Cell lines and culture conditions. All cells were free of mycoplasma.[1] Chinese hamster ovary cell line AA8 and Chinese hamster lung cell line V79 were from ATCC (Manassas, VA). AA8-derived mutant irs1SF (Xrcc3) and the V79-derived mutant V-C8 (Brca2) were provided by Dr. R. Legerski (M.D. Anderson Cancer Center, Houston, TX). 1SFwt8 (irs1SF complemented with human XRCC3 cDNA),[2] 51D1 (Rad51D knockout line) and 51D1.3 (Rad51D complemented line)[3] were gifts from Dr. Larry Thompson (Lawrence Livermore National Laboratory, Livermore, CA). xrs6 (Ku80) and xrs6-hamKu80 (Ku80 repleted) CHO[4] were from the European Collection of Cell Cultures (Salisbury, Wiltshire, UK). Hamster cell lines were maintained in α-MEM supplemented with 10% fetal bovine serum (FBS).

Human acute myelogenous leukemia (AML) cell lines, ML-1 and OCI-AML3 were gifts from Dr. M. Kastan (St. Jude Children's Research Hospital, Memphis, TN) and Dr. M. Andreeff (M.D. Anderson Cancer Center, Houston, TX), respectively, and were grown in RPMI 1640 with 10% FBS. The cervical cancer cell line HeLa CCL2 was purchased from ATCC and cultured in MEM with non-essential amino acids, sodium pyruvate and 10% FBS. Colon carcinoma cell lines with wild type or knock-out p53 (HCT116 $p53^{+/+}$ and $p53^{-/-}$) were provided by Dr. Bert Vogelstein (Johns Hopkins Medical School, MD), and were cultured in McCoy's 5A with 10% FBS. Glioma-derived

cell lines M059-K (wild type) and M059-J (DNA-PKcs deficient), obtained from Dr. M. J. Allalunis-Turner (Brookhaven National Laboratory, Upton, NY), [5,6] were grown in α-MEM supplemented with 20% FBS. hTERT transformed fibroblast lines 1BRhTERT (wild-type control) and F02-98hTERT (ATR-Seckel), gifted from Dr. P. Jeggo (University of Sussex, UK),[7] were cultured in MEM with 15% FBS. AT22IJE-T (AT-C), a fibroblast cell line derived from an ataxia telangiectasia patient, and lines stably transfected with either an episomal expression vector (AT22IJE-TpEBS7, AT-V) or full-length ATM cDNA (AT22IJE-TpEBS7-YZ5, AT-AT) were gifts from Dr. Y. Shiloh (Tel Aviv University, Israel)[8] and were cultured in DMEM with high glucose and 20% FBS.

Chemicals and antibodies. The nucleoside analogue CNDAC was synthesized as described.[9] The DNA-PK inhibitor, NU7441 was obtained from KuDOS Pharmaceuticals (Cambridge, UK). The ATM inhibitor, KU55933 was from Calbiochem (EMD Bioscience, La Jolla, CA). 5-bromo-2'-deoxyuridine (BrdU, #9285), 5-iodo-2'-deoxyuridine (IdU,#I-7125) and 5-chloro-2'-deoxyuridine (CldU, #C-6891) were from Sigma (Sigma-Aldrich, St. Louis, MO).

Sources of antibodies were as follows. Rat-anti-CldU [BU1/75 (ICR1), ab 6326], which does not cross react with IdU: Abcam (Cambridge, MA); FITC-conjugated anti-IdUrd (# 347583): BD Biosciences (San Jose, CA); mouse monoclonal antibody against Orc2 (51-6875GR): BD PharMingen International (San Diego, CA); mouse-anti-β-actin (A1978): Sigma-Aldrich; rabbit-anti-β-tubulin (#2148): Cell Signaling Technology Inc. (Danvers, MA); mouse monoclonal antibody to phospho-Ser139 of H2AX (γ-H2AX) (#05-636): Upstate Biotechnology (Charlottesville, VA); rabbit polyclonal antibody against ATM (ab17995): Abcam Inc. (Cambridge, MA) ; rabbit polyclonal antibody to

Rad 51 (sc-8349) and mouse monoclonal antibody to RhoA (sc-418): Santa Cruz Biotechnology (Santa Cruz, CA). Anti-mouse or anti-rabbit IgG horseradish peroxidase-conjugated antibody: Amersham Biosciences (Piscataway, NJ). FITC-conjugated goat-anti-mouse IgG (115-095-146): Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA).

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Figure S1. Lack of p53 does not sensitize HCT116 cells to CNDAC

HCT116 cells with wild-type p53 (●) and p53 knocked-out (Δ) were washed into drug-free medium after a 24-hour exposure to CNDAC at a range of concentrations and allowed to form colonies in 7-8 days. Results are representative of two independent experiments. All points, mean ± SD of triplicate plates.

Figure S2. CNDAC induces higher levels of sister chromatid exchanges after second S-phase. Continuously exposed to BrdU, AA8 cells were treated with 1 μM CNDAC for either 15 hours (1 cell cycle) or for 30 hours (2 cycles). Colcemid was

added in the final 1.5 hours. Representative images of metaphase chromosome spreads are shown. (A) Untreated; (B) MMC as a positive control; (C) CNDAC treated for 1 cell cycle; (D) CNDAC treated for 2 cell cycles. Arrows indicate SCEs in (A).

Table S1. Characteristics of AML patients

Figure S1. p53 deficiency does not sensitize cells to CNDAC

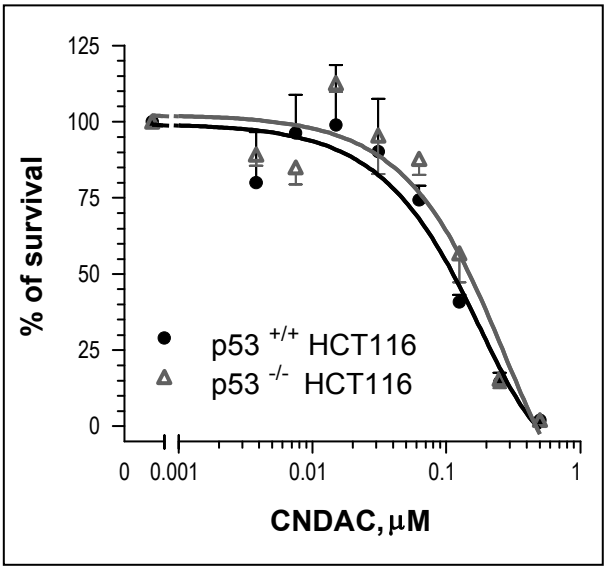

Figure S2. CNDAC induces higher levels of sister chromatid exchanges after second S-phase

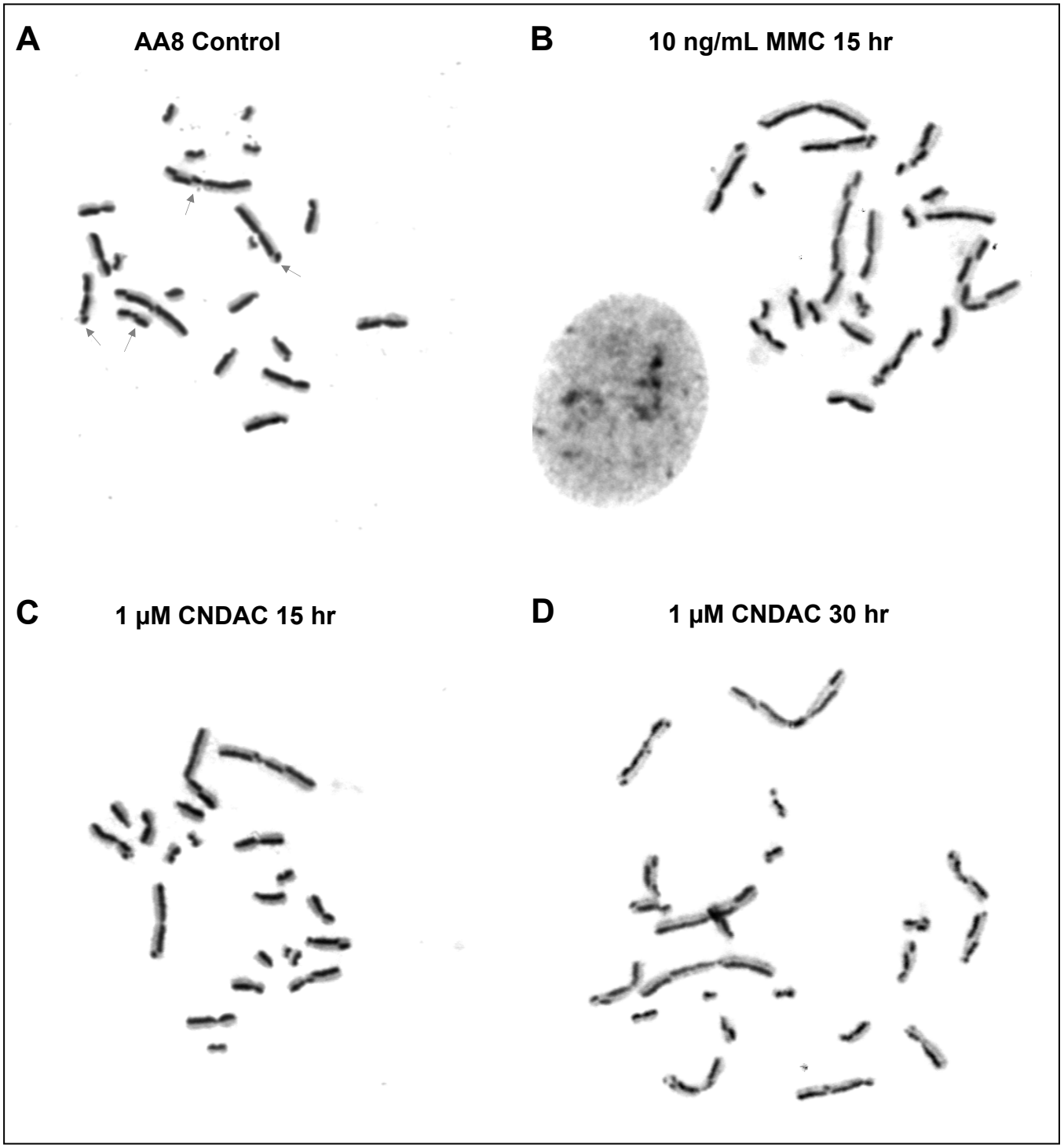

Table S1. Characteristics of AML patients

Study #	Gender	Diagnosis	Age	Prior treatment / Status
Pt #1	Female	AML	51y	Untreated
Pt #2	Female	AML	78y	Relapsed post 3 Rx
Pt #3	Male	AML-M1	50y	Relapsed post 3 Rx
Pt #4	Male	AMML	73y	Relapsed post 2 Rx
Pt #5	Female	AML	21y	Refractory post 2 Rx
Pt #6	Male	AMML	83y	Refractory post 2 Rx
Pt #7	Male	AML	39y	Relapsed post 4 Rx
Pt #8	Female	AML	51y	Relapsed post 2 Rx
Pt #9	Female	AML	51y	Refractory post 2 Rx
Pt #10	Female	AML	58y	Refractory post 1 Rx
Pt #11	Female	AML-M2	76y	Refractory post 2 Rx
Pt #12	Male	AML	53y	Refractory post 2 Rx
Pt #13	Male	AML-M2	18y	Untreated
Pt #14	male	AMML	56y	Untreated
Pt #15	Male	AML -M2	19y	Untreated
Pt #16	female	AML	45y	Relapsed 5 treatments
Pt #17	Female	AML	64y	Refractory post 2 Rx

Note:
Relapsed: Patients had a complete remission at some point and lost it.
Refractory: Patients have never responded.