Table S1. Primers used in gene expression analysis.

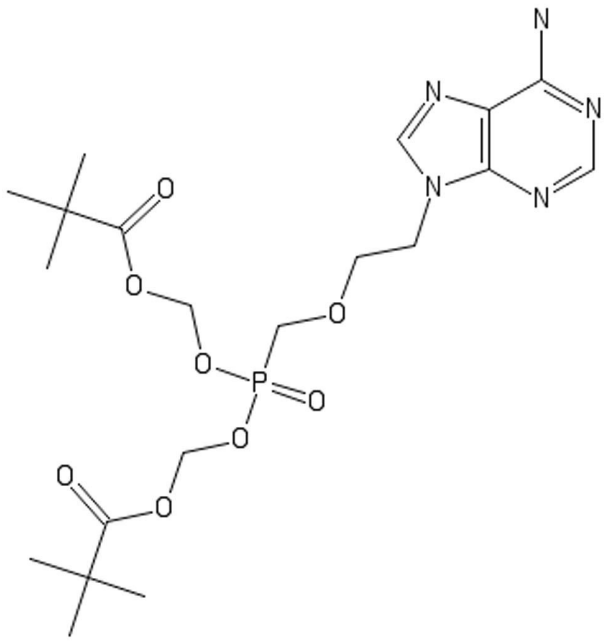
Gene	Forward primer	Reverse primer
P21	GGAAGACCATGTGGACCTGT	GGATTAGGGCTTCCTCTTGG
Bax	GCTGGACATTGGACTTCCTC	CTCAGCCCATCTTCTTCCAG
Puma	GACGACCTCAACGCACAGTA	CACCTAATTGGGCTCCATCT
Noxa	GTTCCAGGAGGCTCTGTCTG	CTGAGTCCAGGCCATTCAG
dCK	AGCAAGGCATTCCTCTTGAA	AACCATTTGGCTGCCTGTAG
hENT1	CAGAAAGTGCCTTCGGCTAC	GGGCTGAGAGTTGGAGACTG
MRP4	CTTGGAGAGGAGTTGCAAGG	GCTGTGTTCAAAGCCACAGA
GAPDH	GAAGGTGAAGGTCGGAGTC	GAAGATGGTGATGGGATTTC

Primers were designed using Primer 3 (version 0.4.0) software. All primers were designed to cross at least one intron.

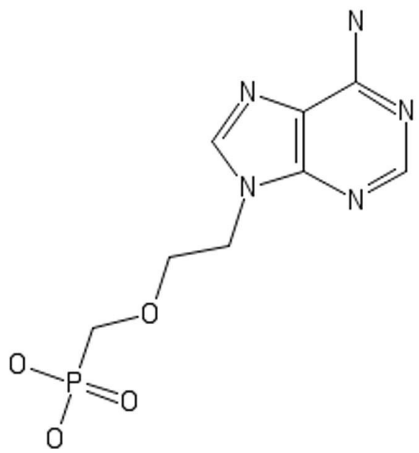
Table S2. BL-CFC in primary AML patient samples.

Sample ID	NT	Adefovir-DP	ODE-adefovir	HDP-Adefovir	Ara-c
P1	125±16	68±4	71±7	105±9	70±7
P2	108±11	67±12	74±10	93±11	56±6
P3	113±13	67±4	64±4	78±5	59±5
P4	110±9	65±11	61±10	78±8	87±12
P5	125±9	67±7	66±6	84±7	54±7
P6	115±12	11±4	10±4	32±4	7±2
P7	150±22	22±5	24±4	65±6	19±5
P8	120±8	21±4	24±5	62±8	15±3
P9	92±12	41±3	41±4	77±4	24±3
P10	90±12	48±5	51±8	76±7	16±3
P11	98±10	51±6	55±5	73±12	25±4
P12	119±5	15±2	17±3	49±4	55±8
P13	125±7	41±7	38±6	74±6	96±7
P14	112±11	28±3	27±6	59±4	101±18
P15	128±14	18±4	14±4	28±7	75±13
P16	134±12	28±4	31±4	67±8	84±15
P17	107±9	27±3	31±4	76±7	64±5
P18	95±8	15±6	13±5	37±9	53±6
P19	97±10	6±2	5±3	16±5	67±6

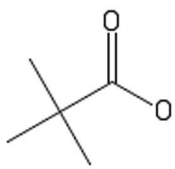
Nineteen primary AML patient samples were treated with adefovir-DP (150nM), ODE-adefovir (150nM), ODE-adefovir (150nM) or ara-c (5μM) for 48h in liquid culture. Then, treated and untreated (NT) cells were washed and cultured in triplicate in methylcellulose medium for 7-14 days. Colonies containing more than 20 cells were counted under an inverted microscope. Results were reported as mean±SD.



Adefovir Dipivoxil



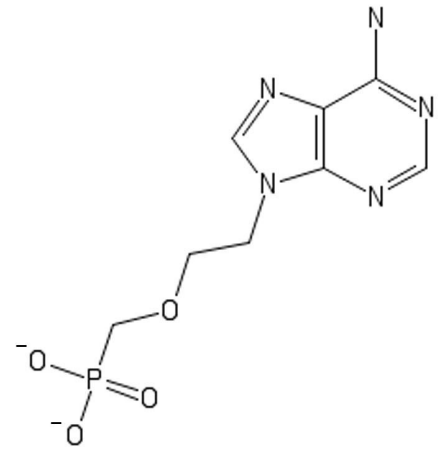
Adefovir



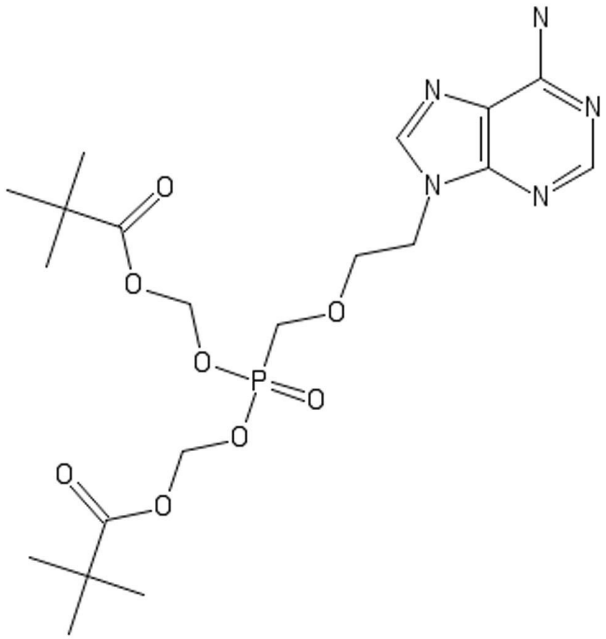
Pivalic acid

Formaldhyde

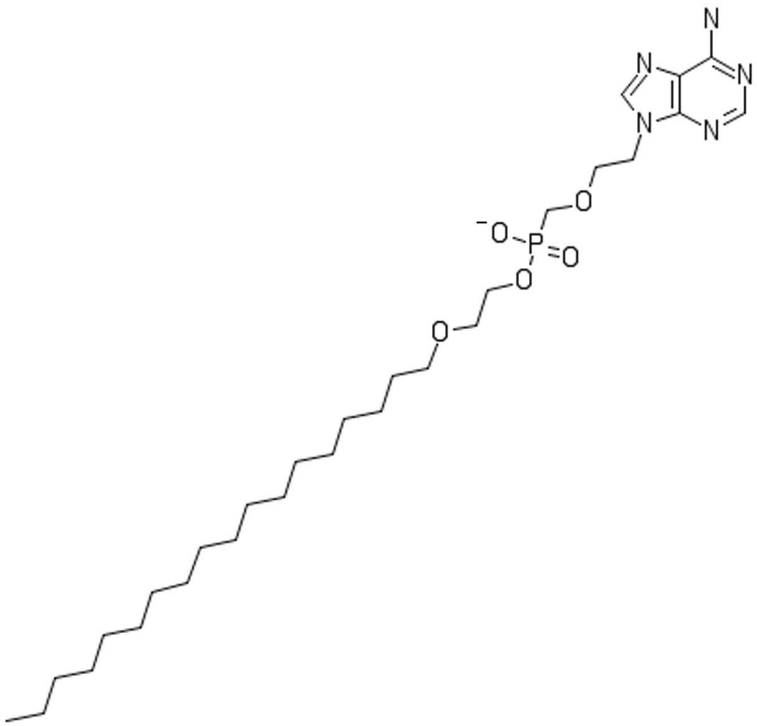
Figure S1. Adefovir dipivoxil and its anabolites. Adefovir dipivoxil, when given orally, is cleaved by non-specific esterases in the gastro-intestinal mucosa into 1 adefovir, 2 pivalic acid and 2 formaldhyde molecules. This process also occurs in vitro in treated cells, of any kind.



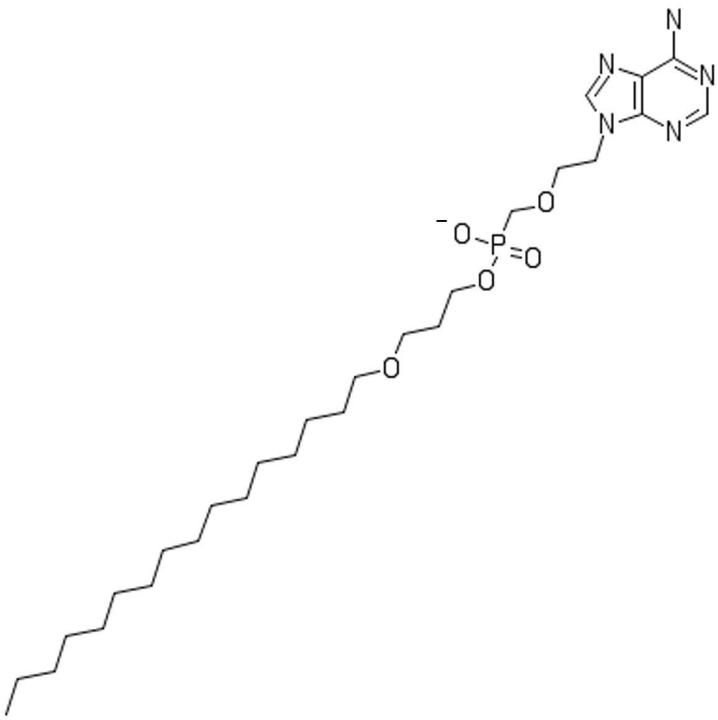
Adefovir



Adefovir dipivoxil



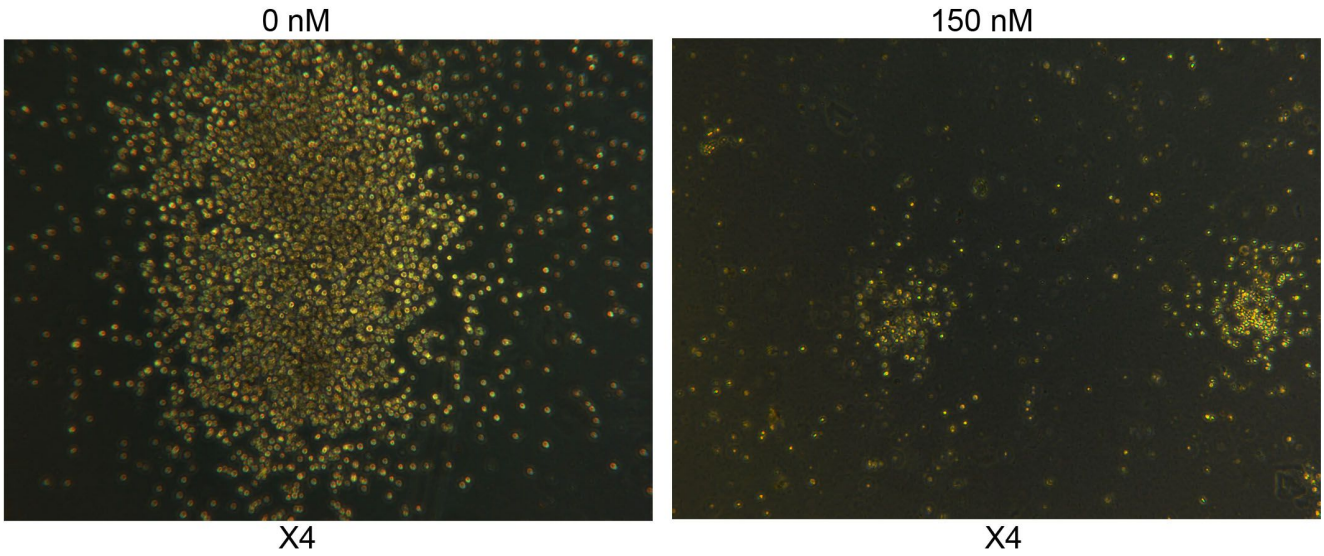
ODE-adefovir



HDP-adefovir

Figure S2. Chemical structure of adefovir, adefovir dipivoxil, ODE-adefovir and HDP-adefovir. Structures were obtained from the Pubchem database.

A.



B.

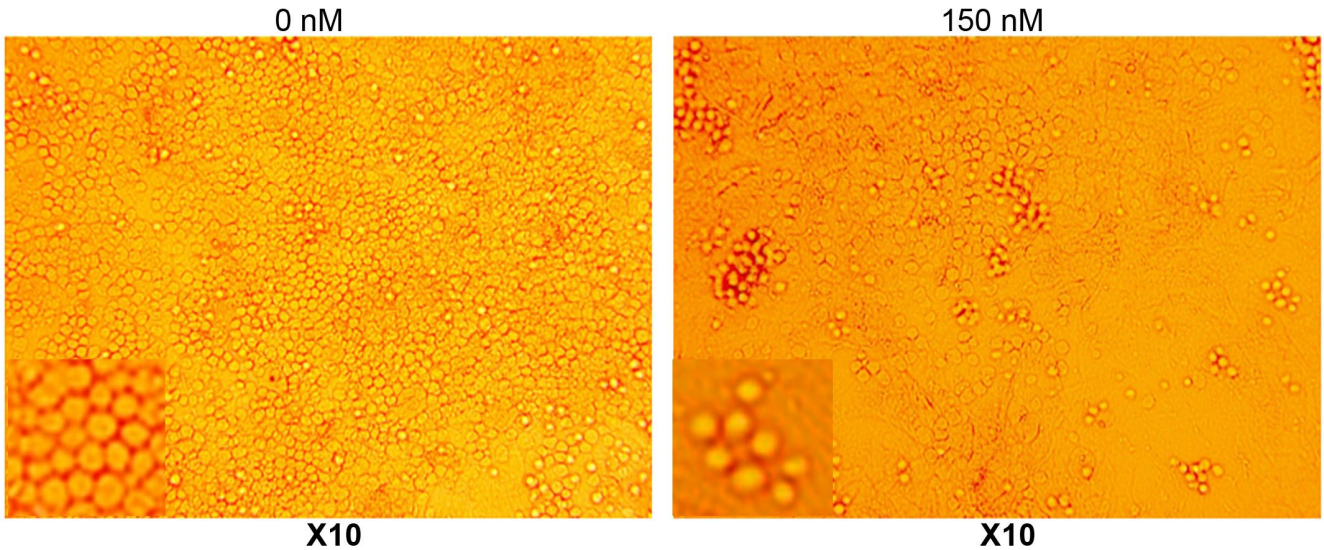


Figure S3. BL-CFC and LTC-IC assays in primary AML patient samples. (A) BL-CFC assay in primary patient sample # p12. Cells were treated with ODE-adefovir 150nM for 48h in liquid culture. Then, untreated (left) and treated (right) cells were cultured in methylcellulose medium for 7 days. Colonies containing more than 20 cells were counted under an inverted microscope. , Interestingly, even when there were colonies in the treated samples, the colonies were smaller size and less compact as compared to the non-treatment control. (B) LTC-IC assay in primary patient sample # p19. Cells were treated with ODE-adefovir 150nM for 48h in liquid culture. Then, untreated (left) and treated (right) cells were seeded on stromal cells for 5 weeks. Cobblestone areas were defined as colonies of 20 cells or more of small polygonal of tightly packed cells that were nonrefractory (dim) when viewed under a phase microscope. Untreated sample (left) is full of areas of small polygonal, tightly packed cells that are embedded within the stromal feeder layer. In treated sample, the cells are mostly round, bright cells that are losely attached to the stromal feeder layer.

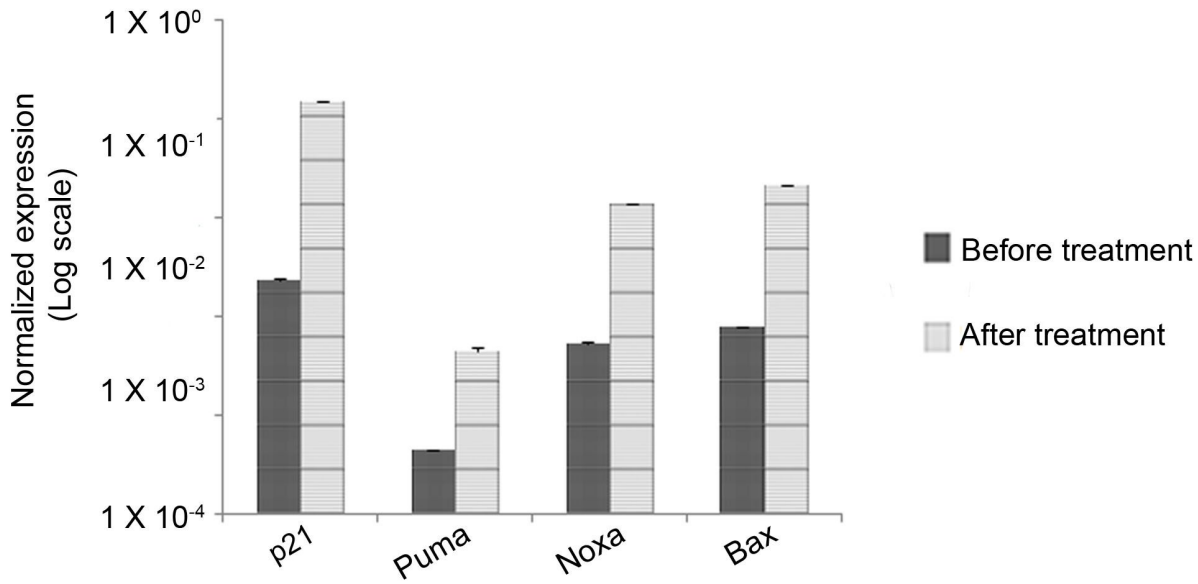


Figure S4. Change in the expression of P53 targets. OCI/AML-2 cells were treated with 1μM of ODE-adefovir for 6 hours. Gene expression was analyzed using real-time RT-PCR. Data was normalized to GAPDH and represented on a log scale.

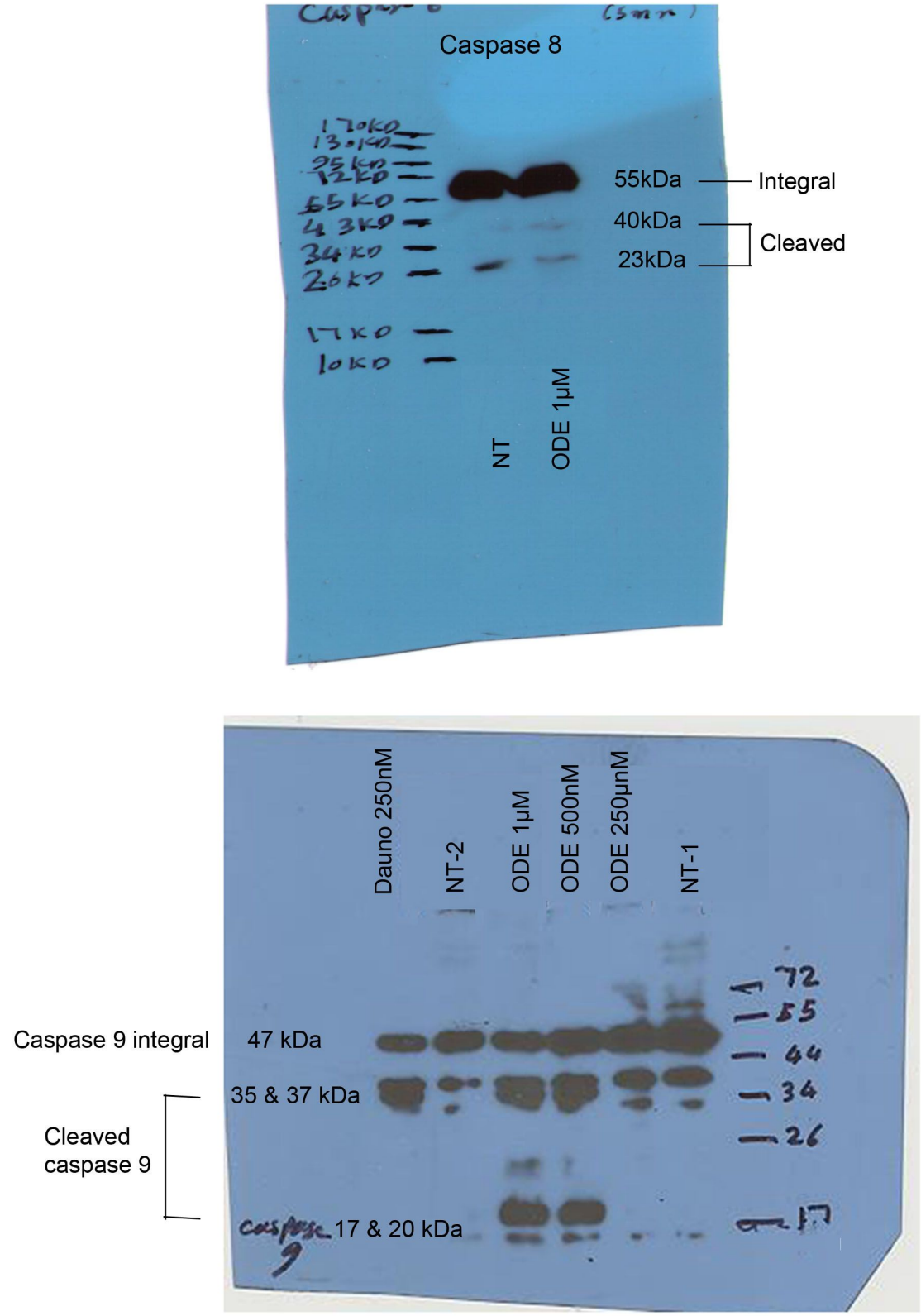


Figure S5. Analysis of caspases 8 & 9 cleavage after ODE-adefovir treatment. (A) OCI/AML-2 cells were treated with 1μM of ODE-adefovir for 24h. Then, lysate from treated and untreated (NT) cells were analyzed for caspase 8 cleavage by western blot using an antibody that recognizes both cleaved and uncleaved forms. (B) OCI/AML-2 cells were treated with ODE-adefovir (1μM, 500nM and 250nM) or daunorubicin (250nM) for 24h. Then, lysates from treated and untreated (NT1 & NT2) cells were analyzed by western blot for caspase 9 cleavage using an antibody that recognizes cleaved and uncleaved forms.

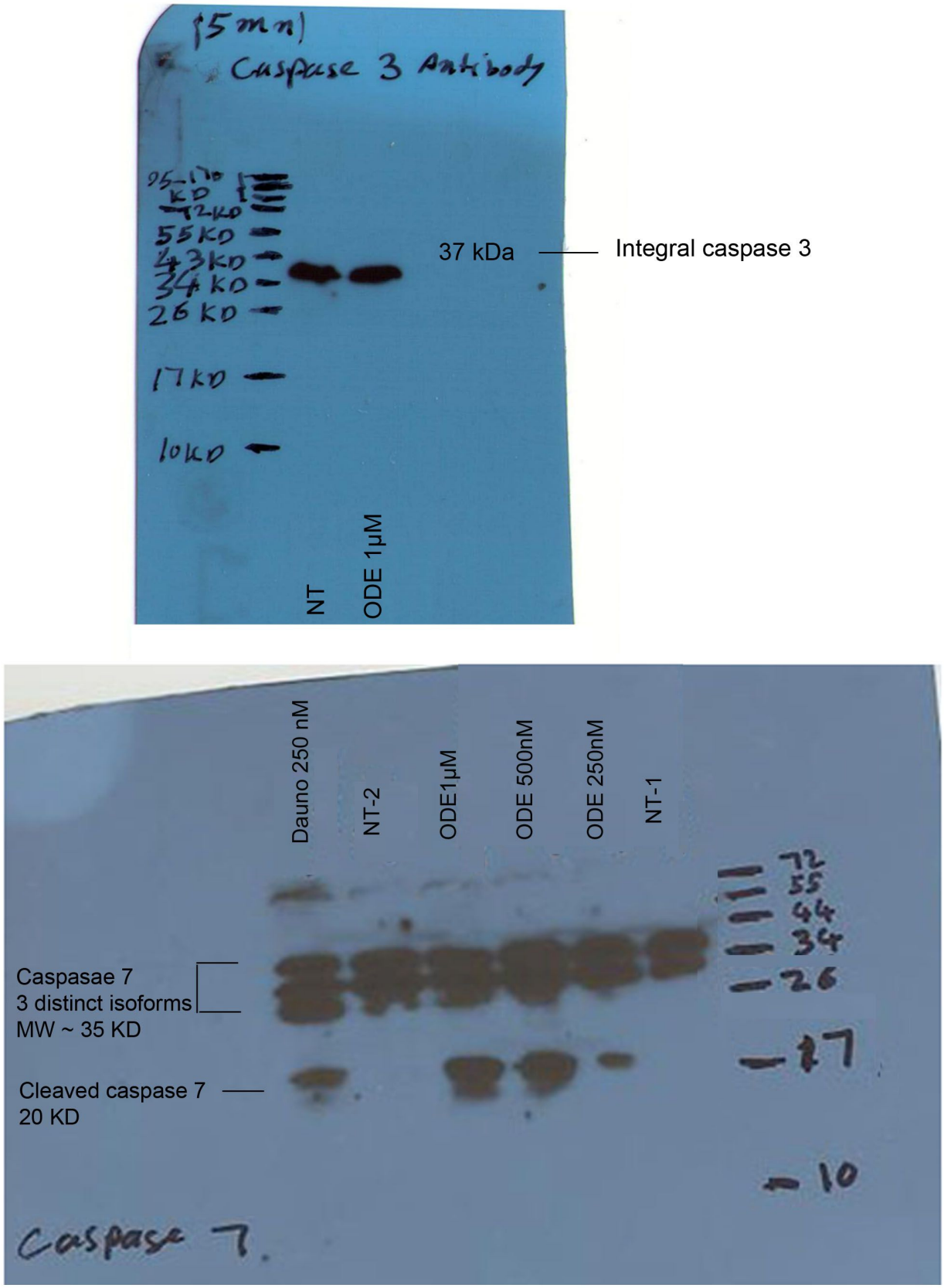


Figure S6. Analysis of caspases 3 & 7 cleavage after ODE-treatment. (A) OCI/AML-2 cells were treated with 1μM of ODE-adefovir for 24h. Then, lysate from treated and untreated (NT) cells were analyzed for caspase 3 cleavage by western blot using an antibody that recognizes both cleaved and uncleaved forms. (B) OCI/AML-2 cells were treated with ODE-adefovir (1μM, 500nM and 250nM) or daunorubicin (250nM) for 24h. Then, lysates from treated and untreated (NT1 & NT2) cells were analyzed by western blot for caspase 7 cleavage using an antibody that recognizes cleaved and uncleaved forms.

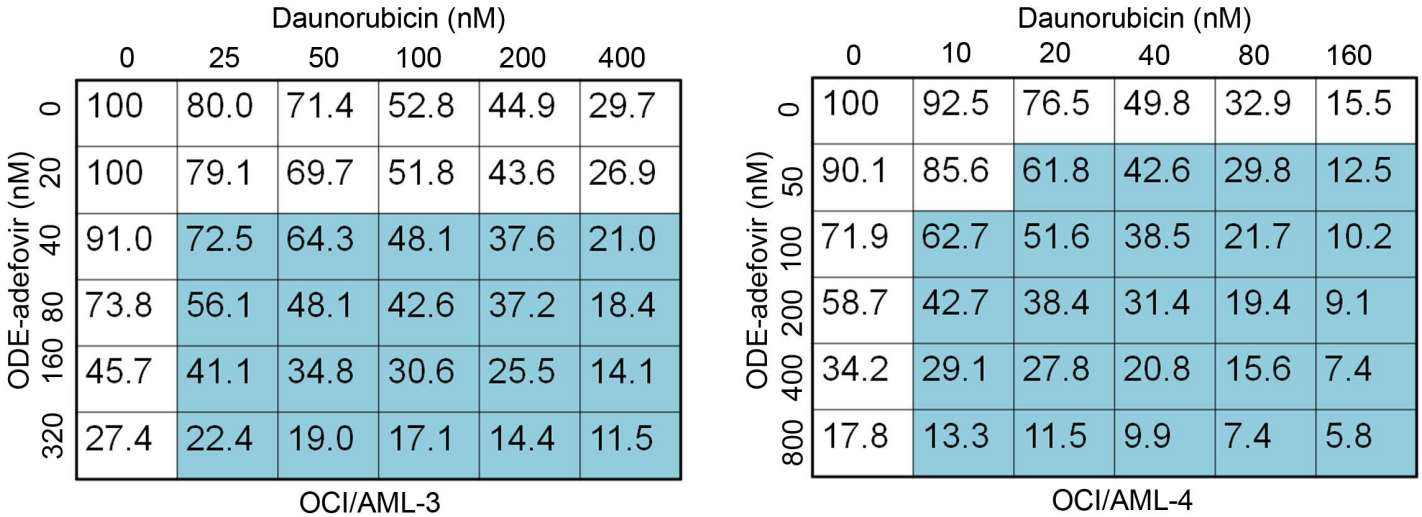


Figure S7. ODE-adefovir does not synergizes with daunorubicin. Additive effect of daunorubicin and ODE-adefovir in OCI/AML-3 and OCI/AML-4, respectively. AML cells were treated with increasing concentrations of ODE-adefovir and increasing concentrations of daunorubicine. Then, viability was assessed using the alamarBlue assay. Combination index (CI) was calculated. Blue squres indicate additive effect (CI ≈ 1).

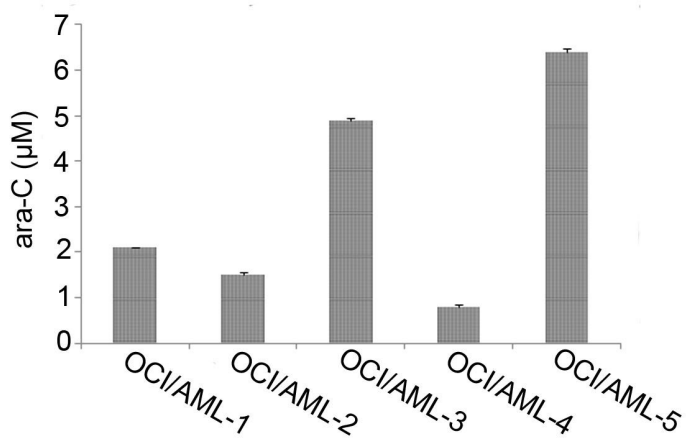


Figure S8. Cytotoxic activity of ara-C in AML cell lines. AML cell lines were cultured with increasing concentrations with ara-C. IC50 was calculated according to the Four-Parameter Logistic Fit model. Data represented as IC50±SD.

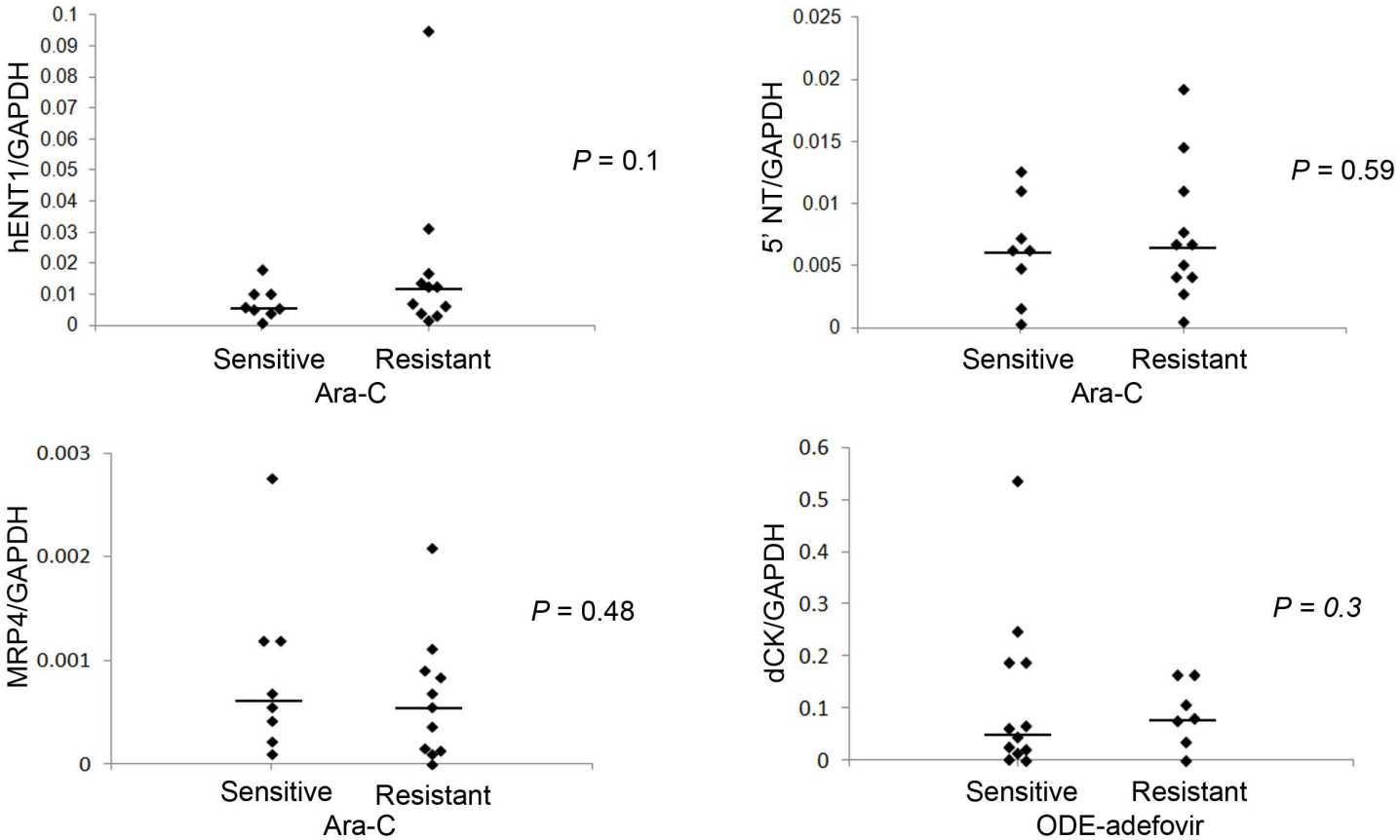


Figure S9. No correlation between hENT1, 5'NT or MRP4 expression levels and ara-C sensitivity or between dCK expression levels and ODE-adefovir sensitivity in primary AML patient samples. Nineteen AML patient samples were classified according to their sensitivity pattern to arc-C. Cases that showed >50% reduction in BL-CFC in response to ara-c were considered as sensitive, whereas as that showed < 50% reduction were considered resistant. Gene (hENT1, 5'NT, MRP4 & dCK) expression was analyzed using real-time RT-PCR and level was normalized to GAPDH.

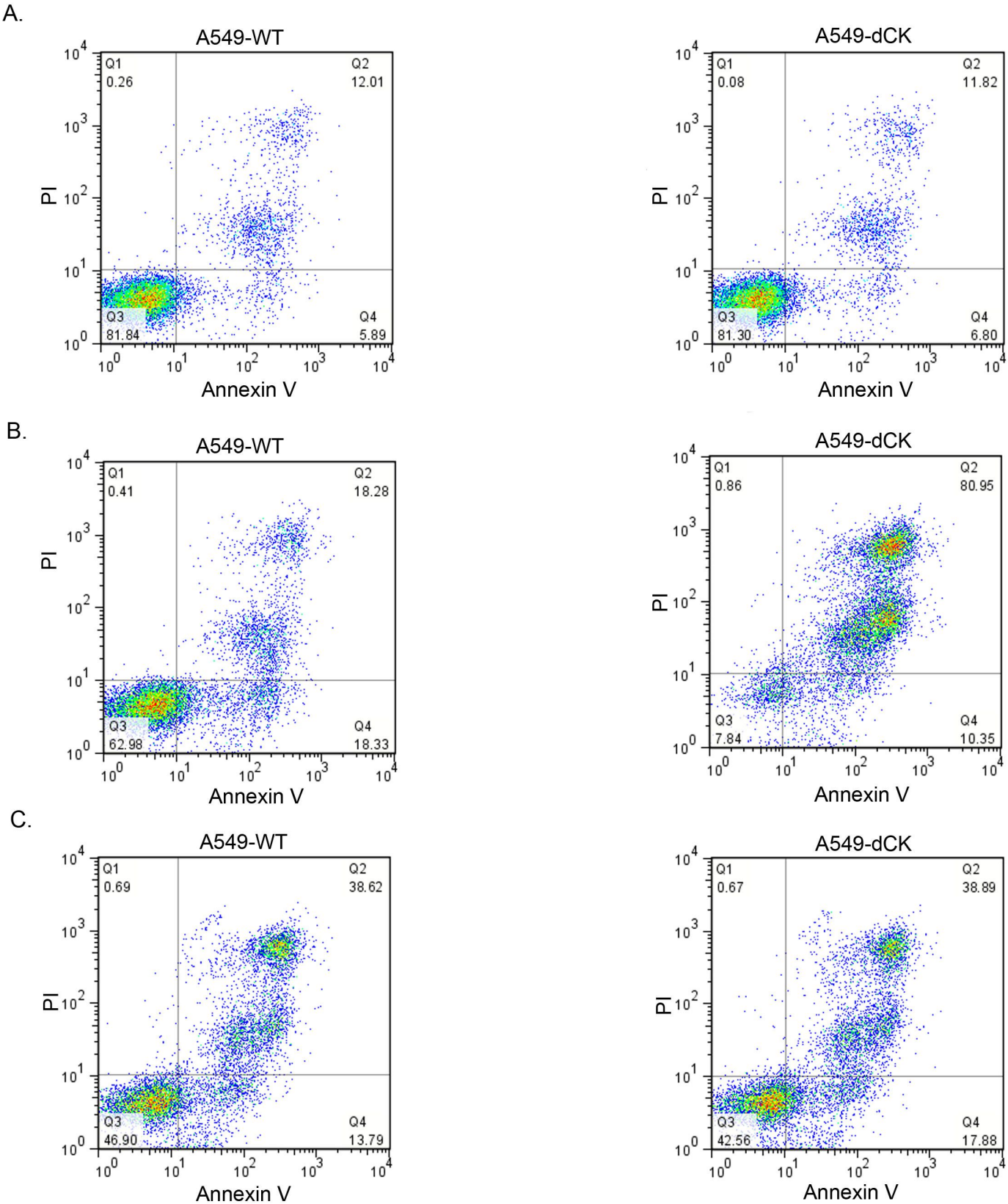


Figure S10. dCK modulates ara-C, but not ODE-adefovir, sensitivity. (A) Untreated Wild-type A549 cells and dCK-transduced A549 were stained with annexin V antibody and propidium iodide. (B) A549-WT and A549-dCK were treated with ara-C (500nM) for 24 hours and then stained with annexin V and PI. (C) A549-WT and A549-dCK were treated with ODE-adefovir (500nM) for 24 hours and then stained with annexin V and PI.

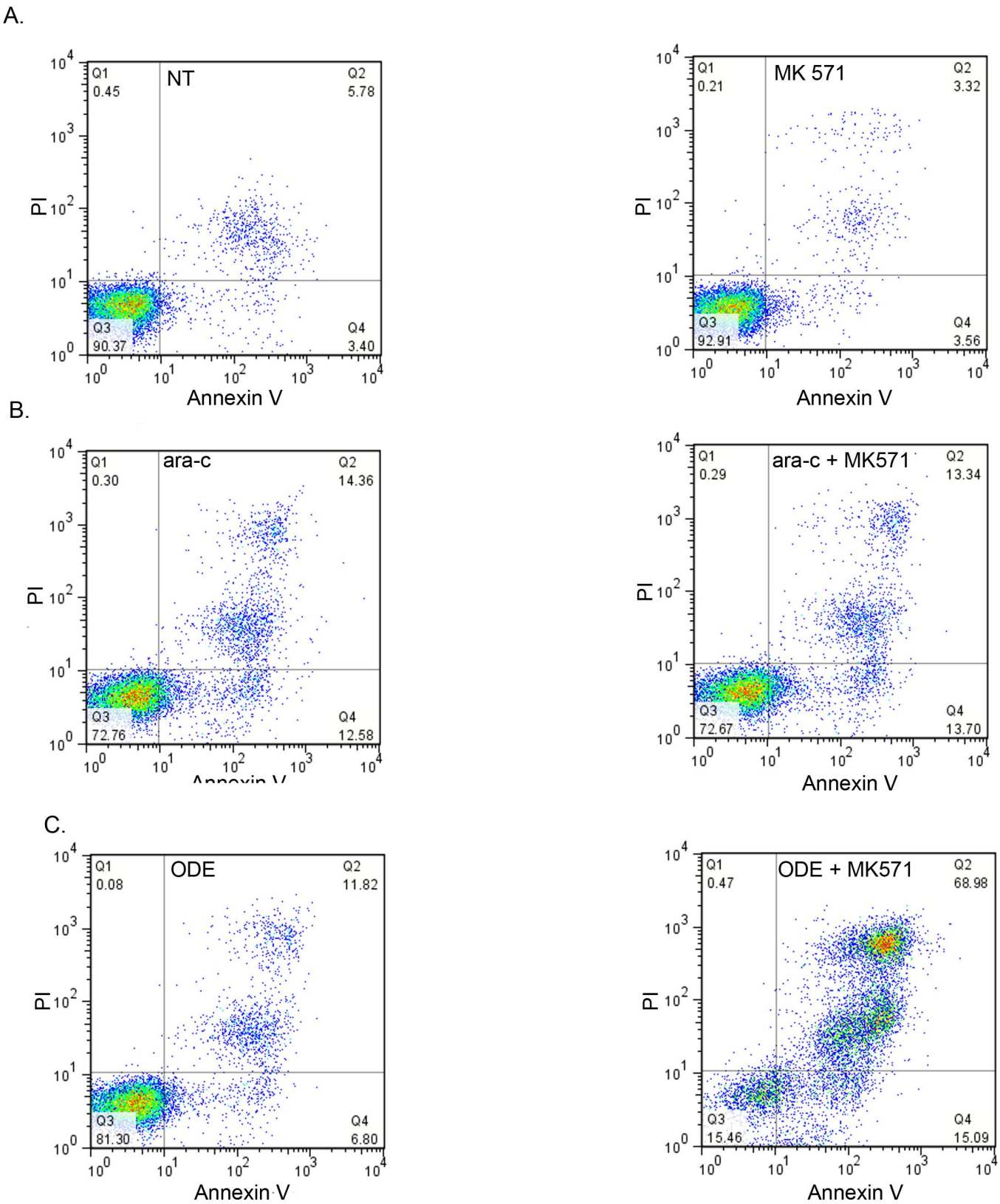


Figure S11. MRP4 inhibition potentiates the cytotoxic effect of ODE-adefovir, but not that of ara-C. (A) Untreated OCI/AML-3 cells (left) or treated with MK571 (5μM) for 24h (right) were stained for annexin V and PI. (B) OCI/AML-3 cells treated with ara-C (2μM) (left) or with ara-C (2μM) + MK571 (5μM) for 24h (right) were stained for annexin V and PI. (C) OCI/AML-3 cells with ODE-adefovir (40nM) (left) or with ODE-adefovir (40nM) + MK571 (5μM) for 24h (right) were stained for annexin V and PI.