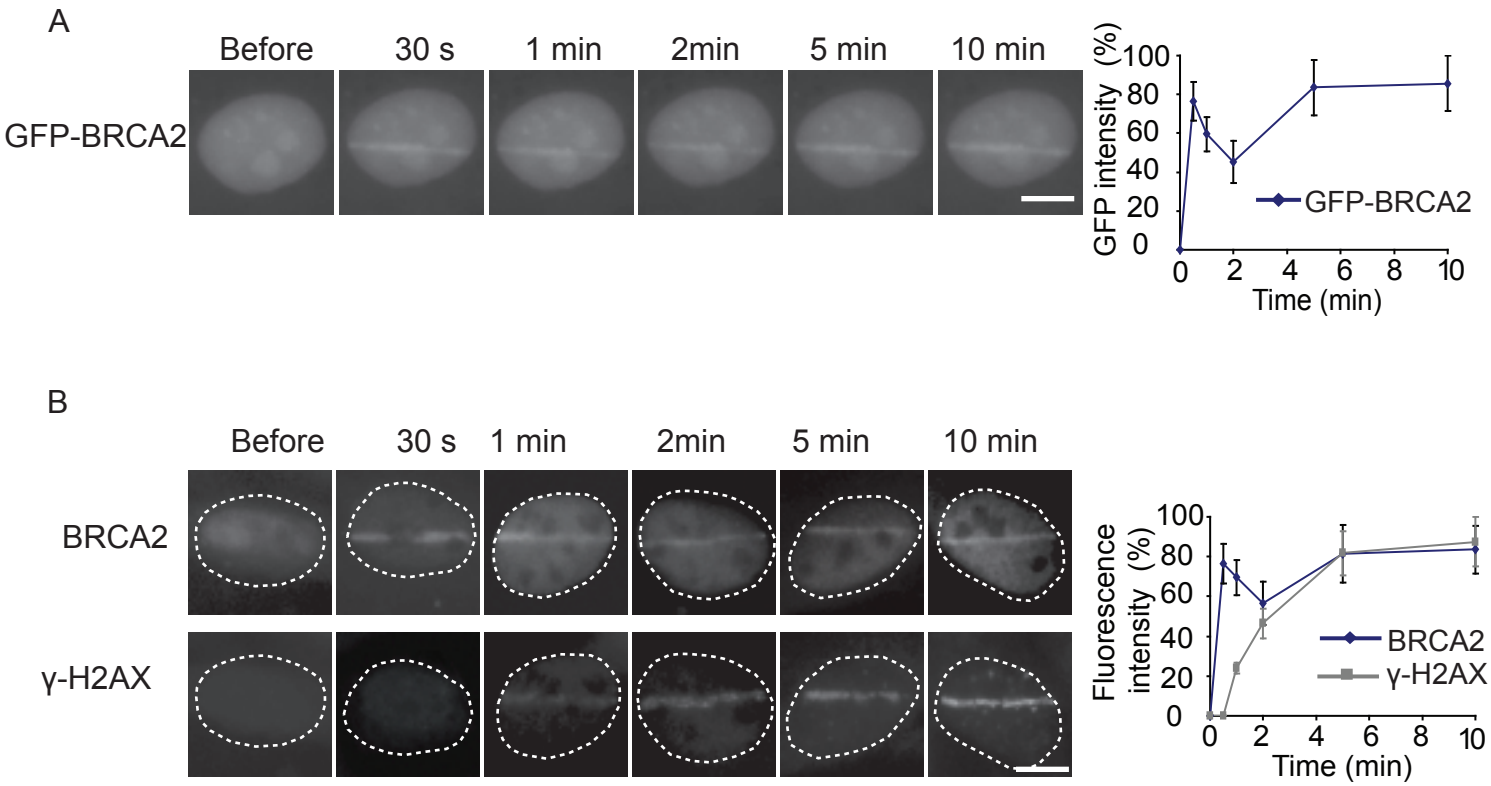

A
Before
30 s
1 min
2min
5 min
10 min
GFP-BRCA2
GFP intensity (%)
100
80
60
40
20
0
0
2
4
6
8
10
Time (min)
GFP-BRCA2
B
Before
30 s
1 min
2min
5 min
10 min
BRCA2
γ-H2AX
Fluorescence intensity (%)
100
80
60
40
20
0
0
2
4
6
8
10
Time (min)
BRCA2
γ-H2AX

Figure S2

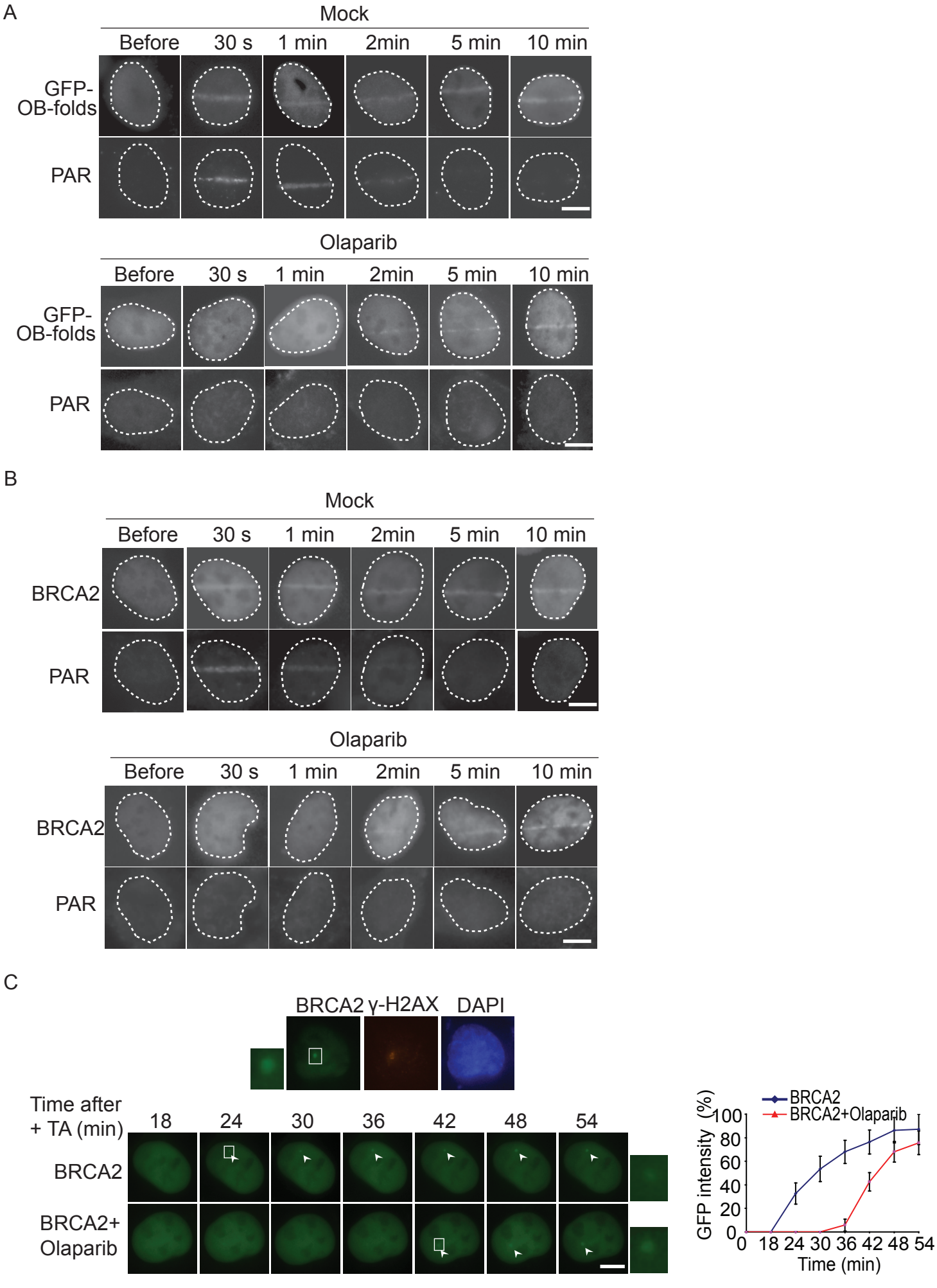

A
Mock
Before
30 s
1 min
2min
5 min
10 min
GFP-OB-folds
PAR
Olaparib
Before
30 s
1 min
2min
5 min
10 min
GFP-OB-folds
PAR
B
Mock
Before
30 s
1 min
2min
5 min
10 min
BRCA2
PAR
Olaparib
Before
30 s
1 min
2min
5 min
10 min
BRCA2
PAR
C
BRCA2
γ-H2AX
DAPI
Time after + TA (min)
18
24
30
36
42
48
54
BRCA2
BRCA2+ Olaparib
BRCA2
BRCA2+Olaparib
GFP intensity (%)
100
80
60
40
20
0
0 18 24 30 36 42 48 54
Time (min)

Figure S3

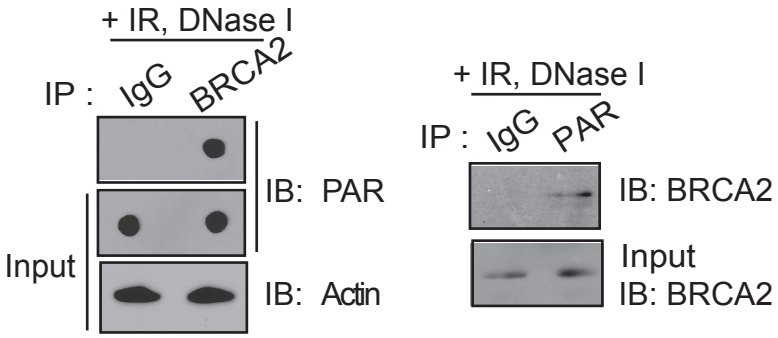

+ IR, DNase I
IP :
IgG
BRCA2
IB: PAR
Input
IB: Actin
+ IR, DNase I
IP :
IgG
PAR
IB: BRCA2
Input
IB: BRCA2

Figure S4

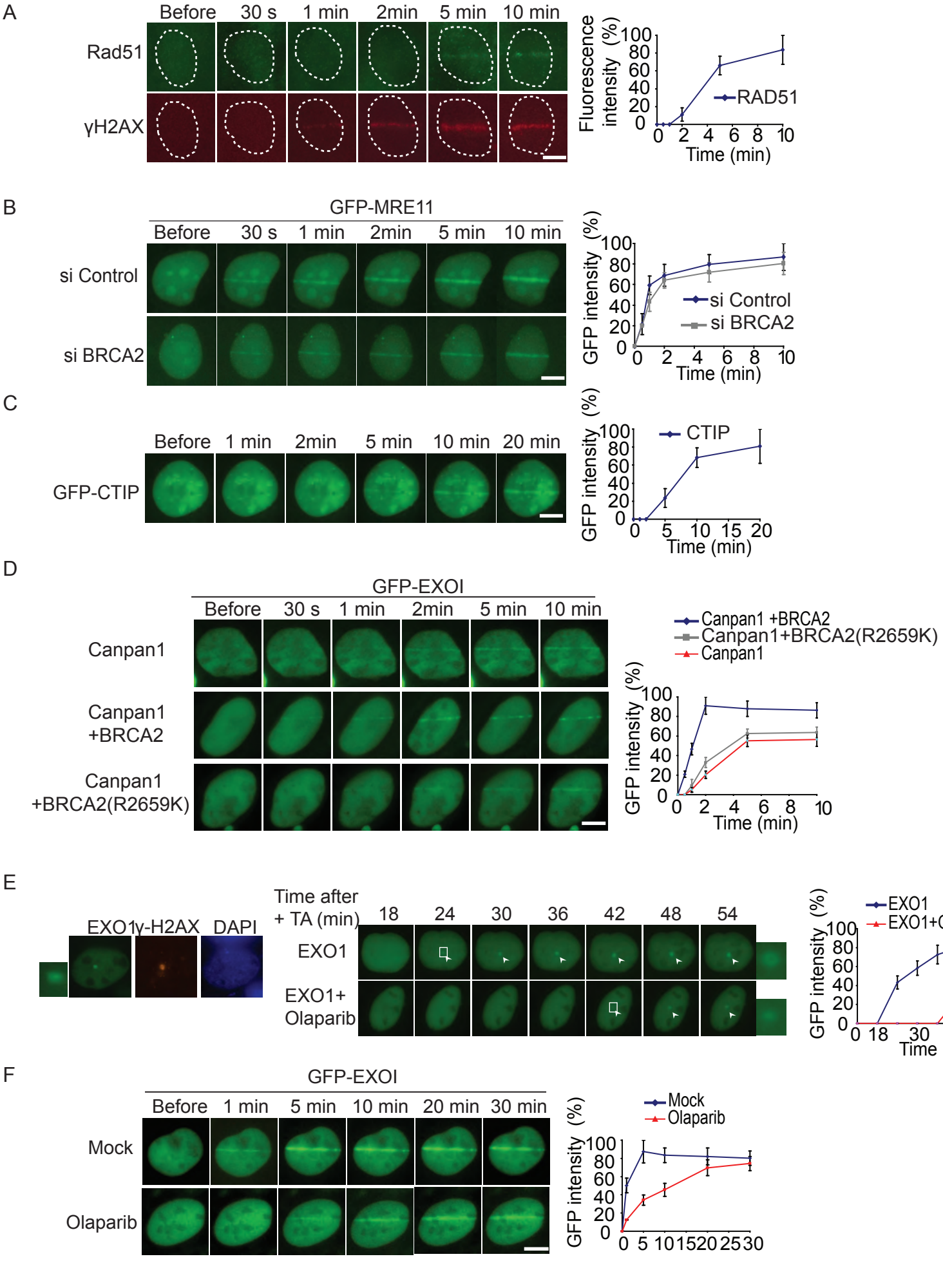
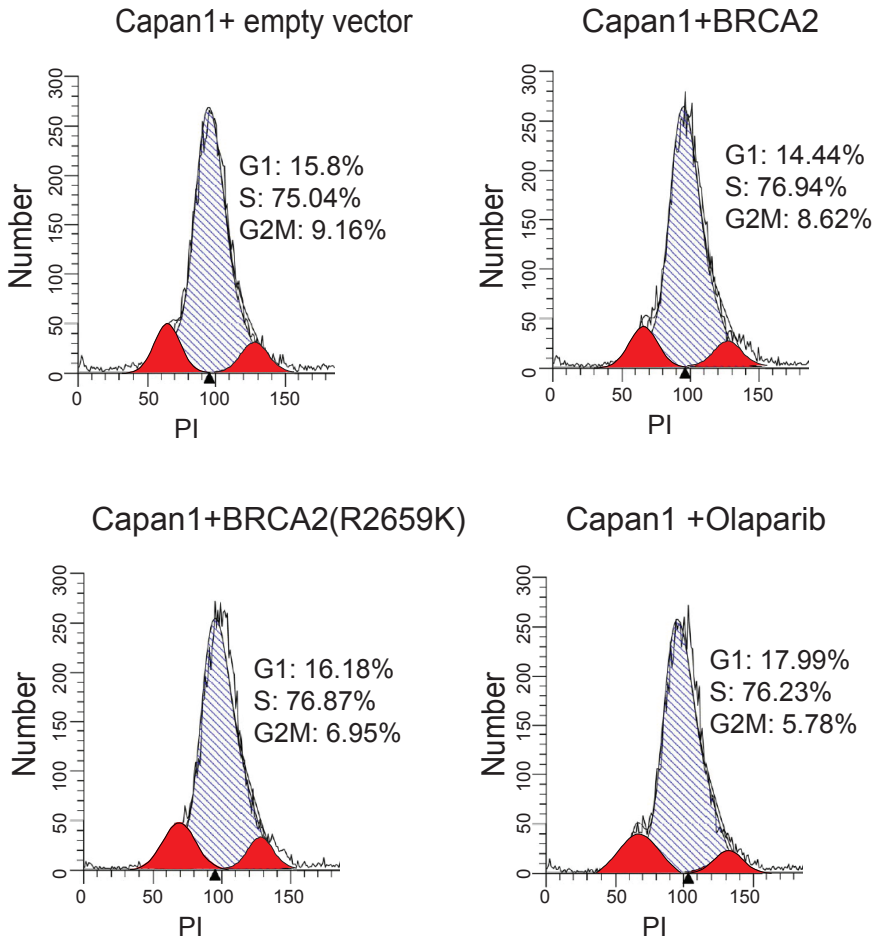
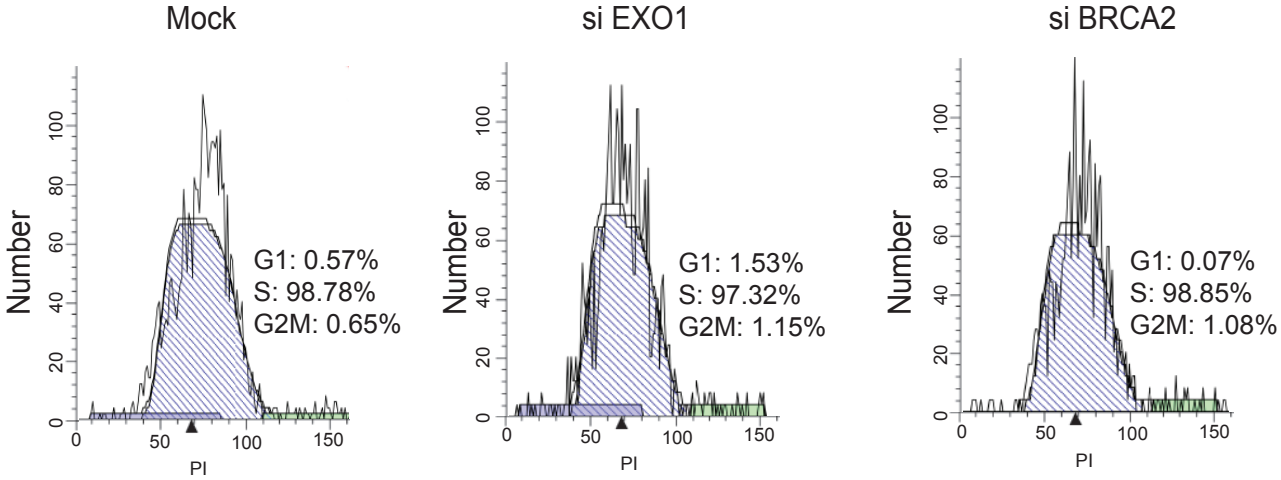

A
Before
30 s
1 min
2min
5 min
10 min
Rad51
γH2AX
Fluorescence intensity (%)
RAD51
Time (min)
B
GFP-MRE11
si Control
si BRCA2
GFP intensity (%)
Time (min)
C
1 min
20 min
GFP-CTIP
CTIP
D
GFP-EXOI
Canpan1
Canpan1 +BRCA2
Canpan1 +BRCA2(R2659K)
E
EXO1
γ-H2AX
DAPI
Time after + TA (min)
18
24
30
36
42
48
54
EXO1+ Olaparib
EXO1+Olaparib
F
Mock
Olaparib

Figure S5

A


Capan1+ empty vector
G1: 15.8%
S: 75.04%
G2M: 9.16%
Capan1+BRCA2
G1: 14.44%
S: 76.94%
G2M: 8.62%
Capan1+BRCA2(R2659K)
G1: 16.18%
S: 76.87%
G2M: 6.95%
Capan1 +Olaparib
G1: 17.99%
S: 76.23%
G2M: 5.78%
Number
PI

B


Mock
G1: 0.57%
S: 98.78%
G2M: 0.65%
si EXO1
G1: 1.53%
S: 97.32%
G2M: 1.15%
si BRCA2
G1: 0.07%
S: 98.85%
G2M: 1.08%
Number
PI

Supplemental Figure legends

Figure S1, Related to Figure 1

(**A**) The relocation kinetics of GFP-BRCA2 to DNA damage sites. Same as Figure 1A except that color images are transformed into gray scale. (B) The relocation kinetics of BRCA2 to DNA damage sites. Same as Figure 1B except that color images are transformed into gray scale.

Figure S2, Related to Figure 2

(**A**) The effect of PARP inhibitor treatment on the recruitment of GFP-OB-folds to DNA damage sites. Same as Figure 2A except that color images are transformed into gray scale. (**B**) PARP inhibitor treatment suppresses the recruitment of BRCA2 to DNA damage sites. Same as Figure 2A except that color images are transformed into gray scale. **(C)** (Upper panel): the localization of BRCA2 after TA induction. The DSB (focus) was marked by γ-H2AX. Magnified boxes denote the colocalization of BRCA2 withγ-H2AX at DSB. (Lower panel): Real-time images of the recruitments of GFP-BRCA2 with or without olaparib treatment in the inducible I-Sce system. Magnified boxes denote the GFP fusion proteins at focus. Error bars represent the SD. Scale bar = 10 μm

Figure S3, Related to Figure 4

The interaction between BRCA2 and PAR is not mediated by DNA. U2OS cells were treated with 0 or 10 Gy of IR. 5 minutes after IR, cells were lysed with NETN-100 (0.5 % NP-40, 2 mM EDTA, 100 mM Tris-HCl pH 7.5, 100 mM NaCl) buffer. Lysed cells were

treated with DNase I (0.1 mg/mL) and analyzed with indicated antibodies. Input or IPed samples were analyzed by Western blotting with the indicated antibodies.

Figure S4, Related to Figure 5

(A) The relocation kinetics of RAD51 to DNA lesions. U2OS cells were examined with laser microirradiation and stained with anti-Rad51 and anti-γH2AX at indicated time points. Scale bar = 10 μm. **(B)** Depletion of BRCA2 does not affect the recruitment of MRE11 to DNA lesions. U2OS cells were treated with siBRCA2, the relocation kinetics of GFP-MRE11 to the sites of DNA damage was examined. **(C)** The relocation kinetics of GFP-CTIP to the sites of DNA damage was examined at indicated time points. **(D)** GFP-EXO1 was expressed in Capan1 cells reconstituted with empty vector, wild type or mutant BRCA2 and the relocation kinetics of GFP-EXO1 to the sites of DNA damage was examined. Scale bar = 10 μm. GFP fluorescence at the laser line was converted into a numerical value (relative fluorescence intensity) using Axiovision software (version 4.5). Normalized fluorescent curves from 20 cells from three independent experiments were averaged. Signal intensities were plotted using Excel. Error bars represent the SD. **(E)** (Upper panel): the localization of EXO1 after TA induction. The DSB (focus) was marked by γ-H2AX. Magnified boxes denote the colocalization of EXO1 withγ-H2AX at DSB. (Lower panel): Real-time images of the recruitments of GFP- EXO1 with or without olaparib treatment in the inducible I-SceI system. Magnified boxes denote the GFP fusion proteins at focus. Error bars represent the SD. Scale bar = 10 μm **(F)** The effect of PARP inhibitor treatment on the recruitment of GFP-EXO1 to DNA damage sites. GFP-EXO1 was expressed in U2OS cells and treated with or without olaparib. The

relocation kinetics was monitored in a time course following laser microirradiation. For quantitative and comparative imaging, signal intensities at the laser line were converted into a numerical value using Axiovision software (version 4.5). Normalized fluorescent curves from 20 cells were averaged. The error bars represent the standard deviation. Scale bar = 10 μm. Signal intensities were plotted using Excel.

Figure S5, Related to Figure 6

(**A**) U2OS cells treated with siEXO1 or siBRCA2 were synchronized by using double thymidine block and harvested for propidium-iodide flow cytometry at 6 hours after release into normal medium. At the time of release, over 97% of cells were at the S Phase. (**B**) Capan1 cells reconstituted with empty vector, wild type or mutant BRCA2 and Capan1 cells treated with olaparib were enriched for S-phase cells by treating an asynchronous culture (70–80% confluence) with 2 mM thymidine for 16 h, then released into fresh medium for 7 h, and arrested again with 2 mM thymidine for 17 h. Over 75% of cells at the S phase were obtained by harvesting cells 4 h after release from the second block.