Supplementary Materials

Supplementary Materials include six figures and two tables.

Supplementary Figures

Figure S1

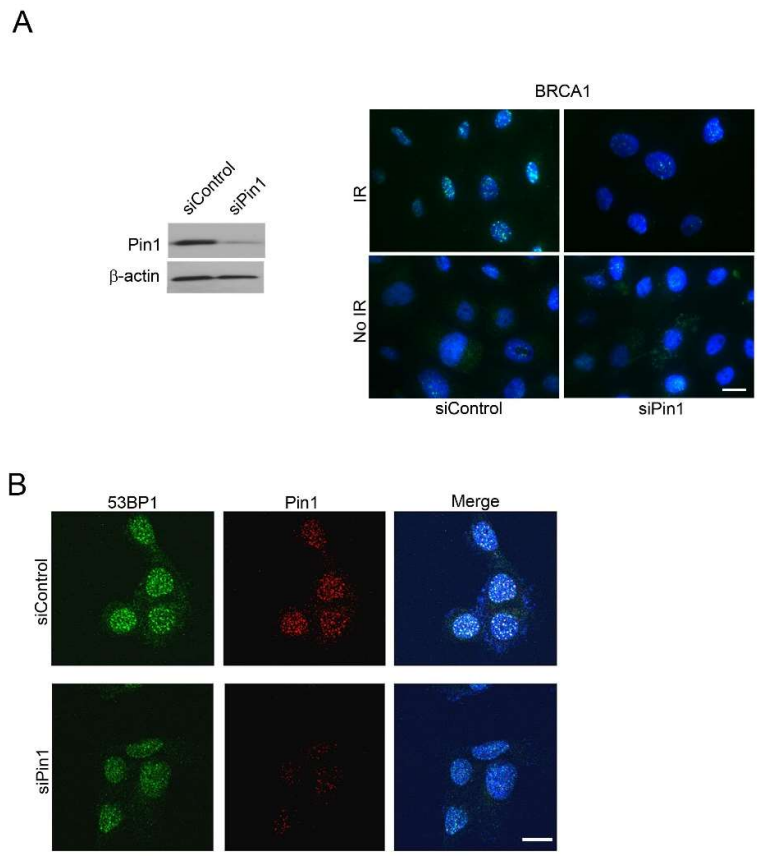

Figure S1. Pin1 is essential for DNA damage foci formation. **A.** Pin1 knockdown prevents BRCA1 from participating the formation of DNA damage foci. Pin1 siRNA was transfected into MCF10A cells for 48 hours. Cells were then fixed for immunofluorescence 5 hours after IR (10G). **B.** SiPin1 prevents the recruitment of 53BP1 into DNA damage foci in MCF-7 cells. Scare bar represents 10 μm. (also see Fig.1)

Figure S2

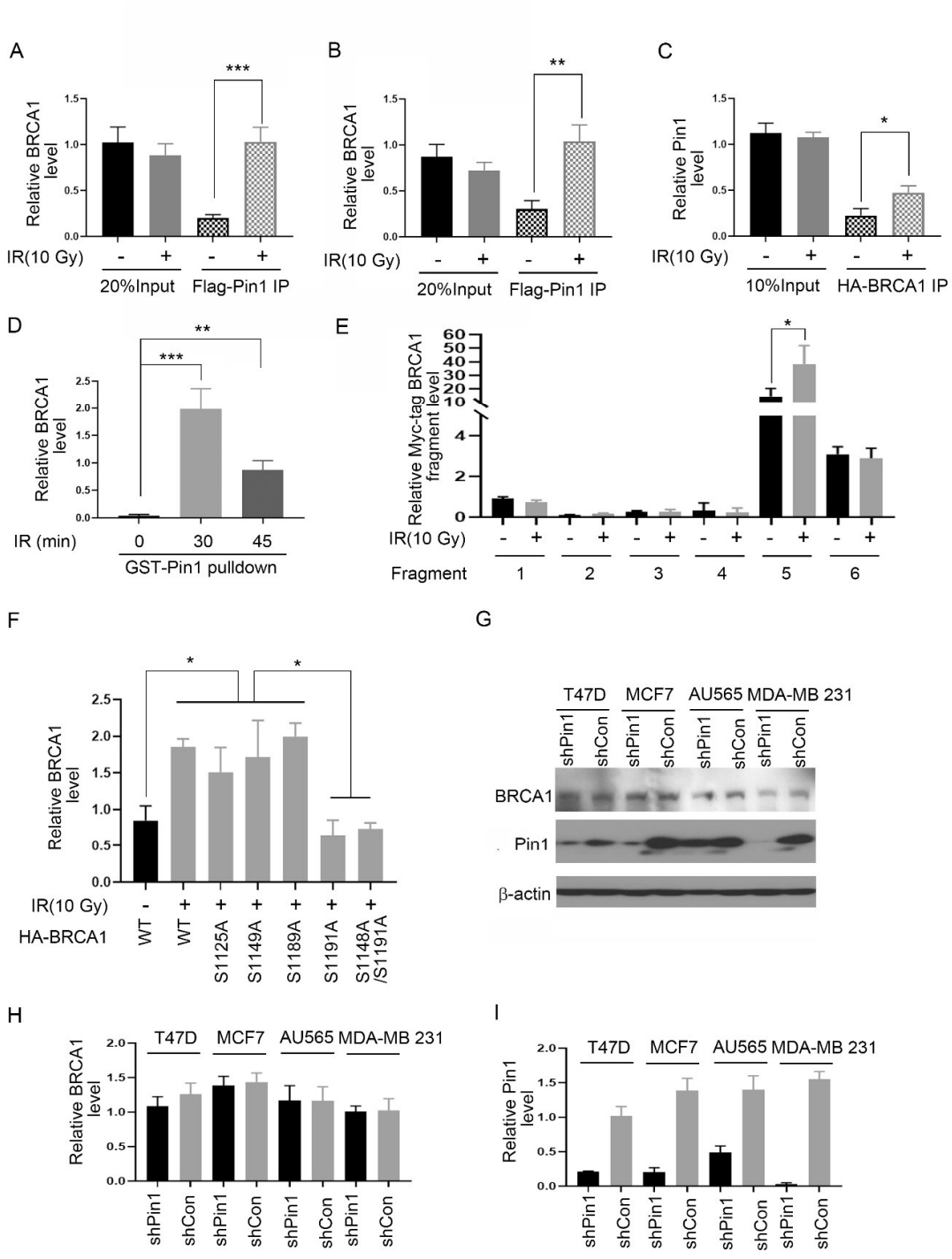
A
Relative BRCA1 level
IR(10 Gy)
20%Input
Flag-Pin1 IP
B
Relative BRCA1 level
IR(10 Gy)
20%Input
Flag-Pin1 IP
C
Relative Pin1 level
IR(10 Gy)
10%Input
HA-BRCA1 IP
D
Relative BRCA1 level
IR (min)
GST-Pin1 pulldown
E
Relative Myc-tag BRCA1 fragment level
IR(10 Gy)
Fragment
F
Relative BRCA1 level
IR(10 Gy)
HA-BRCA1
WT
S1125A
S1149A
S1189A
S1191A
S1148A /S1191A
G
T47D
MCF7
AU565
MDA-MB 231
shPin1
shCon
BRCA1
Pin1
β-actin
H
Relative BRCA1 level
I
Relative Pin1 level

Figure S2. Pin1 binds to BRCA1 in a DNA-damage dependent manner. **A, B.** Quantified Co-IP results of endogenous BRCA1 with Flag-tagged Pin1 in MCF-7 (A) and T47D (B), related to Figure 2A and 2B. **C.** Quantified Co-IP results of endogenous Pin1 with HA-tagged BRCA1 in HEK293 cells, related to Figure 2C. **D.** Quantified pulldown results of GST-Pin1 with BRCA1 in MCF-7 cells, related to Figure 2D. **E.** Quantified pulldown results of GST-Pin1 with myc-tagged BRCA1 fragments, related to Figure 2F. **F.** Quantified pulldown results of GST-Pin1 with BRCA1 mutants, related to Figure 2G. **G-I.** Pin1 depletion does not affect the baseline BRCA1 level in breast cancer cells. ShPin1 or shControl cells were lysed for western blotting (G). Pin1 and BRCA1 levels were quantified in H and I. *P<0.05, **P<0.01, ***P<0.001. (also see Fig.2 and 3)

Figure S3

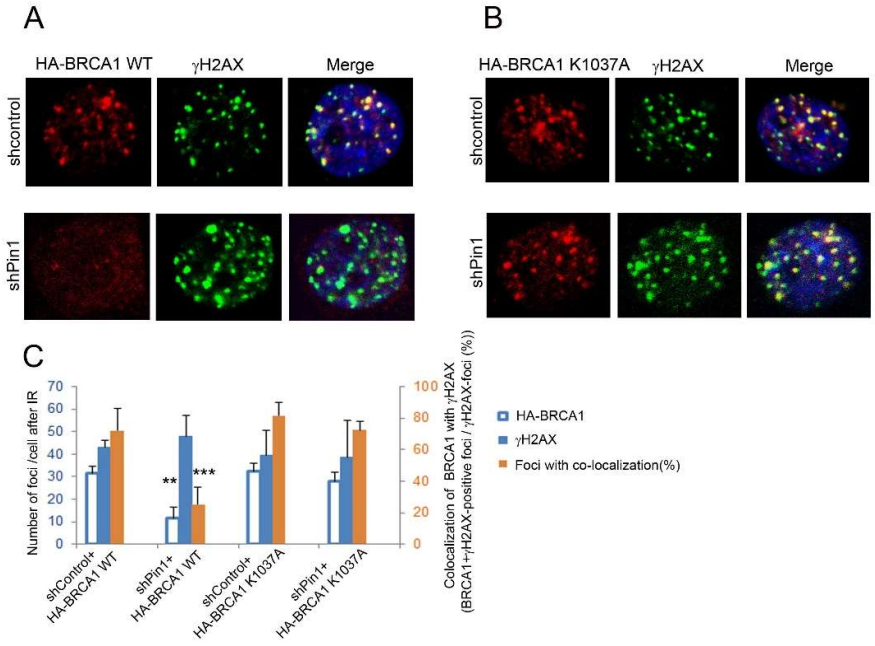

Figure S3. Mutation of Lysine 1037 to Alanine stabilizes BRCA1 after irradiation. **A, B.** Stability of BRCA1 K1037A was not affected by Pin1 knockdown after irradiation with regards to DNA repair foci formation. MCF7 cells were transfected with HA-BRCA1 (WT or K1037A) for 48 hours and then irradiated. Immunofluorescence were performed 5 hours after IR. **C.** Quantification of data in (A and B) of experimental triplicates. *P<0.05, **P<0.01, ***P<0.001. (also see Fig.4)

Figure S4

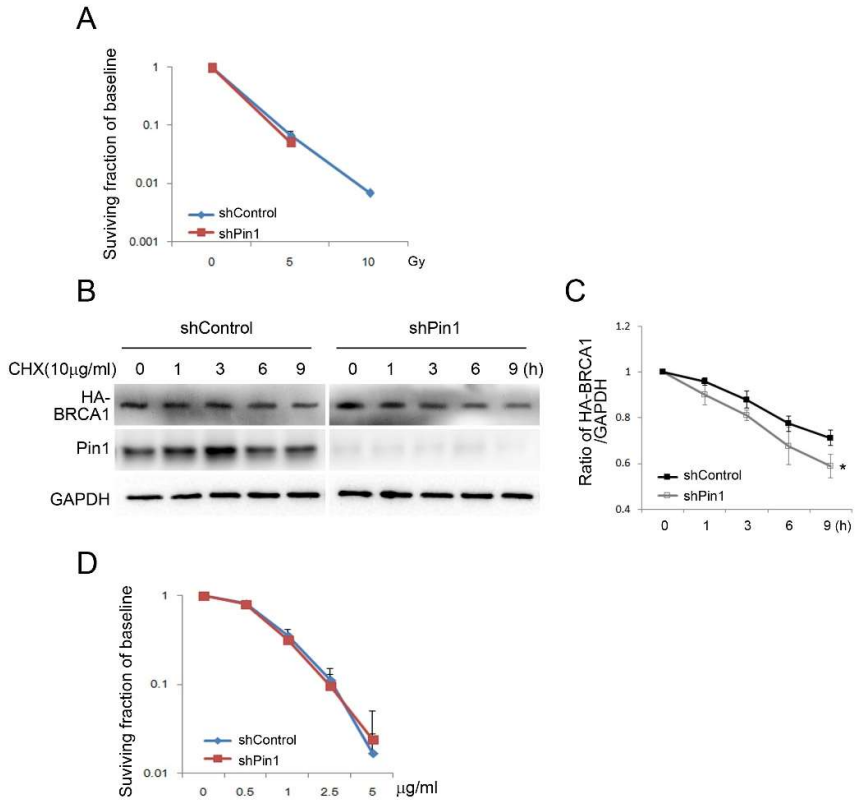

Figure S4. Effects of Pin1 knockdown on BRCA1 mutant and wildtype breast cancer cells. **A.** Pin1 knockdown does not sensitize BRCA1 mutated breast cancer cells to DNA damage. The shPin1 or shControl SUM149 cells with BRCA1 mutant (2288delT) were subjected to irradiation and used for the colony formation assays. **B, C.** BRCA1 degrades faster in Pin1 knockdown cells than in the control cells. ShControl and shPin1 MCF-7 cells were transfected with HA-BRCA1 and treated with 10 μM Olaparib for 72 h, and then subjected to the CHX chase experiment. *P<0.05. **D.** Pin1 knockdown does not sensitize BRCA1 mutated breast cancer cells to the PARP inhibitor. SUM149 cells were treated with Olaparib and used for the colony formation assays. (also see Fig.5)

Figure S5

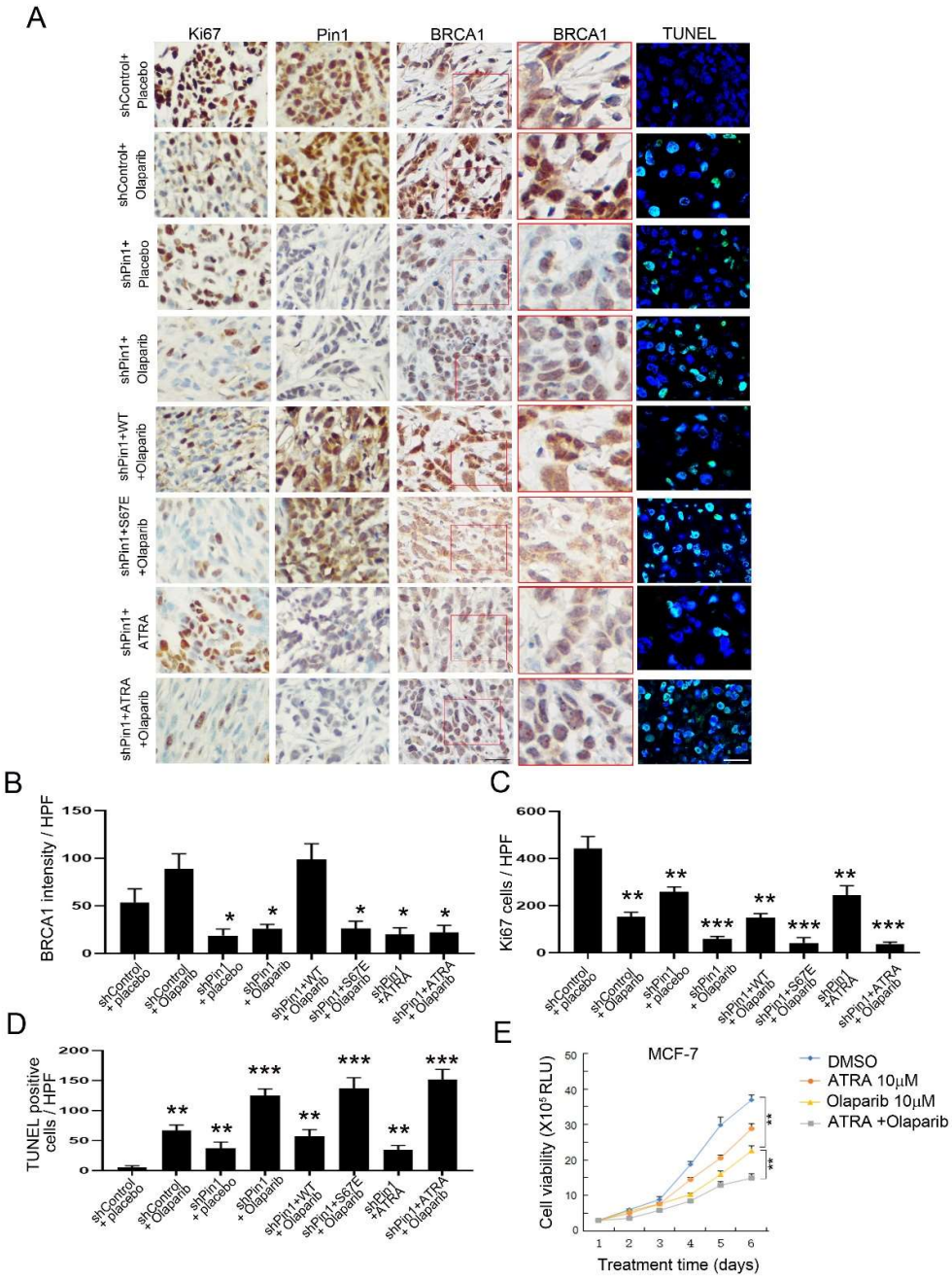
A
Ki67
Pin1
BRCA1
BRCA1
TUNEL
shControl+ Placebo
shControl+ Olaparib
shPin1+ Placebo
shPin1+ Olaparib
shPin1+WT +Olaparib
shPin1+S67E +Olaparib
shPin1+ ATRA
shPin1+ATRA +Olaparib
B
BRCA1 intensity / HPF
C
Ki67 cells / HPF
D
TUNEL positive cells / HPF
E
MCF-7
DMSO
ATRA 10μM
Olaparib 10μM
ATRA +Olaparib
Cell viability (X10⁵ RLU)
Treatment time (days)

Figure S5. Pin1 knockdown sensitizes breast tumors to PARP inhibitor treatment, but not to ATRA treatment. **A.** Immunohistochemistry (IHC) and TUNEL assay of MDA MB-231 xenograft. IHC and TUNEL images of representative tumors at treatment endpoints were shown as indicated (n=6 per group). Red boxed area of BRCA1 staining is shown in a higher magnification on the right. Scale Bars represent 50 μm. **B, C.** Quantification of BRCA1 and Ki67 staining for the IHC images of three tumors at treatment endpoints. **D.** Quantification of apoptotic cells in TUNEL assay of three tumors at treatment endpoints. **E.** Effects of ATRA and Olaparib treatment on MCF-7 cell proliferation. *P<0.05, **P<0.01, ***P<0.001. (also see Fig.5 and 6)

Figure S6

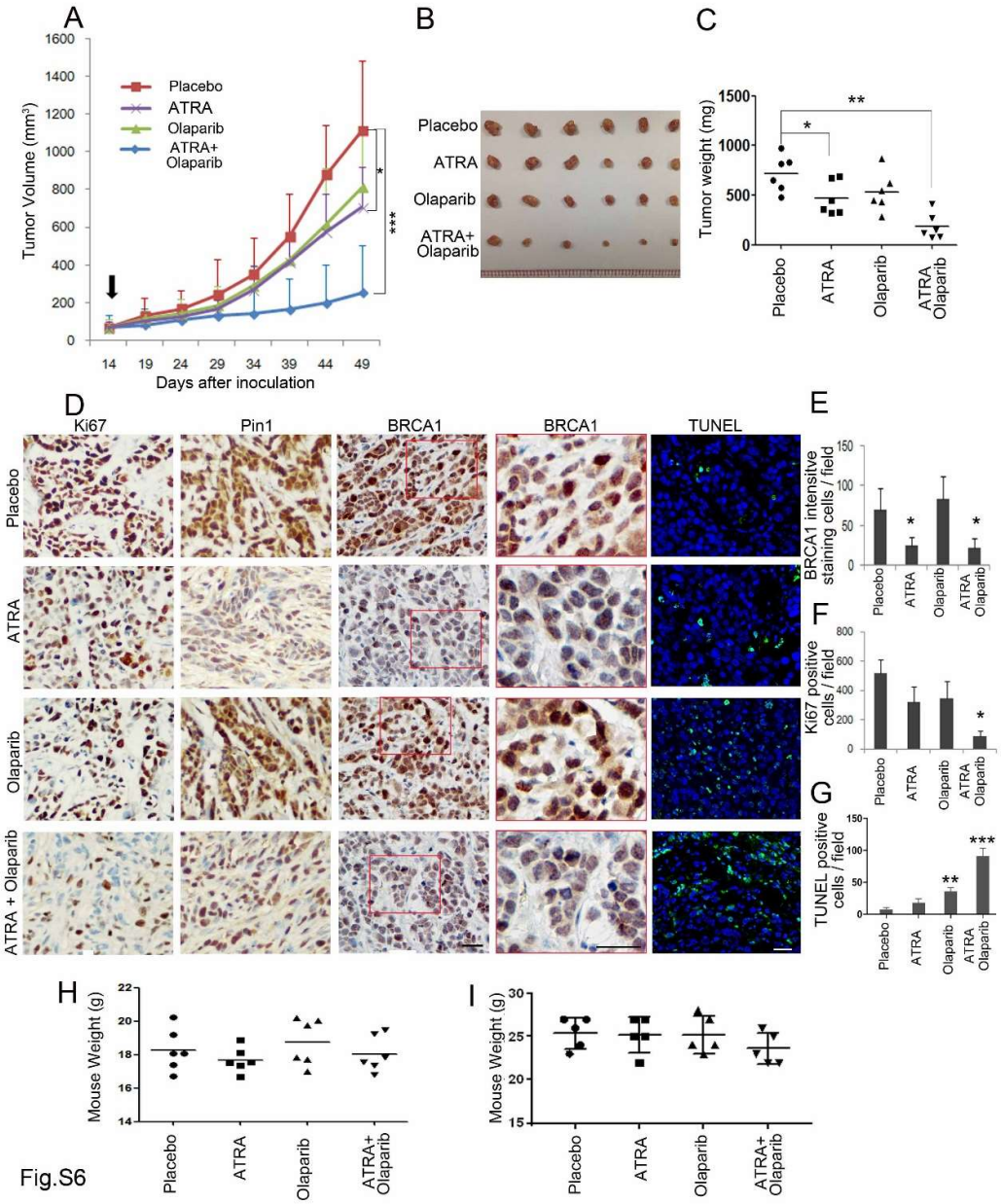

Fig.S6

Figure S6. ATRA sensitizes BRCA1-profidienct breast tumors to PARP inhibitor treatments. **A-C.** ATRA treatments sensitize BRCA1-profidienct breast tumors to PARP inhibitor. MDA-MB 231 cells (1.5 million per mouse) were implanted into the mammary fat pad of NOD-SCID mice. A total of 24 tumor-bearing animals were randomized to four groups (n=6 per group). Treatments as indicated by the arrow were started 2 weeks after inoculation (A). Treatments were continued for 35 days when animals were euthanized and the tumors were weighed (B and C). **D-G.** ATRA treatments mimic Pin1 ablation with regards to extinction of BRCA1 expression, and show dramatic anti-proliferative effects in combination with Olaparib. IHC of Ki67, Pin1 and BRCA1, as well as TUNEL assay in representative tumors at treatment endpoint. Scale Bars represent 50 μm. Red boxed area in BRCA1 is shown in a higher magnification on the right. (D). Positive cells of Ki67 staining (E), BRCA1 staining (F) and TUNEL assay (G) were quantified in three tumor sections. Scale Bars represent 50 μm. **H, I.** The body weights of mice were not affected by the combination of ATRA and BRCA1 treatment. The body weight was measured at the treatment endpoint and the tumor weight was subtracted from the total mouse weight for the net body weight. The mice body weights of breast cancer cell xenografts (H) and the breast cancer PDXs (I). *P<0.05, **P<0.01, ***P<0.001. (also see Fig.6)

Table S1. Data quality of NGS results of PDXs

Sample	DataProduction	TotalReads	Alignment	EffectiveDepth	Coverage	Uniformity	CV
PDX1	978.19M	7,306,226	99.39%	1,036	100.00%	99.59%	0.44
PDX2	943.83M	7,183,328	99.35%	1,506	100.00%	99.92%	0.41
PDX3	790.56M	5,904,656	99.18%	1,174	100.00%	99.84%	0.29

Table S2. Abnormal genes in the PDXs

Sample	Gene	CDS Change	Type	Frequency	ACMG class
PDX1	*TP53*	NM_000546.5:intron4:c.376-2A>G:p.?	Splice	77.70%	4
PDX1	*MRE11*	NM_005591.3:exon12:c.1260G>A:p.(K420=)	Nonsense	10.11%	3
PDX2	*CDH1*	NM_004360.3:exon2:c.123_124insT:p.P42Sfs*17	Frameshift	18.62%	4
PDX2	*FANCL*	NM_018062.3:exon8:c.621C>T:p.(I207=)	Nonsense	48.64%	3
PDX3	*TP53*	NM_000546.5:exon8_intron8:c.915_919+24del29:p.?	Splice	14.81%	4
PDX3	*BRIP1*	NM_032043.2:exon7:c.918C>T:p.(N306=)	Nonsense	9.83%	3
PDX3	*PIK3CA*	NM_006218.2:exon21:c.3140A>G:p.H1047R	Missense	36.08%	-