SUPPLEMENTARY MATERIALS AND METHODS

Generation of HCT116 Artemis-/- cells:

Construction of a pAAV-Artemis exon 2 NEO targeting vector was carried out by PCR followed by restriction enzyme digestion and subsequent DNA ligation. Genomic DNA isolated from HCT116 cells was amplified to create homology arms flaking exon 2 of the Artemis gene.

Primers used to create either the left or right homology arms included:

ART2F: 5′-ATACATAGCGGCCGCGAGCCACCATGTCCAACTGGTTTAG-3′

ART2SacIIR: 5′-TTATCCGCGGTGGAGCTCCAGCTTTTGTTCCCTTTAGAAAAGAACAAAAACTCATGAATATG-3′

ART2KpnIF: 5′-ATGGTACCCAATTCGCCCTATAGTGAGTCGTATTACTATTTTGCTACTTGTGTTTTTAAG-3′

ART2R: 5′-ATACATACGCGGCCGCGTCAATAAGTAAATACAAATAAAGTAATAAAAAATTATTGGC-3′

Fusion PCR was then performed using the PCR-generated left and right homology arms along with a PvuI restriction enzyme fragment (containing NEO) from the pNeDaKO-NEO vector to create a NotI-digestible vector fragment that was subsequently ligated into pAAV-MCS resulting in pAAV-Artemis-Exon2-Neo. In order to perform a second round of targeting with a different drug selection, the NEO (neomycin) cassette in pAAV-Artemis-Exon2-Neo was replaced with a KpnI/SpeI PURO (puromycin) cassette.

In order to complement Artemis-null cells, a pAAV-Artemis-Exon2-Knock-In vector was constructed using a Golden Gate pAAV-MCS-SEPT-Rox-Neo plasmid. Primers used for the construction of pAAV-Artemis-Exon2-KI-SEPT-Rox-Neo included:

ArtExon2KI-LF: 5′-GACGCTCTTCACCGACTGGGTCTAATGATGGCCACACG-3′

ArtExon2KI-LR: 5′-GACGCTCTTCTGAGTCTTTCTTCTGCAAGTGAAGAATCCTCC-3′

ArtExon2KI-RF: 5′-GACGCTCTTCGTCAGCTCATAGCCCATGTGAATCAATTC-3′

ArtExon2KI-RIntR: 5′-GACGCTCTTCTTTTAGAATTACCTGCACTCCAACCTTCTTTTC-3′

ArtExon2KI-RIntF: 5′-GACGCTCTTCTAAAGGGAACAAAAGCTGGAG-3′

ArtExon2KI-RR: 5′-GACGCTCTTCCATGATCAAGAAGACAGAGTATTGCTATC-3′

ART_Exon2KI-LoxPF: 5′-TCAGCTCATAGCCCATGTGAATCAATTCTTATAACTTCGTATAGCA-3′

ART_Exon2KI-LoxPR: 5′-GTATGCTATACGAAGTTATAAGAATTGATTCACATGGCTATGAGC-3′

ART_Exon2KI-LoxP_RF: 5′-GACGCTCTTCCTACATTATACGAAGTTATAAGCAAACCTCATGGGTTTTCTC-3′

All recombinant adeno-associated viral (rAAV)-locus specific targeting viruses were generated using a triple transfection strategy in which the targeting vector (8 μg) was mixed with pAAV-RC and pAAV-helper (8 μg each) plasmids and then transfected onto 4 x 10^6 AAV-293 cells using Lipofectamine LTX (Invitrogen). Virus was isolated from the AAV-293 cells 48 hr later by scraping the cells into 1 ml medium followed by

three rounds of freeze/thawing using liquid nitrogen. Targeting vectors used for this study include rAAV-Artemis exon 2 Neo, rAAV-Artemis exon 2 Puro, rAAV-Artemis exon 2 KI SEPT-Neo, rAAV-HPRT-Exon 3 Neo, and rAAV-CCR5 exon 3 Neo.

HCT116 cells were grown to ~70 to 80% confluency on 6-well tissue culture plates. Fresh medium (1 ml) was added at least 30 min prior to the addition of virus. At that time, the required amount of virus was added drop-wise to the plates. The cells and virus were allowed to incubate for 2 hr before adding back more medium (3 ml). The infected cells were allowed to grow for 2 days before they were trypsinized and plated at 2,000 cells per well onto 96-well plates.

Genomic DNA for PCR was isolated using a PureGene DNA Purification Kit (Qiagen). Cells were harvested from confluent wells of a 24-well tissue culture plate. DNA was resuspended in 50 µl 10 mM Tris, pH 8.0, 1 mM EDTA, 2 µl of which was used for each PCR reaction. For Artemis exon $2^{+/-}$ targeting events, a control PCR was performed for the 3′-side of the targeted locus using the primer set RArmF (5′-CGCCCTATAGTGAGTCGTATTAC-3′) and Art2R while correct targeting was determined using RArmF and Art2R1 (5′-GTCACAGGTGACCAAAAAAAATTACTG-3′). For the second round of targeting, a control PCR for the 5′ side of the targeted locus was performed using the primer pair PuroR (5′-GGCTTGTACTCGGTCATTGCTCAGC-3′) and Art2F-1 (5′-GAGCCACCATGTCCAACTGGTTTAG-3′) while correct targeting was determined by using Art2EF2 (5′-GGTGCTCGTGGTAATTAGAATTGG-3′) and PuroR. Artemis null status was determined by performing PCR using exon 2 flanking primers.

In order to knock out Artemis in HCT116 cells, rAAV-mediated gene targeting was performed using the rAAV-Artemis-Exon2-Neo virus to remove exon 2 of the Artemis gene. Three positive clones (#34, #48 and #168) were identified from 167 first round clones resulting in a relative gene targeting frequency of 1.8%. Once the correctly targeted clones ($Artemis^{flox:NEO/+}$) were identified, the floxed neomycin selection cassette was removed from clones #34 and #48 using Cre recombinase. Briefly, the cells were transfected with the PML-Cre plasmid using Lipofectamine LTX after which they were plated at limited dilutions onto 10 cm dishes and allowed to form colonies. Altogether, a total of four G418-sensitive clones were recovered from each first round targeted clone. A G418-sensitive sub-clone ($Artemis^{-/+}$) from clone 34 (34.16) was then subjected to a second round of targeting using the rAAV-Artemis-Exon2-Neo virus. Thirteen clones from a total of 8 X 96-well plates were identified as being correctly targeted for a targeting frequency of 1.6%. Another round of Cre treatment was then performed on one of these clones 2-12H1 to remove the second neomycin drug selection cassette. These clones were putatively Artemis-null, however, upon further examination it was determined that exon two of Artemis still remained on what was thought to be a third allele. Consequently, a third round of gene targeting was then performed on a neomycin sensitive sub-clone 2-12H1 Cre1 using a rAAV-Artemis-Exon2-Puro virus. Four correctly targeted clones of 90 were identified resulting in a relative gene targeting frequency of 4%. Two of these clones, 18.1 and a Cre-treated sub-clone of 15.1 Cre1, were used for the subsequent characterizations described in this study. It was later discovered that the first round targeted Cre-treated clone 34.16 was actually a randomly targeted clone, therefore, both Artemis-null clones (15.1 and 18.1) each contain a randomly-integrated Cre'd Artemis vector

within their genome.

Complementation of the Artemis$^{-/-}$ 15.1 Cre1 cell line was accomplished using the rAAV-Artemis-SEPT-Rox-Neo virus as described. A total of 2 out of 12 clones were confirmed to be correctly targeted and had incorporated exon 2 of Artemis resulting in a relative targeting frequency of 16.7%.

TDP1 Knockdown:

For the expression of a TDP1 shRNA, the pLSLP lentiviral vector, a distant relative of the pLV vector containing an RNA polymerase III-driven H1 RNA promoter controlling the expression of a small hairpin RNA (shRNA) transcript and harboring the puromycin N-acetyl transferase (pac) gene conferring resistance to puromycin was used (19). Phosphorylated oligonucleotides with sequences 5′-GATCCGGTGATAAGCGAGAGGCTAACTTCGTGTCATTAGCCTCTCGCTTATCACTTTTTG-3′ and 3′-GCCAGTATTCGCTCTCCGATTGAAGGACAGTAATCGGAGAGCGAATAGTGAAAAACTTAA-5′ were annealed at a 1:1 ratio in annealing buffer (10 mM Tris, pH 7.5, 50 mM NaCl, 1 mM EDTA) by heating the mixture at 95°C for 5 min followed by cooling of the mixture at a rate of 1°C/ min and stored at 4°C. The lentiviral pLSLPw backbone construct was restriction enzyme digested using BamHI and EcoRI, dephosphorylated and gel purified. The annealed oligomers were ligated in the digested lentiviral construct. This vector expresses a hairpin that targets the sequence GUGAUAAGCGAGAGGCUA (bases 20300-20319 in exon 6 of the TDP1 gene, GenBank #NG009164). shTDP1 lentiviral constructs were transfected into HEK293T cells along with packaging plasmids pLP1 and pLP-VSVG using a calcium phosphate method. Supernatant containing packaged lentiviral particles was collected, centrifuged at 1200 RPM at room temperature (RT). The viral supernatant was then collected and stored in 1 mL aliquots at -80°C.

HCT116 WT and HCT116 Artemis$^{-/-}$ cells were seeded at 75% confluency in 6-well plates and incubated for 24 hr. The medium was aspirated, cells washed with PBS, incubated with 1 mL of the lentiviral stock in the presence of 4 µg/mL polybrene overnight on a rocker at 37°C in 5% CO_2. The viral supernatant was removed and cells were fed with fresh medium containing 0.8 µg/mL puromycin and selected for 4 days. Cells from each genotype were expanded under selection and cryogenic stocks were stored. Genomic DNA was extracted from a fraction of selected cells using QIAGEN DNeasy Blood and Tissue kit and DNA concentration was measured. 1 µg genomic DNA was used as a template in a 50 µL PCR reaction for the amplification of the puromycin resistance gene using forward primer: 5'-CGAGTACAAGCCCACGGT-3' and reverse primer: 5'- AGACCCTTGCCCTGGTG-3' (synthesised by IDT) and analyzed on a 1% Agarose gel. Using dilution cloning, 5-25 cells from the derivative cell lines were seeded in 15 cm dishes and allowed to form colonies. Single cell clones were harvested using cloning towers and expanded under selection. The selected single-cell clones were analyzed for knockdown efficiency using a TDP1 activity assay.

TDP1 Activity Assay

Cells (2 X 10^6) from each derivative cell line were collected using trypsinization and centrifuged at 1200 RPM for 5 min at room temperature (RT). The cell pellet was washed once in 1X PBS and treated with lysis buffer (10 mM HEPES at pH 7.8, 60 mM KCl, 1 mM EDTA, 0.5% NP-40) in the presence of 2 mM

serine protease inhibitor phenylmethanesulfonyl fluoride (PMSF), 1 mM $NaVO_4$, 1 µg/mL leupeptin, 1 µg/mL aprotinin and 1 µg/mL pepstatin, vortexed until the pellet was disrupted, incubated on ice for 10 min and centrifuged at 13000 RPM for 5 min at 4°C. The supernatant ("cell extract") was collected and serially diluted in dilution buffer (50 mM Tris at pH 8.0, 5 nM DTT, 100 mM NaCl, 5 mM EDTA, 10% glycerol, 500 µg/mL BSA). Serial dilutions of 1 µL of the extract was incubated with 100 attomoles of an 18-base 5′-Cy5 labelled 3′-phosphotyrosyl oligonucleotide with sequence TCCGTTGAAGCCTGCTTT (18Y) (Midland Certified Reagents Midland, TX) in 1X reaction buffer (60 mM KOAc, 10 mM MgOAc, 50 mM triethanolamine-HAc pH 7.5, 2 mM ATP, 1 mM DTT) in total 5 µL reaction volume and incubated at 37°C for 1 hr, denatured at 95°C for 5 min and separated on 20% denaturing polyacrylamide sequencing gels by electrophoresis for around 4 hr at 42 V/cm. Gels were then imaged on a Typhoon 9410 Variable Mode Imager (GE Healthcare) in Fluorescence Acquisition mode with a Cy5 Emission filter using a Red (633 nM) laser at PMT of 800 V and analyzed on ImageQuant 5.1 software.

Flourescence In Situ Hybridization:

Cells were seeded in 6 cm dishes and allowed to grow to ~50-60% confluency. Cells were treated with 1mM caffeine followed by 2 nM NCS treatment for 6 hours. 4 hours into NCS treatment, cells were treated with 1 µg/mL colchicine for 2 hr. After 6 hr NCS treatment, cells were collected by trypsinization, washed with PBS and swollen in 75 mM KCl for 10 min at 37°C, then centrifuged and fixed with ice-cold Carnoy's fixative (3:1 methanol:glacial acetic acid) for 10 min. Samples were centrifuged and washed twice with methanol/acetic acid. Cells were dropped onto ethanol-cleaned cold slides and dried overnight. Slides were dehydrated by immersing for 2 min each in 70%, 90% and 100% ethanol followed by baking at 65°C for 10 min, washing in acetone for 10 min and air drying. Slides were treated with 100 µg/mL RNase A in 2XSSC under a parafilm coverslip for 30 min at 37°C then washed for 5 min in 2XSSC and for 10 min in PBS, dehydrated in an ethanol series and allowed to dry. Chromosomes were denatured by immersing in 70% formamide / 2XSSC (pH =7) for 2 min at 75°C and then in ice-cold 70% ethanol for 2 min. Slides were again dehydrated in an ethanol series, dried and then 20 µL of 200 nM Cy3-labelled PNA CENP-B probe (PNA Bio – F3002) in hybridization buffer (20 mM Tris, pH 7.4, 60% formamide, 0.1 µg/mL salmon sperm DNA) was added. Slides were covered with a 24 mm X 50 mm coverslip and allowed to hybridize for 2 hr at 37°C in dark. Slides were then washed in 2XSSC for 5 min at 37°C, three changes of 0.5×SSC / 0.3% NP-40 for 10 min at 55°C, two changes of 2XSSC / 0.1% NP-40 for 5 min at 22°C, and finally in 2XSSC for 5 min at 22°C. The washed slides were dried and counterstained with Vectashield mounting medium containing DAPI (Vector Laboratories) under a coverslip and was sealed with nailpolish. Metaphases were then imaged using a Zeiss LSM700 Confocal Microscope as described. All washes were performed in 50-mL glass Coplin jars.

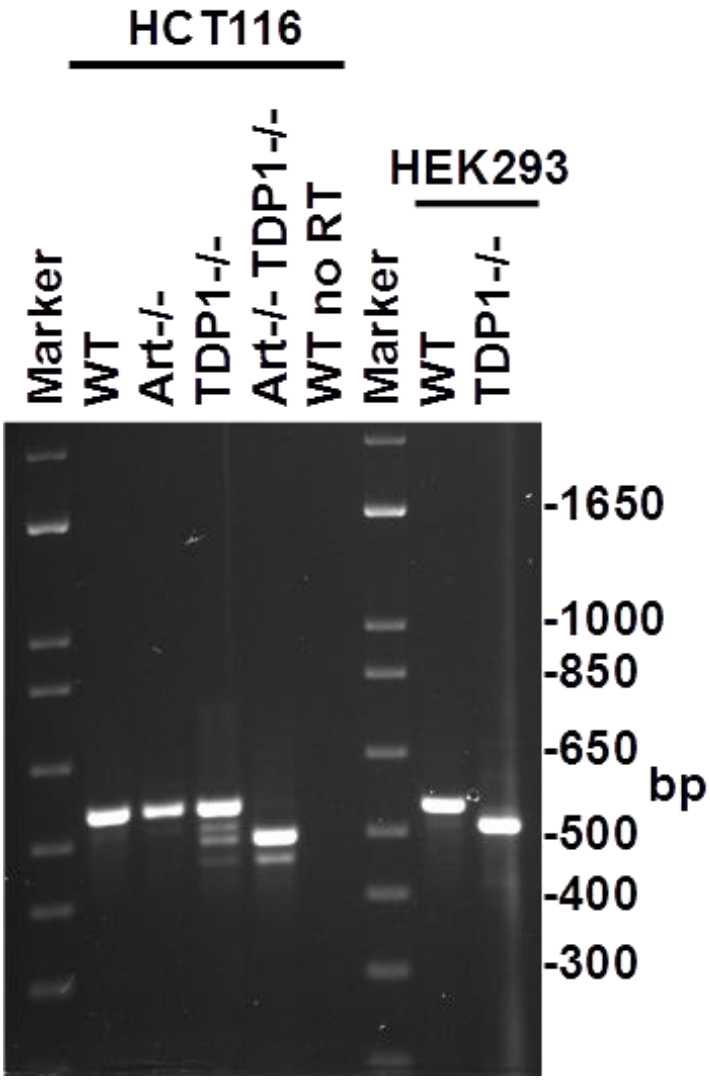
SUPPLEMENTARY FIGURES:

Supplementary Figure 1A. Sequence of CRISPR cassette for disruption of the TDP1 active site. The targeted sequence (bases 24880-24899 in exon 7 of the genomic TDP1 sequence NG_009164, corresponding to bases 757-776 of the TDP1 cDNA NM_018319.3) is shown in bolded lower case and the U6 promoter is underlined. The cassette was synthesized as a double-stranded gBlock from Integrated DNA Technologies and cloned into the SmaI site of pUC19 for transfection and expression.

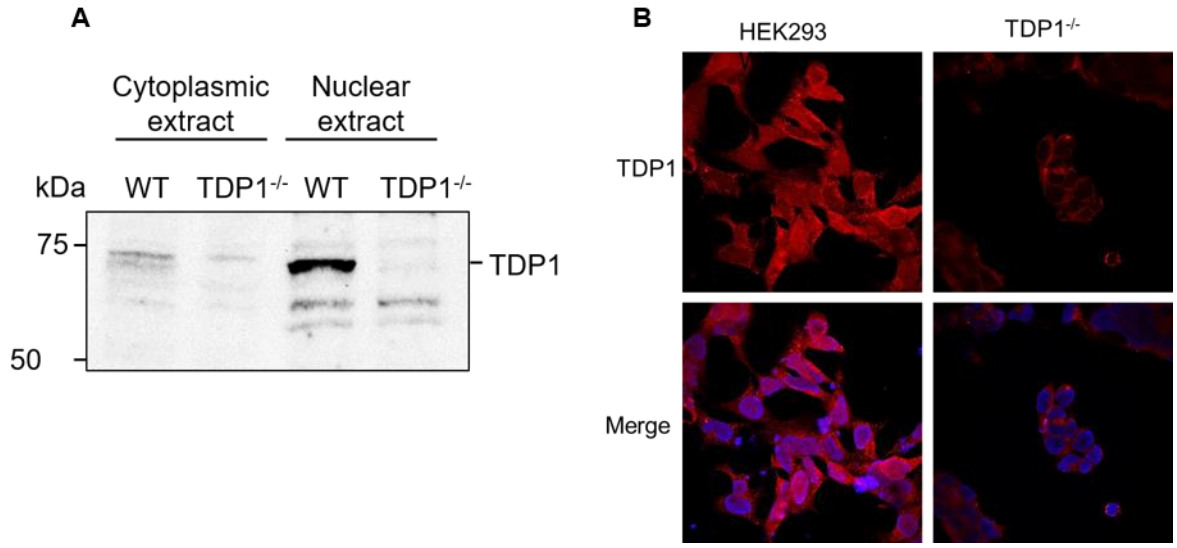
Length 457
Scale: 500ng
Purification: standard desalting
Modifications: 5′ phosphate
SEQUENCE:

TGTACAAAAAAGCAGGCTTTAAAGGAACCAATTCAGTCGACTG
GATCCGGTACCAAGGTCGGGCAGGAAGAGGGCCTATTTCCCAT
GATTCCTTCATATTTGCATATACGATACAAGGCTGTTAGAGAGA
TAATTAGAATTAATTTGACTGTAAACACAAAGATATTAGTACAA
AATACGTGACGTAGAAAGTAATAATTTCTTGGGTAGTTTGCAGT
TTTAAAATTATGTTTTAAAATGGACTATCATATGCTTACCGTAAC
TTGAAAGTATTTCGATTTCTTGGCTTTATATATCTTGTGGAA
AGGACGAAACACCG**gcaaagttggatattgcgtt**GTTTTAGAGCTAGAA
ATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAA
AAGTGGCACCGAGTCGGTGCTTTTTTTCTAGACCCAGCTTTC
TTGTACAAAGTTGGCATTAT

Supplementary Figure 1B. RT-PCR analysis of mRNA from TDP1$^{-/-}$ knockouts. Total cell RNA was isolated from ~2 million cells using an RNAeasy kit (Qiagen) and cDNA was prepared from 2 µg RNA using an Omniscript RT kit (Qiagen) and a dT_{15} primer in a 20 µL incubation. A 1.5 µL aliquot was used as template for a 30-µL PCR containing Roche FastStart Master Mix and the primers ACGGCACTGCCCAAAGAACTGAAA and TCGTGGGTATAAGGGGCTCA (1 µM), which amplify a 551-bp segment of cDNA encompassing TDP1 exons 4-8 and part of exons 3 and 9 (bases 661-1211 of the TDP1 mRNA sequence NM_018319.3). The PCR profile consisted of 5 min at 94°C followed by 35 cycles of 30 s at 94°C, 30 s at 61° and 1 min at 72°. Analysis of a 6-µL aliquot of each reaction by electrophoresis on a 1.3% agarose gel indicated a ~50-base deletion in TDP1 mRNA from HCT116 Arte$^{-/-}$ • TDP1$^{-/-}$ cells and a ~30-base deletion in mRNA from HEK293 TDP1$^{-/-}$ cells, with a complete lack of normal-length message, consistent with the complete lack of tyrosyl-DNA phosphodiesterase activity in both cell lines. The previously described HCT116 TDP1$^{-/-}$ cell line (Ref. 18) did not show a detectable change in amplicon size, but was completely devoid of phosphodiesterase activity and presumably harbored a smaller inactivating mutation.

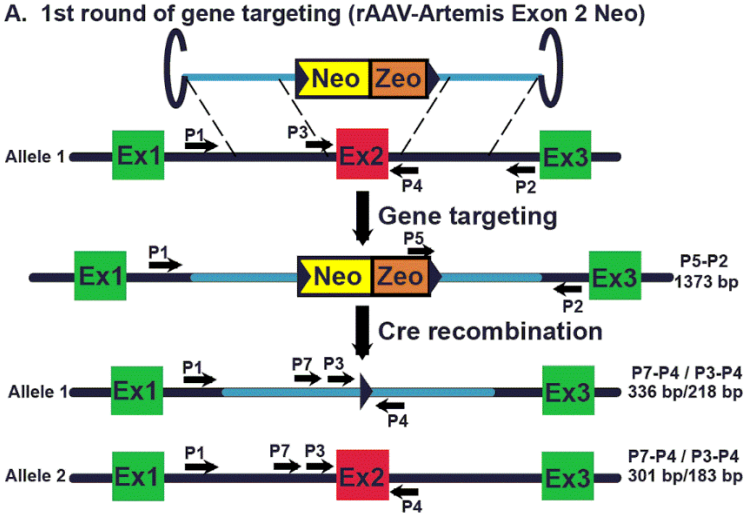


Supplementary Figure 2. (A) Western blot depicting loss of TDP1 protein from the nuclear extracts of HEK293 TDP1$^{-/-}$ cells. (B) Loss of TDP1 in HEK293 TDP1$^{-/-}$ cells as observed from immunolabelling for TDP1 followed by confocal microscopy. Red fluorescence: TDP1 (1:1000, 1° anti-TDP1 Abnova, 2° anti-mouse CFL594, SCBT). Blue fluorescence: DAPI.

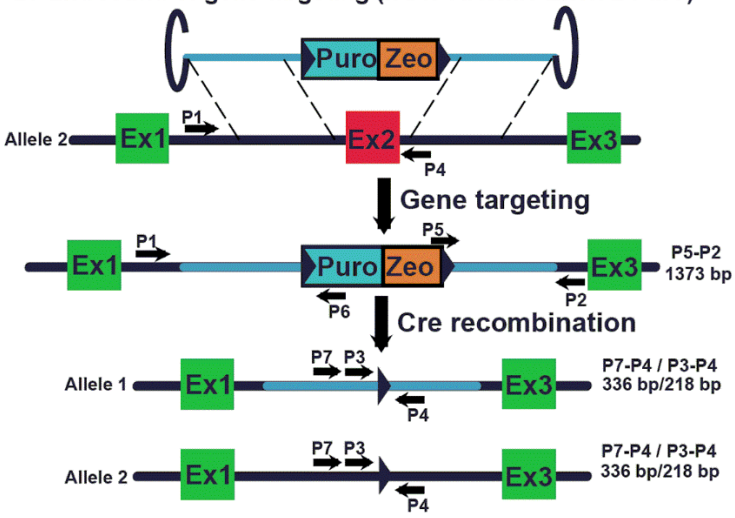


Supplementary Figure 3: Generation of Artemis$^{+/-}$, Artemis$^{-/-}$, and complemented Artemis$^{-/-}$ 116 cell lines. (A) Gene targeting strategy using a rAAV-Artemis exon 2 Neo/Zeo virus to knockout one allele of the Artemis genomic locus in the HCT116 cell line during the first round of gene targeting. Exons are shown (not to scale) as green colored rectangles with the exon to be deleted (exon 2) in red. The rAAV-Artemis exon 2 Neo/Zeo virus is shown above with sequences homologous to Artemis 5′ and 3′ to exon 2 shown in blue with LoxP sites represented as black triangles. The neomycin-resistance (Neo) gene is highlighted as a yellow rectangle while the Zeomycin (Zeo) gene is highlighted as an orange rectangle. The direction and approximate location of vector-specific and gene-specific PCR primers are shown as black horizontal arrows. All steps performed before a second round of targeting are depicted including Cre recombination to remove the neomycin drug selection cassette. Primer pairs and predicted amplicon sizes used for confirmation of modified cell lines for panels (D, E and F), are shown to the right of the two resulting alleles. (B) A diagram representing the rAAV-mediated gene targeting strategy using a rAAV-Artemis exon 2 Puro/Zeo virus to knockout the second allele of Artemis in the HCT116 cell line during a second round of gene targeting. All symbols are the same as (A) except that the Puro gene is represented as a blue rectangle. (C) A diagram representing the strategy to complement Artemis exon 2 null cells by using an rAAV-Artemis exon 2 knockin (KI) Dre-SEPT-Neo virus to reintroduce Artemis exon 2 back onto the chromosome. All symbols are the same as (A and B) except that the Dre recombination sites are represented as red triangles. (D) PCR confirmation of Artemis$^{+/-}$, Artemis$^{-/-}$, and Artemis$^{-/KI\text{-}Neo}$ complemented cell lines. A confirmation PCR was performed using genomic DNA isolated from parental HCT116 (WT), along with Artemis$^{+/+}$ randomly targeted (#34.16), Artemis$^{+/-}$ (#2-12H1), Artemis$^{Puro/-}$ (#18.1), Artemis $^{-/-}$ (#15.1), and two Artemis$^{-/KI\text{-}Neo}$ complemented clones before (#12 and #16) and after (#12-20 and #16-15) Dre recombination to remove the SEPT-Neo drug selection cassette to restore Artemis expression. The primer pair P7 and P4 was used. (E) As in (D) except that the primer pair P3 and P4 sequence were used. (F) As in (D) except that the primer pair P5 and P2 were used to confirm correct targeting. (G) Artemis$^{-/-}$ cells lack detectable Artemis protein. Whole cell extracts, prepared from parental (WT) as well as all cell lines listed in (D), were analyzed by immunoblot for Artemis protein expression.

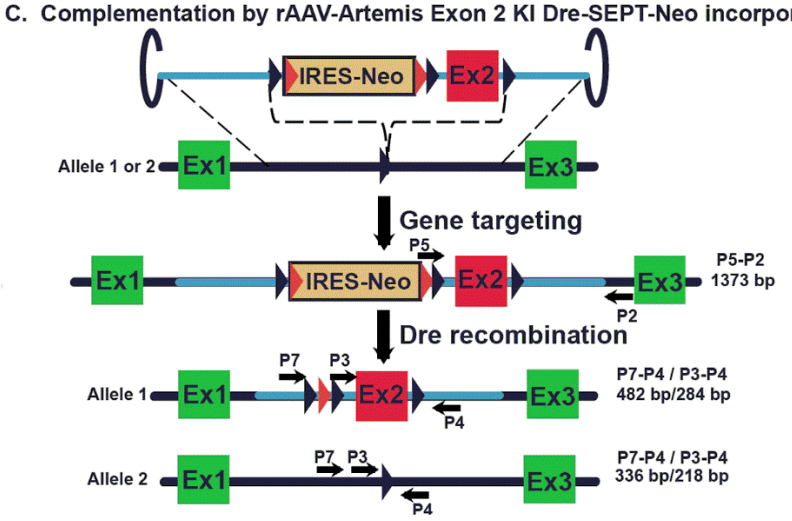
A. 1st round of gene targeting (rAAV-Artemis Exon 2 Neo)

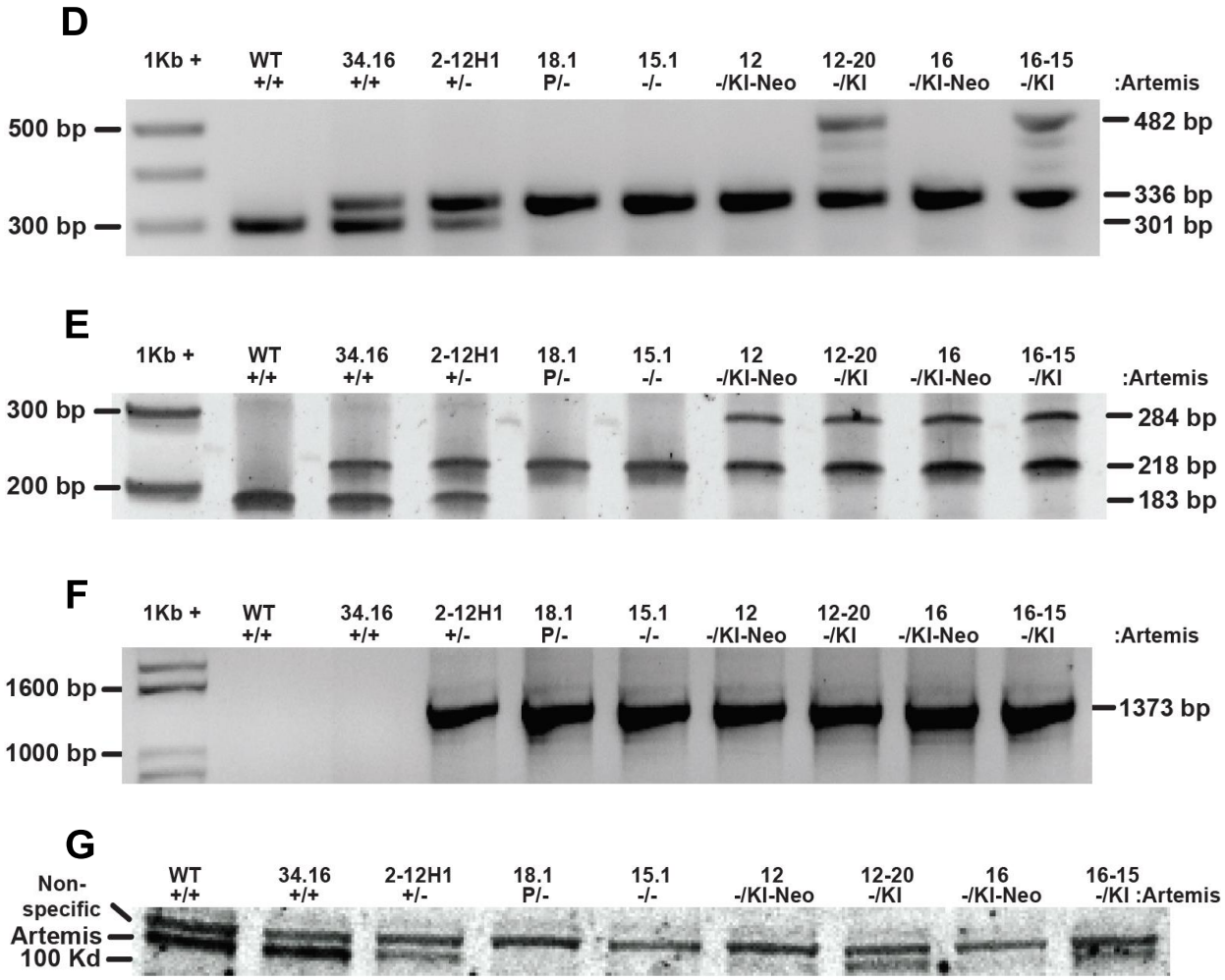


B. 2nd round of gene targeting (rAAV-Artemis Exon 2 Puro)

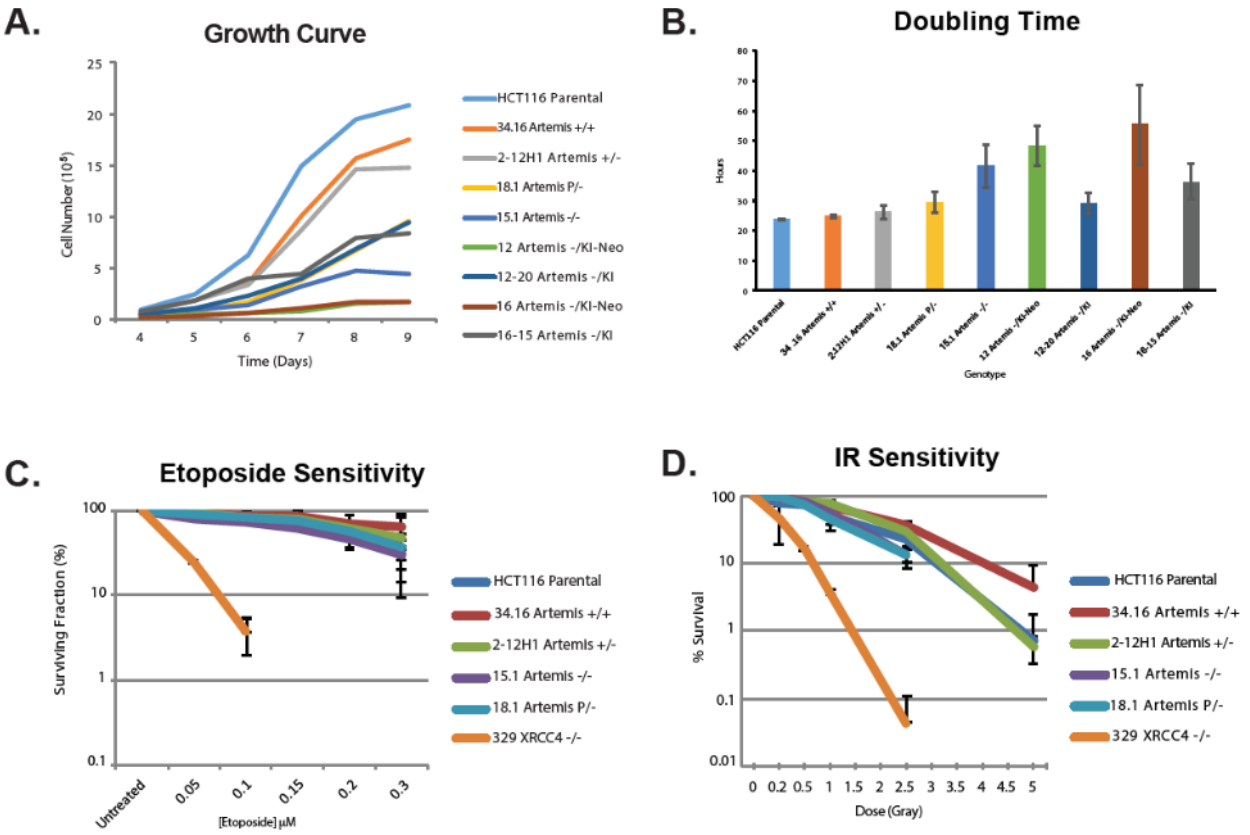


C. Complementation by rAAV-Artemis Exon 2 KI Dre-SEPT-Neo incorporation




D
1Kb +
WT +/+
34.16 +/+
2-12H1 +/-
18.1 P/-
15.1 -/-
12 -/KI-Neo
12-20 -/KI
16 -/KI-Neo
16-15 -/KI
:Artemis
500 bp
300 bp
482 bp
336 bp
301 bp
E
1Kb +
WT +/+
34.16 +/+
2-12H1 +/-
18.1 P/-
15.1 -/-
12 -/KI-Neo
12-20 -/KI
16 -/KI-Neo
16-15 -/KI
:Artemis
300 bp
200 bp
284 bp
218 bp
183 bp
F
1Kb +
WT +/+
34.16 +/+
2-12H1 +/-
18.1 P/-
15.1 -/-
12 -/KI-Neo
12-20 -/KI
16 -/KI-Neo
16-15 -/KI
:Artemis
1600 bp
1000 bp
1373 bp
G
Non-specific
Artemis
100 Kd
WT +/+
34.16 +/+
2-12H1 +/-
18.1 P/-
15.1 -/-
12 -/KI-Neo
12-20 -/KI
16 -/KI-Neo
16-15 -/KI :Artemis

Supplementary Figure 4. Artemis-deficient HCT116 cells exhibit growth defects and are sensitive to DNA damage caused by ionizing radiation. (A) Cell proliferation assay. Either 4,000 parental (WT) #34.16 Artemis$^{+/+}$, #2-12H1 Artemis$^{+/-}$, #18.1 Artemis$^{-/-}$, and #12-20 Artemis$^{-/KI}$, or 8,000 #15.1 Cre1 Artemis$^{-/-}$, #12 Artemis$^{-/KI\text{-}Neo}$, #16 Artemis$^{-/KI\text{-}Neo}$, and #16-15 Artemis$^{-/KI}$ were plated in each well of six-well plates and allowed to grow in growth media without selection for the duration of the experiment. Cell numbers were determined using either a hemocytometer or a Countess cell counter every day in triplicate starting at day 4. The averages of three independent experiments are shown. (B) Doubling time. The average doubling time for each cell line shown in (A) was determined from the endpoint (9 days) cell number compared to the initial (day 0) number of cells plated per 6-well plate. (C) Etoposide sensitivity. For parental, Artemis$^{+/-}$, and Artemis$^{-/-}$ cell lines, 400 cells (while for the XRCC4$^{-/-}$ cell line #329, 4,000 cells) were plated in duplicate and exposed to the indicated levels of etoposide. Surviving cells that grew into colonies of at least 50 cells after ~10 days were scored. The averages (+/- standard deviations) of two experiments are shown. (D) IR sensitivity. For parental, Artemis$^{+/-}$, and Artemis$^{-/-}$ HCT116 cell lines, 400 cells (while for XRCC4$^{-/-}$ cell line #329, 4,000 cells) were plated in duplicate and X-irradiated at the indicated doses. Surviving cells that grew into colonies of at least 50 cells after ~10 days were scored. The averages (+/- standard deviations) of two experiments are shown.

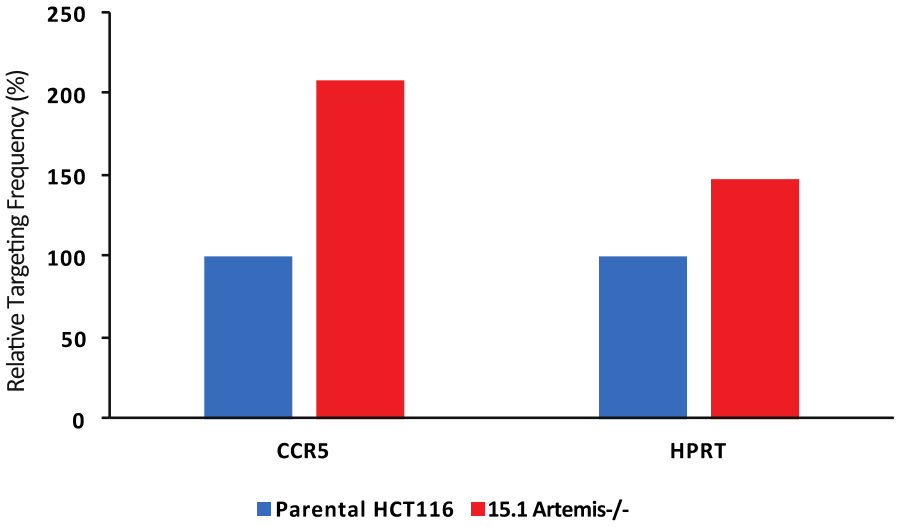


Supplementary Figure 5. **rAAV-mediated gene targeting frequency in Artemis-deficient cells.** (A) A table showing rAAV-mediated gene targeting of CCR5 and HPRT loci for both parental HCT116 and Artemis$^{-/-}$ cell lines. (B) A graph displaying the relative rAAV-mediated gene targeting frequencies observed for each locus for parental HCT116 and Artemis$^{-/-}$ cells.

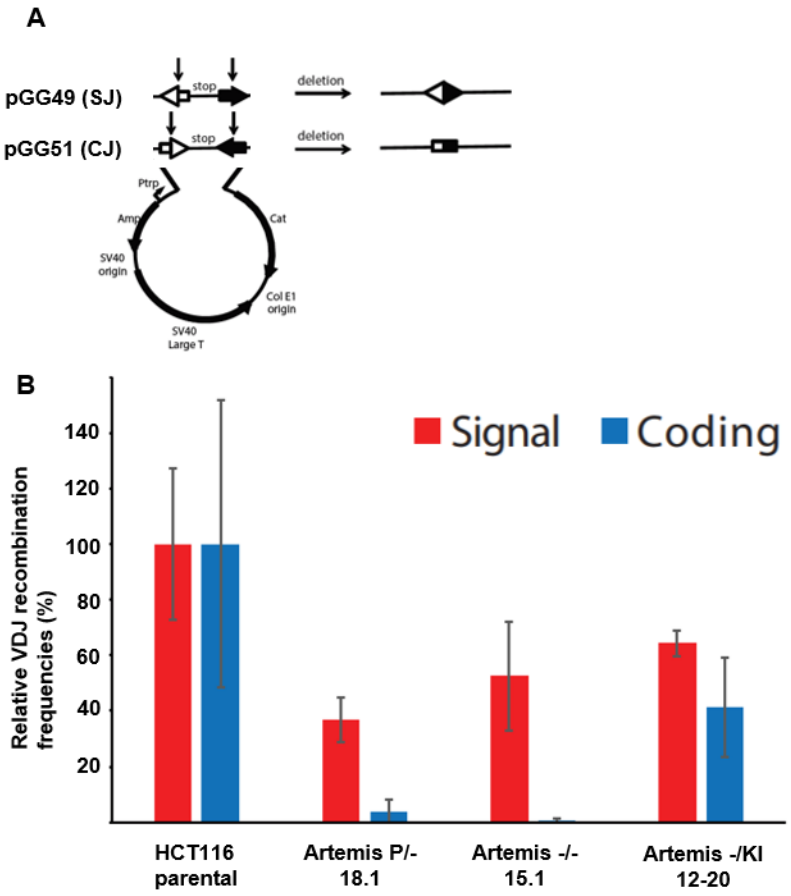
A.

Locus Targeted	Parental	Artemis-/-
CCR5	4/97 = 4.1%	8/93 = 8.6%
HPRT	1/116 = 0.9%	1/79 = 1.3%

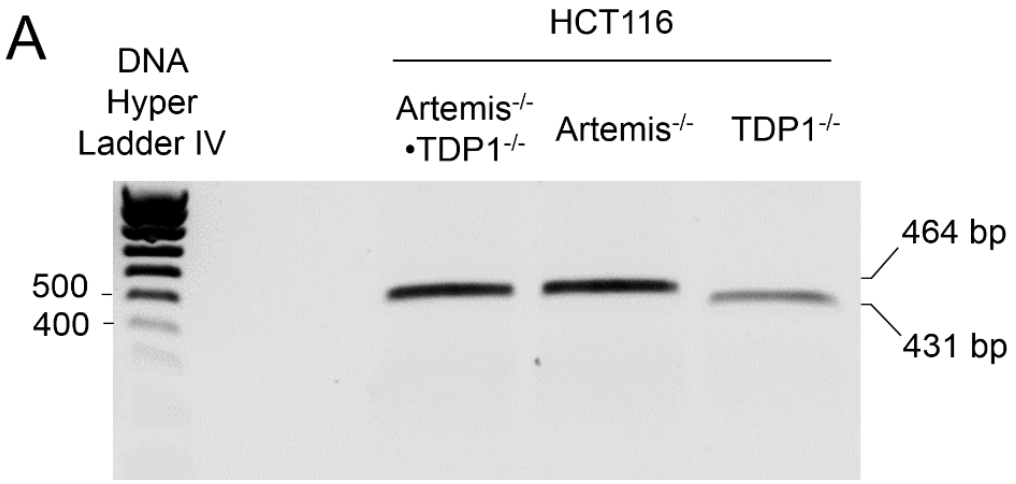
B.



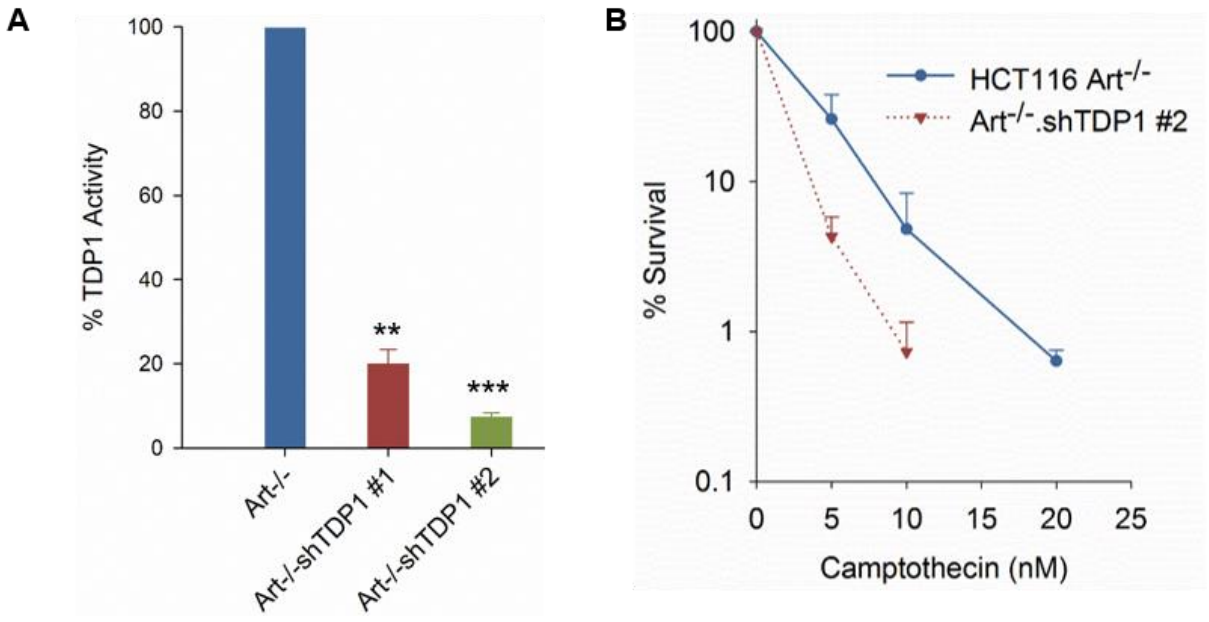
Supplementary Figure 6. HCT116 cells lacking Artemis exhibit reduced levels of V(D)J recombination. (A) A human V(D)J recombination substrate plasmid diagram extirpated from Gauss and Lieber, Mol. Cell. Biol., 1993, 13:3900-3906. Two different plasmids are represented. Each plasmid is a dual drug selection vector that includes an amp (ampicillin resistance) gene as well as a Ptrp (prokaryotic tryptophan promoter) element driving expression of a promoter-less chloramphenicol (cat) resistance gene. Importantly, a λ phage OOP transcription terminator is represented by "stop" in the three cassettes. This terminator is flanked by 12- and 23-bp V(D)J recombination signal sequences (open or closed triangles, respectively) arranged in different orientations for each vector. Transfection of each V(D)J substrate plasmid along with RAG1 and RAG2 expression plasmids into human cells and subsequent active V(D)J recombination will result in the deletion or inversion of the terminator sequence. Plasmids are then recovered from the human cells and propagated in bacteria, where only recombined plasmids confer resistance to both chloramphenicol and ampicillin while non-recombined plasmids confer resistance only to ampicillin. (B) Relative V(D)J recombination frequencies for each cell line were determined from the average of the three individual experiments. Artemis P/-: Artemis knockout with a Puro gene in one allele, Artemis -/KI: Artemis knockout complemented by reintroduction of exon 2. (See Supplementary Materials and Methods, Supplementary Figure 3, and Supplementary Table 1 for details).



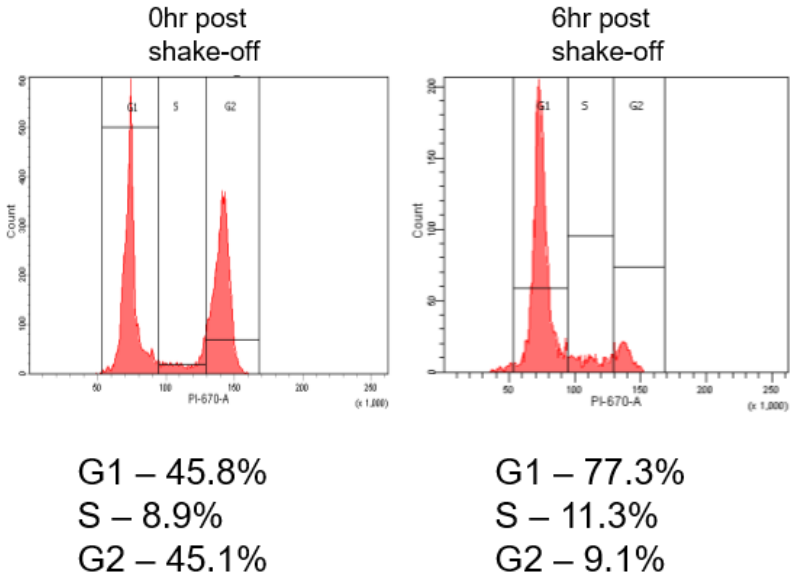
Supplementary Figure 7. Artemis Exon 2 was amplified by PCR and the samples were separated on a 1% Agarose gel. TDP1$^{-/-}$ is used as a control. The Artemis-knockout cells have a larger amplicon due to the insertion of the homology arms in between exons 1 and 3.



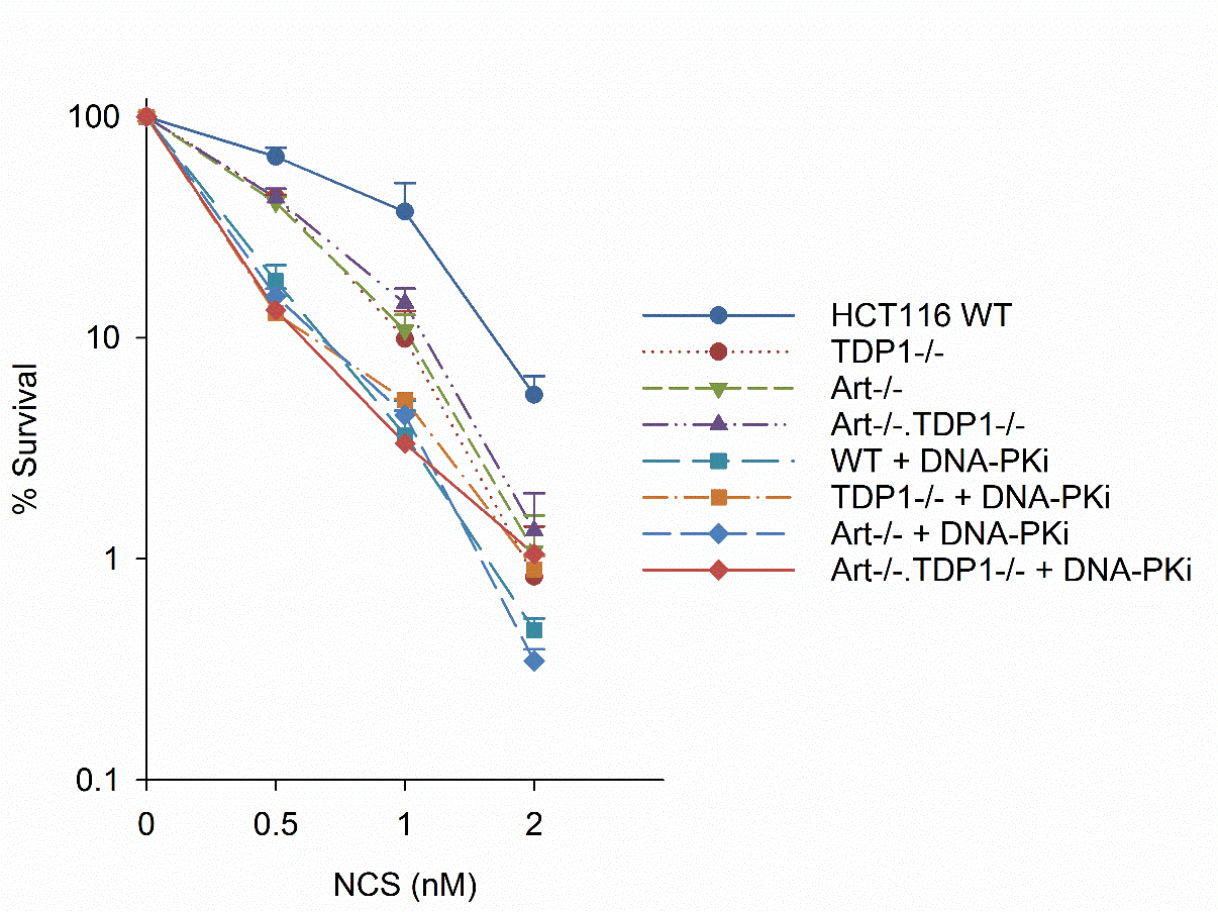
Supplementary Figure 8. (A) Percentage of TDP1 Activity left in while extracts as measured by the conversion of 18-pTyr substrate to 18-p product after shRNA-mediated knockdown of TDP1 in Artemis$^{-/-}$ cells. Error bars represent SEM. n=2. (B) Clonogenic survival assay performed upon camptothecin treatment in Art$^{-/-}$ and Art$^{-/-}$.shTDP1 #2 cells. n=2.



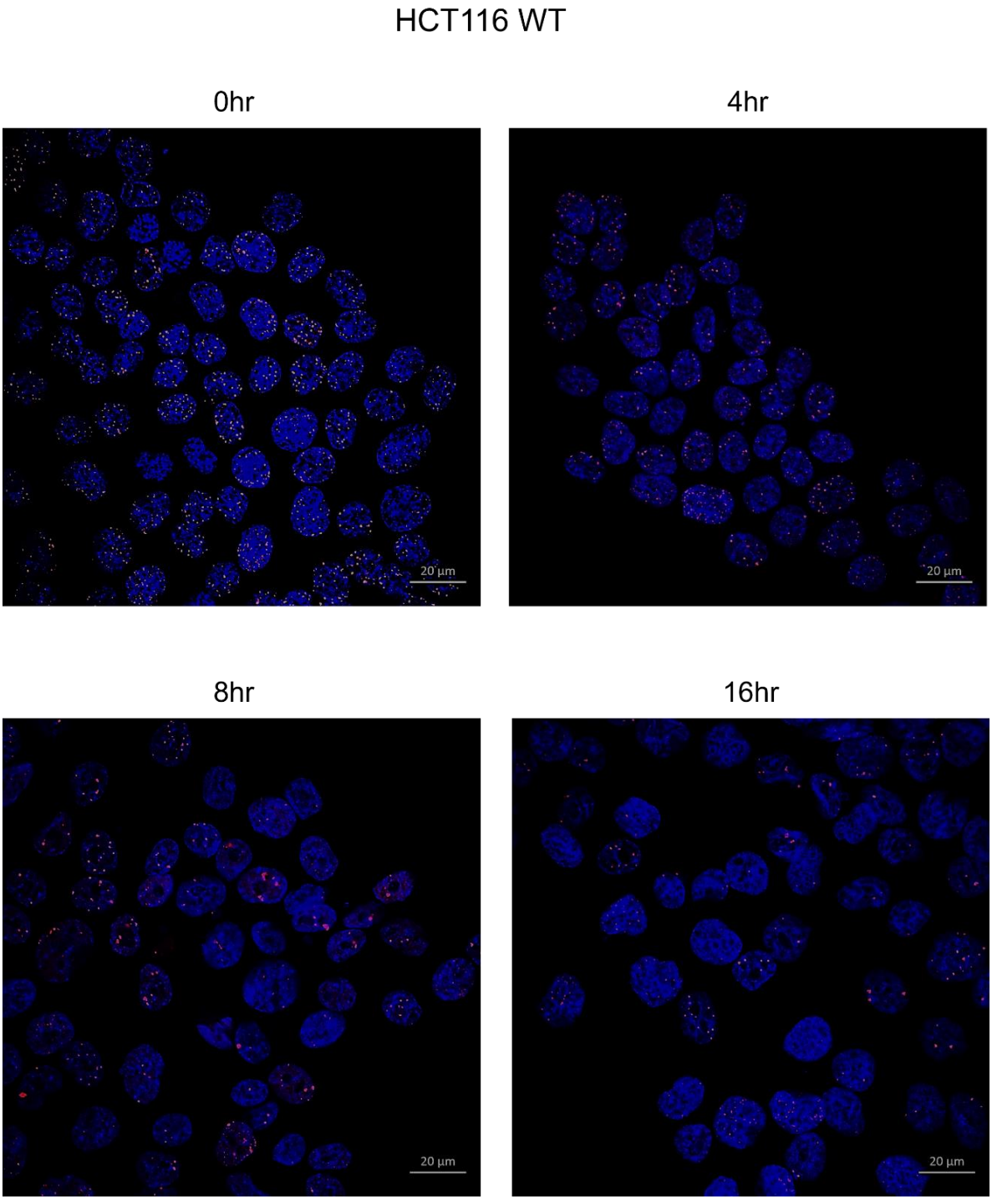
Supplementary Figure 9. Cells were fixed immediately after mitotic shake-off and processed (left panel) or allowed to attach and harvested 6 hr after mitotic shake-off (right panel). Representative FACS histogram plot showing the percentage of cells in each phase of the cell cycle. Horizontal axis depicts the DNA-content. 10,000 events were collected for each sample.



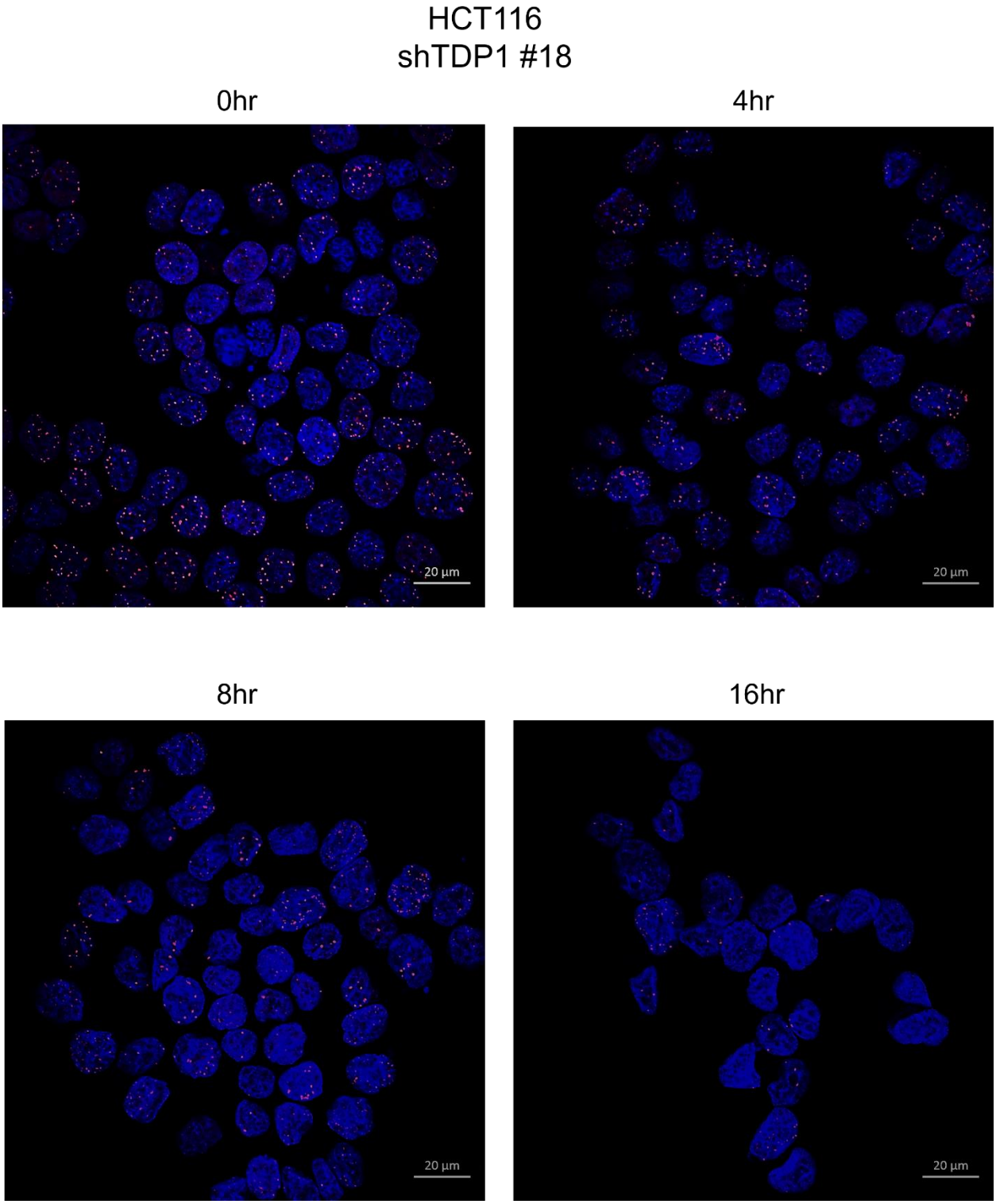
Supplementary Figure 10. Clonogenic Survival assays performed in HCT116 knockout cells (Art-/-, TDP1-/- and Art-/-.TDP1-/-) in the presence and absence of a DNA-PK inhibitor.



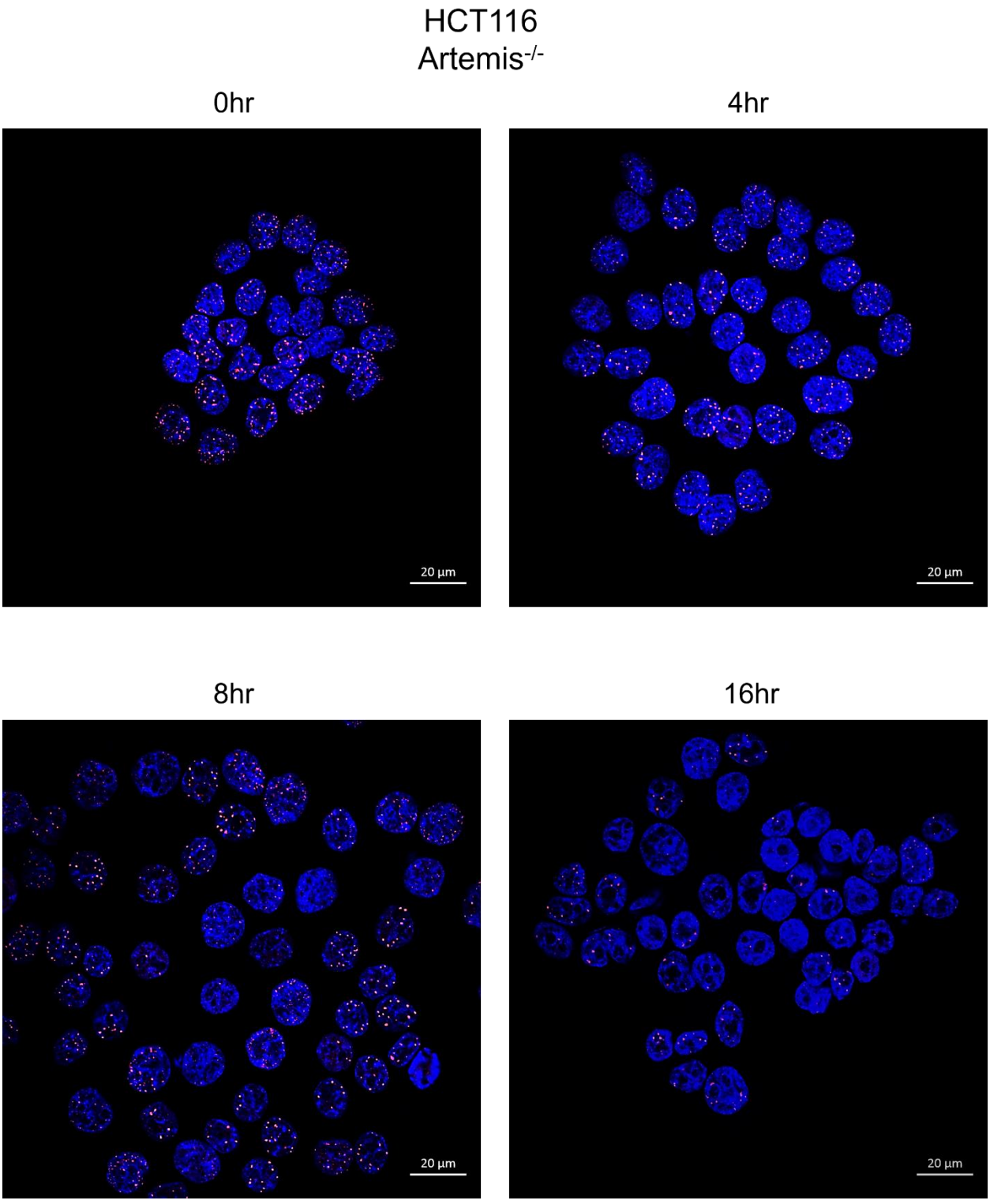
Supplementary Figure 11 (A): Formation and disappearance of 53BP1 foci upon 4nM NCS treatment. Immunofluorescent labelling followed by confocal microscopy was used to obtained images showing representative HCT116 WT (A), shTDP1 #18 (B), Art-/- (C) and Art-/-.shTDP1 (D). Red fluorescence: 53BP1 (BD Transduction Laboratories), blue fluorescence: DAPI (Vectashield).



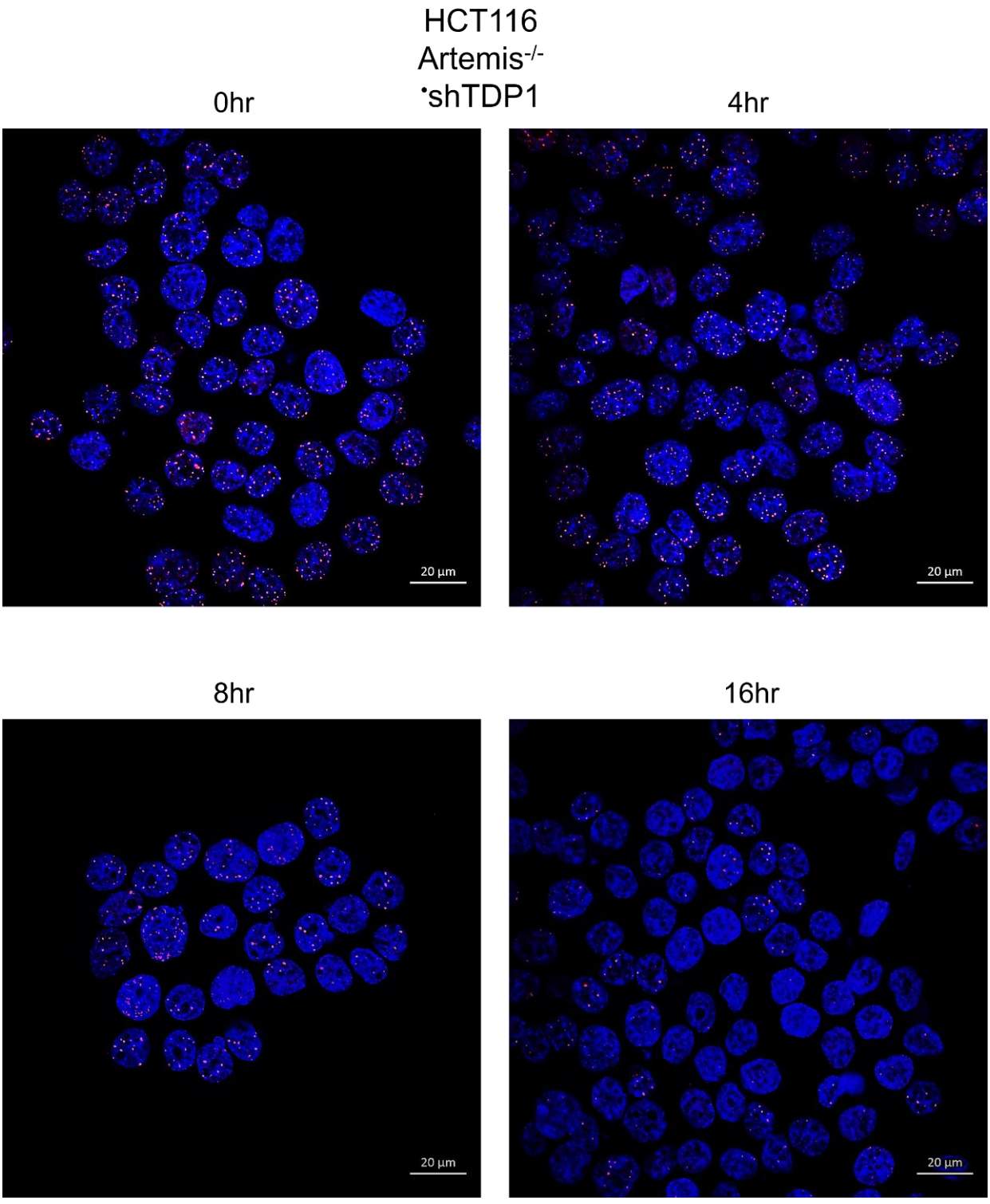
Supplementary Figure 11 (B):



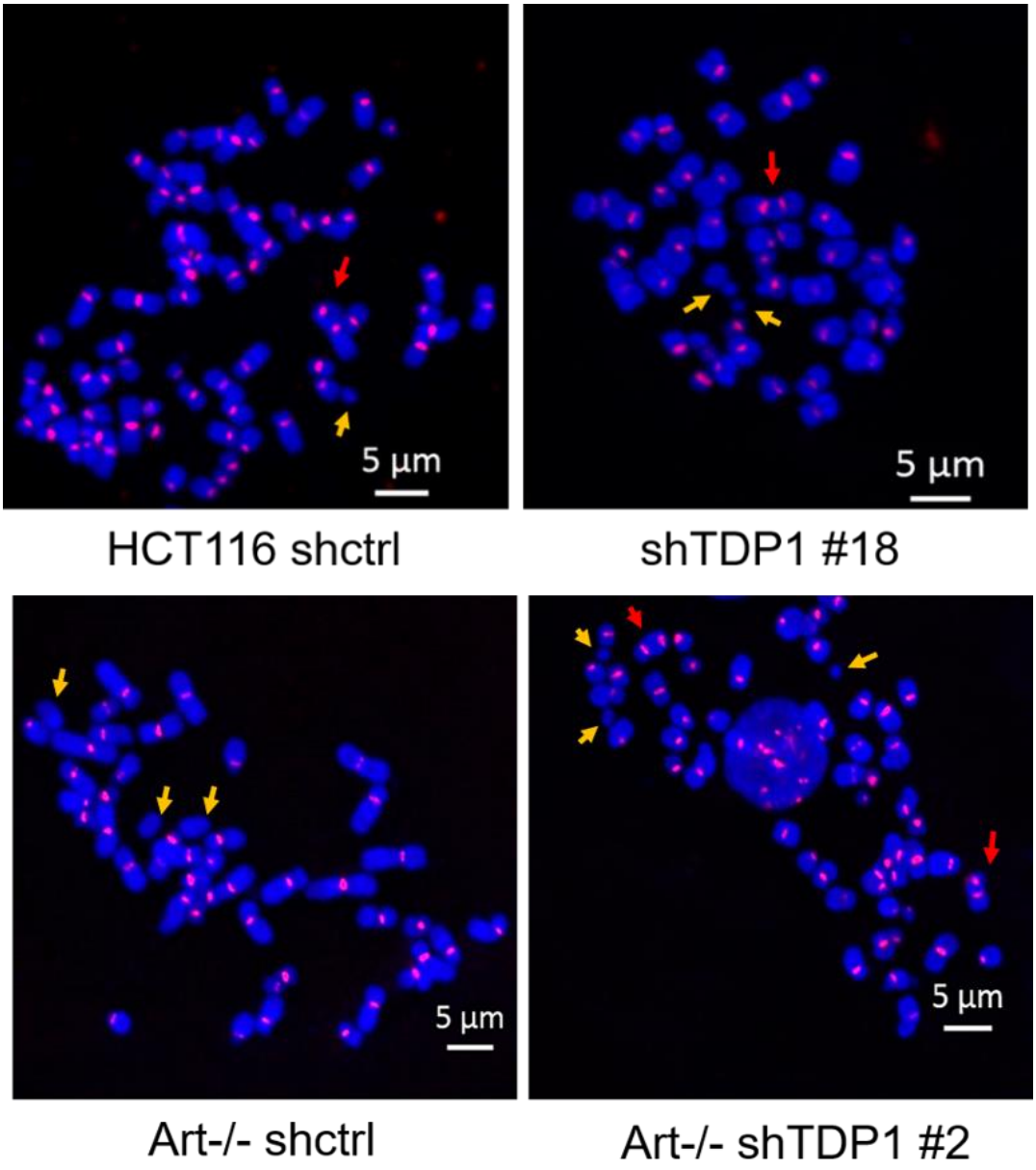
Supplementary Figure 11 (C):



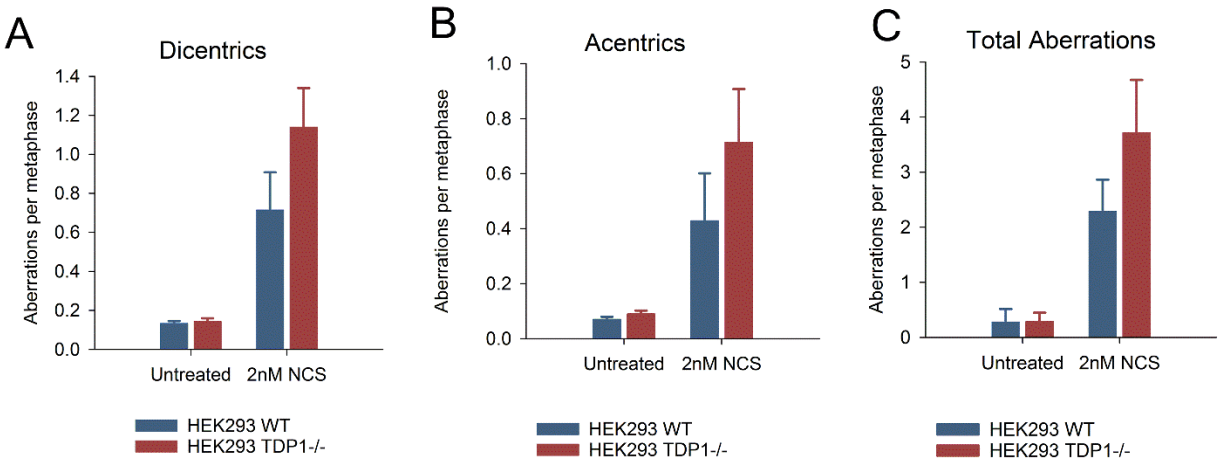
Supplementary Figure 11 (D):



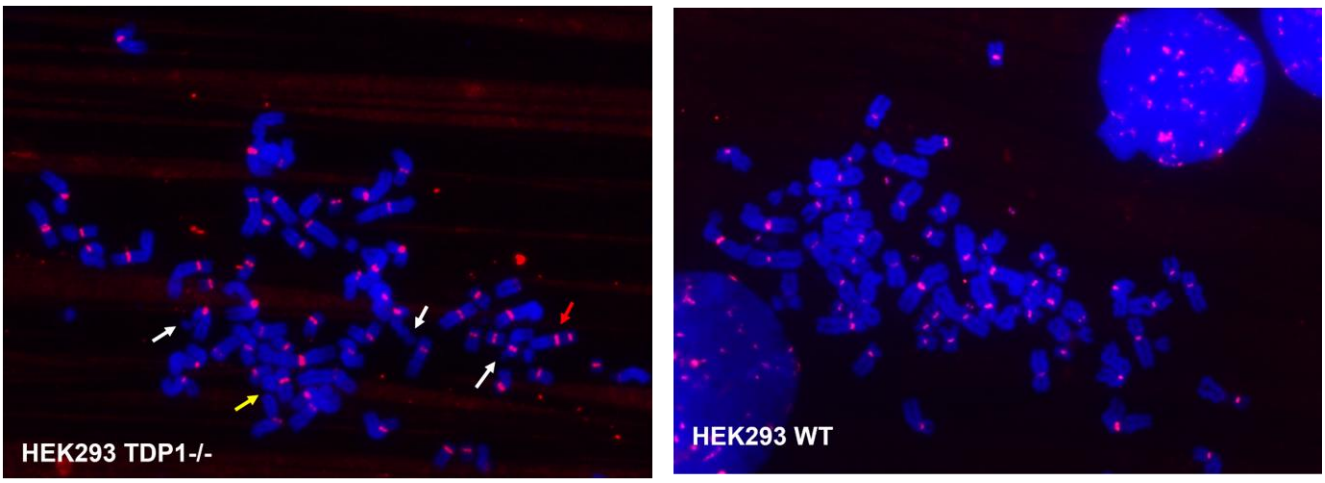
Supplementary Figure 12. Metaphase spreads of HCT116 WT, shTDP1#18, Art-/- and Art-/-.shTDP1 #2 cells. Cells were treated with 2nM NCS for 6 hours. Centromeres were labelled with a Cy3-conjugated fluorescent probe (PNA Bio Inc.). Red arrows represent dicentric chromosomes, yellow arrows represent acentric fragments.



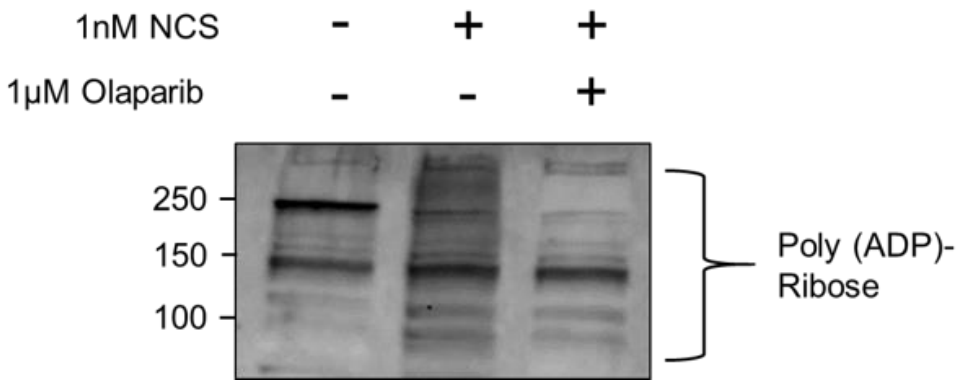
Supplementary Figure 13. HEK293 WT and TDP1-/- cells were treated with 2 nM NCS, harvested in metaphase after 2 hr colchicine (1 µg/mL) treatment and metaphase spreads were prepared followed by hybridization with a fluorescent (Cy3) centromeric probe. A total of approximately 30 metaphases from 2 independent experiments were imaged, scored for the presence of dicentric chromosomes (A), acentric fragments (B) and total aberrations (C) and results plotted as number of aberrations (dicentrics, acentrics or overall aberrations) per metaphase. Total aberrations include acentrics, dicentrics, breaks, gaps, radials, unstructured chromosomal regions. Error bars represent SEM for n=2. (D) Metaphase spreads in HEK293 WT and TDP1-/- cells.



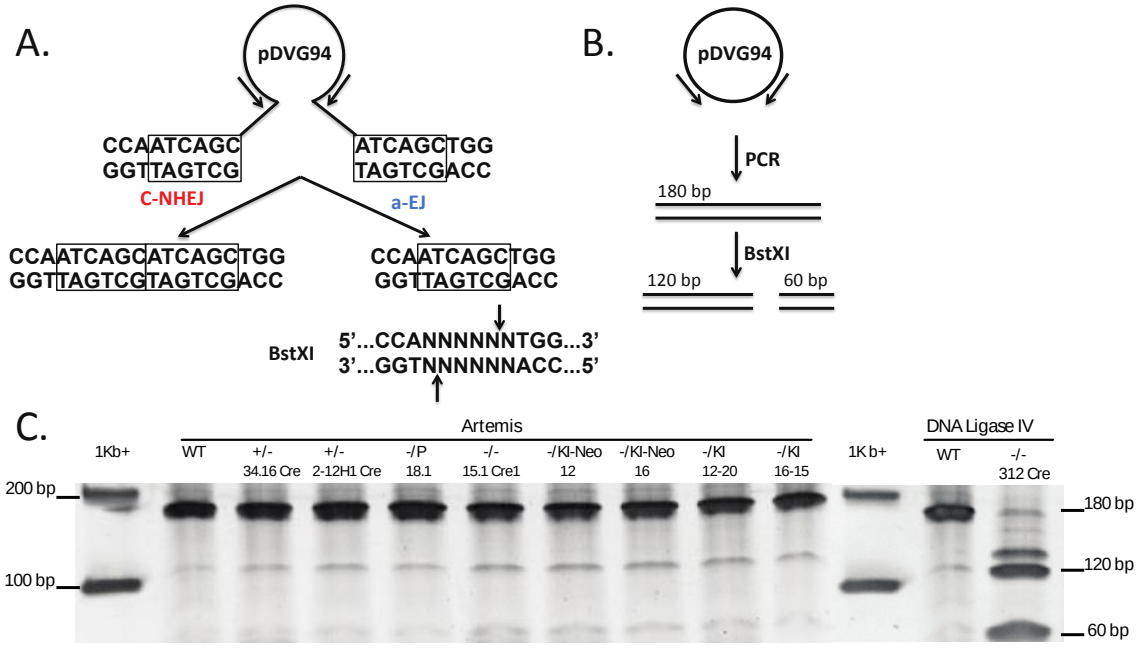
D



Supplementary Figure 14: Cells treated with 1nM NCS in the presence or absence of 1μM Olaparib were harvested, extracts separated on a 10% polyacrylamide gel, transferred onto a nitrocellulose membrane and immunoblotted with Anti Poly(ADP)-Ribose (PAR) antibody (1:10000, BD Pharmingen). NCS-induced poly(ADP-ribosylation) alters the size of target proteins, resulting in detection of a broad smear of PAR-conjugated proteins from 150 to >250 kDa, that is largely eliminated upon PARP inhibition.

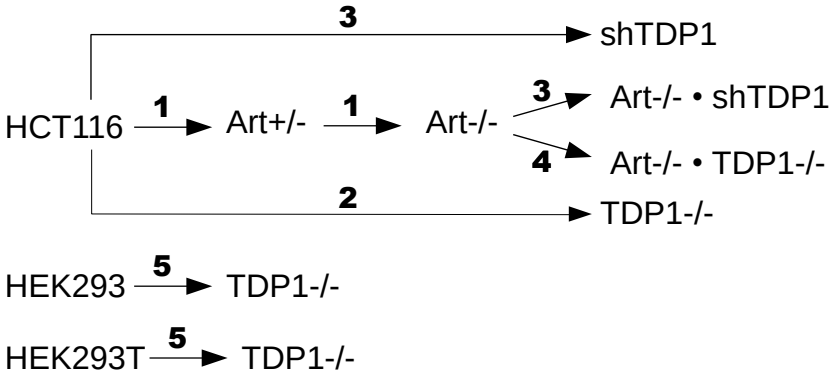


Supplementary Figure 15. **The absence of Artemis in HCT116 cells has no effect on microhomology-mediated end-joining.** (A) A microhomology-directed alternative NHEJ (a-EJ)-biased reporter substrate plasmid that has been engineered so that digestion with AfeI and EcoRV yields a blunt-ended linear substrate with 6-bp repeats (boxes) at each end. Repair of this plasmid by C-NHEJ joining will result in the retention of both repeats while a-EJ should yield a single repeat, which generates a novel BstXI restriction enzyme recognition site. (B) Experimental strategy for analysis of repair events produced in cells transfected with repair plasmid pDVG94. The recovered plasmids were subjected to PCR amplification using primers flanking the repair junction. These 180 bp PCR products were then subjected to BstXI restriction enzyme digestion that will yield both 120 bp and 60 bp products when successfully cleaved before being subjected to polyacrylamide gel electrophoresis and visualized using SYBR gold. (C) The results of one such experiment. DNA Ligase IV$^{-/-}$ cell line (last lane) was used as a positive control for a-EJ events. In Ligase IV-deficient cells, C-NHEJ (180-bp band) is abolished as expected, and joining is dominated by a-EJ (120-bp product). In the various Artemis-deficient cells, the a-EJ band is at least as intense as in WT even though it is still a minor component of overall joining, suggesting that Artemis deficiency does not impair a-EJ.



Supplementary Table 1. Cell lines used in the study.

Cell line	Derivatives	Description	Method
HCT116	WT	Parental cell line	
	shTDP1#18	Single cell clone #18 showing maximum knockdown of TDP1 in HCT116 parental cells	short hairpin RNA via lentiviral infection
	$TDP1^{-/-}$	Knockout of TDP1 showing complete absence of TDP1 activity in HCT116 parental cells	CRISPR
	$Art^{-/-}$	Artemis knockout clone with exon 2 removed from both the alleles of the Artemis gene	Homologous recombination (HR)
	$Art^{P/-}$	Artemis knockout clone with exon 2 removed from both alleles but having a puromycin resistance gene insertion on one of the alleles of the Artemis gene	
	$Art^{-/KI\text{-}neo}$	Artemis knockout clone with exon 2 removed from both alleles followed by reintroduction (knock-in) of exon 2 and a neomycin resistance gene on one of the alleles	
	$Art^{-/KI}$	Artemis knockout clone with exon 2 removed from both alleles followed by reintroduction (knock-in) of exon 2 on one of the alleles	
	$Art^{-/-}$•shTDP1#2	Clone #2 showing maximum knockdown of TDP1 in Artemis-knockout cells	Artemis – Homologous Recombination; TDP1 – short hairpin RNA via lentiviral infection
	$Art^{-/-}$•$TDP1^{-/-}$	Clone with a knockout of both Artemis and TDP1	Artemis – Homologous Recombination; TDP1-CRISPR
HEK293	WT	Parental Cell line	
	$TDP1^{-/-}$	Knockout clone of TDP1 with absence of TDP1 activity	CRISPR
HEK293T	WT	Parental cell line	
	$TDP1^{-/-}$	Knockout clone of TDP1 with absence of TDP1 activity	CRISPR



1 Hendrickson laboratory via AAV-mediated homologous recombination
2 Pommier laboratory via CRISPR/CAS9 with PURO insertion
3 Povirk laboratory via lentiviral transduction
4 Povirk laboratory using Pommier vectors and method
5 Povirk laboratory via straight CRISPR/CAS9