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Supplementary Materials for

Abacavir, an anti–HIV-1 drug, targets TDP1-deficient adult T cell leukemia

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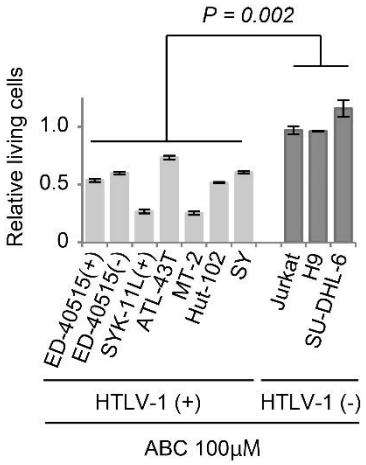

Fig. S1. ABC selectively kills HTLV-1–infected and ATL cell lines. Viability of indicated cells following treatment (2 days) with 100 μM of ABC. MTS values of treated cells relative to untreated cells are shown. Results are expressed as means ± SD of three independent experiments. Statistical analysis was performed using Student's t-test to compare the relative living cells between the HTLV-1(+) cell lines and the non-HTLV-1-infected cell lines.

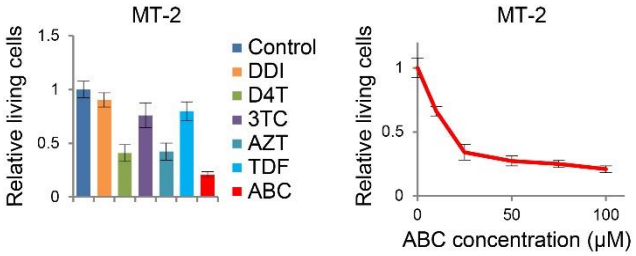

Fig. S2. Cell counting assays showing ABC cytotoxicity to MT-2 cells. MT-2 cells were treated with 100 μM of one of six nucleoside-analog reverse-transcriptase inhibitors (NRTIs, left panel) or escalating doses of ABC (right panel) for 2 days. The cells were counted using Trypan blue staining. Numbers of treated relative to untreated cells are shown. Results expressed as means ± SD of three independent experiments.

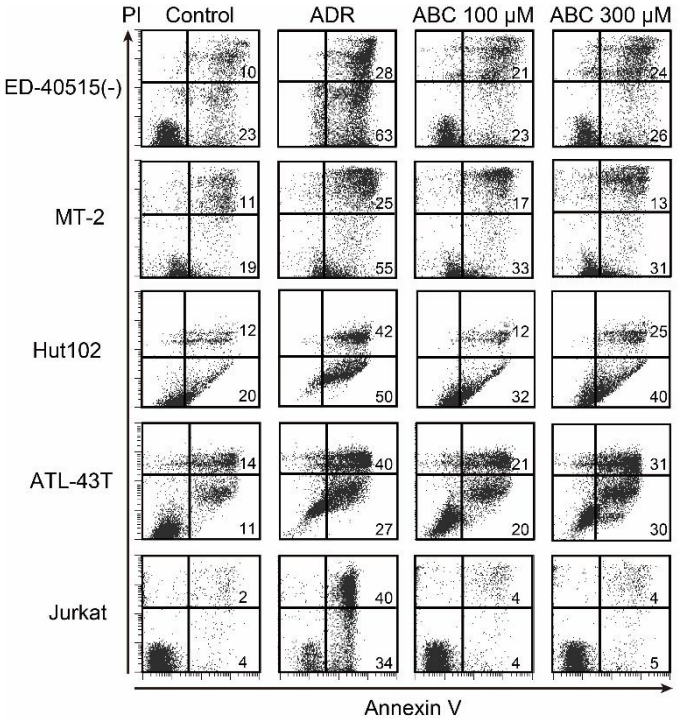

Fig. S3. ABC-induced apoptosis. Apoptosis was analyzed by flow cytometry after Annexin V and PI staining. ED-40515(-), MT-2, Hut-102, ATL-43T, and Jurkat cells were treated with the indicated dose of ABC or 1 μg/ml ADR for 48 hours. The lower-left pael represents viable cells, the lower-right panel represents early apoptotic cells, and the upper-right panel represents late apoptotic cells. The values are expressed as the percentages of cells in each region. ADR treatment was used as a positive control.

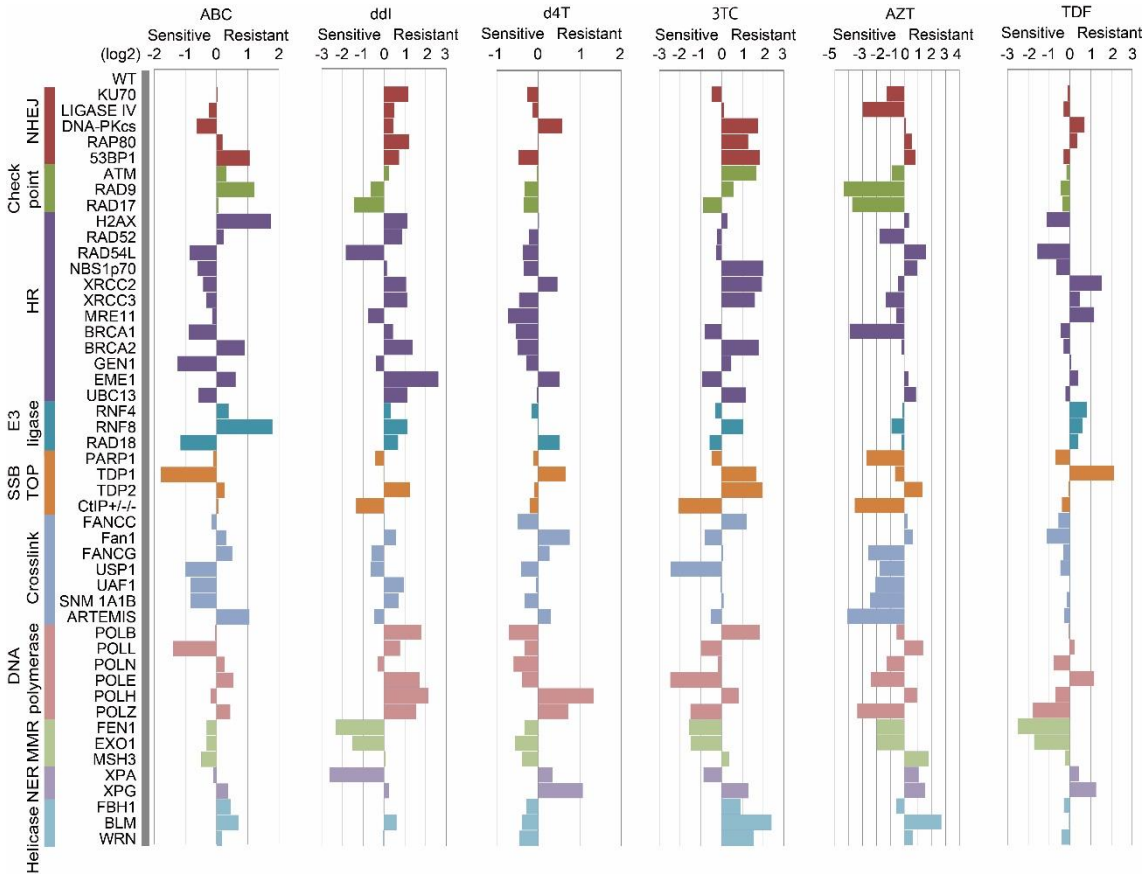

Fig. S4. Relative sensitivities to six NRTIs of DT40 cells deficient in indicated gene.

After 48 hours of treatment, the relative sensitivity of each isogenic mutant chicken DT40 cell line was compared to that of the *wild-type* DT40 cell line. Negative (left) or positive (right) scores indicate that the cell line was either sensitive or resistant to the specified nucleoside-analog reverse-transcriptase inhibitor (NRTI). The relative sensitivities of the selected DNA-repair-deficient DT40 cell lines to 25 μM of the drug are shown. Bars are colored according to the primary DNA-repair function of the deficient gene (shown in Table S2).

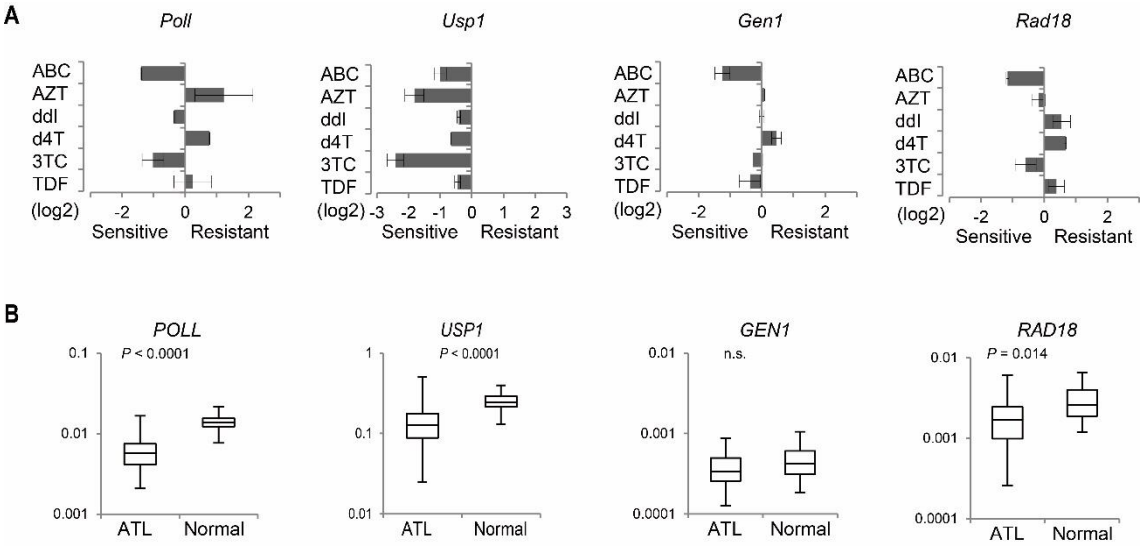

Fig. S5. The relative sensitivity of candidate gene-deficient DT40 cells to the six individual NRTIs and the level of the mRNA expression in ATL cells. (**A**) Relative sensitivities of DT40 cells deficient in *Poll, Usp1, Gen1, Rad18* treated with 6 NRTIs. Results are expressed as means ± SD of three independent experiments. (**B**) Analysis of mRNA-expression levels based on microarray data from the Gene Expression Omnibus (GEO) database (available at http://www.ncbi.nlm.nih.gov/geo/; accession number GSE33615). Relative expression levels of *POLL, USP1, GEN1, RAD18* in peripheral blood cells obtained from ATL patients and normal healthy donor controls are shown. The box plot uses the median, the approximate quartiles, and the lowest and highest data points to convey the level, spread, and symmetry of the distribution of data values. Statistical analysis was performed using Student's t-test to

compare the mRNA expression levels between the ATL cases (n=52) and the normal controls (n=21).

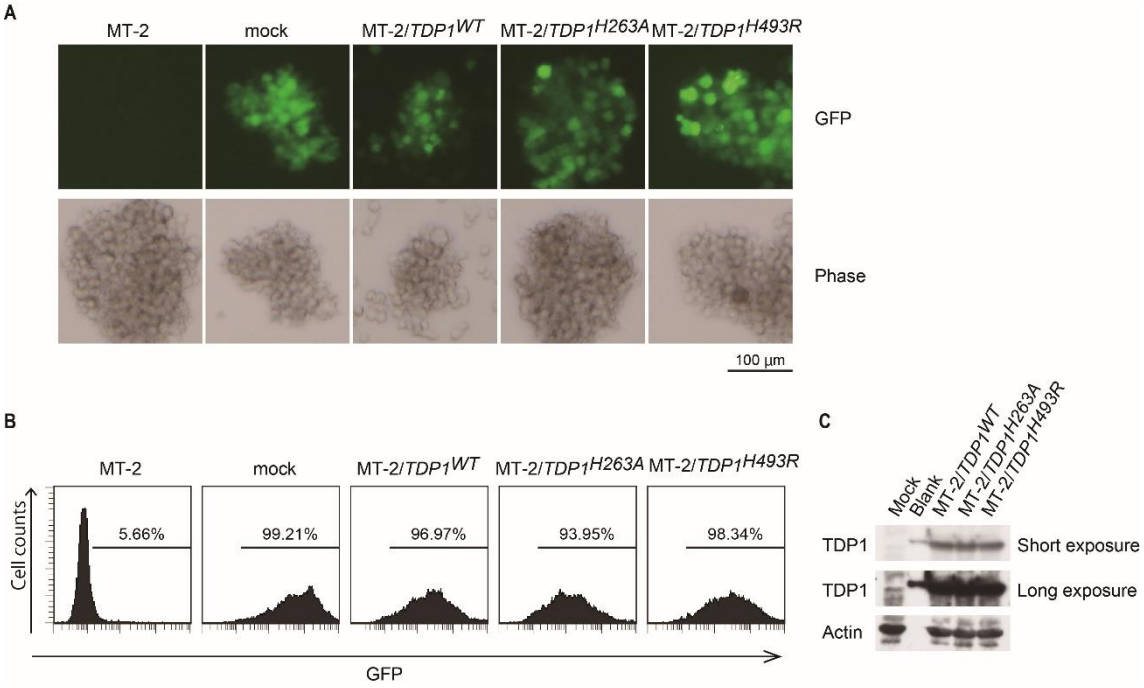

Fig. S6. Ectopic expression of *TDP1*^{WT}, *TDP1*^{H263A}, and *TDP1*^{H493R} in MT-2 cells. MT-2/*TDP1*^{WT}, MT-2/*TDP1*^{H263A} or MT-2/*TDP1*^{H493R} clones ectopically expressed *wild-type*, H263A, or H493R TDP1 in MT2 cells using humanized Renilla reniformis-derived, GFP (hrGFP)-containing lentiviral vectors. (**A**) hrGFP expression based on fluorescence microscopy. (**B**) hrGFP expression based on flow cytometry. (**C**) Western blot analysis of human TDP1 expression.

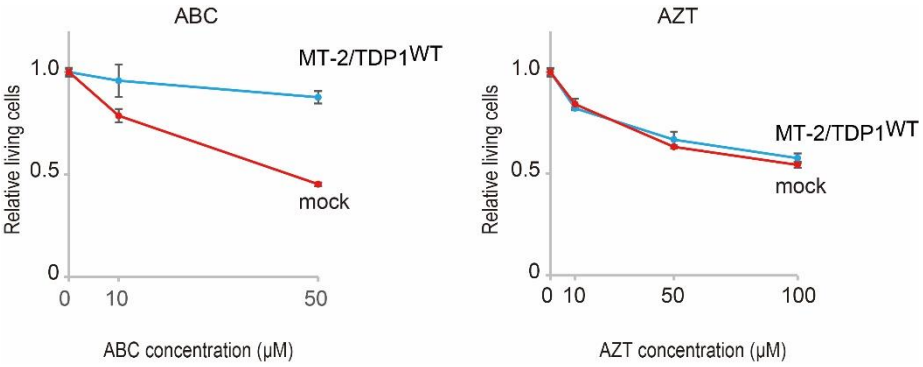

Fig. S7. ABC and AZT cytotoxicity in MT-2/*TDP1*^{WT} cells. MT-2 cells reconstituted with *wild-type* TDP1 or mock-reconstituted cells were treated with the indicated dose of ABC (left panel) or AZT (right panel) for 48 hours. The MTS values relative to those of the untreated cells are shown.

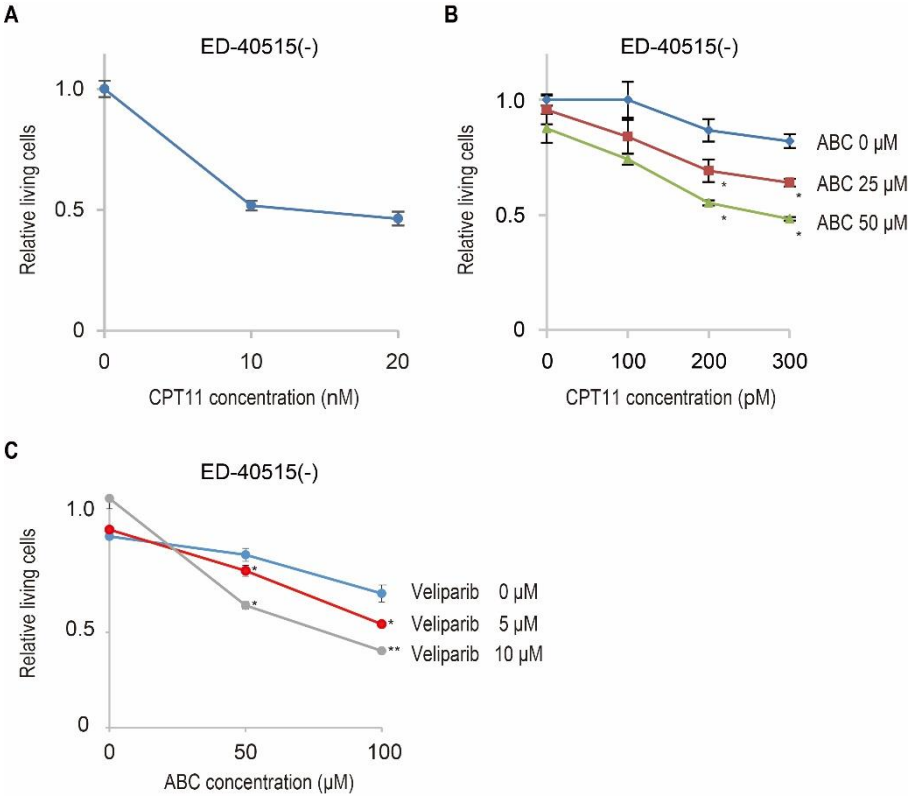

Fig. S8. CPT11 and veliparib enhance the lethality of ABC on ATL cells. ED-40515(-) cells were treated with CPT11 or veliparib for 3 days and analyzed with an MTS assay. Cells were treated with (A) indicated concentrations of CPT11 or (B) indicated concentrations of CPT11 with 0 μM, 25 μM, or 50 μM of ABC. (C) ED-40515(-) cells were treated with the indicated concentrations of ABC and 0 μM, 5 μM, or 10 μM veliparib. MTS values relative to day 0 are shown. Results are expressed as means ± SD of three independent experiments. *CI = 0.3 - 0.7 and **CI = 0.1 - 0.3 are indicated as synergy and strong synergy between the two treatments, respectively.

Table S1. Inhibitory concentration (IC_{50}) values of ABC for each cell line.

	IC_{50} (μM)
cell line	ABC
ED-40515(-)	117.6 ± 3.11
ED-40515(+)	147.00 ± 4.23
SYK-11L(+)	52.75 ± 0.20
ATL-43T	180.08 ± 20.55
MT-2	12.61 ± 1.53
Hut-102	108.4 ± 1.4
SY	>200
Jurkat	>200
H9	>200
SU-DHL-6	>200

All data represent means ± SD of three independent experiments.

Table S2. Isogenic mutant chicken DT40 cell lines used in this study (cell lines are ordered as in fig. S4).

Cell lines	Function of deleted(mutated) gene(s), and annotation	References
KU70	Nonhomologous end-joining	(*48*)
LIGASE IV	Nonhomologous end-joining	(*49*)
DNA-PKcs	Nonhomologous end-joining	(*50*)
RAP80	Functional interaction with Top2, component of BRCA1-a complex, K63 poly-ubiquitin binding protein	(*51*)
53BP1	Inhibition of HR	(*52*)
ATM	Damage check point control	(*53*)
RAD9	Damage check point control	(*54*)
RAD17	Damage check point control	(*54*)
H2AX	HR	(*55*)
RAD52	HR	(*56*)
RAD54L	HR	(*57*)
NBS1p70	HR	(*58*)
XRCC2	HR	(*59*)
XRCC3	HR	(*59*)

Cell lines	Function of deleted(mutated) gene(s), and annotation	References
MRE11	HR	(*60*)
BRCA1	HR	(*61*)
BRCA2	HR	(*62*)
GEN1	HR	Unpublished
EME1	HR	Unpublished
UBC13	E2 ligase, post-replication repair, HR	(*63*)
RNF4	E3 ligase	(*64*)
RNF8	E3 ligase, DSB repair	(*65*)
RAD18	E3 ligase of PCNA, post-replication repair	(*66*)
PARP1	DNA damage sensing, poly(ADP-rybosyl)ation, SSB and DSB repair	(*67*)
TDP1	Removal of Top1 cleavage complex (Top1cc)	(*32*)
TDP2	Removal of Top2 cleavage complex (Top2cc)	(*68*)
CtIP+/-/-	Heterozygous knockout of CtIP gene	(*69*)
FANCC	Interstrand crosslink repair, HR	(*70*)
Fan1	Interstrand crosslink repair, HR	(*71*)
FANCG	Interstrand crosslink repair, HR	(*72*)

Cell lines	Function of deleted(mutated) gene(s), and annotation	References
USP1	Interstrand crosslink repair, HR	(*73*)
UAF1	Interstrand crosslink repair, HR, USP1 association factor	(*73*)
SNM 1A1B	Interstrand crosslink repair	(*74*)
ARTEMIS	5'–3' exonuclease, nonhomologous end-joining	(*74*)
POLB	Base excision repair	(*75*)
POLL	DNA polymerase, base excision repair	(*75*)
POLN	Translesion synthesis DNA polymerase	(*76*)
POLE	Nuclear excision repair, mismatch repair	Unpublished
POLH	Translesion synthesis DNA polymerase	(*77*)
POLZ	Translesion synthesis DNA polymerase	(*78*)
FEN1	5' flap endonuclease, base excision repair, HR	(*79*)
EXO1	Mismatch repair	Unpublished
MSH3	Mismatch repair	Unpublished
XPA	Nuclear excision repair	(*80*)
XPG	Nuclear excision repair	(*81*)
FBH1	DNA helicase, phenotype similar to BLM	(*82*)

Cell lines	Function of deleted(mutated) gene(s), and annotation	References
BLM	RecQ helicase responsible for Bloom syndrome	(*83*)
WRN	RecQ helicase responsible for Bloom syndrome	(*84*)

HR **=** homologous recombination.

Table S3. Characteristics of two ATL patients (cells ordered as in Fig. 5F).

	Age	Gender	Type	Disease status	Cell isolation site	ATL cell %
ATL 1	61	Male	acute	Refractory	Peripheral blood	94
ATL 2	60	Male	acute	Relapse after SCT	Skin tumor	93

Table S4. List of HTLV-1–infected and ATL cell lines.

Cell line	Leukemic origin (ATL cells)	HTLV-1 replication	IL-2 dependency
ED-40515(-)	+	–	-
ED-40515(+)	+	–	+
SYK-11L(+)	+	–	+
ATL-43T	+	–	+
MT-2	–	+	–
Hut-102	+	+	–
SY	-	–	+