Supplementary Data

Figure Legends

Supplemental Figure S1. Expression of FLAG-BRCA2 proteins used for the homologous recombination assay.

293T cells were transfected with indicated FLAG-tagged BRCA2 constructs using Lipofectamine Plus according to the manufacturer's protocol. After 48 hours, cells were lysed in NETN (0.5% NP-40; 20mM Tris pH 8.0; 100mM NaCl; 1mM EDTA) buffer and western blotting was done using anti FLAG (Sigma F1804) or anti β-actin (Sigma A1978).

Supplemental Figure S2. BRCA2 restoration is critical for acquired drug resistance of cisplatin-selected cells. (Full-length blots are presented in Supplemental Fig. S5E)

A. BRCA2 western blotting of PEO1, PEO4, C4-2, C4-5 and C4-11 cells treated with indicated siRNA. BRCA2 siRNA #2 was used in this experiment.

B. Cisplatin sensitivity assessed by crystal violet assay. BRCA2 siRNA (#2)- treated C4-2 and C4-5 cells were cisplatin-sensitive compared to control siRNA-treated cells ($p<0.05$, LD_{50} data were compared by unpaired t test). BRCA2 siRNA (#2)-treated PEO4 cells tended to be cisplatin-sensitive compared to control siRNA-treated cells, but the difference is not statistically significant ($p=0.073$). BRCA2 siRNA #2 had no effect on cisplatin sensitivity of PEO1 and C4-11 cells. (mean ± SEM, n=3)

C. AG14361 sensitivity assessed by crystal violet assay. BRCA2 siRNA (#2)-treated PEO4, C4-2 and C4-5 cells were AG14361-sensitive compared to control siRNA-treated cells ($p<0.05$, LD_{50} data were compared by unpaired t test). BRCA2 siRNA #2 did not

sensitize PEO1 or C4-11 cells to AG14361. (mean ± SEM, n=3)

Supplemental Figure S3. Restoration of functional BRCA2 by secondary *BRCA2* mutation in PARP inhibitor-selected PEO1 clones. (Full-length blots are presented in Supplemental Fig S5F-G)

*A***.** BRCA2 western blotting of the 3 clones (AG4-8, AG4-11 and AG4-12) of PEO1 generated by selecting the cells in the presence of AG14361. A double asterisk indicates a band presumed to be non-specific.

B. Genomic DNA sequence of *BRCA2* in AG4-11. In addition to the original mutation (5193C>G), a secondary mutation (5192A>T) was observed. The same mutation was observed in AG4-8 and AG4-12 (data not shown). The mutation was confirmed in cDNAs (data not shown).

C. Ionizing radiation (IR)-induced RAD51 foci formation is restored in the BRCA2-restored PEO1 clones. Quantification of the cells with at least five RAD51 foci before (-, white bars) and 12 hours after IR (+, grey bars) is shown. Asterisks (*) indicate significant difference with irradiated parental PEO1 cells ($p<0.05$, unpaired t test). (mean ± SEM, n=3)

D. Cisplatin/PARP inhibitor sensitivity assay of AG14361-selected PEO1 clones. The 3 BRCA2-restored clones were resistant to both cisplatin and AG14361 compared to the parental PEO1 cells. The cells were treated with cisplatin or AG14361 at the indicated concentrations for 8 days, and survival fraction was measured by crystal violet assay. All of the AG14361-selected PEO1 clones and PEO4 are cisplatin/AG14361 resistant compared to parental PEO1 ($p<0.05$, LD_{50} data were compared by unpaired t test).

(mean ± SEM, n=3)

E. BRCA2 western blotting of AG4-8, AG4-11 and AG4-12 cells treated with indicated siRNA. BRCA2 siRNA #1 was used in this experiment.

F. Cisplatin/PARP inhibitor sensitivity assay. Depletion of BRCA2 by siRNA sensitized AG4-8, AG4-11 and AG4-12 to cisplatin and AG14361. BRCA2 siRNA (#1)- treated AG4-8, AG4-11 and AG4-12 cells were cisplatin/AG14361-sensitive compared to control siRNA-treated cells ($p<0.05$, LD_{50} data were compared by unpaired t test). (mean ± SEM, n=3).

Supplemental Figure S4. BRCA2 restoration is critical for acquired drug resistance of AG14361-selected cells. (Full-length blots are presented in Supplemental Fig. S5H)

A. BRCA2 western blotting of PEO1, AG4-8, AG4-11, and AG4-12 cells treated with indicated siRNA. BRCA2 siRNA #2 was used in this experiment.

B. Cisplatin/PARP inhibitor sensitivity assessed by crystal violet assay. BRCA2 siRNA (#2)- treated AG4-8, AG4-11 and AG4-12 cells were cisplatin/AG14361-sensitive compared to control siRNA-treated cells ($p<0.05$, LD_{50} data were compared by unpaired t test). (mean ± SEM, n=3).

Supplemental Figure S5. Non-cropped pictures of blots.

(A-B) Non-cropped pictures of blots presented in Figure 1A.

(C) Non-cropped pictures of blots presented in Figure 2A.

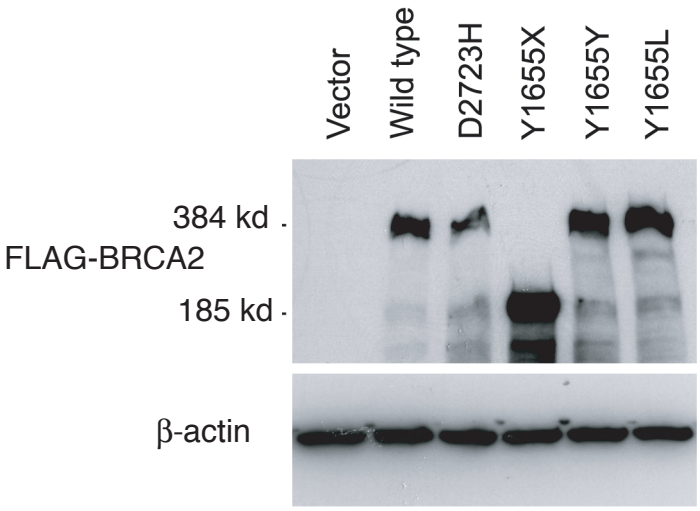
(D) Non-cropped pictures of blots presented in Figure 4A.

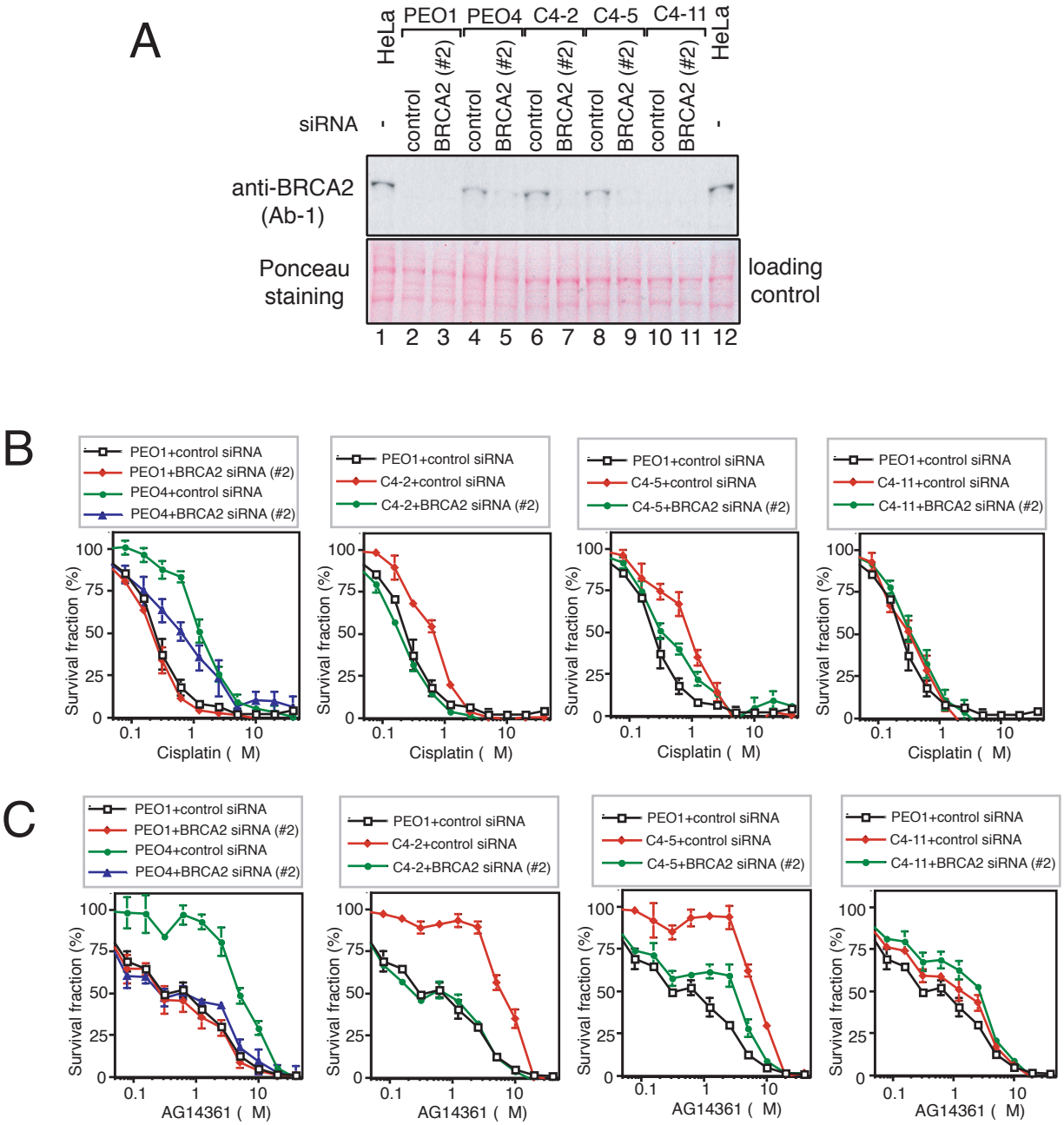
(E) Non-cropped pictures of blots presented in Supplemental Figure S1A.

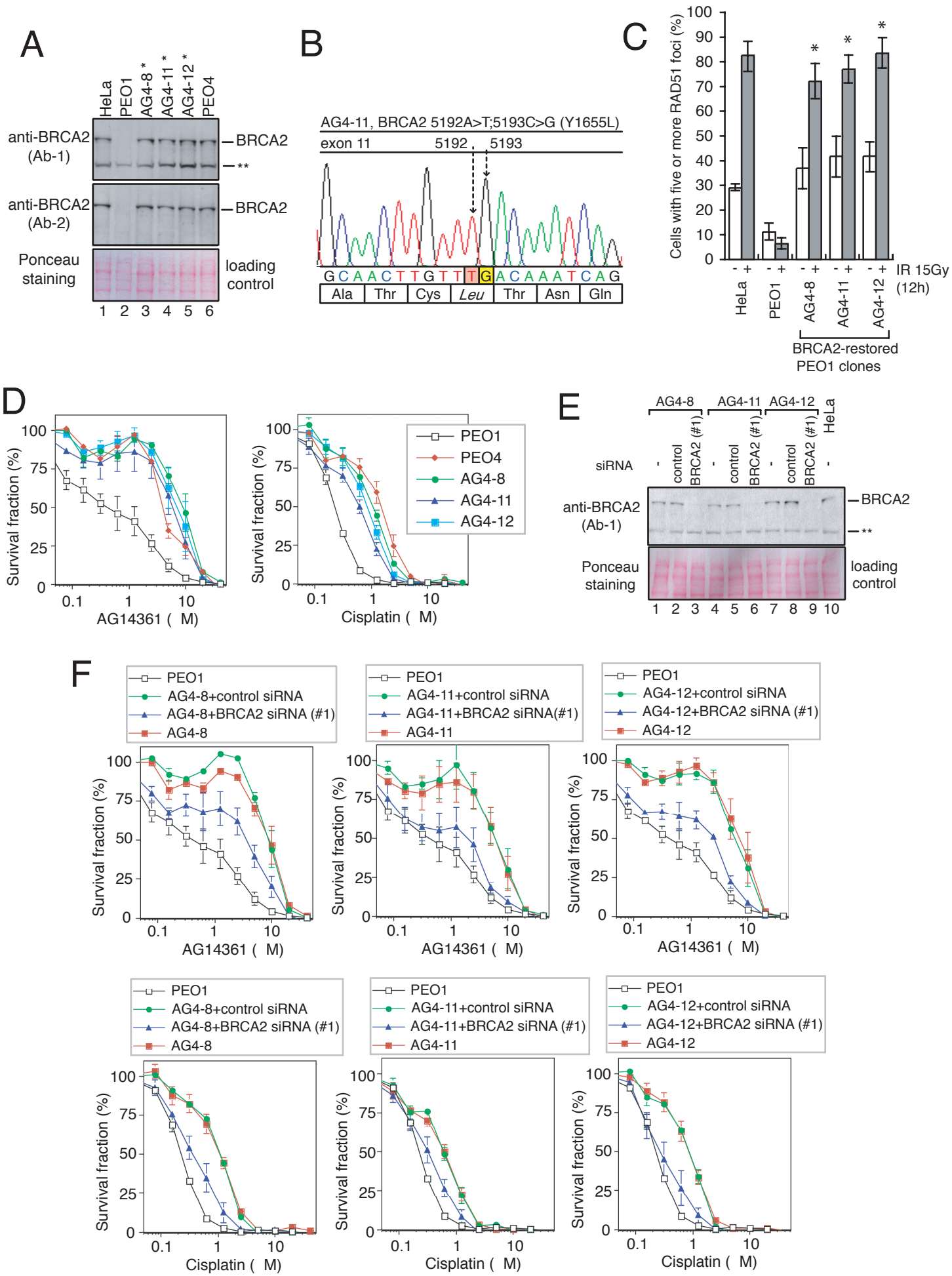
(F) Non-cropped pictures of blots presented in Supplemental Figure S2A.

(G) Non-cropped pictures of blots presented in Supplemental Figure S2E.

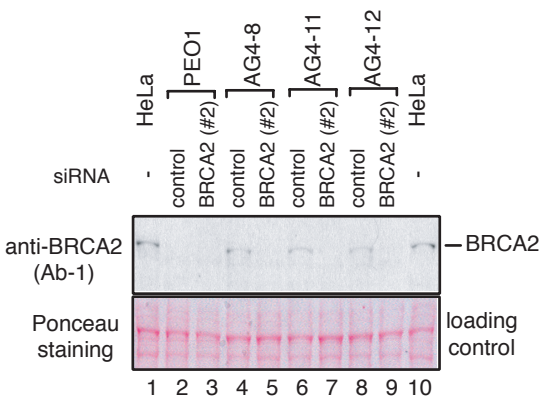
(H) Non-cropped pictures of blots presented in Supplemental Figure S3A.


Vector
Wild type
D2723H
Y1655X
Y1655Y
Y1655L
384 kd
FLAG-BRCA2
185 kd
β-actin

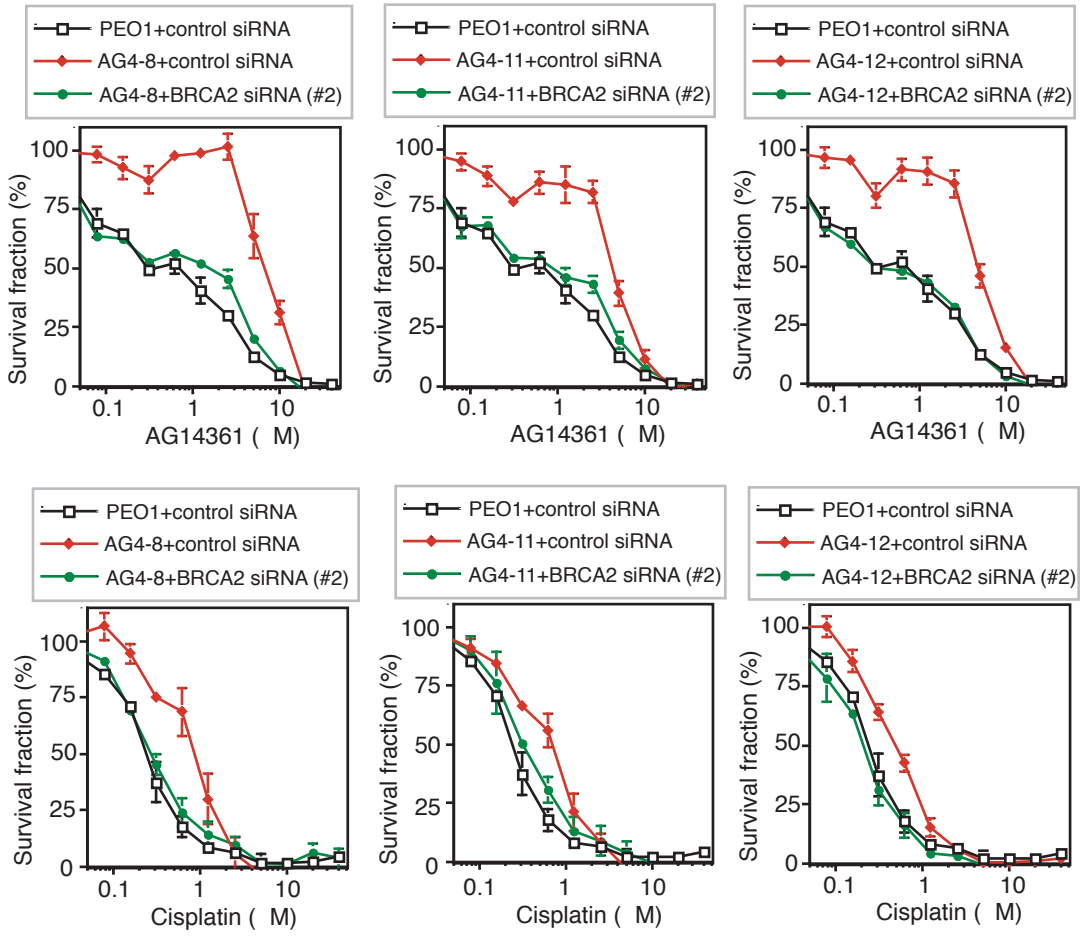

A
HeLa
PEO1
PEO4
C4-2
C4-5
C4-11
HeLa
siRNA
-
control
BRCA2 (#2)
anti-BRCA2 (Ab-1)
Ponceau staining
loading control
1 2 3 4 5 6 7 8 9 10 11 12
B
PEO1+control siRNA
PEO1+BRCA2 siRNA (#2)
PEO4+control siRNA
PEO4+BRCA2 siRNA (#2)
C4-2+control siRNA
C4-2+BRCA2 siRNA (#2)
C4-5+control siRNA
C4-5+BRCA2 siRNA (#2)
C4-11+control siRNA
C4-11+BRCA2 siRNA (#2)
Survival fraction (%)
Cisplatin (M)
C
AG14361 (M)


A
HeLa
PEO1
AG4-8 *
AG4-11 *
AG4-12 *
PEO4
anti-BRCA2 (Ab-1)
BRCA2
**
anti-BRCA2 (Ab-2)
BRCA2
Ponceau staining
loading control
1 2 3 4 5 6
B
AG4-11, BRCA2 5192A>T;5193C>G (Y1655L)
exon 11
5192
5193
G C A A C T T G T T T G A C A A A T C A G
Ala Thr Cys Leu Thr Asn Gln
C
Cells with five or more RAD51 foci (%)
IR 15Gy (12h)
HeLa
PEO1
AG4-8
AG4-11
AG4-12
BRCA2-restored PEO1 clones
D
Survival fraction (%)
AG14361 (M)
Cisplatin (M)
PEO1
PEO4
AG4-8
AG4-11
AG4-12
E
AG4-8
AG4-11
AG4-12
HeLa
siRNA
control
BRCA2 (#1)
anti-BRCA2 (Ab-1)
BRCA2
**
Ponceau staining
loading control
1 2 3 4 5 6 7 8 9 10
F
PEO1
AG4-8+control siRNA
AG4-8+BRCA2 siRNA (#1)
AG4-8
AG4-11+control siRNA
AG4-11+BRCA2 siRNA(#1)
AG4-11
AG4-12+control siRNA
AG4-12+BRCA2 siRNA (#1)
AG4-12
Survival fraction (%)
AG14361 (M)
Cisplatin (M)

A


HeLa
PEO1
AG4-8
AG4-11
AG4-12
HeLa
siRNA
-
control
BRCA2 (#2)
anti-BRCA2 (Ab-1)
BRCA2
Ponceau staining
loading control
1 2 3 4 5 6 7 8 9 10

B


PEO1+control siRNA
AG4-8+control siRNA
AG4-8+BRCA2 siRNA (#2)
AG4-11+control siRNA
AG4-11+BRCA2 siRNA (#2)
AG4-12+control siRNA
AG4-12+BRCA2 siRNA (#2)
Survival fraction (%)
AG14361 (M)
Cisplatin (M)

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Supplemental Figure S5