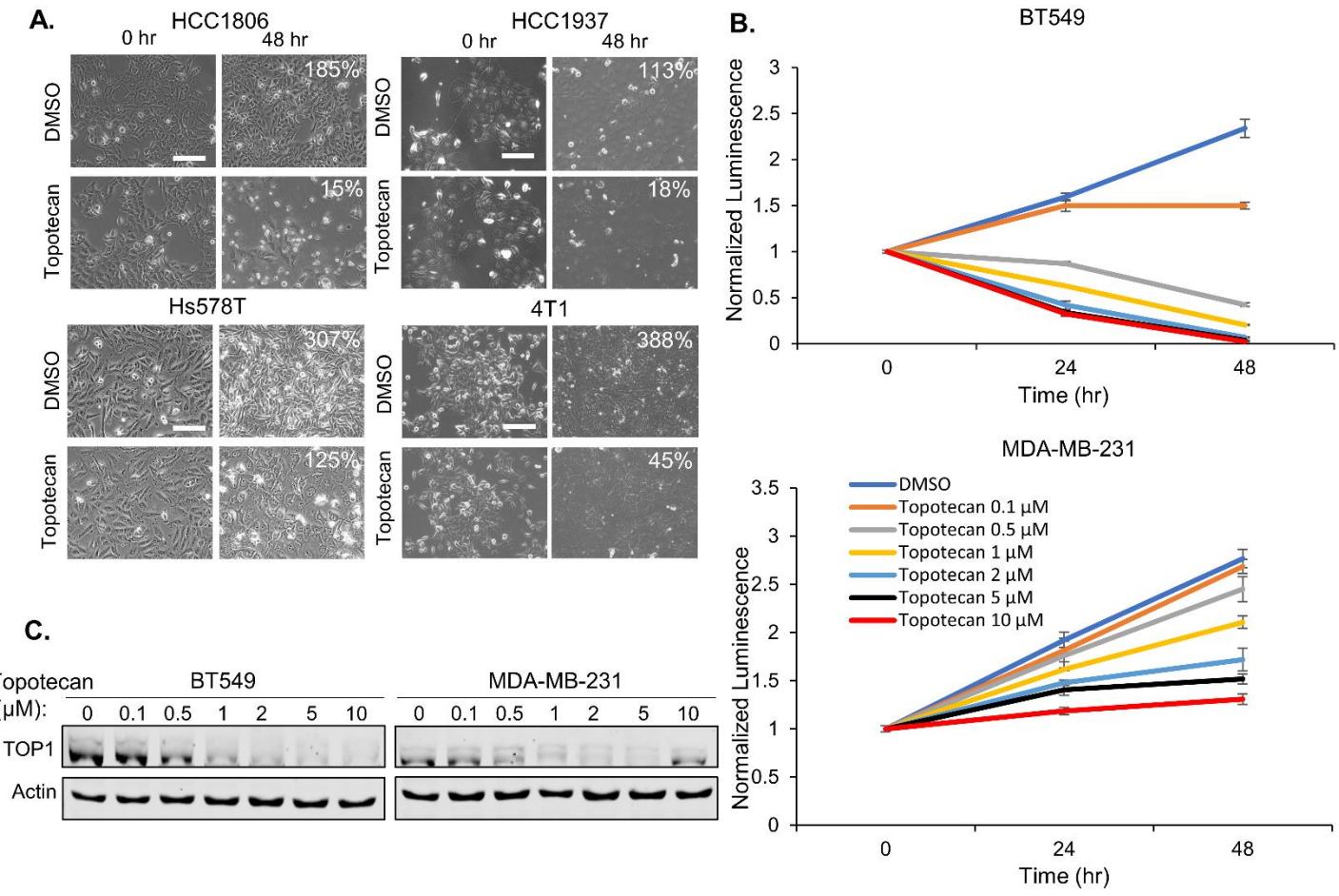
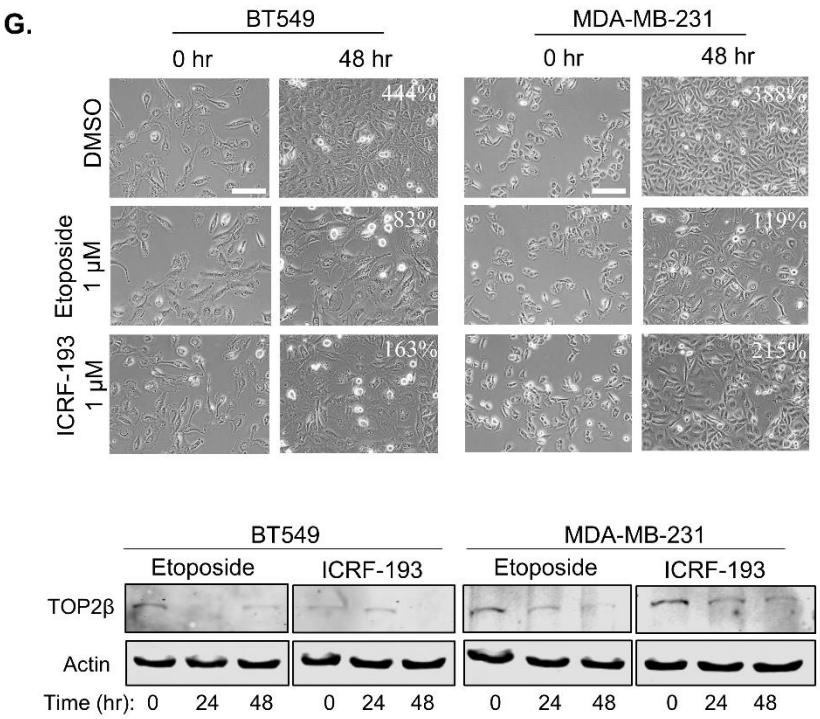
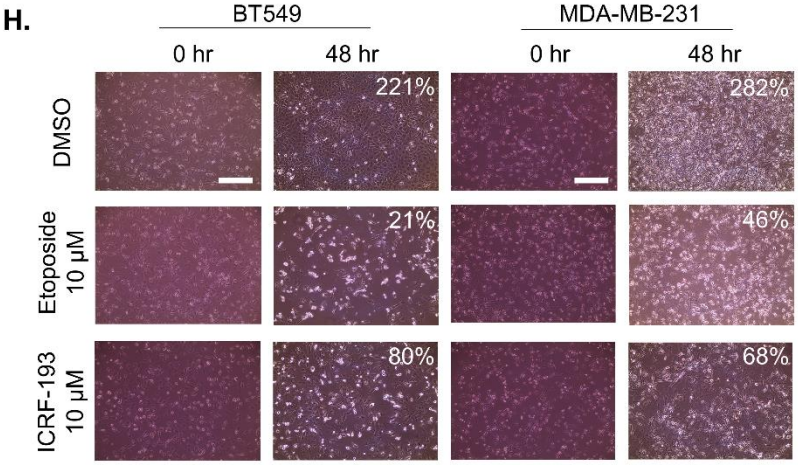

A.
HCC1806
0 hr
48 hr
DMSO
Topotecan
185%
15%
HCC1937
113%
18%
Hs578T
307%
125%
4T1
388%
45%
B.
BT549
Normalized Luminescence
Time (hr)
MDA-MB-231
DMSO
Topotecan 0.1 μM
Topotecan 0.5 μM
Topotecan 1 μM
Topotecan 2 μM
Topotecan 5 μM
Topotecan 10 μM
C.
Topotecan (μM):
0 0.1 0.5 1 2 5 10
TOP1
Actin
D.
BT549
MDA-MB-231
Camptothecin
E.
DMSO
Camptothecin
*
F.
PDX6 p8
PDX4 p7
PDX5 p6
218%
253%
76%
44%
261%
299%
118%
90%
228%
354%
101%
105%


G.
BT549
MDA-MB-231
0 hr
48 hr
0 hr
48 hr
DMSO
444%
388%
Etoposide 1 μM
83%
119%
ICRF-193 1 μM
163%
215%
BT549
MDA-MB-231
Etoposide
ICRF-193
Etoposide
ICRF-193
TOP2β
Actin
Time (hr): 0 24 48 0 24 48 0 24 48 0 24 48


H.
BT549
MDA-MB-231
0 hr
48 hr
0 hr
48 hr
DMSO
221%
282%
Etoposide 10 μM
21%
46%
ICRF-193 10 μM
80%
68%

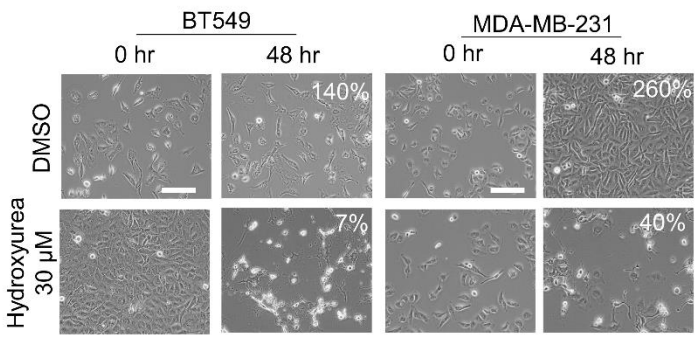
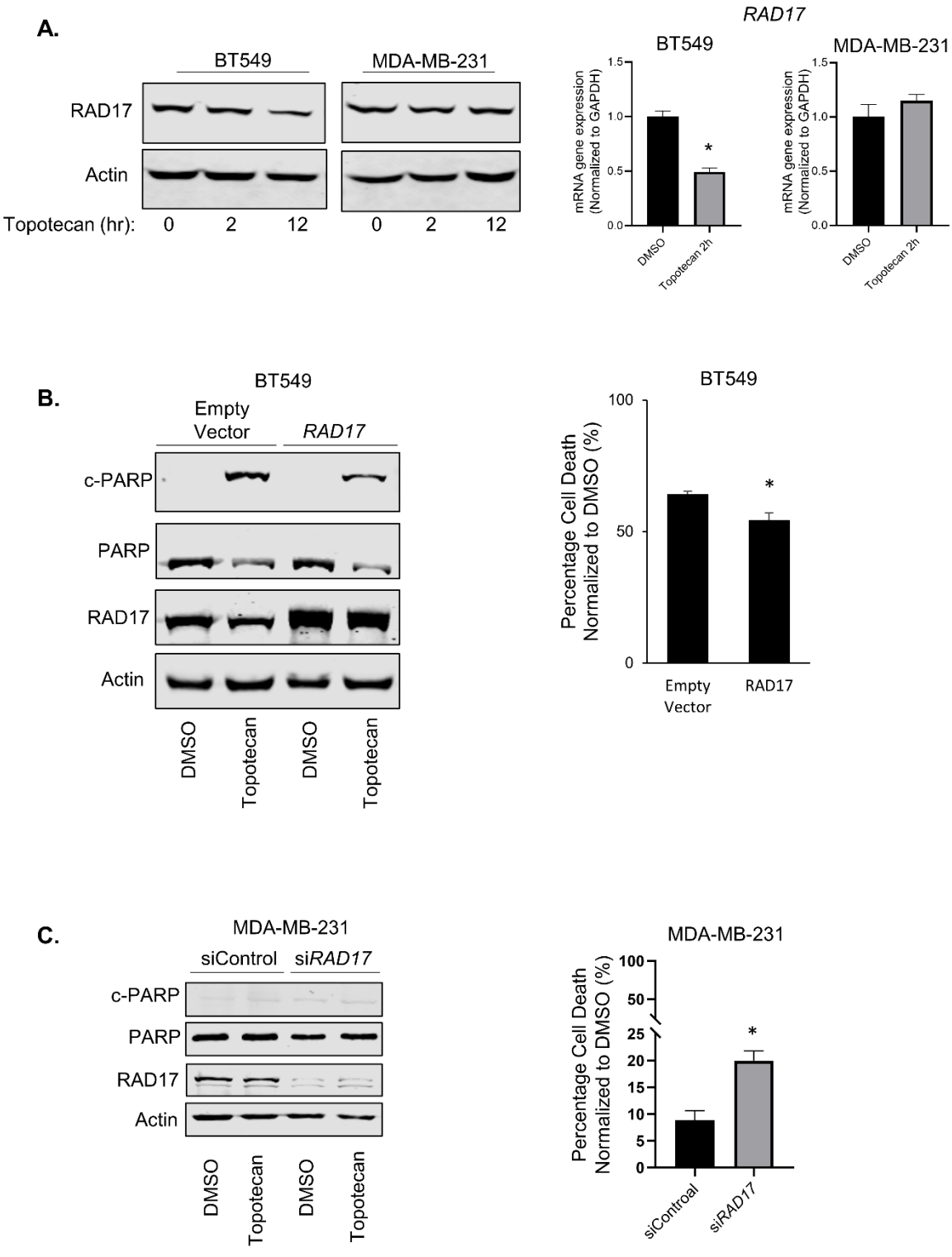
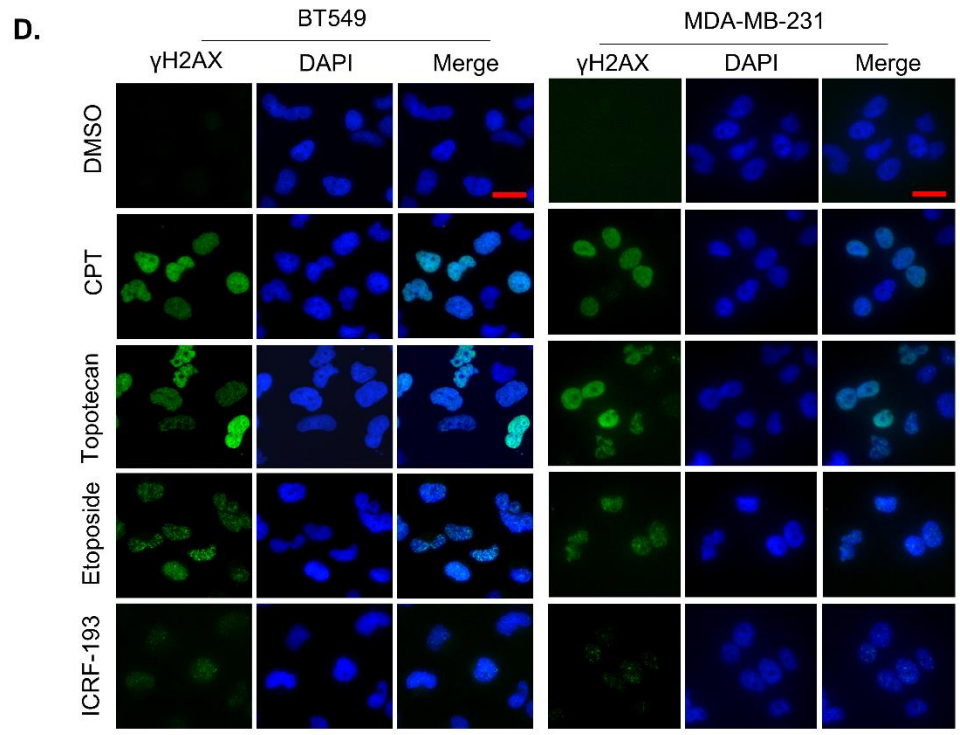
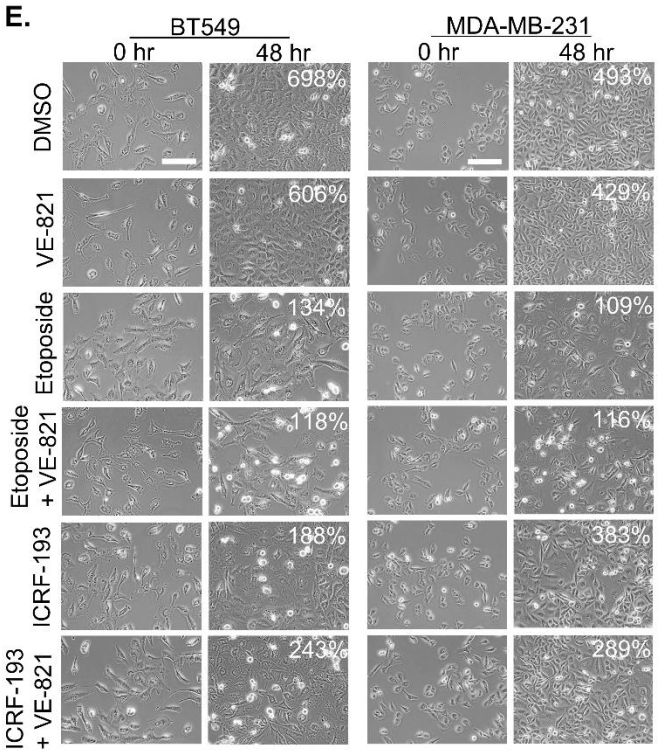
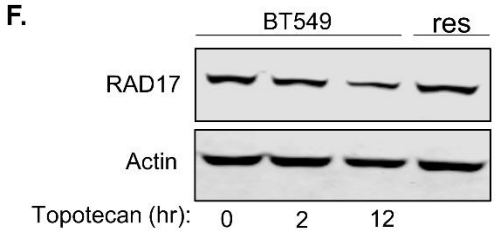

I.
BT549
MDA-MB-231
0 hr
48 hr
0 hr
48 hr
DMSO
140%
260%
Hydroxyurea 30 μM
7%
40%

**Figure S1. TNBC cells show distinct responses to TOP1 and TOP2 inhibitors.**
(A) Microscopy of TNBC cell lines HCC1806, Hs578T, HCC1937, and 4T1 (mouse). Cells were treated with DMSO or 1 μM topotecan.
(B) Viability of BT549 and MDA-MB-231 cells treated with DMSO or topotecan at the indicated concentrations.
(C) TOP1 protein levels were measured by western blotting. Cells were treated with DMSO or topotecan at the indicated concentrations.
(D) Microscopy of BT549 and MDA-MB-231 cells treated with DMSO or 1 μM camptothecin.
(E) Viability of BT549 and MDA-MB-231 cells treated with DMSO or 1 μM camptothecin (n = 3).
(F) Microscopy of primary human ovarian cancer cells (PDX6 p8, PDX4 p7, and PDX5 p6). Cells were treated with DMSO or 1 μM topotecan.
(G) Microscopy and western blotting of BT549 and MDA-MB-231 cells treated with DMSO, 1 μM etoposide, or 1 μM ICRF-193.
(H) Microscopy of BT549 and MDA-MB-231 cells treated with DMSO, 10 μM etoposide, or 10 μM ICRF-193.
(I) Microscopy of BT549 and MDA-MB-231 cells treated with DMSO or 30 μM hydroxyurea.
Data are presented as mean ± SD. Significance was calculated using two-tailed, unpaired Student's t test. *$p < 0.05$. Scale bars, 100 μm. Percentages in the microscopic images represent relative cell number changes versus the 0-h time point.


A.
BT549
MDA-MB-231
RAD17
Actin
Topotecan (hr): 0 2 12 0 2 12
RAD17
BT549
MDA-MB-231
mRNA gene expression (Normalized to GAPDH)
DMSO
Topotecan 2h
B.
BT549
Empty Vector
RAD17
c-PARP
PARP
RAD17
Actin
DMSO
Topotecan
DMSO
Topotecan
BT549
Percentage Cell Death Normalized to DMSO (%)
Empty Vector
RAD17
C.
MDA-MB-231
siControl
siRAD17
c-PARP
PARP
RAD17
Actin
DMSO
Topotecan
DMSO
Topotecan
MDA-MB-231
Percentage Cell Death Normalized to DMSO (%)
siControal
siRAD17


D.
BT549
MDA-MB-231
γH2AX
DAPI
Merge
γH2AX
DAPI
Merge
DMSO
CPT
Topotecan
Etoposide
ICRF-193


E.
BT549
MDA-MB-231
0 hr
48 hr
0 hr
48 hr
DMSO
698%
493%
VE-821
606%
429%
Etoposide
134%
109%
Etoposide + VE-821
118%
116%
ICRF-193
188%
383%
ICRF-193 + VE-821
243%
289%


F.
BT549
res
RAD17
Actin
Topotecan (hr):
0
2
12

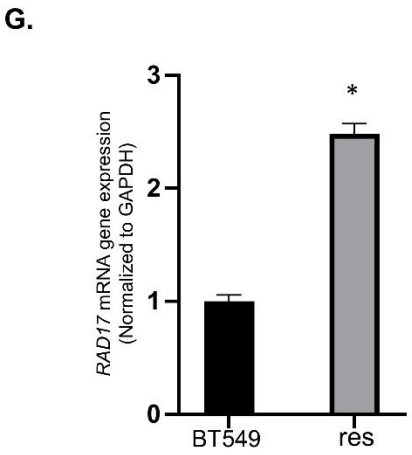

G.
RAD17 mRNA gene expression (Normalized to GAPDH)
3
2
1
0
*
BT549
res

**Figure S2. ATR activation and RAD17 correlate with cell sensitivity to TOP1 inhibition.**
(A) Western blotting and qRT-PCR (n = 3) of RAD17. BT549 and MDA-MB-231 cells treated with 1 μM topotecan for 2 and 12 hours.
(B) BT549 cells were transfected with pCMV6-entry or pCMV6-RAD17. RAD17 Overexpression and c-PARP levels were examined by western blotting (left) and cell death was measured by cell viability assays (right) after 48-h transfection and 24-h treatment with 1 μM topotecan.
(C) MDA-MB-231 cells were transfected with scrambled siRNA (siControl) or *RAD17* siRNA (si*RAD17*). Knockdown was validated by western blotting and cell death was measured by cell viability assays after 48-h transfection and 24-h treatment with 1 μM topotecan.
(D) DNA damage was evaluated by p-γ-H2AX immunofluorescent staining. BT549 and MDA-MB-231 cells were treated with DMSO, 1 μM camptothecin, 1 μM topotecan, 1 μM etoposide, or 1 μM ICRF-193 for 4 hours. Cell nuclei were stained by DAPI.
(E) Microscopy of BT549 and MDA-MB-231 cells treated with DMSO, 1 μM VE-821, 1 μM etoposide, 1 μM etoposide combined with 1 μM VE-821, 1 μM ICRF-193, or 1 μM ICRF-193 combined with VE-821 for 48 hours.
(F) Western blotting of RAD17. BT549-res cells were grown in the presence of 1 μM topotecan treatment. BT549 cells treated with 1 μM topotecan for 2 and 12 hours. This blot membrane was used in Fig.S2A, which does not show the result of BT549-res cells.
(G) qRT-PCR analysis of RAD17 expression in BT549 and BT549-res cells (n = 3).
Data are presented as mean ± SD. Significance was calculated using two-tailed, unpaired Student's t test. *$p < 0.05$. Red Scale bars, 25 μm. White Scale bars, 100 μm. Percentages in the microscopic images represent relative cell number changes versus the 0-h time point.

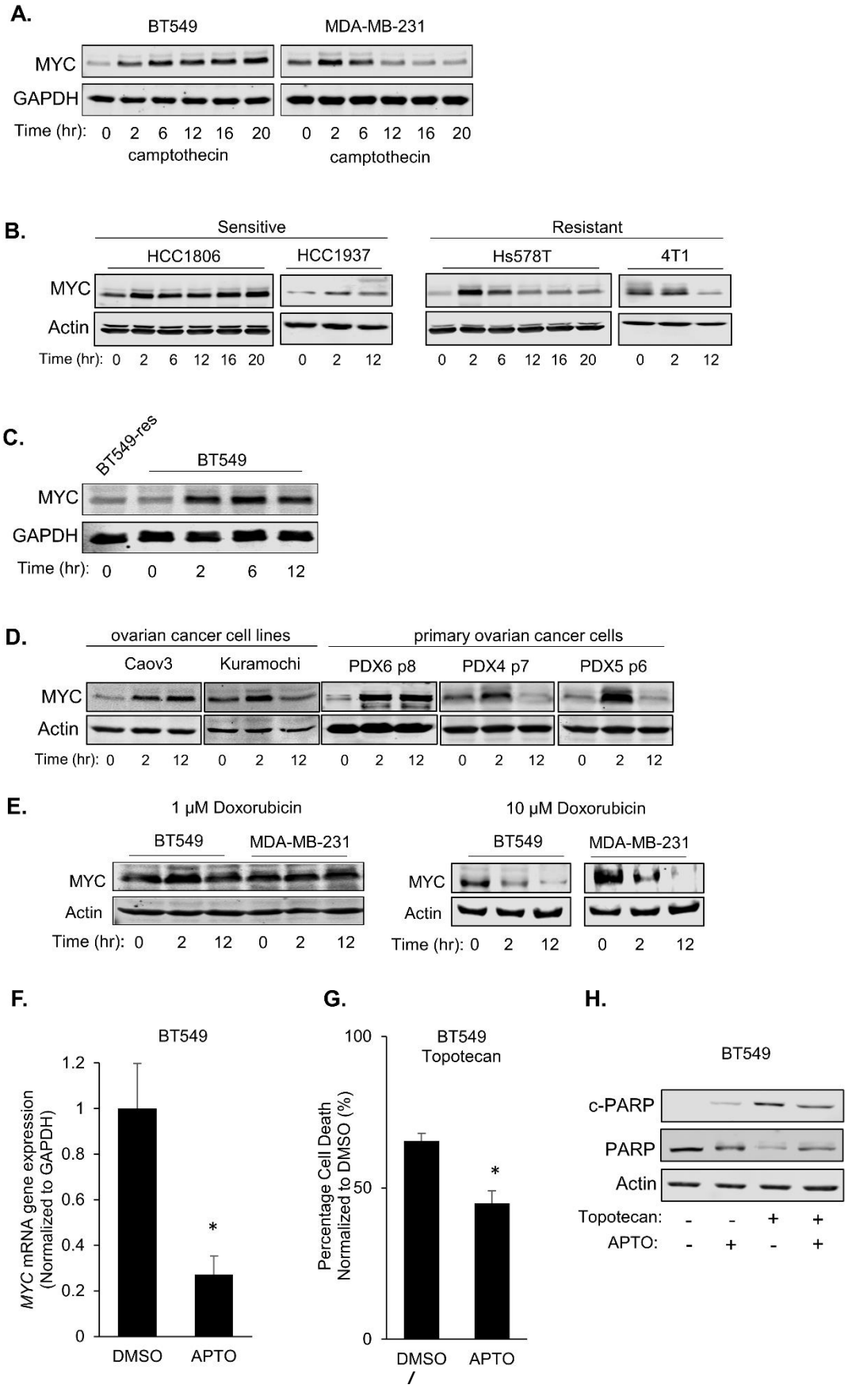

A.
BT549
MDA-MB-231
MYC
GAPDH
Time (hr): 0 2 6 12 16 20 0 2 6 12 16 20
camptothecin
camptothecin
B.
Sensitive
Resistant
HCC1806
HCC1937
Hs578T
4T1
MYC
Actin
Time (hr): 0 2 6 12 16 20 0 2 12 0 2 6 12 16 20 0 2 12
C.
BT549-res
BT549
MYC
GAPDH
Time (hr): 0 0 2 6 12
D.
ovarian cancer cell lines
primary ovarian cancer cells
Caov3
Kuramochi
PDX6 p8
PDX4 p7
PDX5 p6
MYC
Actin
Time (hr): 0 2 12 0 2 12 0 2 12 0 2 12 0 2 12
E.
1 μM Doxorubicin
10 μM Doxorubicin
BT549
MDA-MB-231
BT549
MDA-MB-231
MYC
Actin
Time (hr): 0 2 12 0 2 12 0 2 12 0 2 12
F.
BT549
MYC mRNA gene expression (Normalized to GAPDH)
1.2
1
0.8
0.6
0.4
0.2
0
*
DMSO
APTO
G.
BT549
Topotecan
Percentage Cell Death Normalized to DMSO (%)
100
50
0
*
DMSO
APTO
H.
BT549
c-PARP
PARP
Actin
Topotecan: - - + +
APTO: - + - +

**Figure S3. MYC induction is associated with breast cancer cell sensitivity to TOP1 inhibition.**
(A) MYC protein levels in BT549 and MDA-MB-231 cells were measured by immunoblotting. Cells were treated with 1 μM camptothecin for 0, 2, 6, 12, 16, and 20 hours.
(B) MYC protein levels in HCC1806, HCC1937, Hs578T, and 4T1 cells were measured by immunoblotting. Cells were treated with 1 μM topotecan for the indicated hours.
(C) Immunoblotting of MYC in BT549-res cells cultured in the presence of 1 μM topotecan and parental BT549 cells treated with 1 μM topotecan for 0, 2, and 12 hours.
(D) MYC protein levels in Caov3, Kuramochi, PDX6 (p8), PDX4 (p7), and PDX5 (p6) ovarian cancer cells were measured by immunoblotting. Cells were treated with 1 μM topotecan for 0, 2, and 12 hours.
(E) MYC protein levels in BT549 and MDA-MB-231 cells were measured by immunoblotting. Cells were treated with 1 μM or 10 μM doxorubicin for 0, 2, and 12 hours.
(F) MYC expression was measured by qRT-PCR. BT549 cells were treated with the 0.5 μM MYC inhibitor APTO-253 (n = 3).
(G) BT549 cells were pretreated with DMSO or 0.5 μM APTO-253 for one hour followed by 1 μM topotecan treatment for 24 hours. Cell death was measured by viability assays (n = 3).
(H) c-PARP and PAPR expression was measured by immunoblotting. BT549 cells were treated with DMSO, topotecan, APTO-253, or topotecan combined with APTO-253.
Data are presented as mean ± SD. Significance was calculated using two-tailed, unpaired Student's t test. *$p < 0.05$.

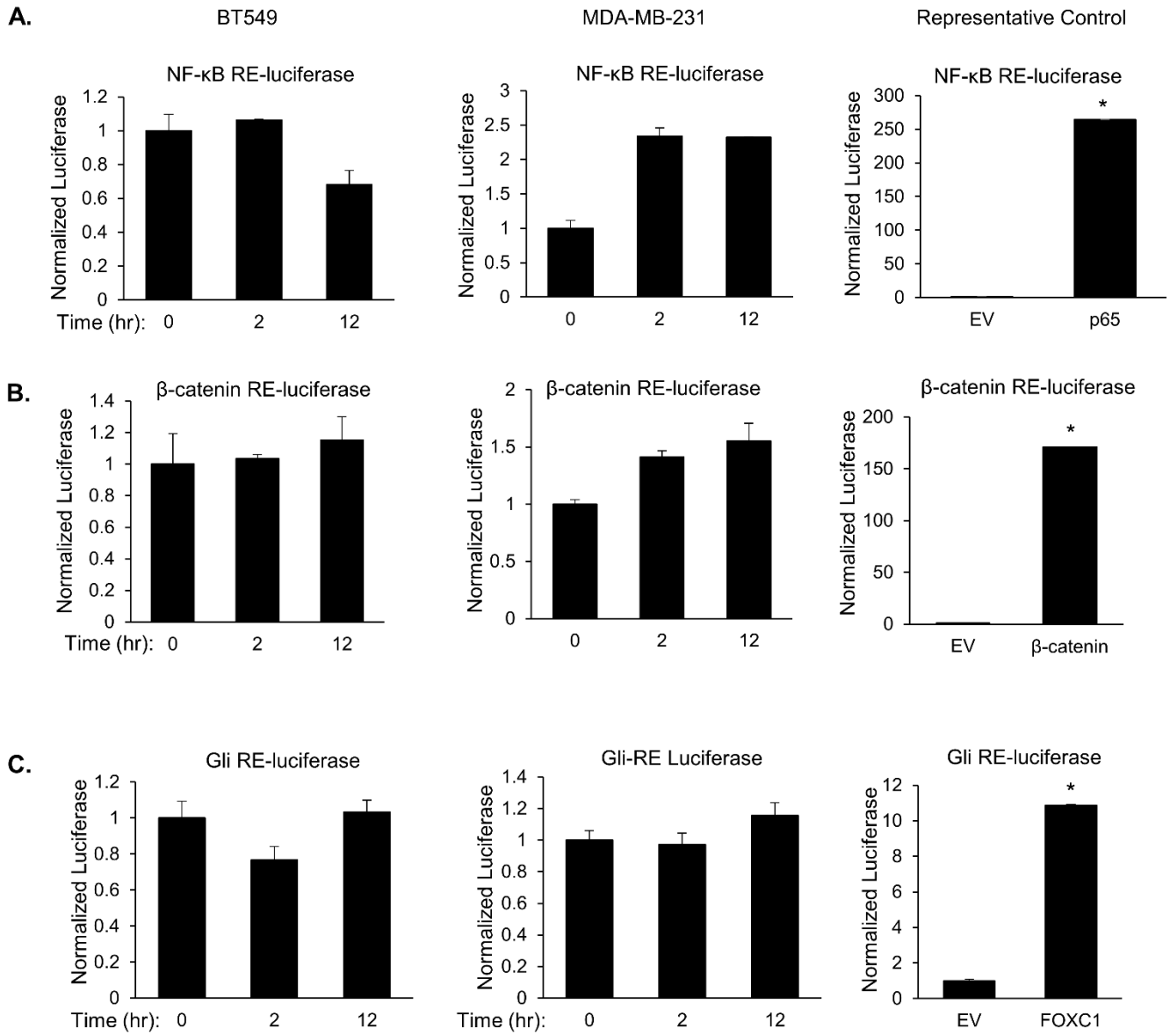



**Figure S4. Reporter assays for the activities of transcriptional factors involved in MYC regulation.**
(A) NF-kB, (B) Beta-catenin, and (C) Gli responsive luciferase reporter assays of BT549 and MDA-MB231 cells treated with topotecan for 2 and 12 hours. BT549 cells were transfected with plasmids overexpressing p65, beta-catenin, or FOXC1 as positive controls for inducing luciferase reporter activity (n = 3). Data are presented as mean ± SD. Significance was calculated using two-tailed, unpaired Student's t test. *$p < 0.05$.

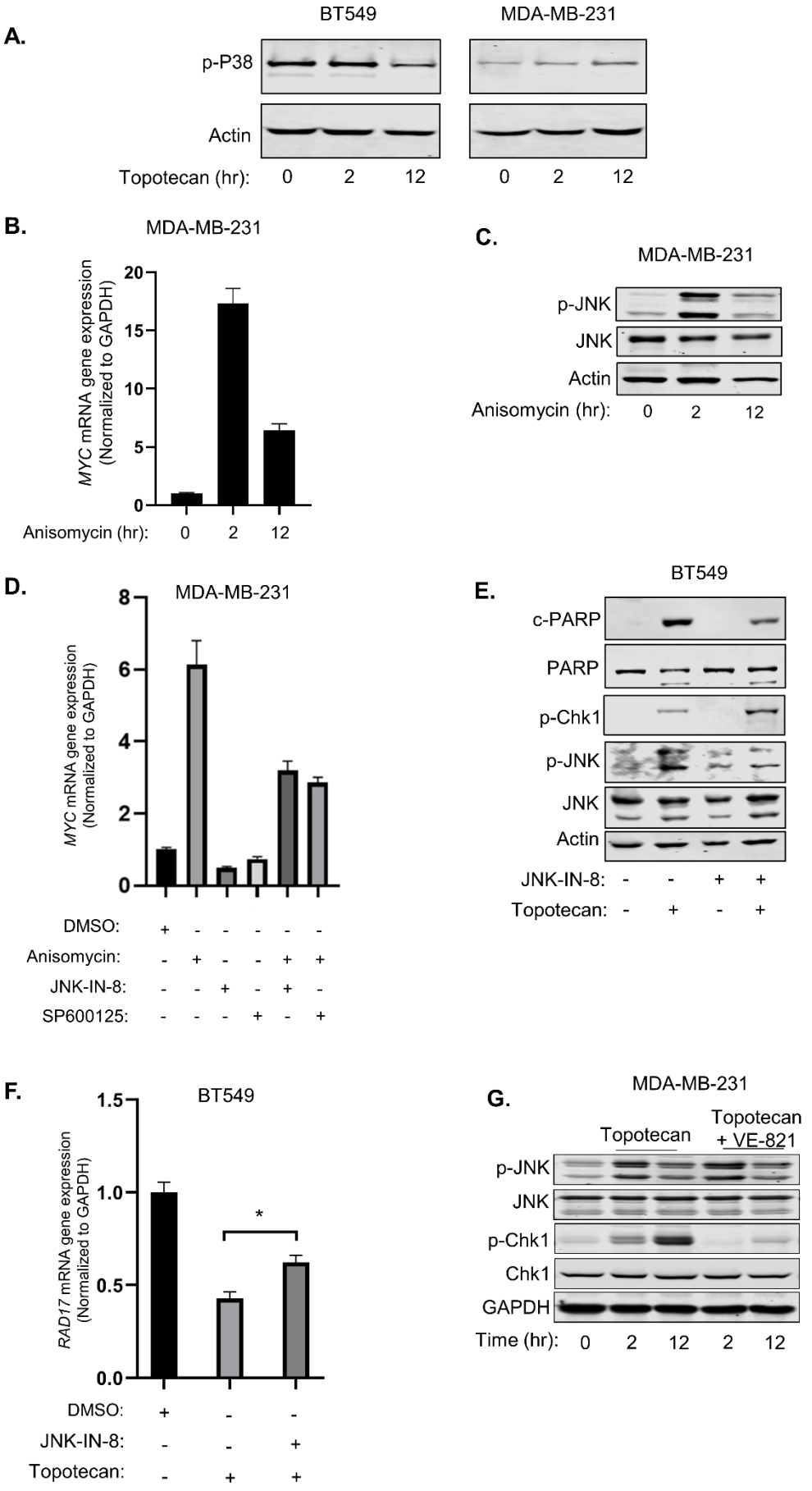

A.
BT549
MDA-MB-231
p-P38
Actin
Topotecan (hr): 0 2 12 0 2 12
B.
MDA-MB-231
MYC mRNA gene expression (Normalized to GAPDH)
Anisomycin (hr): 0 2 12
C.
MDA-MB-231
p-JNK
JNK
Actin
Anisomycin (hr): 0 2 12
D.
MDA-MB-231
MYC mRNA gene expression (Normalized to GAPDH)
DMSO:
Anisomycin:
JNK-IN-8:
SP600125:
E.
BT549
c-PARP
PARP
p-Chk1
p-JNK
JNK
Actin
JNK-IN-8:
Topotecan:
F.
BT549
RAD17 mRNA gene expression (Normalized to GAPDH)
DMSO:
JNK-IN-8:
Topotecan:
G.
MDA-MB-231
Topotecan
Topotecan + VE-821
p-JNK
JNK
p-Chk1
Chk1
GAPDH
Time (hr): 0 2 12 2 12

**Figure S5. JNK mediates the induction of MYC in response to topotecan treatment.**
(A) Immunoblotting of p-P38 in BT549 and MDA-MB-231 cells treated with 1 μM topotecan for 2 and 12 hours. This is the same blot membrane used in supplementary figure 2A for examining RAD17 levels. Thus, the actin signal images are the same.
(B) qRT-PCR analysis of *MYC* and (C) immunoblotting of p-JNK and total JNK in MDA-MB-231 cells treated with 5 μM anisomycin for 2 and 12 hours (n = 3).
(D) qRT-PCR analysis of *MYC* in MDA-MB-231 cells treated with the indicated agents for 4 hours (n = 3).
(E) Immunoblotting of c-PARP, total PARP, p-Chk1, p-JNK, and total JNK. BT549 cells were pretreated with DMSO or 5 μM JNK-IN-8 for 1 hour, followed by DMSO or 1μM topotecan treatment for 12 hours.
(F) qRT-PCR analysis of *RAD17* expression. BT549 cells were treated with DMSO, topotecan, or JNK-IN-8 combined with topotecan for 6 hours (n = 3).
(G) Immunoblotting of p-JNK, total JNK, p-Chk1, and total Chk1. MDA-MB-231 cells were treated with DMSO, 1 μM topotecan, or 1 μM topotecan combined with 1 μM VE-821 for 2 and 12 hours.
Data are presented as mean ± SD. Significance was calculated using two-tailed, unpaired Student's t test. *$p < 0.05$.

**Table S1**

| Gene Name | BT549 Fold change vs DMSO control | | MDA-MB-231 Fold change vs DMSO control | |
|---|---|---|---|---|
| | 2 hr Topotecan | 12 hr Topotecan | 2 hr Topotecan | 12 hr Topotecan |
| *MYC* | 2.755547 | 2.580747 | 2.235605 | 0.57208 |
| *RAD17* | 0.77797 | 0.58709 | 0.86197 | 1.07529 |
| *RB1* | 0.838 | 0.4477 | 0.6692 | 0.5385 |
| *SLFN11* | 1.0047 | 1.5825 | 1.5833 | 0.8333 |
| *ATR* | 0.57112 | 0.633809 | 0.576332 | 0.574526 |
| *RPA1* | 0.792714 | 0.943856 | 0.870374 | 1.120331 |
| *ATRIP* | 0.614184 | 1.331915 | 0.747134 | 1.335032 |
| *RAD9* | 1 | 1.45285 | 1.170816 | 1.363636 |
| *HUS1* | 0.7492 | 1.275112 | 1.150814 | 1.002571 |
| *RAD1* | 0.855467 | 1.644742 | 0.93768 | 1.427804 |
| *TOPBP1* | 0.69881 | 1.018824 | 0.794288 | 1.05218 |
| *ATM* | 0.809367 | 0.523007 | 0.510072 | 0.492252 |
| *RAD50* | 0.788154 | 0.605656 | 0.58827 | 0.548578 |
| *MDC1* | 1.023588 | 1.182273 | 0.903834 | 0.842238 |
| *MRE11* | 0.826438 | 0.895388 | 0.773994 | 0.689241 |
| *RNF168* | 0.894596 | 1.103264 | 0.969619 | 1.329024 |
| *RNF8* | 0.7628 | 1.290143 | 0.929852 | 1.522416 |
| *BRCA1* | 0.642515 | 0.724864 | 0.705663 | 1.06375 |
| *53BP1* | 0.873838 | 0.811531 | 0.926033 | 1.043708 |

**Table S1. Fold changes of selected ATR, ATM, and DNA repair-associated genes from RNA-seq analysis.** ATR pathway genes: *ATR, RPA1, ATRIP, RAD9, HUS1, RAD1, TOPBP1*; ATM pathway genes: *ATM, RAD50, MDC1, MRE11, RNF168, RNF8, BRCA1, 53BP1*.

**Table S2. Drug information.**

| Drug compound | Company | Catalog # |
|---|---|---|
| DMSO | Sigma | D2650 |
| Camptothecin | Sigma | C9911 |
| Topotecan | Cayman Chemical | 14129 |
| Etoposide | Cayman Chemical | 12092 |
| ICRF-193 | Sigma | I4659 |
| VE-821 | Selleckchem | S8007 |
| KU-60019 | ApexBio Technology | A8336 |
| JNK-IN-8 | Selleckchem | S4901 |
| SP600125 | Cayman Chemical | 10010466 |
| DRB | Cayman Chemical | 10010302 |
| Hydroxyurea | Sigma | H8627 |
| (+)-JQ1 | ApexBio Technology | A1910 |
| Anisomycin | Cayman Chemical | 11308 |
| Doxorubicin | MedKoo | 100280 |
| APTO-253 | MedChemExpress | HY-16291 |

**Table S3. Primary antibody information.**

| Antibody | Company | Catalog # |
|---|---|---|
| Cleaved Caspase 3 | Cell Signaling Technology | 9664S |
| Cleaved PARP1 | Cell Signaling Technology | 9546S |
| PARP1 | Cell Signaling Technology | 9532 |
| Phospho-Bcl2 (Ser70) | Cell Signaling Technology | 2827T |
| Beta-Actin | Santa Cruz Biotechnology | sc-69879 |
| RAD17 | Santa Cruz Biotechnology | sc-17761 |
| Topoisomerase I | Santa Cruz Biotechnology | sc-32736 |
| Topoisomerase II beta | Santa Cruz Biotechnology | Sc-365071 |
| TDP1 | Santa Cruz Biotechnology | sc-365674 |
| PNKP | Santa Cruz Biotechnology | sc-271505 |
| XRCC1 | Santa Cruz Biotechnology | sc-56254 |
| DNA Ligase III | Santa Cruz Biotechnology | sc-135883 |
| Phospho-Chk1 (Ser345) | Cell Signaling Technology | 2348T |
| Chk1 | Santa Cruz Biotechnology | sc-8408 |
| Phospho-Chk2 (Thr68) | Cell Signaling Technology | 2197T |
| Phospho-p53 (Ser15) | Cell Signaling Technology | 9286T |
| BRCA1 | Santa Cruz Biotechnology | sc-6954 |
| GAPDH | Santa Cruz Biotechnology | sc-47724 |
| MYC | Santa Cruz Biotechnology<br>Abcam | sc-373712<br>ab32072 |
| TWIST | Abcam | ab49254 |
| SNAIL | Cell Signaling Technology | 3895S |
| Phospho-ERK (Tyr204) | Santa Cruz Biotechnology | sc-7383 |
| Phospho-Akt (Ser473) | Cell Signaling Technology | 4060 |
| Phospho-JNK (Thr183/Tyr185) | Cell Signaling Technology<br>Santa Cruz Biotechnology | 9255S<br>sc-6254 |
| JNK | Cell Signaling Technology | 9252 |
| Phoshpo-P38(Thr180/Tyr182) | Cell Signaling Technology | 4511 |
| Phospho-CTD Ser5 | Bethyl Laboratories | A304-408A-T |
| Phospho-CTD Ser2 | Abcam | ab5095 |
| RNA Polymerase II | Bethyl Laboratories | A300-653A-T |
| Phospho-Histone H2A.X (Ser139) | Millipore Sigma | 05-636 |