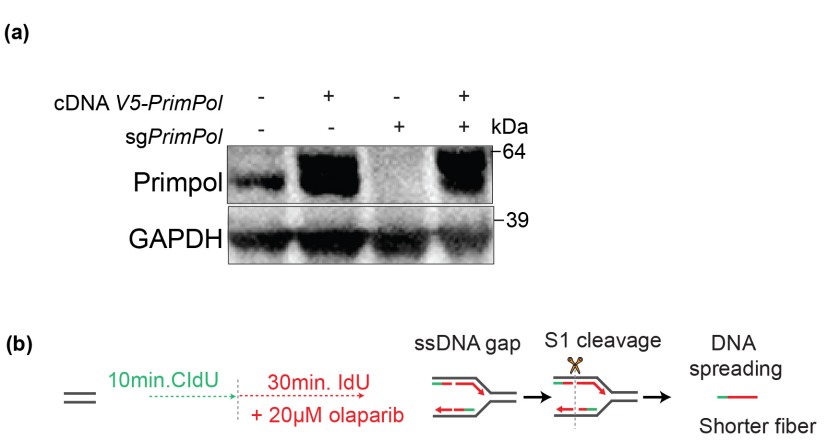
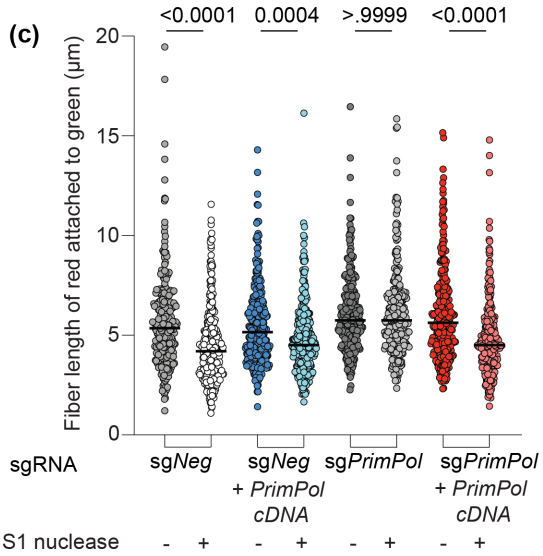

(a)
cDNA V5-PrimPol - + - +
sgPrimPol - - + + kDa
Primpol
64
GAPDH
39

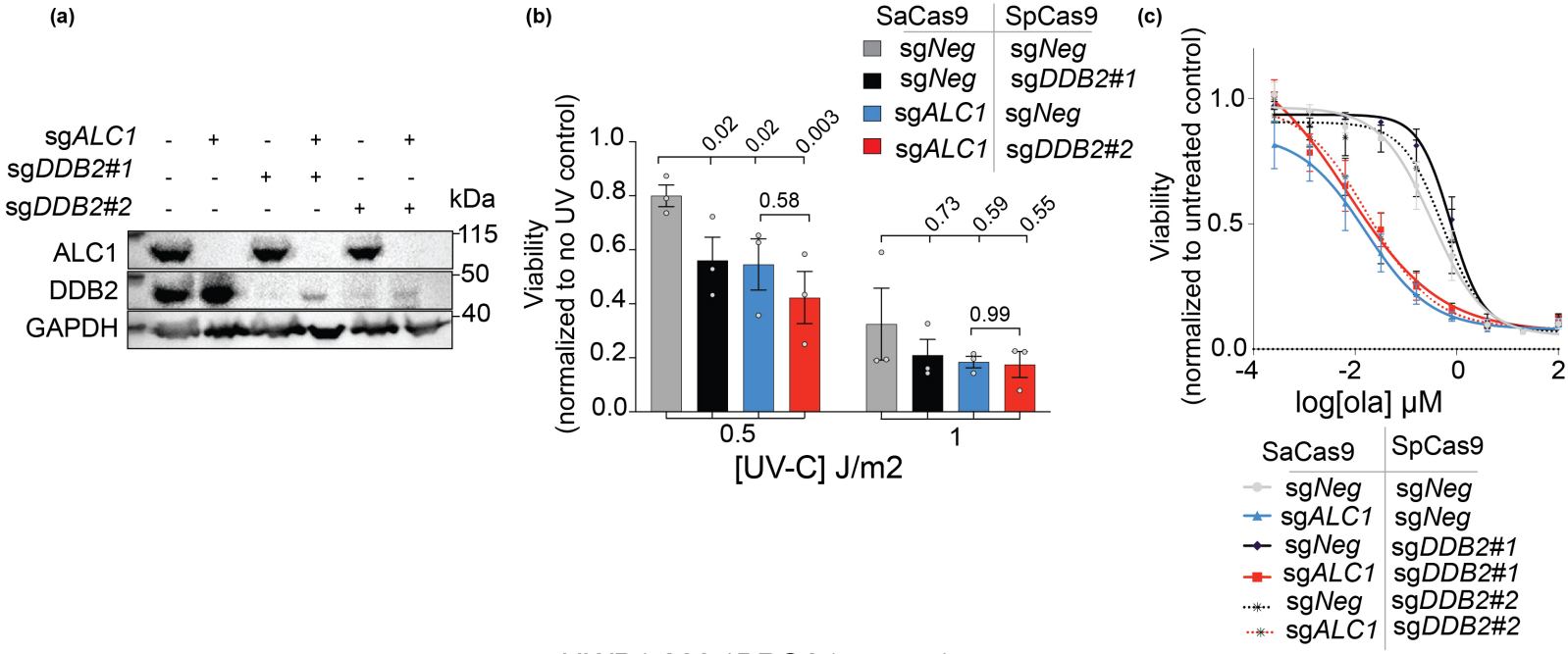
(b)
10min.CldU
30min. IdU
+ 20μM olaparib
ssDNA gap
S1 cleavage
DNA spreading
Shorter fiber


(c)
<0.0001
0.0004
>.9999
<0.0001
Fiber length of red attached to green (μm)
20
15
10
5
0
sgRNA
sgNeg
sgNeg + PrimPol cDNA
sgPrimPol
sgPrimPol + PrimPol cDNA
S1 nuclease - + - + - + - +

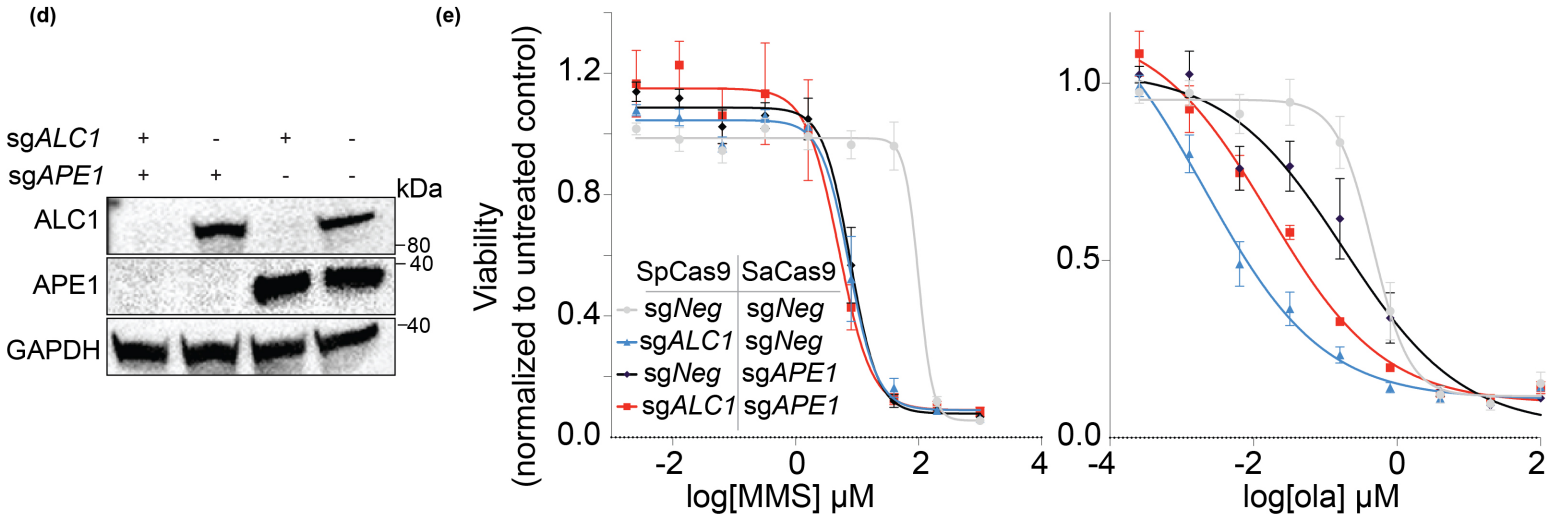
Supplementary Figure 1. Complementation of *PrimPol* knockout cells with *PrimPol* cDNA rescues gap formation.
(a) Immunoblot showing depletion of PrimPol and complementation with V5-tagged *PrimPol* cDNA. Representative of two blots.
(b) Schematic of S1-nuclease fiber assay. CldU: 5-chloro-2'-deoxyuridine; IdU: 5-iodo-2'-deoxyuridine.
(c) Fiber length analysis of the indicated cell lines treated with olaparib. Median values are indicated. *P* values derived by Kruskal-Wallis test from >250 fibers collected over n=2 biologically independent experiments.

Supplementary Figure 2

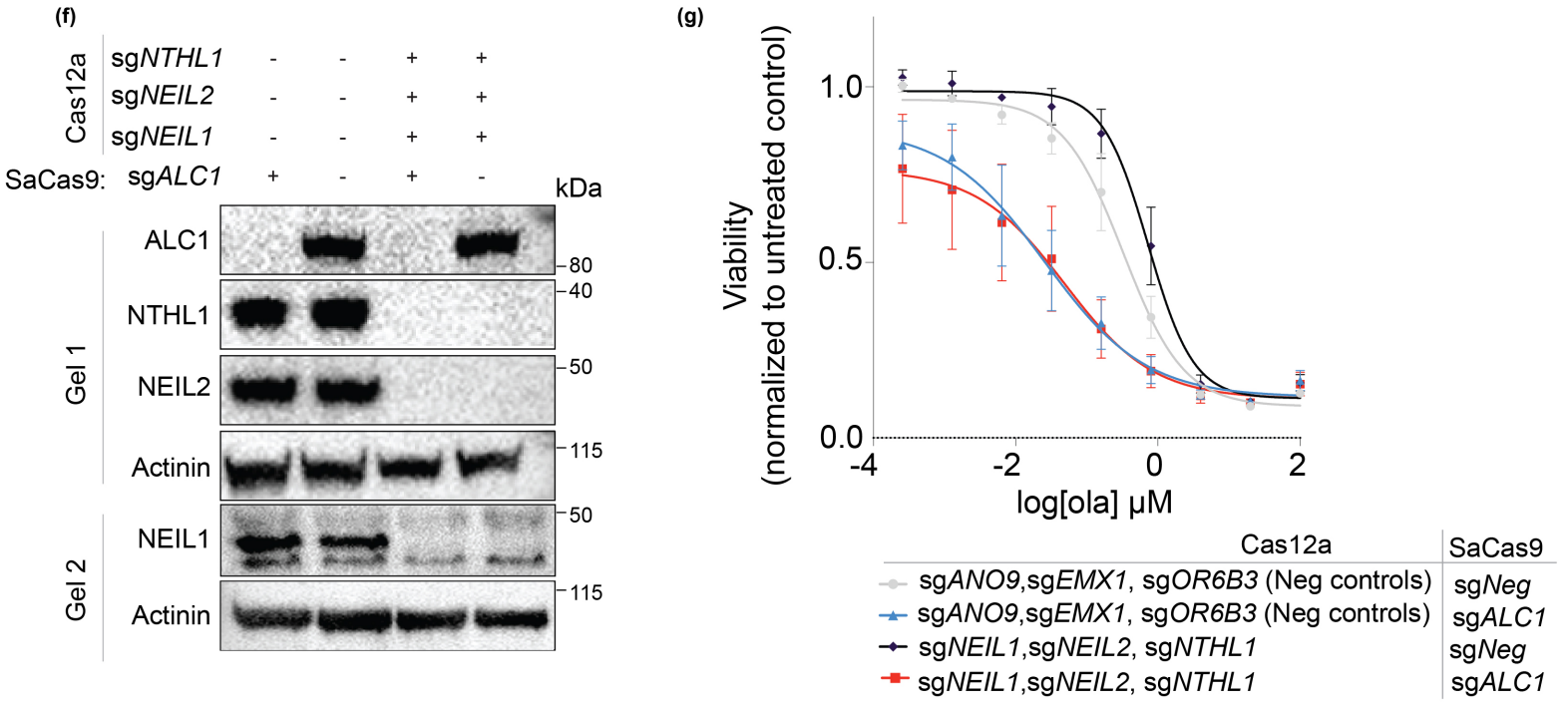
SUM149PT (*BRCA1* mutant)



UWB1.289 (*BRCA1* mutant)



SUM149PT (*BRCA1* mutant)



Supplementary Figure 2. Epistasis between ALC1 and BER/NER factors in response to UV-C and olaparib in *BRCA1* mutant cells.

(a) Immunoblot showing depletion of ALC1 and DDB2. Representative of three blots

(b) Sensitivities of the indicated SUM149PT cell lines to UV-C. Data are presented as mean ± s.e.m from n-3 biologically independent experiments. *P* values derived by Ordinary one-way ANOVA.

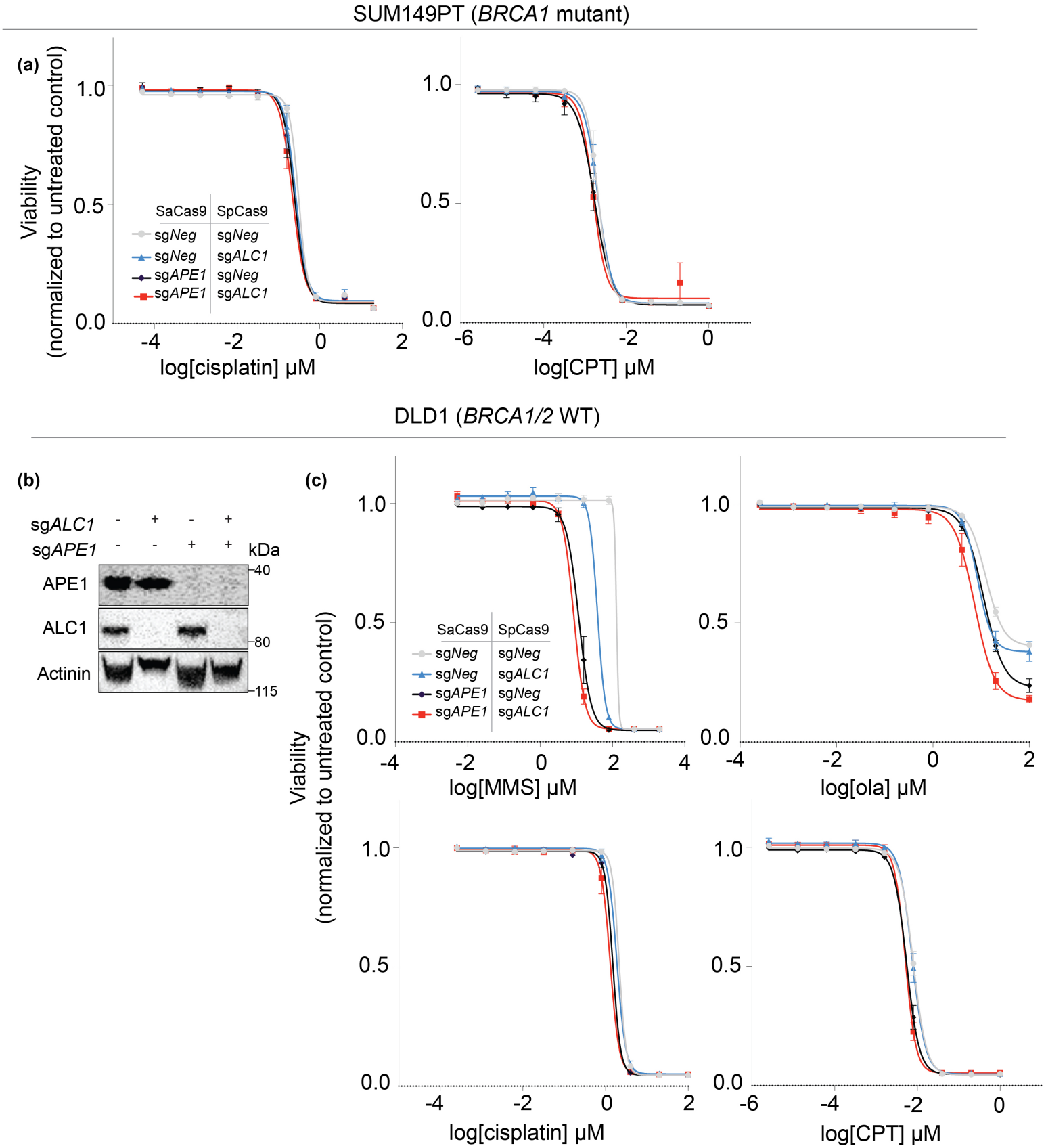
(c) Sensitivities of the indicated cell lines to olaparib. Data are presented as mean ± s.e.m from n=3 biologically independent experiments.

(d) Immunoblot showing depletion of ALC1 and APE1 in UWB1.289. Representative of three blots

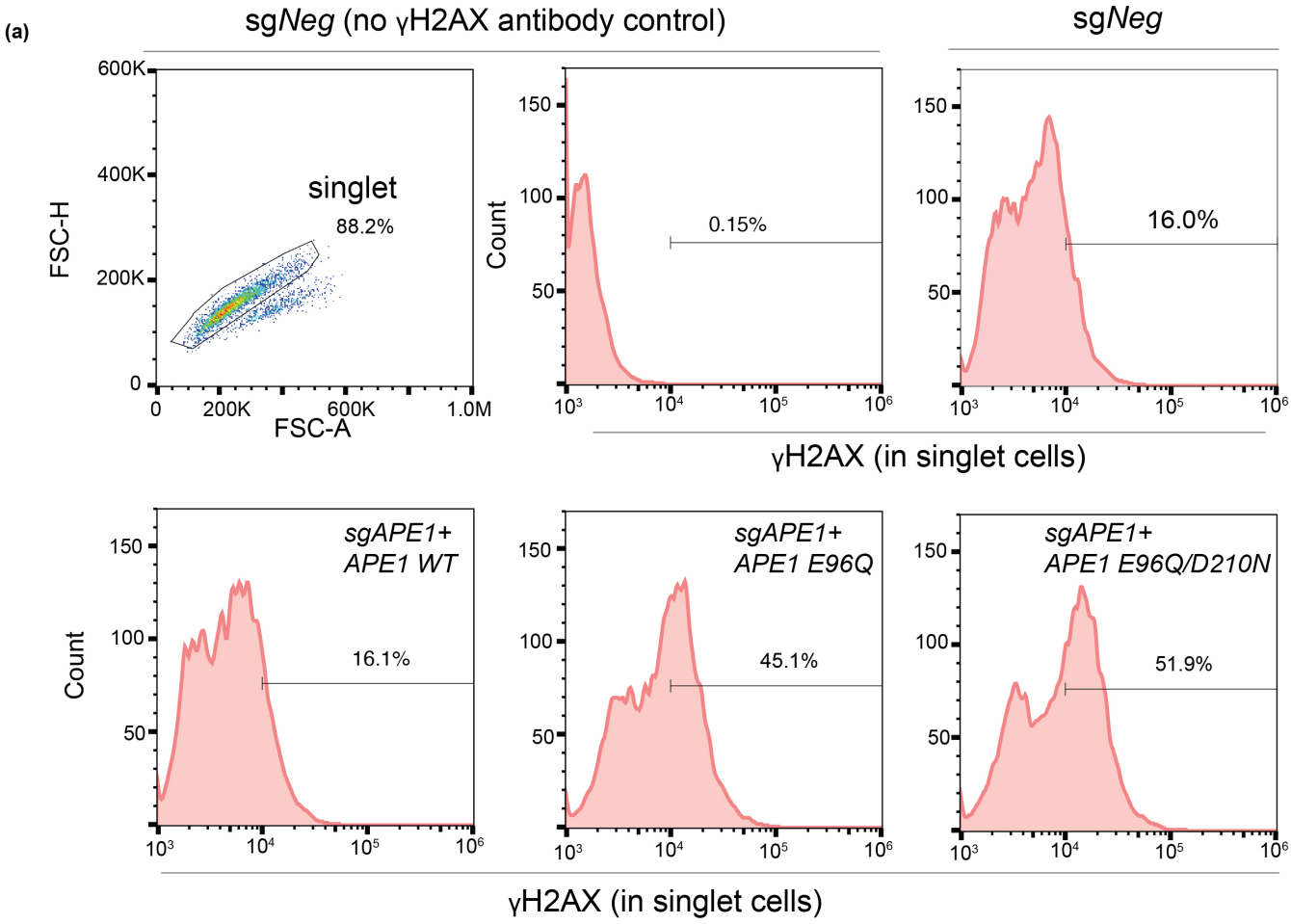
(e) Sensitivities of the indicated UWB1.289 cell lines to MMS (left) and olaparib (right). Data are presented as mean ± s.e.m from n=3 biologically independent experiments.

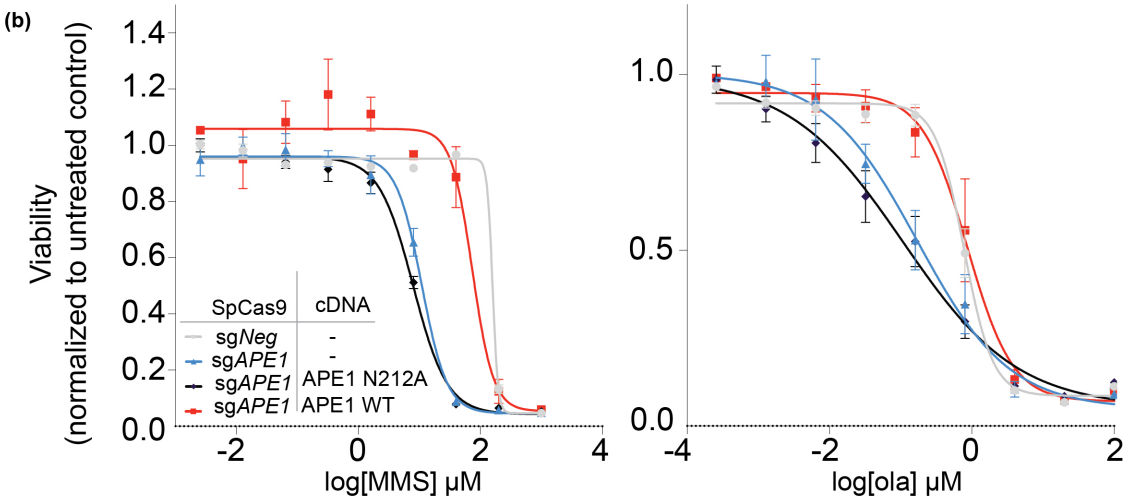
(f) Immunoblot showing depletion of NTHL1, NEIL2, NEIL1, ALC1 in SUM149PT cells. Representative of three blots.

(g) Sensitivities of the indicated SUM149PT cell lines to olaparib. Data are presented as mean ± s.e.m from n=3 biologically independent experiments.


SUM149PT (*BRCA1* mutant)
(a)
Viability (normalized to untreated control)
1.0
0.5
0.0
SaCas9 SpCas9
sg*Neg* sg*Neg*
sg*Neg* sg*ALC1*
sg*APE1* sg*Neg*
sg*APE1* sg*ALC1*
-4 -2 0 2
log[cisplatin] µM
-6 -4 -2 0
log[CPT] µM
DLD1 (*BRCA1/2* WT)
(b)
sg*ALC1* - + - +
sg*APE1* - - + +
kDa
APE1
ALC1
Actinin
40
80
115
(c)
Viability (normalized to untreated control)
log[MMS] µM
log[ola] µM
log[cisplatin] µM
log[CPT] µM

Supplementary Figure 3. Epistasis between ALC1 and APE1 in response to various DNA damaging agents.
(a) Sensitivities of the indicated SUM149PT cell lines to cisplatin (left) and camptothecin (CPT, right). Data are presented as mean ± s.e.m from n=3 biologically independent experiments.
(b) Immunoblot showing depletion of ALC1 and APE1 in DLD1 *BRCA1/2* WT cells. Representative of three blots.
(c) Sensitivities of the indicated DLD1 cell lines to MMS, olaparib, cisplatin and camptothecin (CPT). Data are presented as mean ± s.e.m from n=3 biologically independent experiments.


(a)
sgNeg (no γH2AX antibody control)
sgNeg
FSC-H
FSC-A
singlet
88.2%
Count
0.15%
16.0%
γH2AX (in singlet cells)
sgAPE1+
APE1 WT
16.1%
sgAPE1+
APE1 E96Q
45.1%
sgAPE1+
APE1 E96Q/D210N
51.9%
γH2AX (in singlet cells)


(b)
Viability
(normalized to untreated control)
SpCas9
cDNA
sgNeg -
sgAPE1 -
sgAPE1 APE1 N212A
sgAPE1 APE1 WT
log[MMS] μM
log[ola] μM

Supplementary Figure 4. Impact of various APE1-mutants on genome stability and genotoxin response in *BRCA1* mutant SUM149PT cells.
(a) Representative flow images showing γH2AX signal in singlet cells after expression of various APE1 mutants.
(b) Sensitivities of the indicated SUM149PT cell lines to MMS (left) and olaparib (right). Data are presented as mean ± s.e.m from n=3 biologically independent experiments.

Supplementary Table 1: List of sgRNAs and oligos used in the study

Name of the oligo	Sequence (5'-3')
sgRNA for APE1 _SaCas9	ATATGCTGTTACCAGCACAA
sgRNA#1 for DDB2_SpCas9	TGGTCACAGGAGACAACGTG
sgRNA#2 for DDB2_SpCas9	AAAACTGGTTGGTATTGAGA
sgRNA for PNKP _SpCas9	CCTGGGTCCCGGTAGTTGAG
sgRNA for PrimPol _SpCas9	CTGCCAAGCAAGTCAAGAGC
sgRNA#1 for XPC_SpCas9	GCATAGCTGGTATAGACCAG
sgRNA#2 for XPC_SpCas9	GCTGTAGAGACAATACCAGC
sgRNA for ALC1_SaCas9	TCCACAACAAGAACACTCCA
sgRNA for ALC1_SpCas9	GTCGCCTGCATATGTTACAC
sgRNA for AAVS1_SpCas9 (Negative Control)	GCCAGTAGCCAGCCCCGTCC
sgRNA for HPRT_SaCas9 (Negative Control)	GTCCCAACAGCAATTCCTAA
Cas12a guides for ANO9; EMX1; OR6B3 (Negative control)	AGATGCCTGTACCGCACTCTCCTCTAATTTCTACT, GTCGTAGATCCTCCGACTGCGGGCTCCCTTAATTTCT, ACTATCGTAGATATTCTGGCCTTCATCATCCT
Cas12a guide for NEIL1; NEIL2; NTHL1	AGATGAGCCTGGCTGCGCTGCTATTAATTTCTACT, GTCGTAGATGTCTCCCCCTTTGTGGGTCATAATTTCT, ACTATCGTAGATAGAGCAAGGTGAAATACATC
AP-NCPdyad oligo 1	GGTAATTGTAATCGGATGTATATATCTGACACGTGCCTGGAGACT AGGGAGTAATCCCCTTGGCGGTTAAAACGCGGGGGACAGCGCGT ACGTGCGTTTAAGCGGTGCTAGAGCTGTCTACGACCAATTGAGCG GCCTCGGCACCGGGATTCTCGATTCGAACTGGGTTGCTGAGTTCT CAACGTGGCTCATGTGTC
AP-NCPdyad oligo 2	GACACATGAGCCACGTTGAGAACTCAGCAACCCAGTTCGAATCGA GAATCCCGGTGCCGAGGCCGCTCAATTGGTCGTAGACAGCTCTAG CACCGCTTAAACGCACG
AP-NCPdyad oligo 3	/5Phos/TACGC/idSp/CTGTCCCCCGCGTTTTAACCGCCAAGGGGATT ACTCCCTAGTCTCCAGGCACGTGTCAGATATATACATCCGAT TACAATTACC /3Cy5Sp/
Nucleofection guide for APE1	mG*mU*mC*rUrGrGrUrArCrGrArCrUrGrGrArGrUrArCrGrUrUrUrUrArGrArGrCrUrArGrArArArUrArGrCrArArGrUrUrArArArArUrArArGrGrCrUrArGrUrCrCrGrUrUrArUrCrArArCrUrUrGrArArArArArGrUrGrGrCrArCrCrGrArGrUrCrGrGrUrGrCmU*mU*mU*rU
Nucleofection guide for ALC1	mG*mU*mC*rGrCrCrUrGrCrArUrArUrGrUrUrArCrArCrGrUrUrUrUrArGrArGrCrUrArGrArArArUrArGrCrArArGrUrUrArArArArUrArArGrGrCrUrArGrUrCrCrGrUrUrArUrCrArArCrUrUrGrArArArArArGrUrGrGrCrArCrCrGrArGrUrCrGrGrUrGrCmU*mU*mU*rU

Supplementary Table 2: List of cDNA generated in the study

Name of the construct	Details of the vector backbone
V5-tagged PrimPol cDNA resistant to sg*PrimPol* in pCDH-CMV-Blast	pCDH-CMV was a gift from Kazuhiro Oka (Addgene plasmid # 72265; http://n2t.net/addgene:72265; RRID:Addgene_72265)
HA-tagged APE1 WT cDNA resistant to sgAPE1 in LentiV_Neo	LentiV_Neo was a gift from Christopher Vakoc (Addgene plasmid # 108101; http://n2t.net/addgene:108101; RRID:Addgene_108101)
HA-tagged APE1 N21A cDNA resistant to sgAPE1 in LentiV_Neo	LentiV_Neo was a gift from Christopher Vakoc (Addgene plasmid # 108101; http://n2t.net/addgene:108101; RRID:Addgene_108101)
HA-tagged APE1 E96Q/D210N cDNA resistant to sgAPE1 in LentiV_Neo	LentiV_Neo was a gift from Christopher Vakoc (Addgene plasmid # 108101; http://n2t.net/addgene:108101; RRID:Addgene_108101)
HA-tagged APE1 E96Q/D210N cDNA resistant to sgAPE1 in pInducer20	pInducer20 was a gift from Stephen Elledge (Addgene plasmid # 44012; http://n2t.net/addgene:44012; RRID:Addgene_44012)

Supplementary Table 3: Details of antibodies used in the study

Antibody	Company	Catalogue Number	Lot Number	Dilution for western	Dilution for IF	Dilution for FACS
γH2AX	Sigma	05-636-I	3748823		1 : 2000	1 : 200
PrimPol	Gift from Alessandro Vindigni lab	N/A	N/A	1 : 1000		
ALC1	Cell Signaling Technology	13460S	1	1 : 1000		
ALC1	Santa Cruz Biotechnology	sc-81065 Sample	H1721	1 : 100		
GAPDH	Cell Signaling	2118S and 97166T	1	1 : 2000		
PNKP	Abcam	ab170954	YJ122604DS	1 : 1000		
APE1	Thermo Fisher	MA5-31586	WI3388209	1 : 1000		
Actinin	Cell Signaling Technology	D6F6	4	1 : 2000		
IdU	Becton Dickinson and Company	347580	1210802		1 : 200	
CldU	Abcam	6326	1009715-13		1 : 200	
HA	Biolegend	901514	B272772		1 : 1000	
XPC	Cell Signaling Technology	12701T	1	1 : 1000		
DDB2	Abcam	ab181136	GR3315250-6	1 : 1000		
Biotin	Cell Signaling	5597S	1		1 : 200	
NTHL1	Santa Cruz Biotechnology	sc-130644	92822	1:200		
NEIL1	Life Technologies	12145-1-AP	2807	1:500		
NEIL2	Gentex	GTX132565	42970	1:500		
PARP1	Abcam	ab227244	1035485-20		1:500	
Rad51	Cosmo Bio	BAM-70-001-EX	4		1:500	