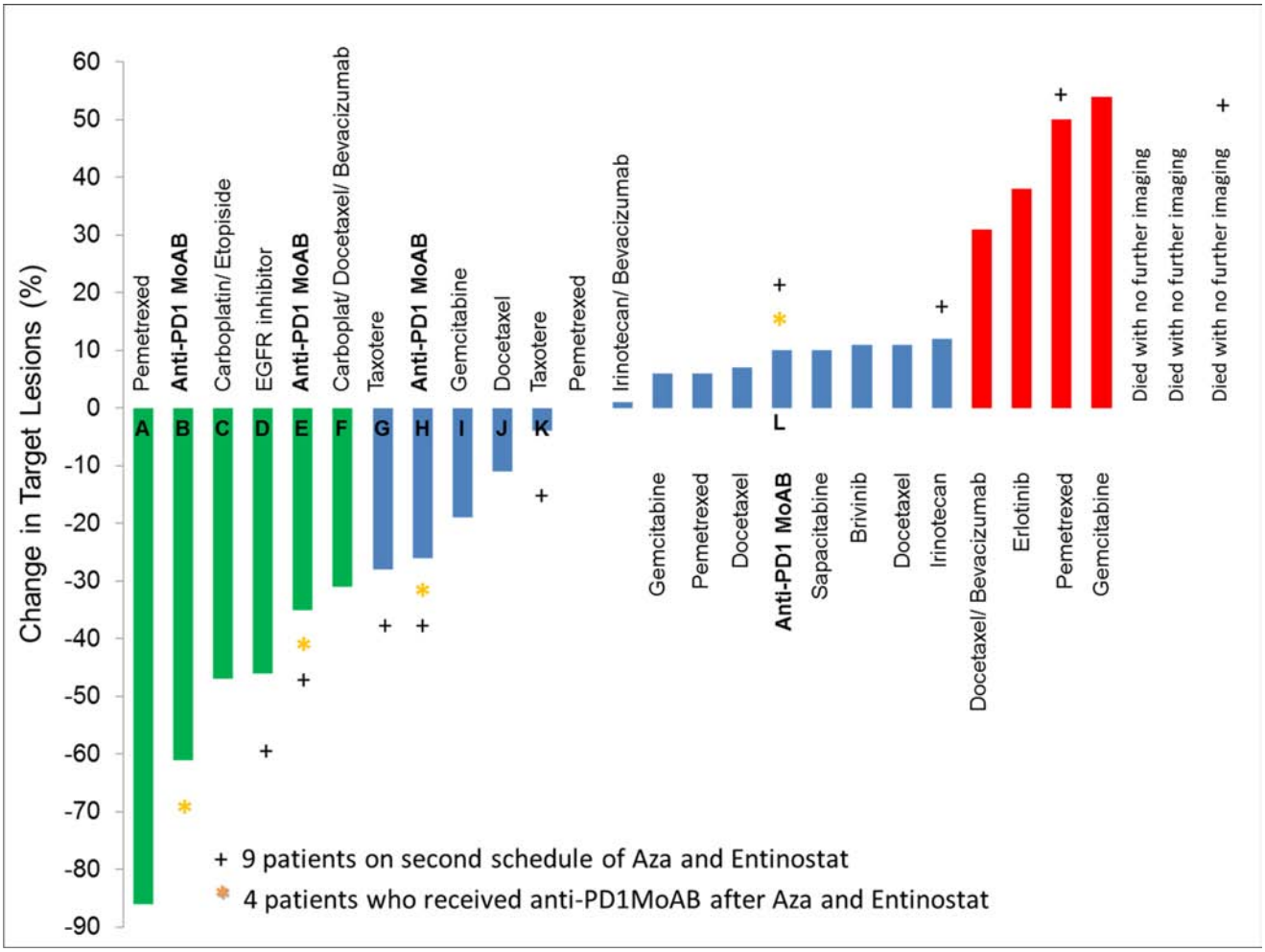
SUPPLEMENTARY METHODS, FIGURES AND TABLE

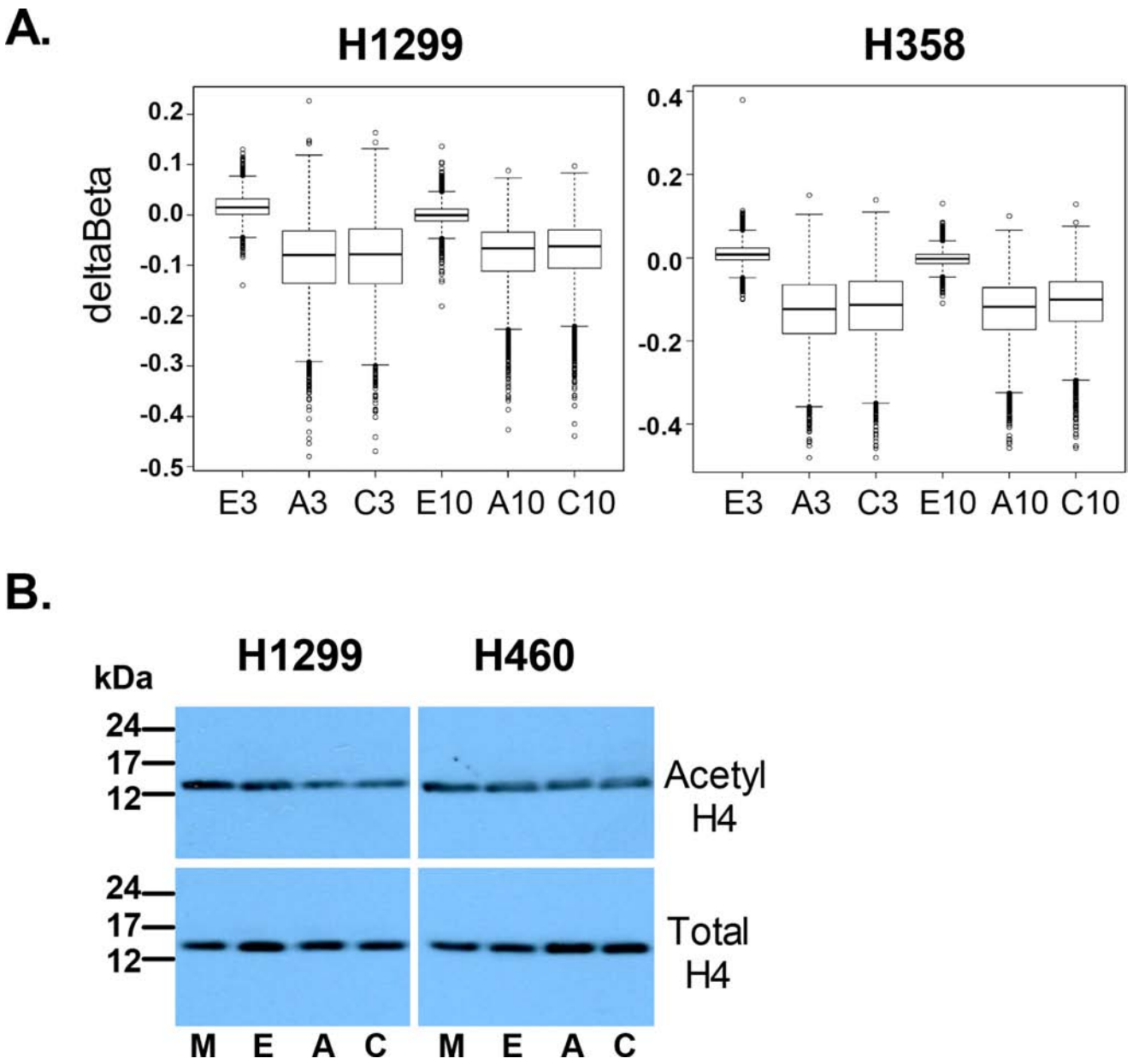
Cell fractionation and sample preparation for Western Blot

Adherent cells were washed with 1x PBS containing 5 mM sodium butyrate (NaB) and directly lysed in 10 mM Tris-HCl (pH 7.9), 0.34 M sucrose, 3 mM CaCl2, 2 mM magnesium acetate, 0.1 mM EDTA, 0.5% Nonidet P-40, protease inhibitors, and 5 mM NaB for 15 min on ice to isolate nuclei. Nuclei were collected by centrifugation (1500 x g for 15 min at 4°C), washed in cytoplasmic lysis buffer without Nonidet P-40, and lysed in 20 mM HEPES (pH 7.9), 3 mM EDTA, 10% glycerol, 150 mM potassium acetate, 1.5 mM MgCl2, 0.1% Nonidet P-40, protease inhibitors, and 5 mM NaB. The insoluble histone-containing pellet was retained and nuclease treated in 150 mM HEPES (pH 7.9), 10% glycerol, 150 mM potassium acetate, 1.5 mM MgCl2, protease inhibitors, and benzonase (250 units) for 10 min at 37°C. After nuclease incubation, an equal volume of 2x Laemmli sample buffer (Bio-Rad) was added. Immediately prior to gel loading, β-mercaptoethanol was added and samples were heated at 95°C for 5 min to reduce and denature proteins. Samples were electrophoresed on NuPage 12% Bis-Tris gels with 1 × MOPS running buffer (Novex, Life Technologies), transferred to 0.2 μm pore PVDF membranes.

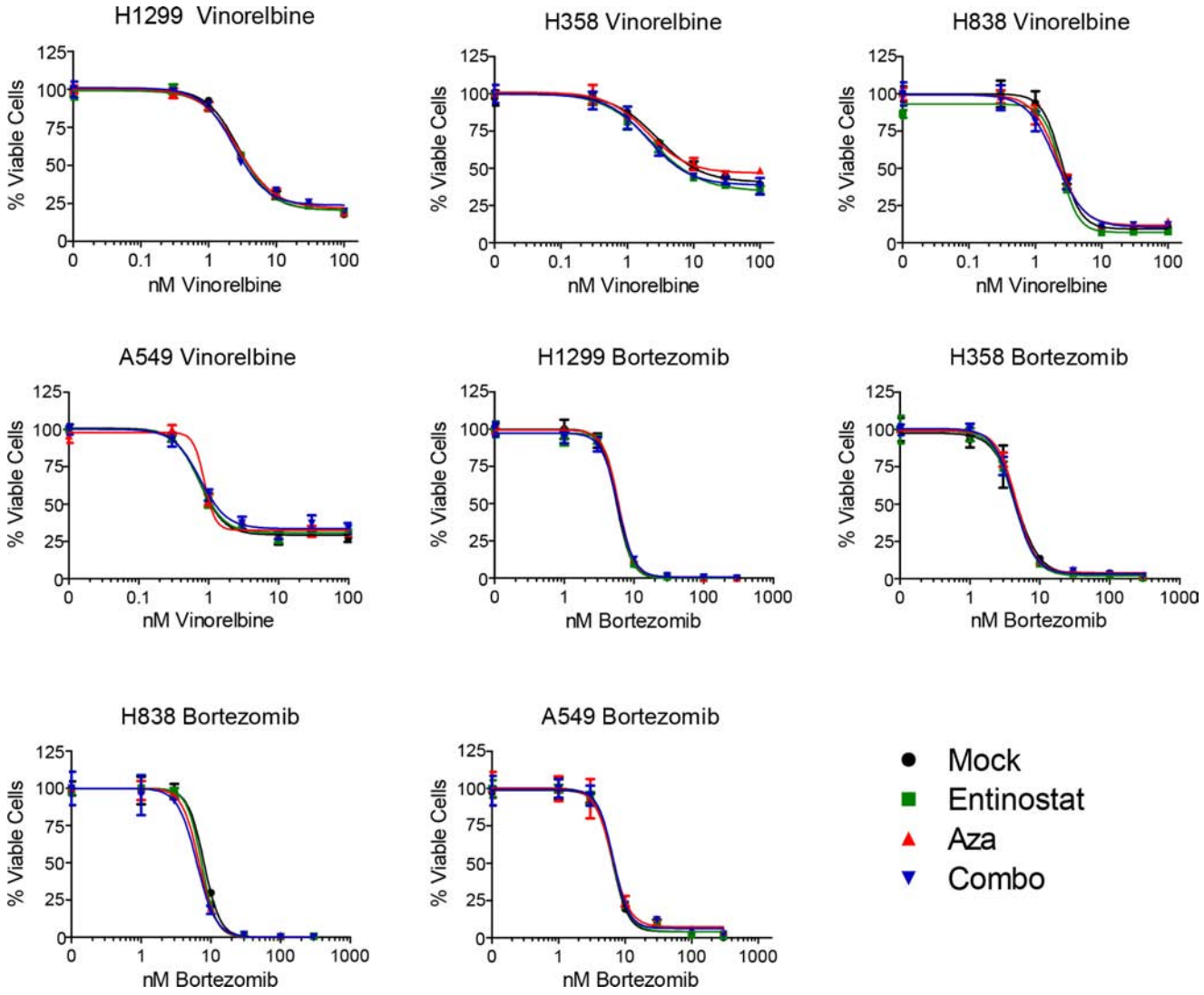
SUPPLEMENTARY FIGURES AND TABLE



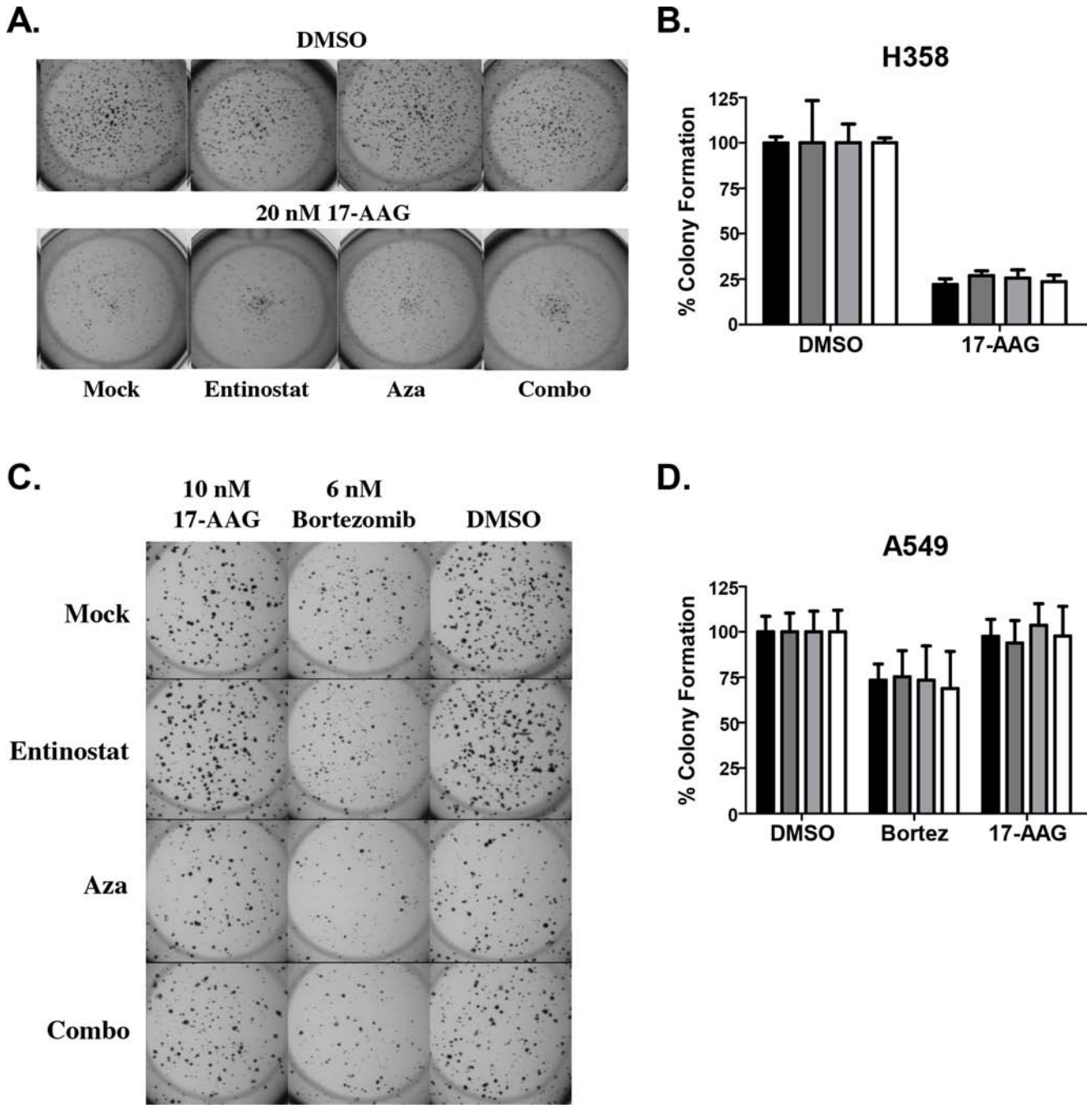
Supplementary Figure S1: Maximal change in tumor size in response to subsequent chemotherapy following combinatorial epigenetic therapy. Green bars represent objective responses by RECIST criteria to specified subsequent treatment regimen as measured by the percent change in maximal diameters of target lesions. Blue bars represent disease stabilization, while red bars indicate disease progression. Graphs updated from Juergens et al., 2011.



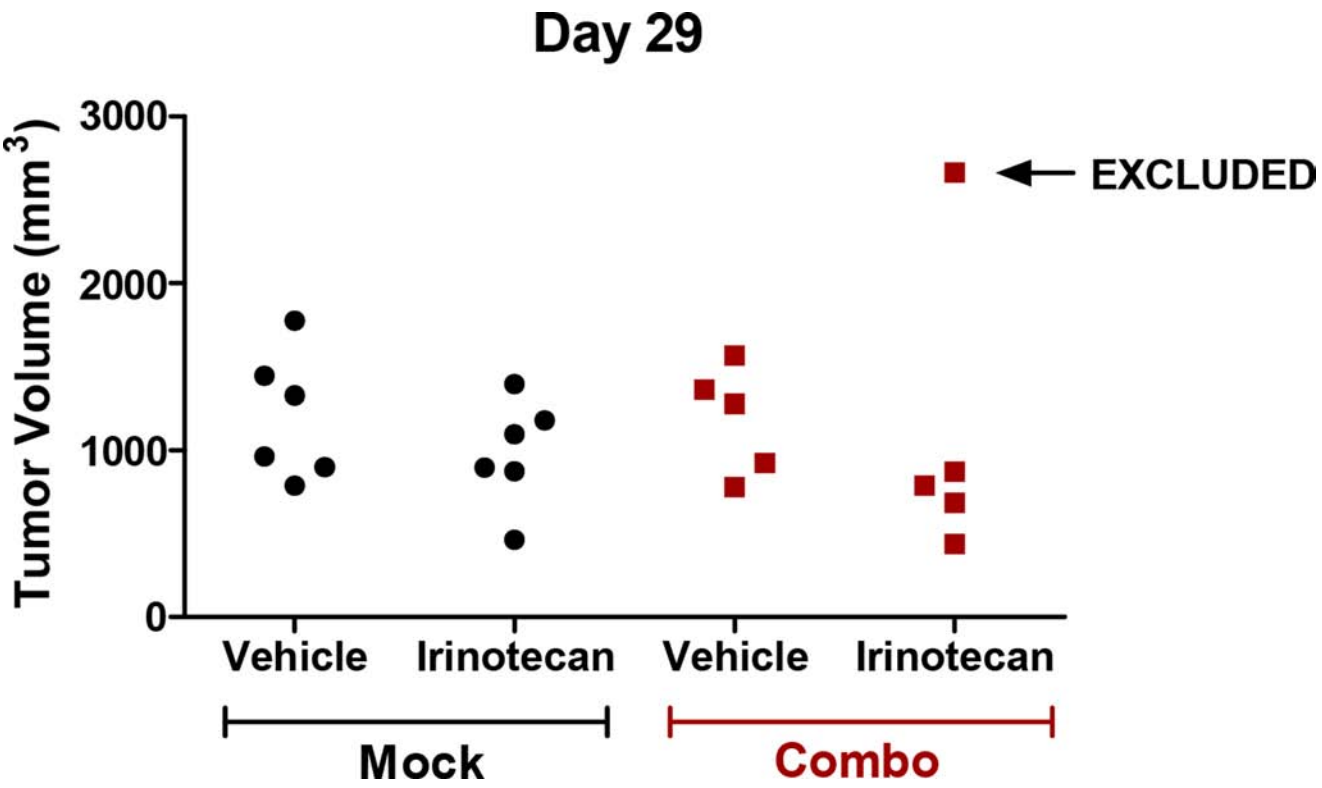
Supplementary Figure S2: Epigenetic changes associated with azacitidine and entinostat treatment. **(A)** Box plots of deltaBeta values depicting promoter region (+/− 1500 bp of transcription start site) demethylation (negative deltaBeta) relative to mock control (probes with Beta >0.5) at day 3 and day 10 following treatment with entinostat (E), Aza (A), or combo (C). **(B)** Western blots depicting acetylated histone H4 (lysine 5, 8, 12, 16) and total histone H4 levels at the end of treatment (day 3) with mock (M), entinostat (E), Aza (A), or combo (C).



Supplementary Figure S3: Epigenetic priming does not alter chemosensitivity of NSCLC cell lines. Log dose response curves for NSCLC cell lines treated with bortezomib or vinorelbine for 72 hours one week post epigenetic therapy. Individual curves represent the percentage of viable cells (+/− standard deviation) for each epigenetic pretreatment condition normalized to its own untreated control cells, such that the highest values for each pretreatment condition represent 100%, and 0 = 0%. Data shown from representative experiments.



Supplementary Figure S4: Epigenetic therapy does not potentiate the effects of chemotherapy on colony growth on Matrigel. H358 and A549 cells were seeded on a solidified Matrigel layer six days after epigenetic therapy. The following day, cells were treated with chemotherapy for 72 hours. Drug was then removed and colonies were permitted to grow 2 – 4 additional days. **(A)** Representative H358 colonies following treatment with 20 nM 17-AAG. **(B)** H358 percent colony formation (+/− standard deviation) relative to untreated control (DMSO), calculated from one representative experiment with five replicates. **(C)** Representative A549 colonies following treatment with 6 nM bortezomib or 10 nM 17-AAG. **(D)** A549 percent colony formation (+/− standard deviation) relative to untreated control (DMSO), averaged from two independent experiments (total nine replicates). Statistical significance by ANOVA with Tukey's multiple comparison test denoted as follows: $*p < 0.05$, $**p < 0.01$.



Supplementary Figure S5: Response of A549 xenografts to irinotecan is augmented by epigenetic therapy. NOD/SCID mice bearing subcutaneous hind flank tumors established from A549 cells treated *in vitro* with mock or the combination of Aza and entinostat (combo) were randomized to receive 10 mg/kg irinotecan (days 2 & 5) or saline vehicle for three one-week cycles. Individual volumes for each animal at day 29 are shown. One tumor was deemed an outlier and excluded from subsequent statistical analysis.

Supplementary Table S1. Calculated IC_{50} values for chemotherapy following epigenetic priming. IC_{50}, 95% CI, and R^2 calculated from representative experiments with three replicates per dose tested. ND denotes IC_{50} values were not determined.

Cell Line	Condition	IC50	95% CI	R^2
Cisplatin				
H1299	Mock	1.78 μM	1.40, 2.26	0.971
	Entinostat	1.72 μM	1.21, 2.44	0.964
	Aza	1.40 μM	1.10, 1.77	0.980
	Combination	1.53 μM	0.93, 2.51	0.940
H358	-	ND	-	-
H838	-	ND	-	-
A549	Mock	2.98 μM	2.34, 3.80	0.972
	Entinostat	3.35 μM	1.83, 6.13	0.962
	Aza	3.60 μM	1.64, 7.89	0.878
	Combination	4.21 μM	0.92, 19.36	0.911
Docetaxel				
H1299	Mock	2.35 nM	1.90, 2.89	0.986
	Entinostat	2.30 nM	1.91, 2.78	0.986
	Aza	2.16 nM	1.81, 2.59	0.989
	Combination	2.40 nM	2.11, 2.72	0.992
H838	Mock	1.38 nM	1.15, 1.65	0.986
	Entinostat	1.12 nM	1.01, 1.24	0.995
	Aza	1.26 nM	1.10, 1.44	0.992
	Combination	1.07 nM	0.77, 1.49	0.962
A549	Mock	1.12 nM	0.74, 1.69	0.970
	Entinostat	0.99 nM	0.74, 1.32	0.982
	Aza	1.50 nM	1.09, 2.07	0.981
	Combination	1.08 nM	0.69, 1.69	0.967
Gemcitabine				
H1299	Mock	7.74 nM	6.70, 8.94	0.989
	Entinostat	6.94 nM	5.89, 8.17	0.989
	Aza	5.88 nM	4.38, 7.87	0.966
	Combination	6.08 nM	5.02, 7.34	0.985
H838	Mock	38.55 nM	26.08, 56.98	0.927
	Entinostat	42.75 nM	31.31, 58.38	0.958
	Aza	38.94 nM	27.84, 54.45	0.946
	Combination	44.80 nM	31.63, 63.45	0.951

(*Continued*)

Cell Line	Condition	IC50	95% CI	R^2
Vinorelbine				
H1299	Mock	2.84 nM	2.55, 3.17	0.994
	Entinostat	2.78 nM	2.48, 3.12	0.994
	Aza	2.73 nM	2.40, 3.10	0.993
	Combination	2.39 nM	2.067, 2.76	0.990
H358	Mock	2.84 nM	2.27, 3.56	0.985
	Entinostat	2.35 nM	1.87, 2.95	0.987
	Aza	2.03 nM	1.54, 2.68	0.974
	Combination	2.08 nM	1.55, 2.78	0.972
H838	Mock	2.48 nM	2.21, 2.78	0.990
	Entinostat	2.42 nM	2.14, 2.74	0.990
	Aza	2.17 nM	1.93, 2.45	0.992
	Combination	2.03 nM	1.73, 2.39	0.987
A549	Mock	0.74 nM	0.64, 0.85	0.989
	Entinostat	0.73 nM	0.64, 0.83	0.990
	Aza	0.87 nM	0.37, 2.04	0.981
	Combination	0.75 nM	0.621, 0.91	0.979
17-AAG				
H1299	Mock	99.29 nM	92.06, 107.1	0.995
	Entinostat	94.44 nM	86.83, 102.7	0.996
	Aza	93.99 nM	82.25, 107.4	0.991
	Combination	102.0 nM	91.60, 113.6	0.990
H358	Mock	42.19 nM	29.45, 60.43	0.921
	Entinostat	31.56 nM	28.13, 35.41	0.985
	Aza	41.49 nM	33.84, 50.88	0.971
	Combination	37.57 nM	33.71, 41.87	0.990
A549	Mock	46.38 nM	37.55, 57.29	0.983
	Entinostat	44.42 nM	35.82, 55.08	0.981
	Aza	34.28 nM	28.15, 41.74	0.971
	Combination	37.62 nM	31.07, 45.56	0.990
Bortezomib				
H1299	Mock	5.62 nM	5.06, 6.24	0.997
	Entinostat	6.00 nM	5.25, 6.86	0.996
	Aza	6.00 nM	5.71, 6.29	0.999
	Combination	5.87 nM	5.27, 6.52	0.996

(*Continued*)

Cell Line	Condition	IC50	95% CI	R^2
H358	Mock	4.65 nM	3.88, 5.56	0.983
	Entinostat	4.34 nM	3.88, 4.86	0.993
	Aza	4.62 nM	4.31, 4.96	0.998
	Combination	4.33 nM	3.98, 4.71	0.996
H838	Mock	8.08 nM	6.89, 9.47	0.994
	Entinostat	7.58 nM	6.84, 8.40	0.999
	Aza	6.80 nM	6.18, 7.48	0.997
	Combination	6.49 nM	5.30, 7.95	0.985
A549	Mock	6.40 nM	5.54, 7.40	0.993
	Entinostat	6.41 nM	5.67, 7.26	0.994
	Aza	6.38 nM	5.06, 8.03	0.978
	Combination	6.62 nM	5.40, 8.12	0.987