Supplemental Table 1.

Supplemental Table 1. Primary Patient Sample Characteristics					
Sample Name	Sample type	Sex	Age	FAB	Karyotype
B1	Bone Marrow	Female	60	M5	Trisomy 8, Trisomy 9
B2	Bone Marrow	Female	68	Non-M3	46XX
B3	Bone Marrow	Female	79	M1	46XX
A1	leukophoresis	Male	55	M5	Trisomy 8
A2	leukophoresis	Female	65	M5	Trisomy 8, Isochromosome 13
A3	leukophoresis	Female	66	M2	Deletion 6q, +Flt3 ITD
A4	leukophoresis	Female	59	M4Eo	Inversion 16
A5	leukophoresis	Male	33	Non-M3	Monosomy 7, inversion 3
A6	leukophoresis	Male	53	M5	46XY, +Flt3 ITD
A7	leukophoresis	Female	89	M4	Complex
A8	leukophoresis	Female	59	Non-M3	46XX
A9	leukophoresis	Male	69	Non-M3	46XY, +Flt3 ITD
A10	Bone Marrow	Female	39	Non-M3	Trisomy 8

A11	Bone Marrow	Female	67	Non-M3	t(8;21)
A12	Leukophoresis	Male	39	M5b	46XY, Flt3 ITD+

Supplementary Figure Legends:

Figure S1. FdUMP[10] is effective against cells cultured in human serum. HL60 and KG1a cells were cultured in either 10% fetal bovine serum (FBS) or 10% human serum (HS) and exposed to FdUMP[10] as indicated for 72 hours. Viability was determined and normalized to untreated controls.

Figure S2. p53 shRNA efficiently suppresses p53 induction. Murine AML cells expressing either a p53 shRNA or control vector were exposed to 500 ng/ml doxorubicin for 4 hours, then lysed and blotted for p53, p21, and actin as shown. An uninfected control is shown in the far right lane.

Figure S3. FdUMP[10] is effective against murine leukemia stem cells. (A) Murine AML cells expressing MLL-ENL and the Flt3 ITD (MFL2) or NRasG12D (MR2) were incubated for 24 hours with the indicated concentration of FdUMP[10]. Following exposure cell viability was determined by trypan blue exclusion. Animals were injected with $1x10^6$ viable cells and imaged as indicated. (B) Kaplan-Meier plots of the survival in days of the animals imaged in (A).

Figure S4. TS and Top1 are expressed in AML cells. TS and Top1 Western blots. HL60 (H), K562 (K), two MLL-ENL driven murine AML lines (M1 and M2), and nine primary samples from patients with AML samples (A1-A9) were blotted with the indicated antibody.

Figure S5. FdUMP[10] induces DNA damage. (A) Immunofluorescence for γH2AX foci. Jurkat cells were treated with the indicated drug for 24 hours and then assayed for the presence of γH2AX foci. Assay was done and images captured as in Figure 5A. (B) K562 cells were incubated with 100 nM FdUMP[10] for 24 hours with or without 20 μM FMK-ZVAD as indicated. Assay was done and images captured as above except the LPlanFl 20X objective was used.

Figure S6. FdUMP[10] induces apoptosis in AML cells. (A) Caspase 3 Western blot results. HL60 cells were treated with the indicated amount of FdUMP[10] for 48 hours. Cells were then lysed and blotted for full length caspase 3. Actin blot serves as a loading control. Relative densitometry values are listed below each lane. (B) Flow cytometry results of Annexin V assays. The indicated MLL-ENL driven leukemia cells were treated with drug for 24 hours analyzed by flow cytometry, as in Figure 5. (B) MLL-ENL and NRas containing AML cells were exposed to the indicated drug for 24 hours and analyzed as in (B).

Figure S7. FdUMP[10] is cytostatic at low doses and cytotoxic at higher doses. (A) HL60 cells were seeded at 400,000 cells per ml and incubated with

the indicated amount of FdUMP[10] through 72 hours. Cells were assessed for viability by trypan blue exclusion and counted every 24 hours. The experiment was carried in triplicate and viable cells per ml are shown. Error bars represent the standard error. (B) The murine cell line MFL2 was seeded at 500,000 cells per ml and incubated with the indicated amount of FdUMP[10]. Cells were assessed for viability as above.

Figure S8. FdUMP[10] is effective *in vivo*. (A) Bioluminescence signals of mice on day 6 following treatment. C57/Bl6 mice were sublethally irradiated to 4.5 Gy and injected with an MLL-ENL and NRas syngeneic leukemia. Once engraftment was established by bioluminescence imaging, mice were treated with 300 mg/kg FdUMP[10] via jugular vein injection on days 1 and 4. (B) Kaplan-Meier curves for animals treated with FdUMP[10] as above versus no treatment. P value was obtained by log rank test. (C) Kaplan-Meier curves for animals treated with FdUMP[10] at 300 mg/kg every other day for 4 doses by tail vein injection. P value was obtained by log rank test.

Figure S9. FdUMP[10] is synergistic with Ara-C and doxorubicin. HL60 cells were incubated with the indicated drugs for 72 hours and viability determined. Viability was normalized to untreated controls. Error bars represent the standard error. Dox=doxorubicin, Fd=FdUMP[10], Ara=Ara-C.

Supplemental Figures:

Figure S1.

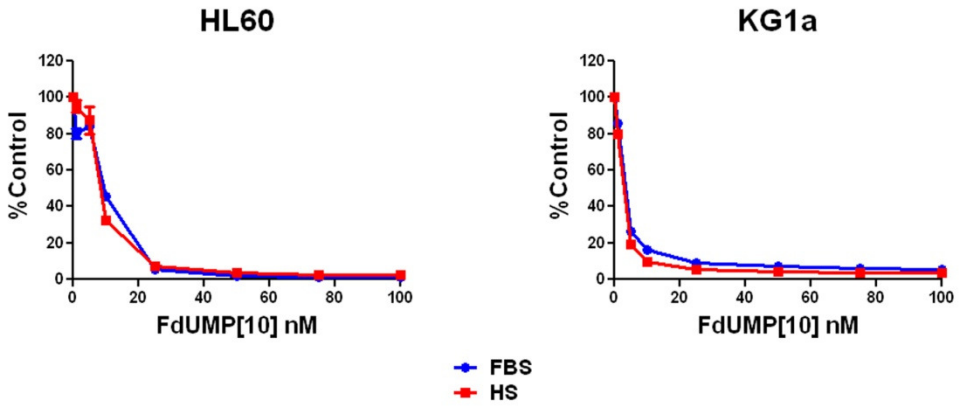


Figure S2.

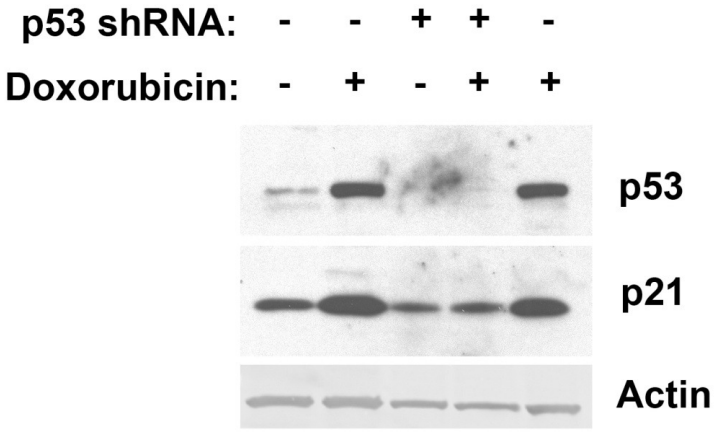
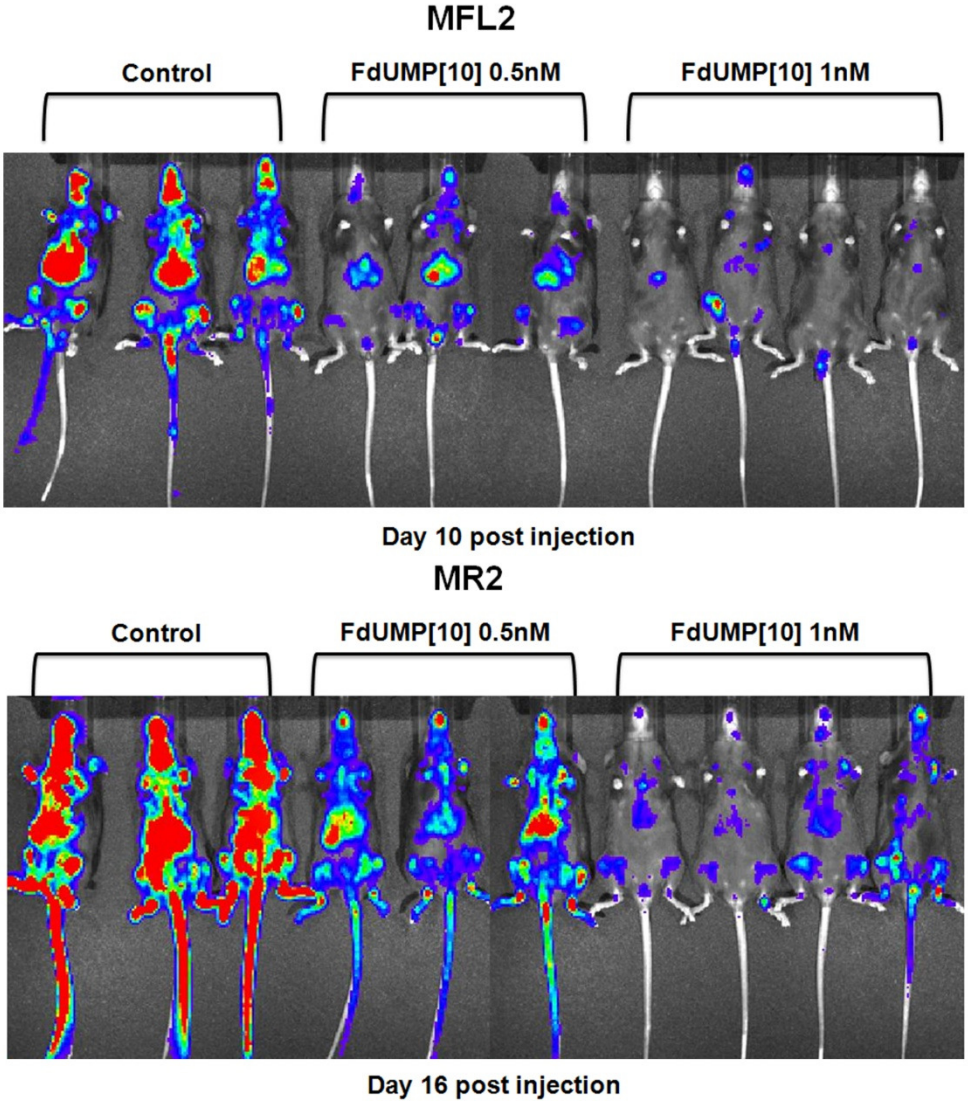


Figure S3.

A


MFL2
Control
FdUMP[10] 0.5nM
FdUMP[10] 1nM
Day 10 post injection
MR2
Control
FdUMP[10] 0.5nM
FdUMP[10] 1nM
Day 16 post injection

B

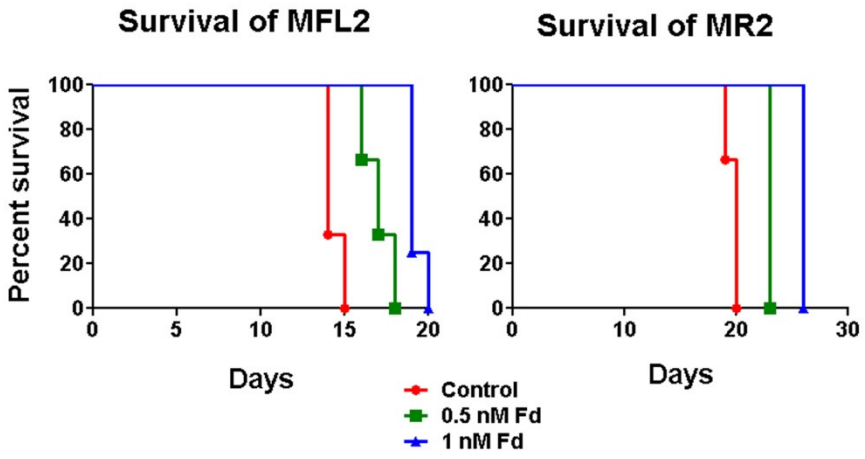

Survival of MFL2
Survival of MR2
Percent survival
100
80
60
40
20
0
0
5
10
15
20
30
Days
Control
0.5 nM Fd
1 nM Fd

Figure S4.

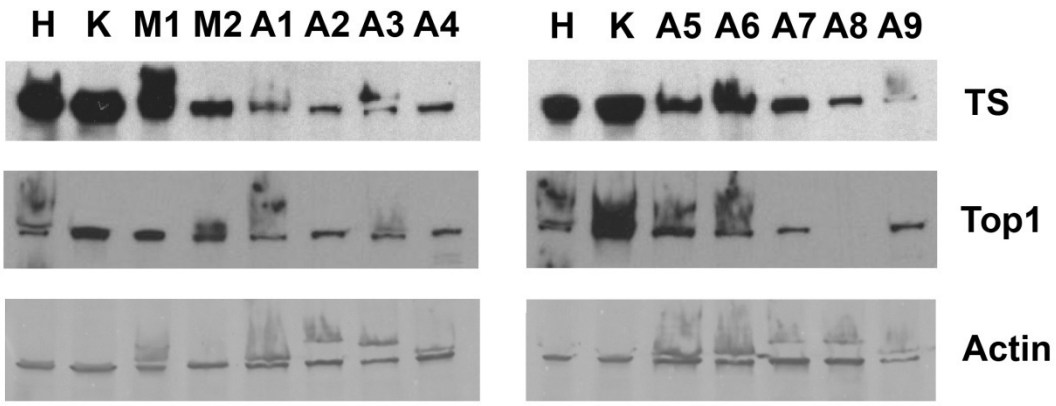


Figure S5

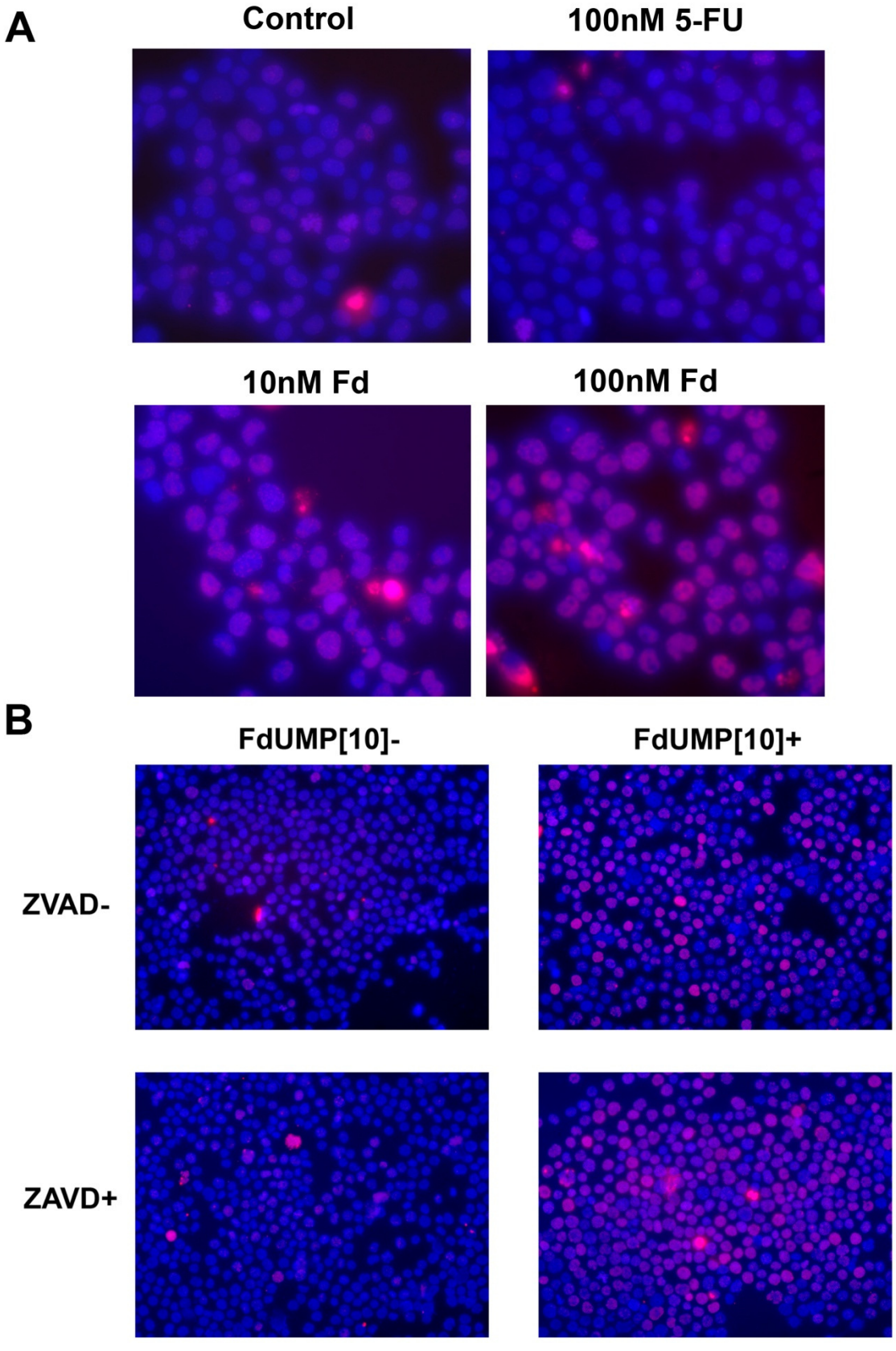

A
Control
100nM 5-FU
10nM Fd
100nM Fd
B
FdUMP[10]-
FdUMP[10]+
ZVAD-
ZAVD+

Figure S6.

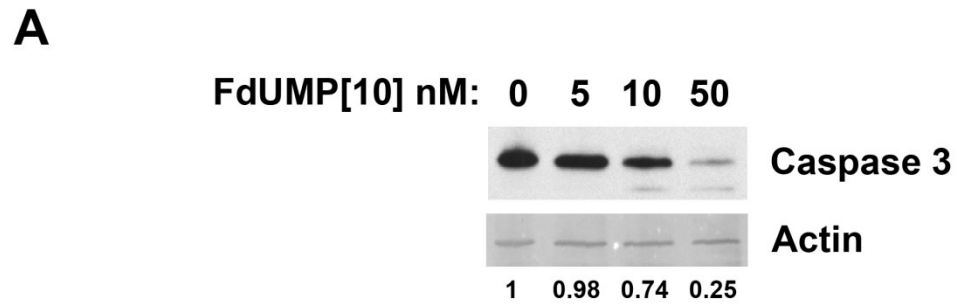
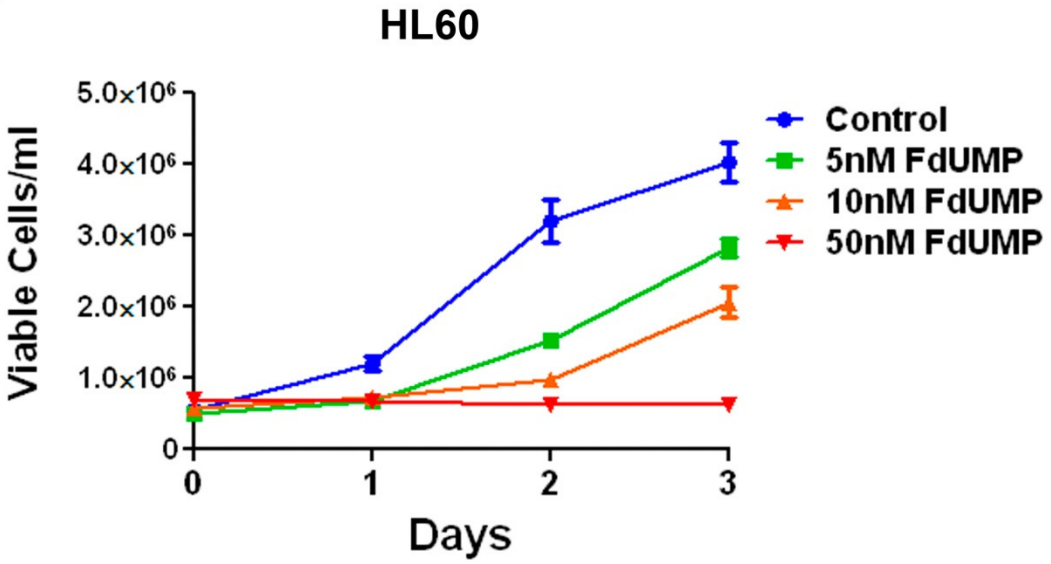

A
FdUMP[10] nM: 0 5 10 50
Caspase 3
Actin
1 0.98 0.74 0.25
B
Control
100nM 5-FU
1nM FdUMP
MLL-ENL
Flt3 ITD
MLL-ENL
NRas G12D
PI
Annexin V
C
Control
100nM Ara-C
1μM Ara-C
100nM FdUMP
PI
Annexin V

Figure S7.

A


HL60
Viable Cells/ml
5.0x10⁶
4.0x10⁶
3.0x10⁶
2.0x10⁶
1.0x10⁶
0
0
1
2
3
Days
Control
5nM FdUMP
10nM FdUMP
50nM FdUMP

B

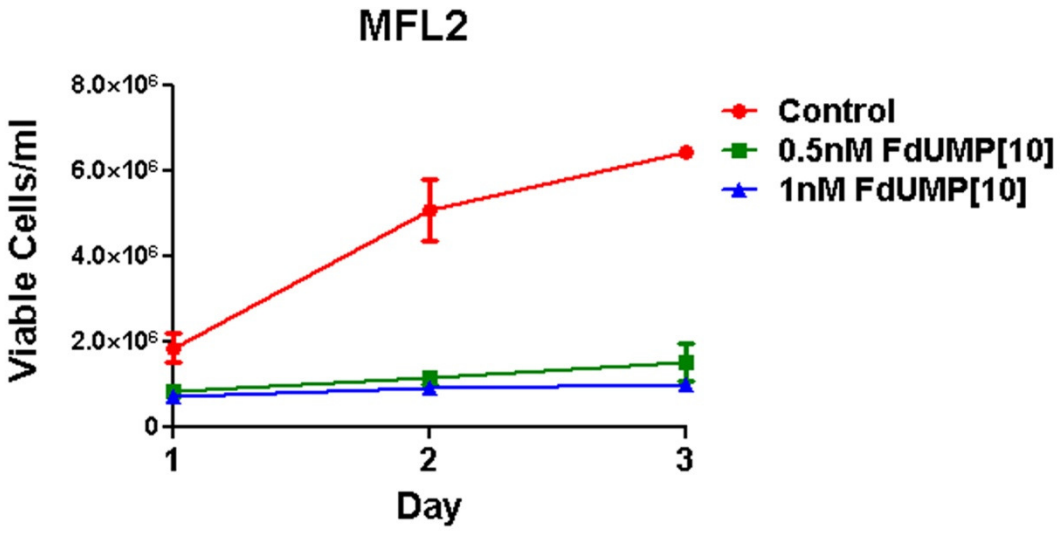
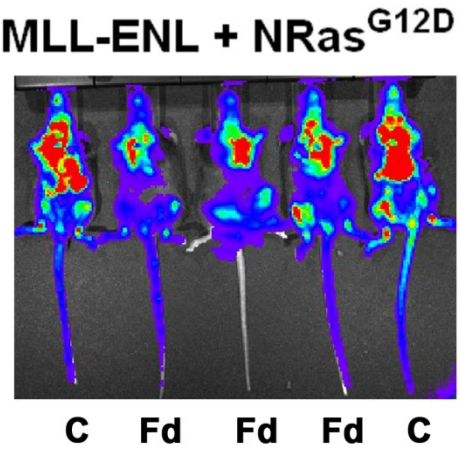
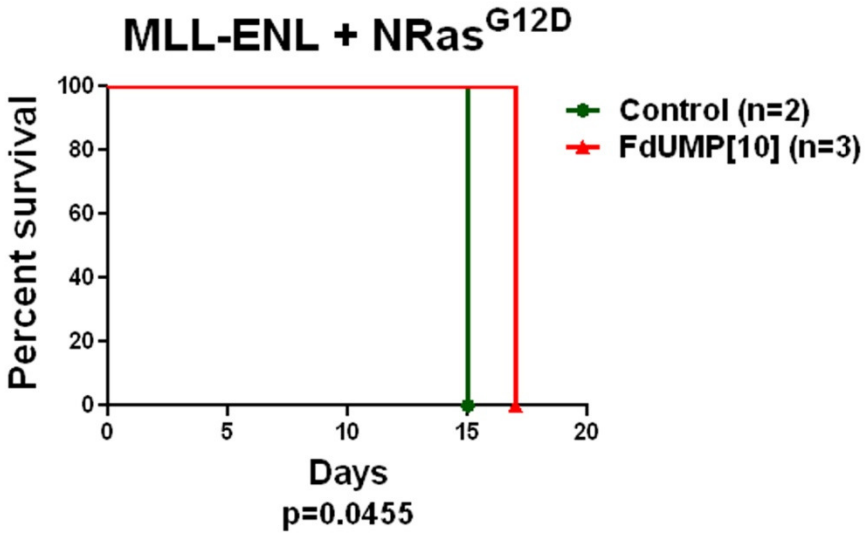

MFL2
Viable Cells/ml
8.0x10⁶
6.0x10⁶
4.0x10⁶
2.0x10⁶
0
1
2
3
Day
Control
0.5nM FdUMP[10]
1nM FdUMP[10]

Figure S8.

A



B



C

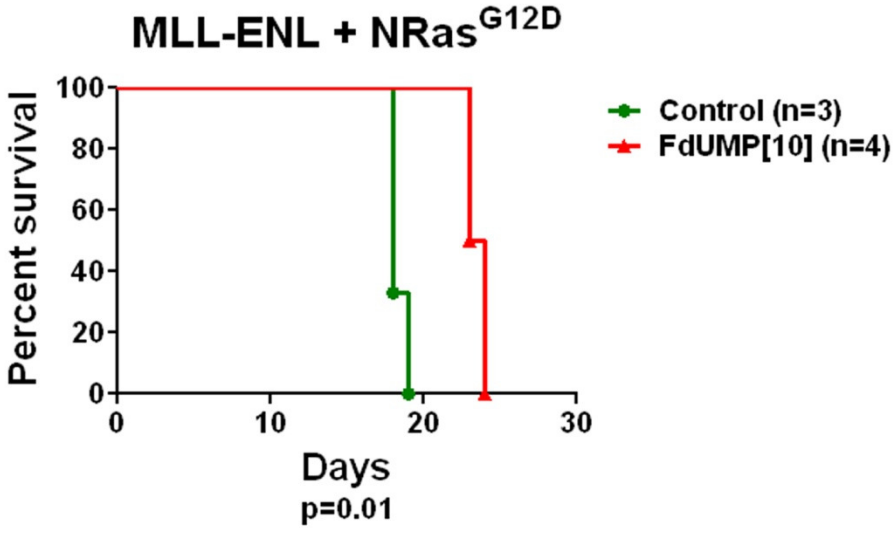
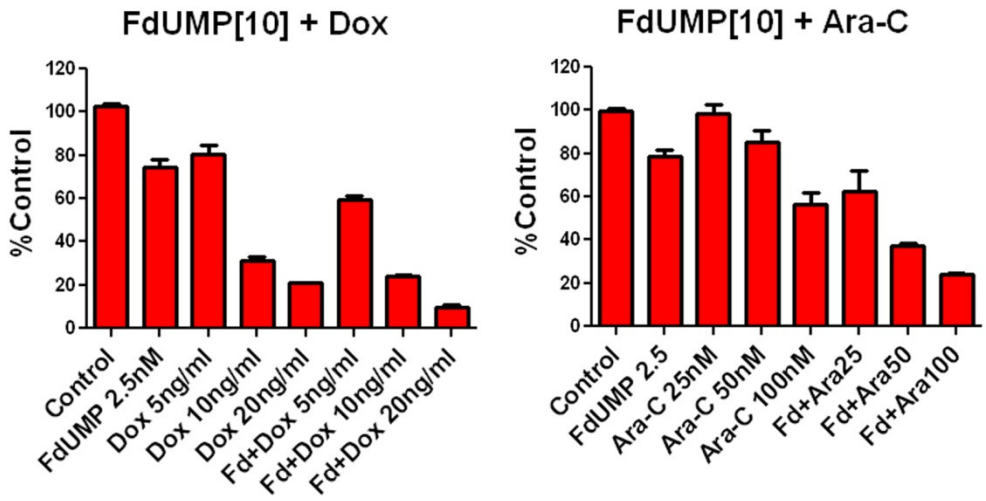


Figure S9.


FdUMP[10] + Dox
% Control
120
100
80
60
40
20
0
Control
FdUMP 2.5nM
Dox 5ng/ml
Dox 10ng/ml
Dox 20ng/ml
Fd+Dox 5ng/ml
Fd+Dox 10ng/ml
Fd+Dox 20ng/ml
FdUMP[10] + Ara-C
% Control
120
100
80
60
40
20
0
Control
FdUMP 2.5
Ara-C 25nM
Ara-C 50nM
Ara-C 100nM
Fd+Ara25
Fd+Ara50
Fd+Ara100