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Supplemental information

Replication gaps are a key determinant of PARP inhibitor synthetic lethality with BRCA deficiency

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Figure S1

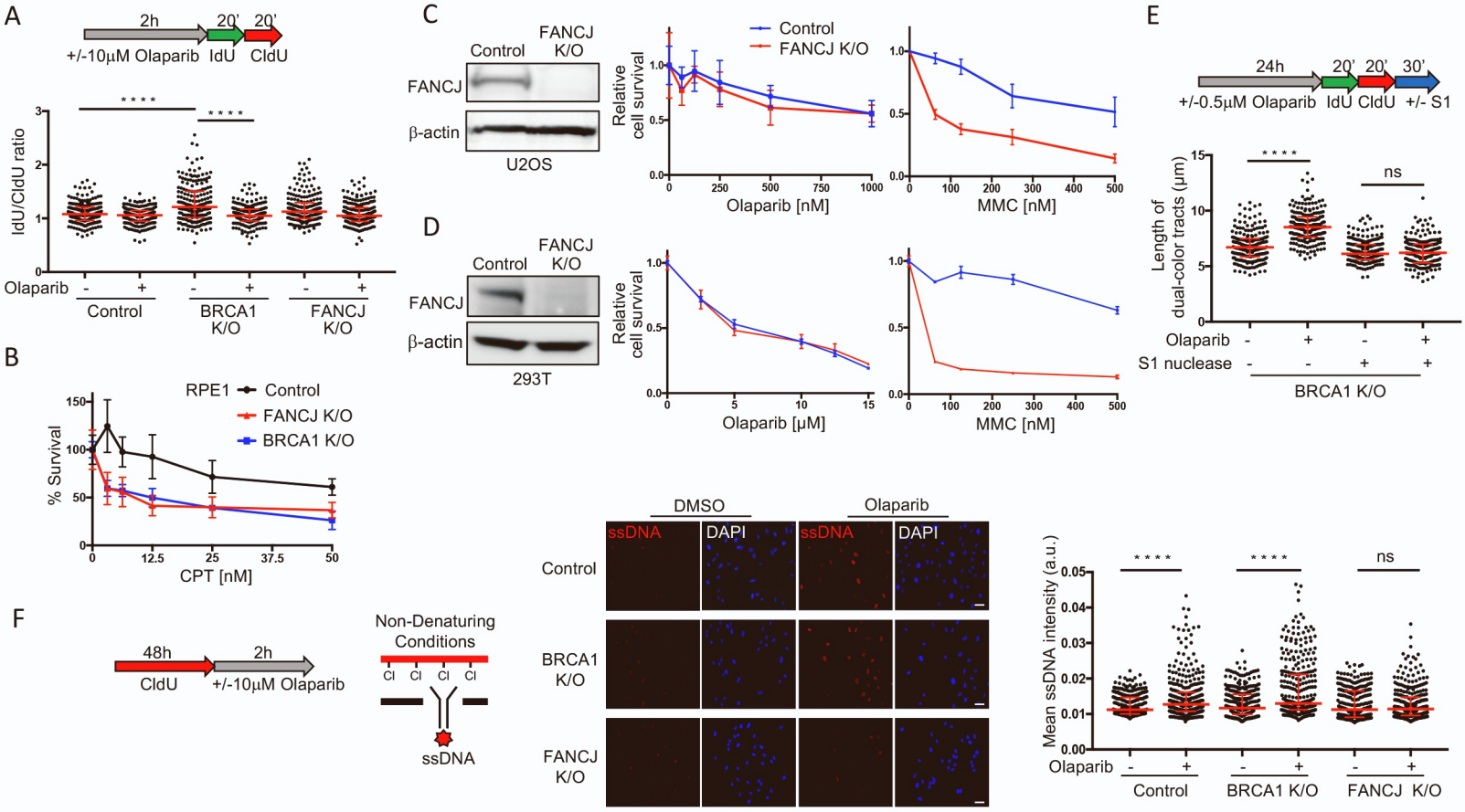
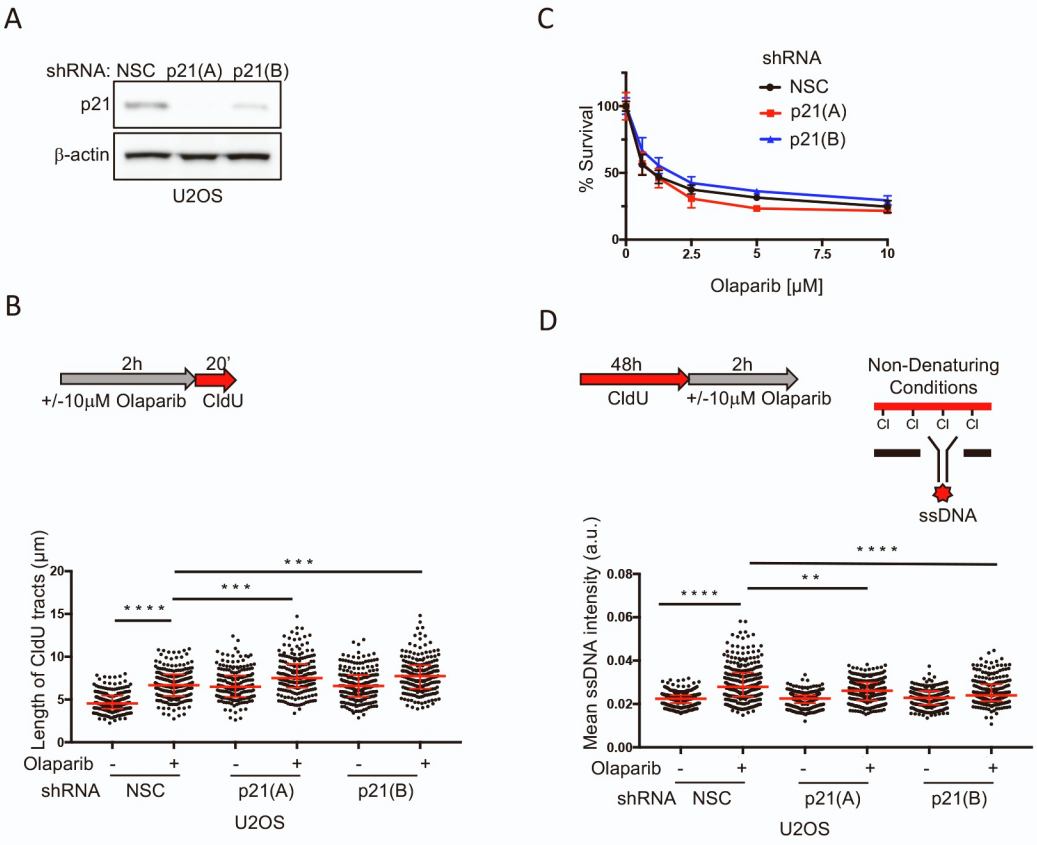

A
2h
20'
20'
+/-10μM Olaparib
IdU
CldU
IdU/CldU ratio
****
****
Olaparib
Control
BRCA1 K/O
FANCJ K/O
B
RPE1
Control
FANCJ K/O
BRCA1 K/O
% Survival
CPT [nM]
C
Control
FANCJ K/O
FANCJ
β-actin
U2OS
Relative cell survival
Olaparib [nM]
MMC [nM]
D
293T
Olaparib [μM]
E
24h
+/-0.5μM Olaparib
IdU
CldU
+/- S1
Length of dual-color tracts (μm)
ns
S1 nuclease
F
48h
CldU
2h
+/-10μM Olaparib
Non-Denaturing Conditions
ssDNA
DMSO
Olaparib
DAPI
Mean ssDNA intensity (a.u.)

Figure S2


A
shRNA: NSC p21(A) p21(B)
p21
β-actin
U2OS
C
shRNA
NSC
p21(A)
p21(B)
% Survival
Olaparib [μM]
B
2h
20'
+/-10μM Olaparib
CldU
Length of CldU tracts (μm)
Olaparib
shRNA
NSC
p21(A)
p21(B)
U2OS
D
48h
2h
CldU
+/-10μM Olaparib
Non-Denaturing Conditions
ssDNA
Mean ssDNA intensity (a.u.)
Olaparib
shRNA
NSC
p21(A)
p21(B)
U2OS

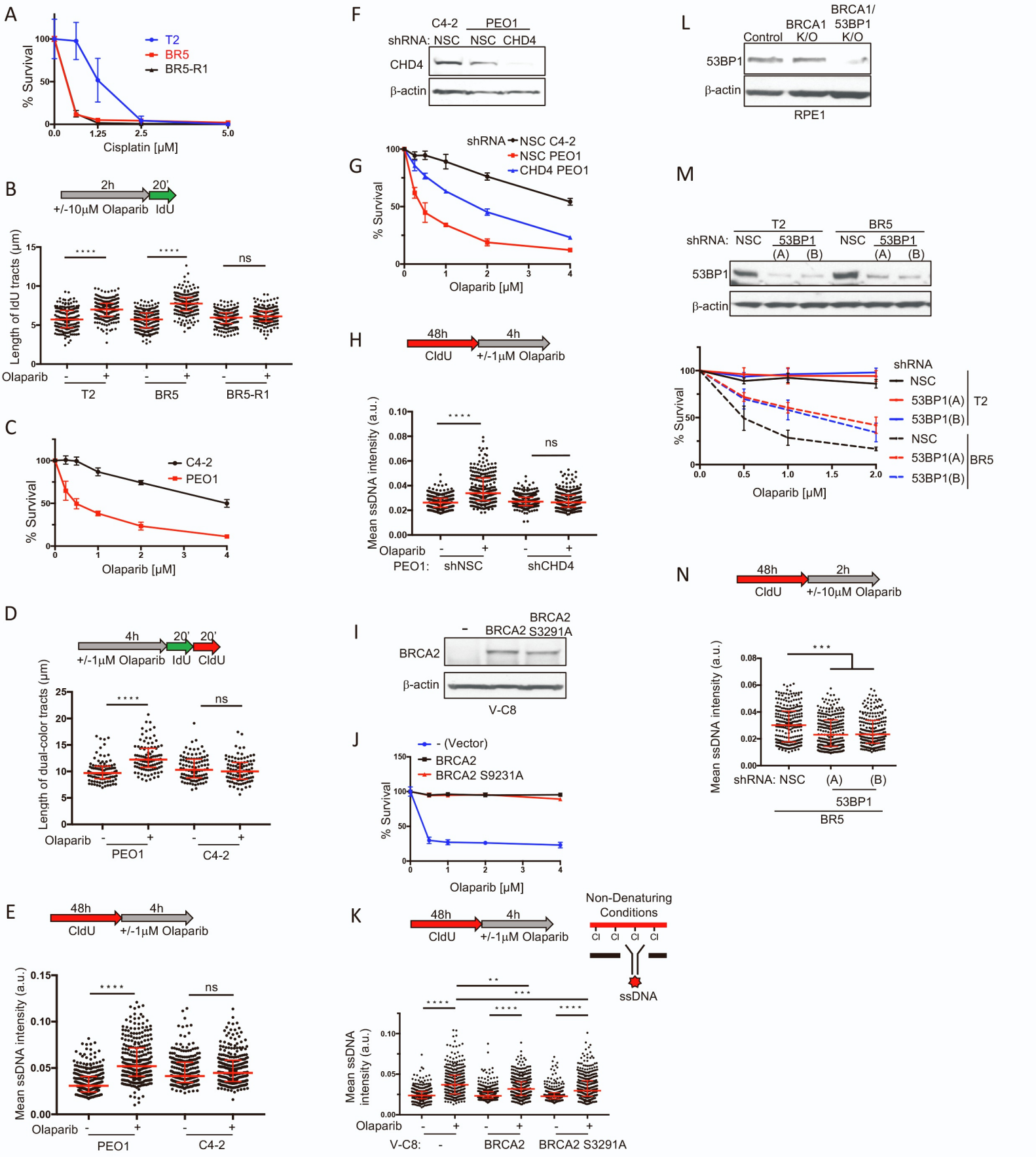

A
% Survival
T2
BR5
BR5-R1
Cisplatin [μM]
B
2h
20'
+/-10μM Olaparib
IdU
Length of IdU tracts (μm)
Olaparib
T2
BR5
BR5-R1
C
C4-2
PEO1
% Survival
Olaparib [μM]
D
4h
20'
20'
+/-1μM Olaparib
IdU
CldU
Length of dual-color tracts (μm)
Olaparib
PEO1
C4-2
E
48h
4h
CldU
+/-1μM Olaparib
Mean ssDNA intensity (a.u.)
Olaparib
PEO1
C4-2
F
C4-2
PEO1
shRNA: NSC NSC CHD4
CHD4
β-actin
G
shRNA
NSC C4-2
NSC PEO1
CHD4 PEO1
% Survival
Olaparib [μM]
H
48h
4h
CldU
+/-1μM Olaparib
Mean ssDNA intensity (a.u.)
Olaparib
PEO1:
shNSC
shCHD4
I
BRCA2
BRCA2 S3291A
BRCA2
β-actin
V-C8
J
- (Vector)
BRCA2
BRCA2 S9231A
% Survival
Olaparib [μM]
K
48h
4h
CldU
+/-1μM Olaparib
Non-Denaturing Conditions
ssDNA
Mean ssDNA intensity (a.u.)
Olaparib
V-C8:
BRCA2
BRCA2 S3291A
L
BRCA1/ 53BP1 K/O
Control
BRCA1 K/O
53BP1
β-actin
RPE1
M
T2
BR5
shRNA: NSC 53BP1 (A) (B) NSC 53BP1 (A) (B)
53BP1
β-actin
shRNA
NSC
53BP1(A)
53BP1(B)
T2
NSC
53BP1(A)
53BP1(B)
BR5
% Survival
Olaparib [μM]
N
48h
2h
CldU
+/-10μM Olaparib
Mean ssDNA intensity (a.u.)
shRNA: NSC (A) (B)
53BP1
BR5

Figure S4

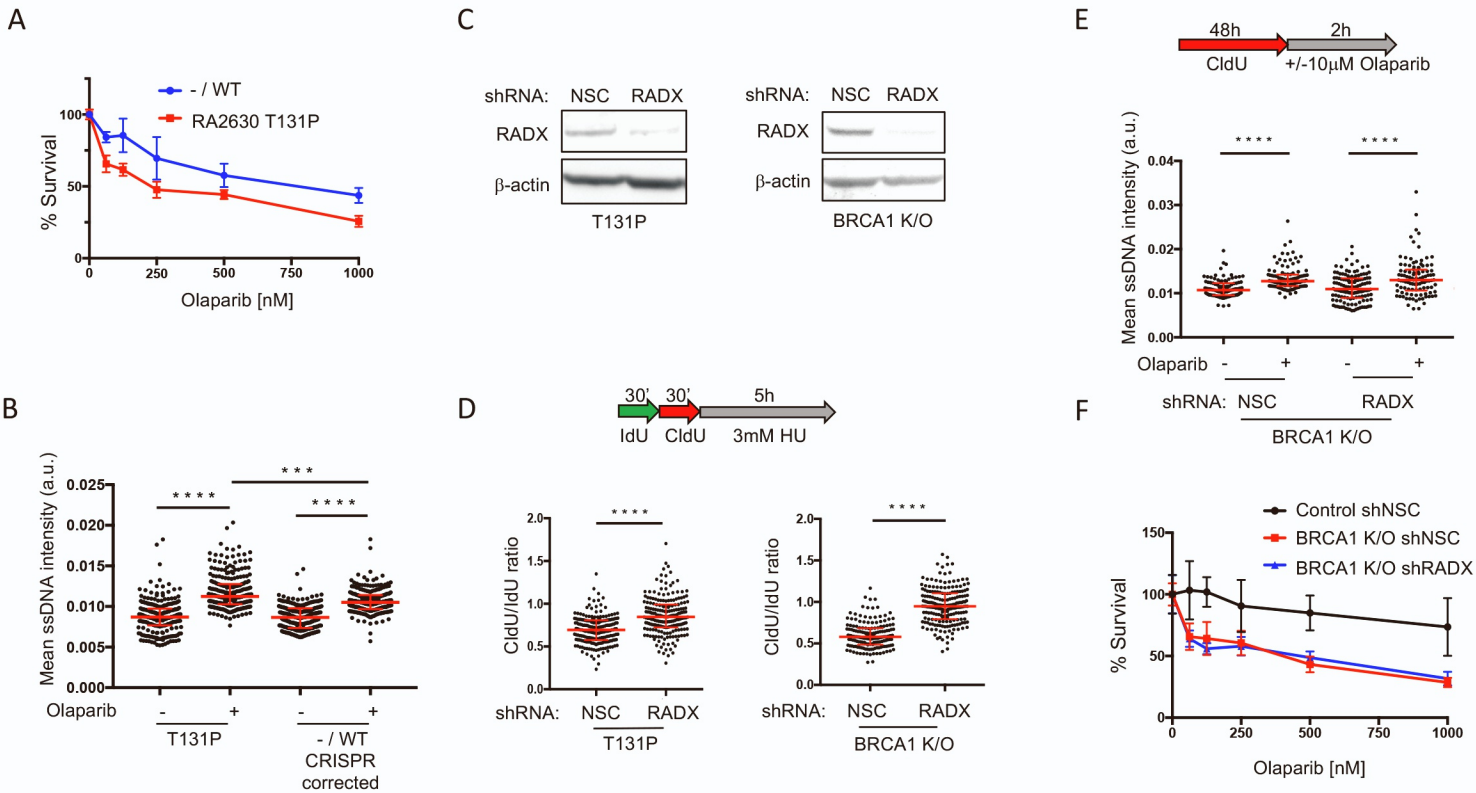

A
- / WT
RA2630 T131P
% Survival
Olaparib [nM]
C
shRNA: NSC RADX
RADX
β-actin
T131P
shRNA: NSC RADX
RADX
β-actin
BRCA1 K/O
E
48h
2h
CldU
+/-10μM Olaparib
Mean ssDNA intensity (a.u.)
Olaparib
shRNA: NSC RADX
BRCA1 K/O
B
Mean ssDNA intensity (a.u.)
Olaparib
T131P
- / WT CRISPR corrected
D
30'
30'
5h
IdU
CldU
3mM HU
CldU/IdU ratio
shRNA: NSC RADX
T131P
CldU/IdU ratio
shRNA: NSC RADX
BRCA1 K/O
F
Control shNSC
BRCA1 K/O shNSC
BRCA1 K/O shRADX
% Survival
Olaparib [nM]

Figure S5

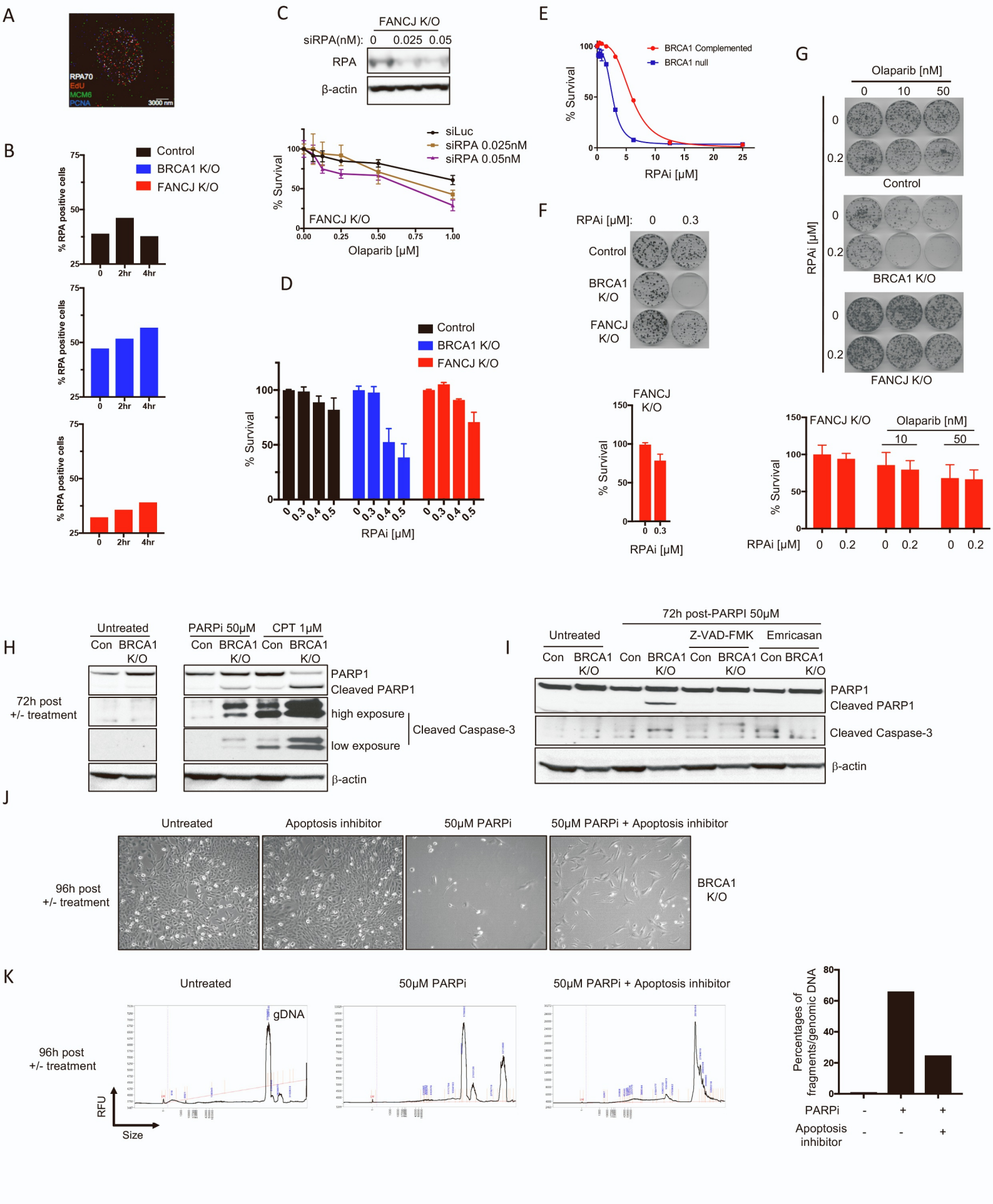

A
RPA70
EdU
MCM6
PCNA
3000 nm
B
Control
BRCA1 K/O
FANCJ K/O
% RPA positive cells
0
2hr
4hr
C
FANCJ K/O
siRPA(nM): 0 0.025 0.05
RPA
β-actin
siLuc
siRPA 0.025nM
siRPA 0.05nM
% Survival
FANCJ K/O
Olaparib [μM]
D
Control
BRCA1 K/O
FANCJ K/O
% Survival
RPAi [μM]
E
BRCA1 Complemented
BRCA1 null
% Survival
RPAi [μM]
F
RPAi [μM]: 0 0.3
Control
BRCA1 K/O
FANCJ K/O
FANCJ K/O
% Survival
RPAi [μM]
G
Olaparib [nM]
0 10 50
RPAi [μM]
Control
BRCA1 K/O
FANCJ K/O
FANCJ K/O
Olaparib [nM]
10
50
% Survival
RPAi [μM] 0 0.2 0 0.2 0 0.2
H
Untreated
PARPi 50μM
CPT 1μM
Con BRCA1 K/O
72h post +/- treatment
PARP1
Cleaved PARP1
high exposure
low exposure
Cleaved Caspase-3
β-actin
I
72h post-PARPI 50μM
Untreated
Z-VAD-FMK
Emricasan
Con BRCA1 K/O
PARP1
Cleaved PARP1
Cleaved Caspase-3
β-actin
J
Untreated
Apoptosis inhibitor
50μM PARPi
50μM PARPi + Apoptosis inhibitor
96h post +/- treatment
BRCA1 K/O
K
Untreated
50μM PARPi
50μM PARPi + Apoptosis inhibitor
96h post +/- treatment
gDNA
RFU
Size
Percentages of fragments/genomic DNA
PARPi - + +
Apoptosis inhibitor - - +

Figure S6

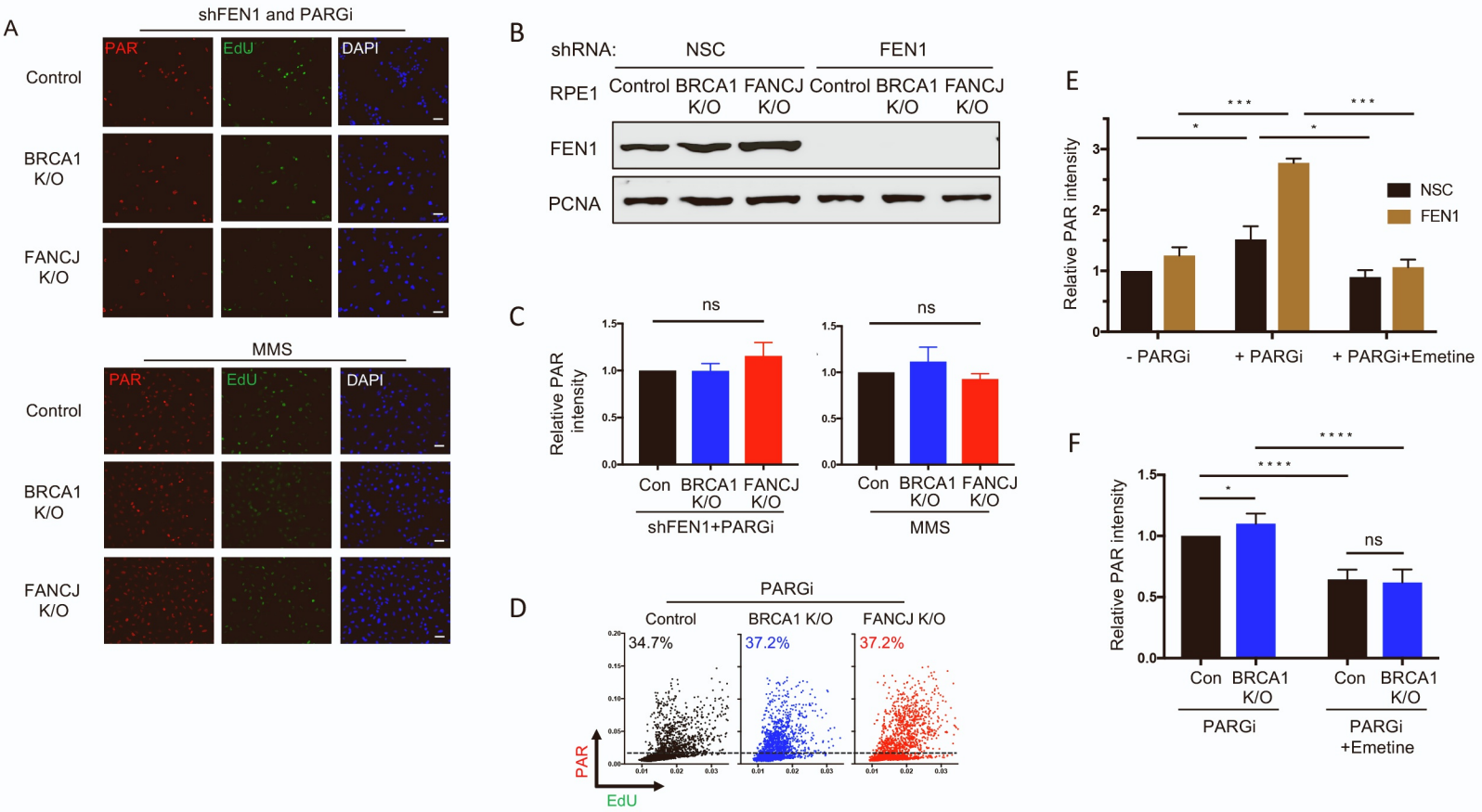

A
shFEN1 and PARGi
PAR
EdU
DAPI
Control
BRCA1 K/O
FANCJ K/O
MMS
B
shRNA:
NSC
FEN1
RPE1
Control
BRCA1 K/O
FANCJ K/O
FEN1
PCNA
C
Relative PAR intensity
ns
Con
shFEN1+PARGi
MMS
D
PARGi
34.7%
37.2%
37.2%
PAR
EdU
E
Relative PAR intensity
NSC
FEN1
- PARGi
+ PARGi
+ PARGi+Emetine
F
Relative PAR intensity
ns
PARGi
PARGi +Emetine

Figure S7

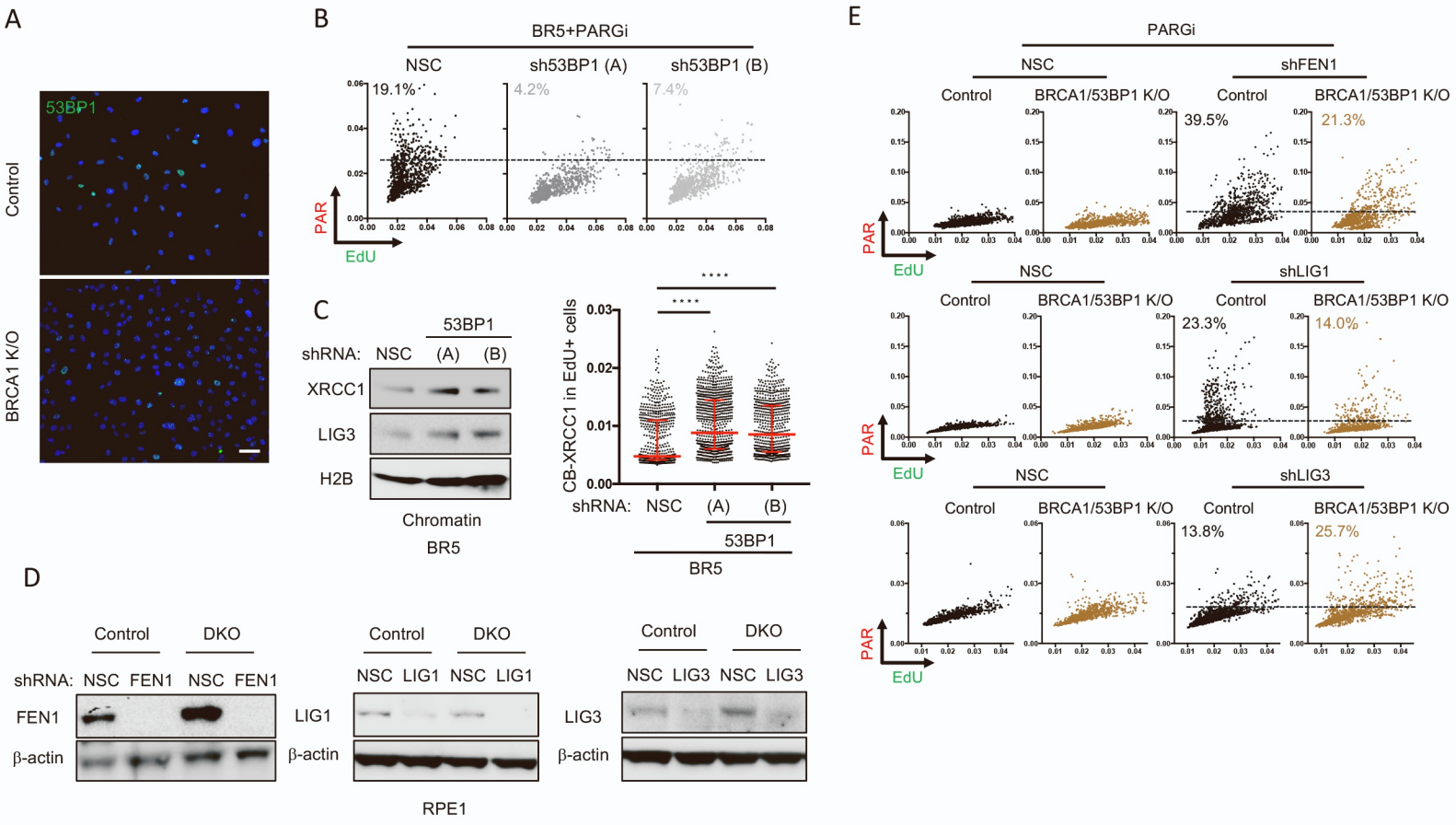

A
53BP1
Control
BRCA1 K/O
B
BR5+PARGi
NSC
sh53BP1 (A)
sh53BP1 (B)
19.1%
4.2%
7.4%
PAR
EdU
C
53BP1
shRNA: NSC (A) (B)
XRCC1
LIG3
H2B
Chromatin
BR5
CB-XRCC1 in EdU+ cells
****
****
shRNA: NSC (A) (B)
53BP1
BR5
D
Control
DKO
shRNA: NSC FEN1 NSC FEN1
FEN1
β-actin
NSC LIG1 NSC LIG1
LIG1
β-actin
NSC LIG3 NSC LIG3
LIG3
β-actin
RPE1
E
PARGi
NSC
shFEN1
Control
BRCA1/53BP1 K/O
39.5%
21.3%
shLIG1
23.3%
14.0%
shLIG3
13.8%
25.7%
PAR
EdU

Table S1

Cell line	HR*	FP*	GS*	PARPi response
BRCA1/2 proficient	+	+	+	resistant
FANCJ deficient	-	-	+	resistant
BRCA1 and 53BP1 deficient	+	-	+	resistant
BRCA2 and CHD4 deficient	-	+	+	resistant
BRCA2 S3291A mutant	+	-	+	resistant
BRCA1/2 deficient	-	-	-	sensitive
RAD51 T131P mutant	+	-	-	sensitive
RAD51 T131P mutant and RADX deficient	+	+	-	sensitive
BRCA1 and RADX deficient	-	+	-	sensitive

* HR: homologous recombination, FP: fork protection, GS: gap suppression

Table S2

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Oligonucleotides		
FANCJ CRISPR K/O gRNA #1: GGGTCGAGGAAAGGTAACGG	This paper	N/A
FANCJ CRISPR K/O gRNA #2: GGCAATCACCACACCCTTCA	This paper	N/A
shp21(CDKN1A): (A) 5′-TAAGGCAGAAGATGTAGAGCG-3′	This paper	N/A
shp21(CDKN1A): (B) 5′-AAAGTCGAAGTTCCATCGCTC-3′	This paper	N/A
sh53BP1 (TP53BP1): (A) 5′-AAACCAGTAAGACCAAGTATC-3′	This paper	N/A
sh53BP1 (TP53BP1): (B) 5′- AATCAATACTAATCACACTGG-3′	This paper	N/A
shCHD4: 5'-AATTCATAGGATGTCAGCAGC-3';	This paper	N/A
shRADX(CXorf57): 5'-ATTTCCGTGGAATACTTTCAG-3'	This paper	N/A
shFEN1: 5'-TACTCTCACAGTAGTCACTGC-3'	This paper	N/A
shLIG1: 5'-TTCACGGACTCGAATAAACCG-3'	This paper	N/A
shLIG3: 5'-AATGTAGTCCTTAAAGTGGGC-3'	This paper	N/A

Supplemental Figure titles and legends

Figure S1: PARPi-induced fork lengthening and ssDNA gaps are enhanced in BRCA1-deficient cells and suppressed in FANCJ-deficient cells. Related to Figure 1.

(A) Schematic and quantification of the ratio of IdU/CldU in Control and BRCA1 K/O RPE1 cells following olaparib treatment (10 μM, 2 h).

(B) Cell survival assays for Control, FANCJ K/O and BRCA1 K/O RPE1 cells under increasing concentrations of camptothecin (CPT). Data represent the mean percent ± SD of survival for each dot.

(C) and (D) Western blot analysis with the indicated antibodies of lysates from Control, FANCJ K/O U2OS cells (C) and 293T cells (D). Cell survival assays for indicated cells under increasing concentrations of olaparib and mitomycin C (MMC). Data represent the mean percent ± SD of survival for each dot.

(E) Schematic and quantification of the length of dual-color tracts with or without S1 nuclease incubation in BRCA1 K/O RPE1 cells following olaparib treatment (0.5 μM, 24 h). For (A), (B) and (F), each dot represents one fiber; at least 200 fibers are quantified from two biological independent experiments (n=2). Red bars represent the median. Statistical analysis according to Kruskal-Wallis test followed by Dunn's test. All p values are described in Statistical methods.

(F) Left: Treatment strategy and scheme for the non-denaturing immunofluorescence of mean ssDNA intensity per nucleus.

Middle: Representative images of ssDNA immunofluorescence for indicated cells following treatment. Scale bars, 50μm.

Right: Quantification of mean ssDNA intensity for indicated cells following CldU pre-labeling and olaparib release in 10 μM, 2 h. At least 300 cells are quantified from n=3. Red bars represent the median ± interquartile range, a.u. for arbitrary units. All statistical analysis according to Kruskal-Wallis test followed by Dunn's test. ****$p < 0.0001$, ns: not significant.

Figure S2: PARPi-induced fork lengthening and sensitivity are uncoupled. Related to Figure 2.

(A) Western blot analysis with the indicated antibodies of lysates from U2OS cells expressing shRNA against non-silencing control (NSC), p21(A) and p21(B).

(B) Schematic and quantification of the length of CldU tracts in indicated U2OS cells following olaparib treatment (10 μM, 2 h). Each dot represents one fiber; at least 200 fibers are quantified from two biological independent experiments (n=2).
(C) Cell survival assays for indicated U2OS cells under increasing concentrations of olaparib. Data represent the mean percent ± SD of survival for each dot.
(D) Schematic and quantification of mean ssDNA intensity for indicated U2OS cells following CldU pre-labeling and olaparib release (10 μM, 2 h). At least 200 cells are quantified from n=2. Red bars represent the median ± interquartile range. Statistical analysis according to Kruskal-Wallis test followed by Dunn's test. All p values are described in Statistical methods.

Figure S3: Survival, fork lengthening, and gap suppression in known and *de novo* models of PARPi resistance. Related to Figure 3.
(A) Cell survival assays for T2, BR5 and BR5-R1 cells under increasing concentrations of cisplatin. Data represent the mean percent ± SD of survival for each dot.
(B) Schematic and quantification of the length of IdU tracts in T2, BR5 and BR5-R1 following olaparib treatment (10 μM, 2 h). Each dot represents one fiber; at least 200 fibers are quantified from two biological independent experiments (n=2).
(C) Cell survival assays for C4-2 and PEO1 cells under increasing concentrations of olaparib.
(D) Schematic and quantification of the length of dual-labeled tracts in PEO1 and C4-2 cells following olaparib treatment (1 μM, 4 h). Each dot represents one fiber, at least 100 fibers are quantified for each.
(E) Schematic and quantification of mean ssDNA intensity for indicated cell lines following CldU pre-labeling and olaparib release (1 μM, 4 h).
(F) Western blot analysis with the indicated antibodies of lysates from C4-2 NSC, PEO1 NSC and PEO1 shCHD4 cells.
(G) Cell survival assays for C4-2 NSC, PEO1 NSC and PEO1 shCHD4 cells under increasing concentrations of olaparib.
(H) Schematic and quantification of mean ssDNA intensity for PEO1 cells expressing shRNA against NSC and CHD4 following CldU pre-labeling and olaparib release (1 μM, 4 h).
(I) Western blot analysis with the indicated antibodies of lysates from V-C8, V-C8+BRCA2 and V-C8+BRCA2 S3291A cells.

(J) Cell survival assays for V-C8, V-C8+BRCA2 and V-C8+BRCA2 S3291A cells under increasing concentrations of olaparib.
(K) Schematic and quantification of mean ssDNA intensity for V-C8 and indicated cells following CldU pre-labeling and olaparib release (1 μM, 4 h).
(L) Western blot analysis with the indicated antibodies of lysates from Control, BRCA1 K/O and BRCA1/53BP1 K/O RPE1 cells.
(M) Western blot analysis for T2 and BR5 cells expressing shRNA against NSC, 53BP1(A) and 53BP1(B), and cell survival assays for indicated cells under increasing concentrations of olaparib.
(N) Schematic and quantification of mean ssDNA intensity for BR5 cells expressing shRNA against NSC, 53BP1(A) and 53BP1(B) following CldU pre-labeling and olaparib release (10 μM, 2 h). All red bars represent the mean ± interquartile range. For (E), (H), (K) and (N), at least 200 cells are quantified from n=2. Statistical analysis according to Kruskal-Wallis test followed by Dunn's test. All p values are described in Statistical methods.

Figure S4: Gaps predict PARPi sensitivity in HR- and FP-proficient cells. Related to Figure 4.
(A) Cell survival assays for patient fibroblasts (RA2630) RAD51 T131P (T131P/WT) and RAD51 single-allele CRISPR deleted cells (-/WT) under increasing concentrations of olaparib.
(B) Quantification of mean ssDNA intensity for indicated cell lines in (A) following CldU pre-labeling and olaparib release (10 μM, 2 h), at least 200 cells are quantified from from two independent biological experiments (n=2).
(C) Western blot analysis with the indicated antibodies of lysates from patient fibroblasts (RA2630) RAD51 T131P cells and BRCA1 K/O RPE1 cells expressing shRNA against non-silencing control (NSC) and RADX.
(D) Schematic and quantification of the CldU/IdU ratio after 3 mM HU treatment for 5 h in indicated cells, at least 200 fibers are quantified from n=2.
(E) Schematic and quantification of mean ssDNA intensity for indicated cell lines following CldU pre-labeling and olaparib release (10 μM, 2 h), at least 100 cells are quantified from n=2. All red bars represent the mean ± interquartile range. Statistical analysis according to Kruskal-Wallis test followed by Dunn's test. All p values are described in Statistical methods.

(F) Cell survival assays for indicated cell lines under increasing concentrations of olaparib. For all cell survival assays, data represent the mean percent ± SD of survival for each dot.

Figure S5: PARPi synthetic lethality in BRCA1-deficient cells is augmented by targeting RPA and corresponds with apoptosis and DSB formation. Related to Figure 5.

(A) Sample image for STORM analysis with staining of RPA70: white, EdU: red, MCM6: green, PCNA: blue. Scale bar, 3000nm.

(B) Bar graphs show percentages of RPA positive cells for different cells and treatments in Figure 5C.

(C) Western blot analysis and cell survival assays for FANCJ K/O cells expressing indicated siRPA under increasing concentrations of olaparib.

(D) Cell survival assays for indicated cell lines with sensitivity normalized to untreated respectively at different concentrations of RPA inhibitor (0.3, 0.4, 0.5 μM for NERx-329). For cell survival assays, data represent the mean percent ± SD of survival for each bar.

(E) Sensitivity of BRCA1-deficient UWB1 and complemented ovarian cancer cell lines to RPA inhibitor NERx-329. Data represent the mean percent ± SD of survival for each dot.

(F) Representative images and quantification for clonogenic assays with indicated cells treated with RPA inhibitor (RPAi, NERx-329) under indicated doses. Mean survival percentages of four biological independent experiments (n=4) with SEM are collected for all cells.

(G) Representative images and quantification for clonogenic assays for indicated cells under increasing olaparib with sublethal dose of RPA inhibitor (RPAi, NERx-329). Mean survival percentages of n=3 with SEM are collected for all cells.

(H) Apoptosis is induced in BRCA1 K/O cells by 72h post-PARPi (Olaparib, 50μM) and CPT (positive control, 1μM) as noted by cleavage of PARP1 and Caspase-3 by Western blot analysis.

(I) Apoptosis proteins in (H) are suppressed by apoptosis inhibitors Z-VAD-FMK (50μM) or Emricasan (50μM) with 2h pretreatment and maintained for the duration of the experiment (72h).

(J) Inhibition of apoptosis with Emricasan (50μM) enhances cell survival of BRCA1 K/O cells treated with PARPi (50μM).

(K) Pulsed field capillary electrophoresis analysis (left) and quantitation (right) for fragments of genome DNA (gDNA) after PARPi treatment in BRCA1 K/O cells reveal DNA fragmentation is suppressed by Emricasan (50μM). Left: X-axis: DNA sizes (base pair), Y-axis: Relative fluorescence units (RFU); purple numbers embedded indicate the sizes for each peak.

Figure S6: Poly(ADP-ribose) validation for Okazaki fragment processing defects. Related to Figure 6.

(A) Top: Representative images of immunofluorescence for shFEN1 Control, BRCA1 K/O and FANCJ K/O RPE1 cells following PARG inhibitor (10 μM) and EdU for 20min, stained for poly (ADP-ribose) (PAR) and EdU. Scale bars, 50μm. Bottom: Immunofluorescence images for Control, BRCA1 K/O and FANCJ K/O RPE1 cells following MMS (0.2mg/ml) and EdU for 20min, stained for poly (ADP-ribose) (PAR) and EdU. Scale bars, 50μm.

(B) Western blot analysis with the indicated antibodies of lysates from Control, FANCJ K/O and BRCA1 K/O RPE1 cells cells expressing shRNA against non-silencing control (NSC) and FEN1.

(C) Quantification of relative PAR intensity across indicated cells incubated with PARG inhibitor (10 μM) or MMS (0.2 mg/ml) for 20min. Average values of five biological independent experiments (n=5) with SEM are calculated for all interphase cells.

(D) Quantification of immunofluorescence for shFEN1 Control, BRCA1 K/O and FANCJ K/O RPE1 cells following PARG inhibitor (10 μM) and EdU for 20min, stained for poly (ADP-ribose) (PAR) and EdU. Dashed lines indicate maximum PAR level in untreated Control cells. Cells higher than those are calculated for percentages respectively. Each dot represents one cell; at least 2000 cells are collected from n=5.

(E) Quantification of relative PAR intensity across indicated Control cells incubated with or not with emetine (10 μM, 1 h), with or without PARG inhibitor (10 μM) and EdU added during the final 20min. Average values of n=3 with SEM are calculated for all interphase cells.

(F) Quantification of relative PAR intensity for Control and BRCA1 K/O RPE1 cells incubated with or without emetine (10 μM, 1 h), with PARG inhibitor (10 μM) and EdU added during the final 20min. Average values of n=5 with SEM are calculated for all interphase cells. For (C), (E) and (F), at least 200 cells are measured for each experiment. Statistical analysis according to t test, all p values are described in Statistical methods.

Figure S7: Analysis for 53BP1 depletion and alternative OFP. Related to Figure 7.

(A) Representative images (20x) for 53BP1 chromatin foci in untreated Control and BRCA1 K/O RPE1 cells. More cells with ≥ 1 foci can be observed in BRCA1 K/O cells. Scale bar, 50μm.

(B) Mean PAR and EdU intensity per nucleus for indicated cells were measured after PARG inhibitor (10 μM, 20min) and EdU incubation. For quantification, PAR positive cells were gated by maximum PAR level of untreated BR5 cells in Figure 6B. Each dot represents one cell; at least 800 cells are collected from two biological independent experiments (n=2) for each.

(C) Left: Western blot analysis for indicated antibodies in chromatin extraction from untreated BR5 cells expressing shRNA against NSC, 53BP1(A) and 53BP1(B).

Right: Quantification of CB-XRCC1 for untreated BR5 cells expressing shRNA against NSC, 53BP1(A) and 53BP1(B) with EdU incubated for 20min. For quantification, EdU+ cells were gated according to positive EdU incorporation. At least 600 cells are quantified from n=2 for each. Bars represent the median ± interquartile range. Statistical analysis according to Kruskal-Wallis test followed by Dunn's test. ****$p < 0.0001$.

(D) Western blot analysis in Control and BRCA1/53BP1 K/O (DKO) RPE1 cells expressing shRNA against NSC, FEN1, LIG1 and LIG3.

(E) Mean PAR and EdU intensity per nucleus for indicated cells were measured after PARG inhibitor (10 μM, 20min) and EdU incubation. For quantification, PAR positive cells were gated by mean maximum PAR level in Control NSC cells. Each dot represents one cell; at least 800 cells are collected from n=4 for each.

Table S1. PARPi response in different genetic backgrounds. Related to Figures 1, 3 and 4.

Table S2. Sequences of CRISPR guides and shRNAs. Related to Oligonucleotides in KEY RESOURCES TABLE.