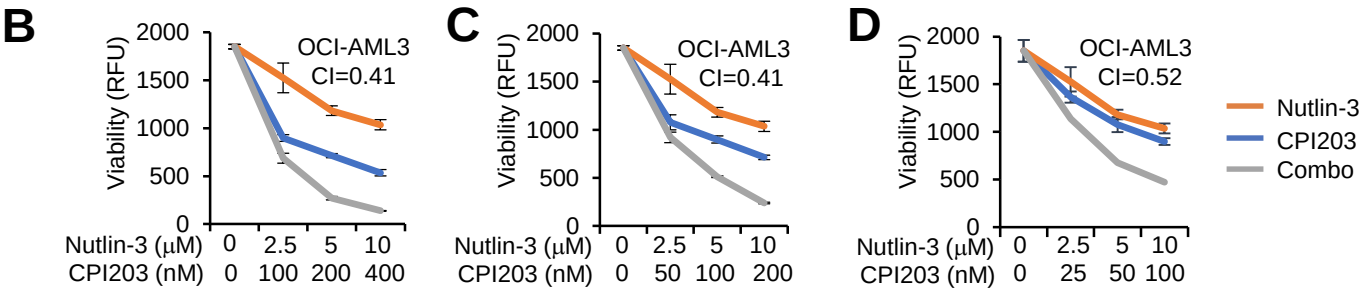
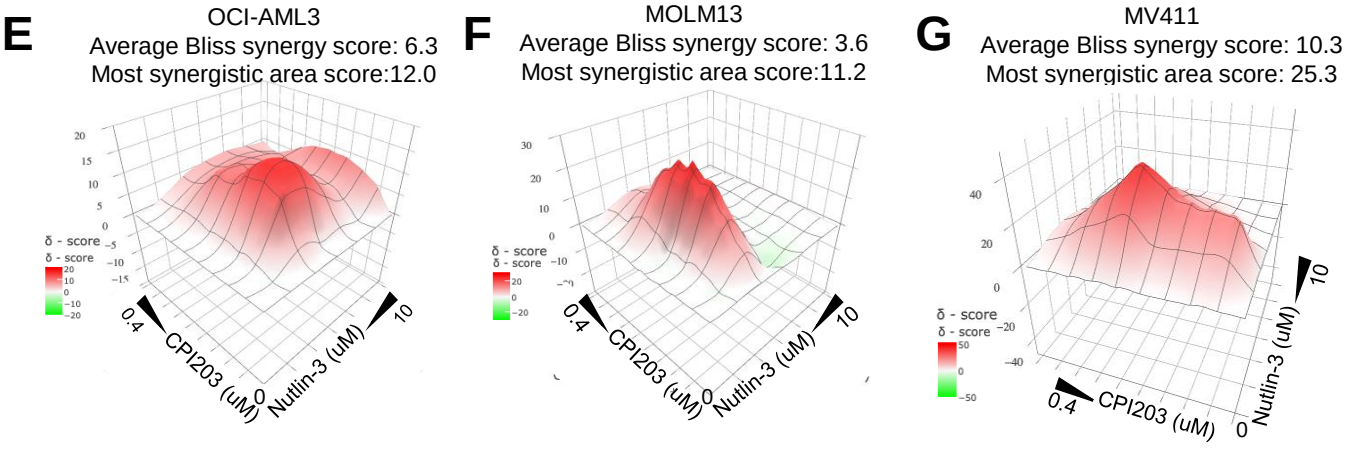
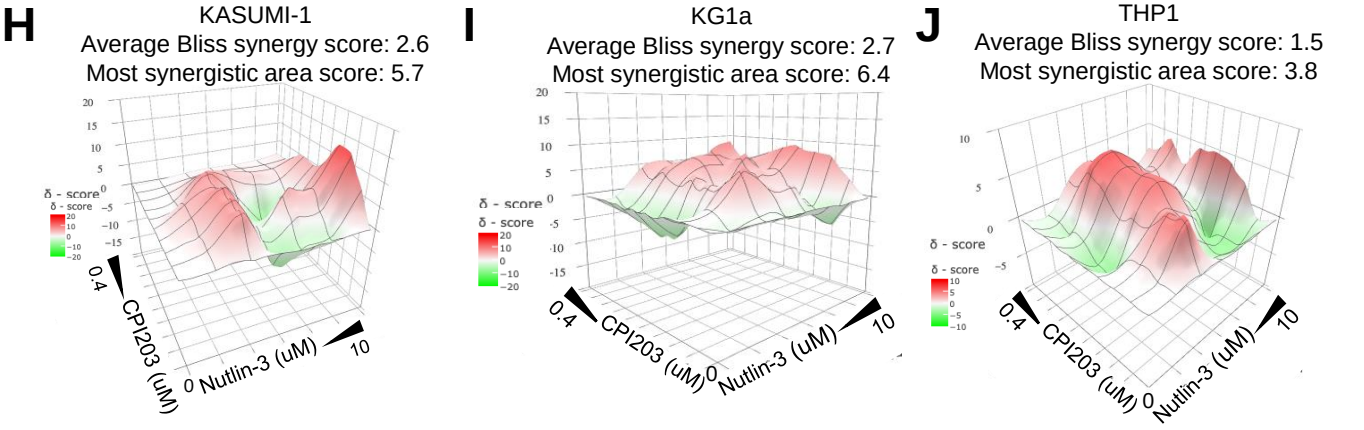
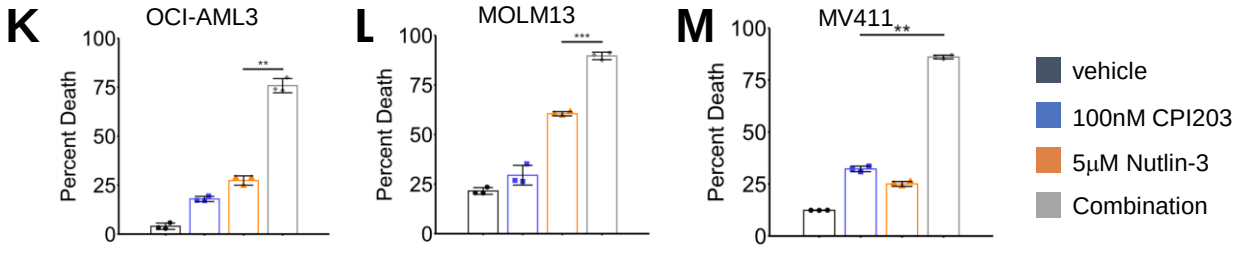
A

	Sample	Cytogenetics	Molecular analysis	TP53 Status
1	BSMS 71	46XY, inv(16)	FLT3-ve, NPM1-ve, CBFB-MYH11	No mutation detected
2	BSMS 74	46XY	FLT3+ve, NPM1+ve	ND
3	BSMS 82	46XY	FLT3-ve, NPM1-ve	ND
4	BSMS 84	46XY	FLT3-ve, NPM1-ve	ND
5	BSMS 87	46XY, del(5)	FLT3-ve, NPM1-ve	ND
6	BSMS 103	46XY	FLT3+ve, NPM1+ve	No mutation detected
7	BSMS 104	48XY, +19, +21	FLT3-ve, NPM1-ve	No mutation detected
8	BSMS 131	46XX	FLT3-ve, NPM1-ve	ND
9	BSMS 136	46XX	N/A	No mutation detected
10	BSMS 256	46XY, del(7)	FLT3-ve, NPM1-ve	ND
11	BSMS 257	46XX	FLT3-ve, NPM1-ve, TET2+ve	ND
12	BSMS 267	46XY	FLT3-ve, NPM1+ve	ND
13	BSMS 269	46XY, del (7)	FLT3-ve, NPM1-ve	ND
14	BSMS 270	46XY	N/A	ND
15	BSMS 273	46XY	N/A	ND

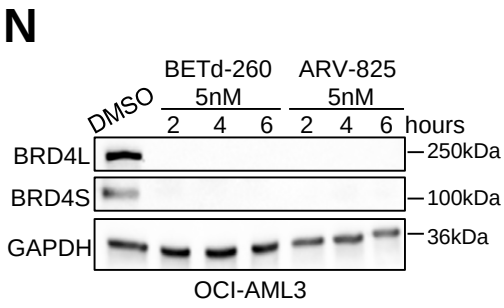


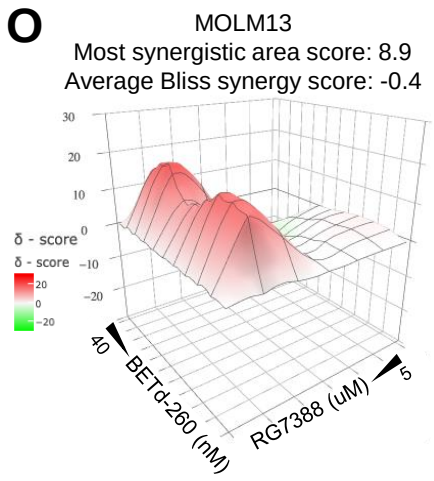


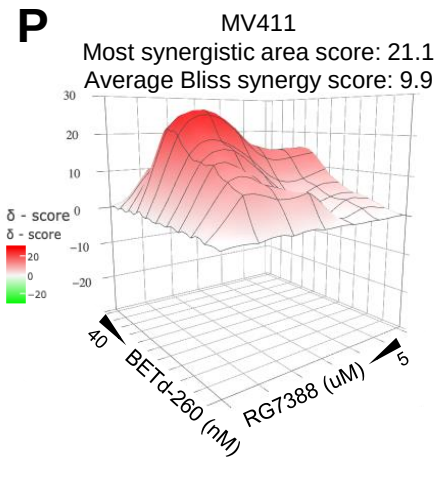


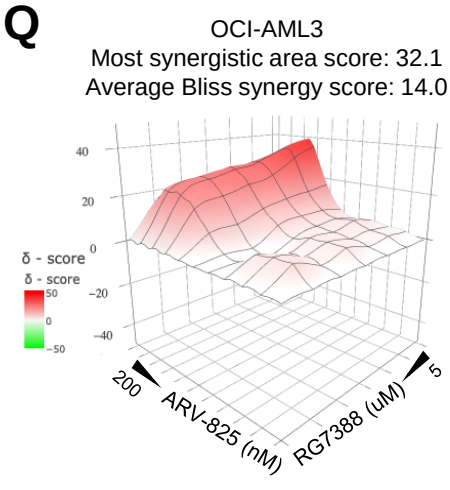


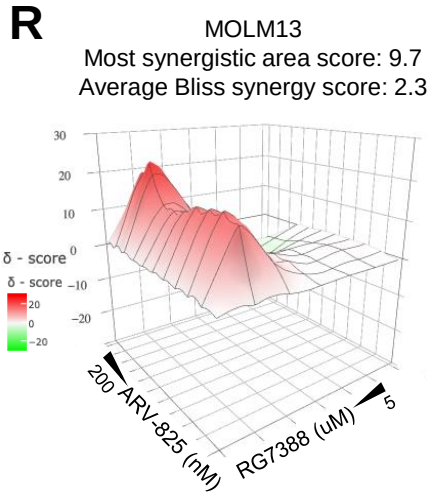
Supp Figure 1 (continued)

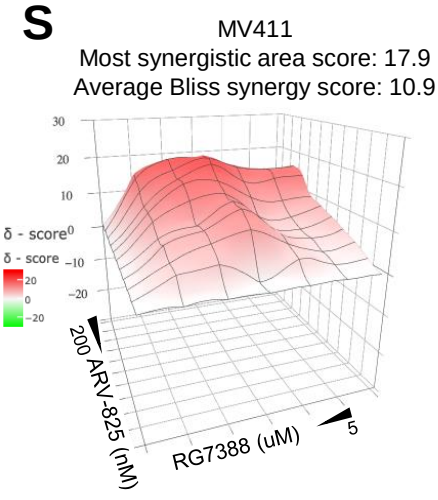












Supp Figure 1. MDM2 and BET inhibitors are synergistically lethal to primary human AML blasts and AML cell lines with wild-type *TP53*.

A. Summary of the cytogenetic, molecular features and *TP53* status of the 15 primary human AML samples in Figure 1A. FLT3+ve denotes mutation for FLT3 (FLT3-ITD) and FLT3-ve denotes WT. NPM1+ve denotes recurrent AML mutation. NMP1-ve denotes WT. For the 4 indicated samples, all exons of TP53 were sequenced, but no mutations detected. ND: not determined. N/A: not available.

B-D. OCI-AML3 cell viability, resazurin assay 72 hours, treatment ratio CPI203:nutlin-3 indicated drug concentrations (Means +/- SD, n=3).

E-J. Excess Over Bliss (EOB) plot between Nutlin-3 and CPI203 in indicated cells. Cell viability by CellTiter-Glo, 72 hours. Bliss score, n=4.

K. Cell kill in OCI-AML3 cells using annexin-V and propidium iodide, (72hrs) (***=p≤0.001). Percent death = sum of percent single (annexin-V+ or propidium iodide+) and double positive cells (two tailed unpaired t-test, Means +/- SD, n=3).

L. Cell kill in the MOLM13 cells using annexin-V and propidium iodide, (72hrs) (***=p≤0.001). Percent death = sum of percent single (annexin-V+ or propidium iodide+) and double positive cells (two tailed unpaired t-test, Means +/- SD, n=3).

M. Cell kill in the MV411 cells using annexin-V and propidium iodide, (72hrs) (**=p≤0.01). Percent death = sum of percent single (annexin-V+ or propidium iodide+) and double positive cells (two tailed unpaired t-test, Means +/- SD, n=3).

N. Western blot of BRD4 in OCI-AML3 cells treated with BETd-260 or ARV-825 at 5nM.

O. EOB plot between RG7388 and BETd-260 in MOLM13 cells. Cell viability by CellTiter-Glo after 24 hours. Bliss score, n=4.

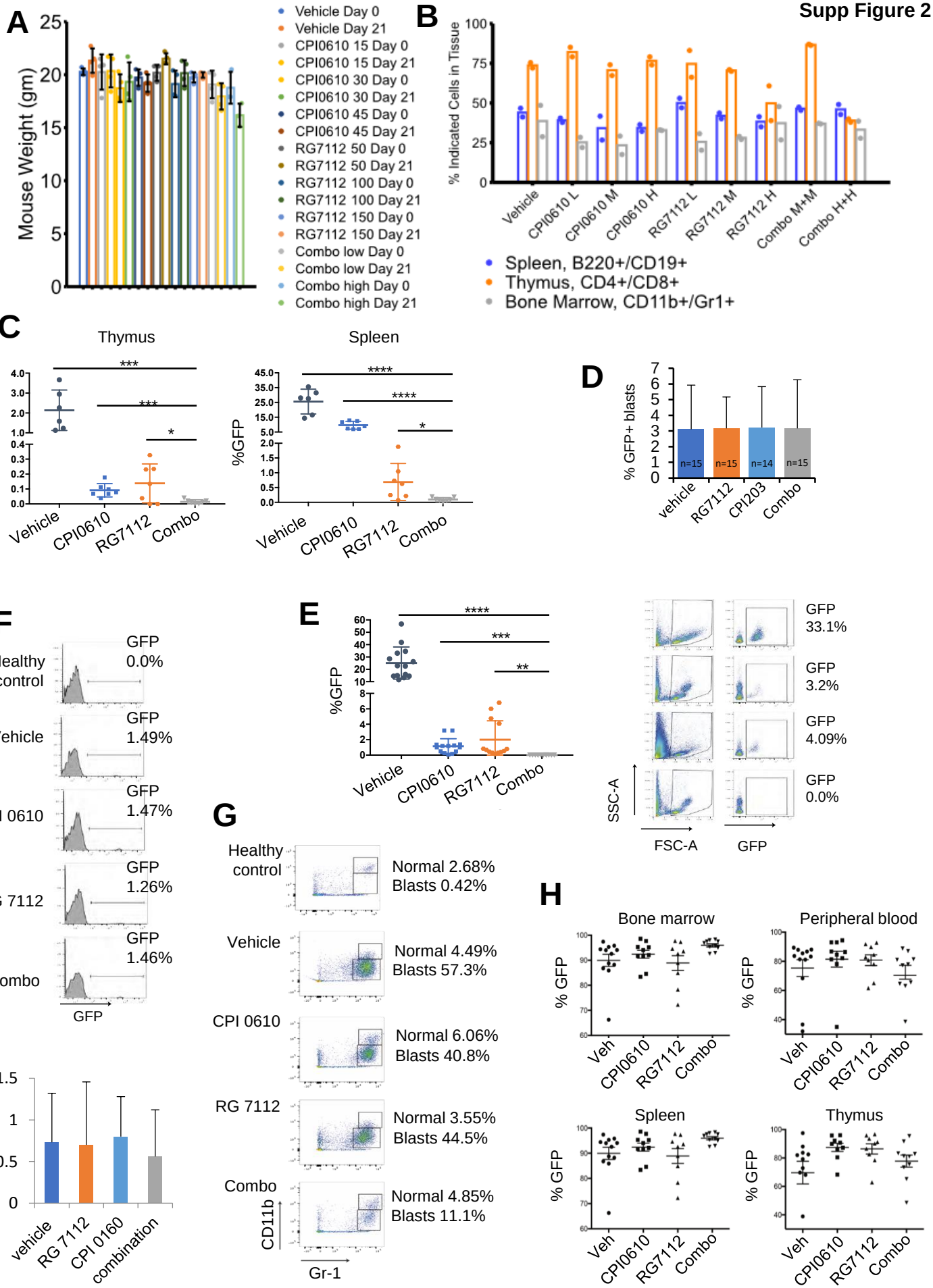
P. EOB plot between RG7388 and BETd-260 in MV411 cells. Cell viability by CellTiter-Glo after 24 hours. Bliss score, n=4.

Q. EOB plot showing synergistic effects between RG7388 and ARV-825 in OCI-AML3 cells. Cell viability by CellTiter-Glo after 24 hours. Bliss score, n=4.

R. EOB plot between RG7388 and ARV-825 in MOLM13 cells. Cell viability by CellTiter-Glo after 24 hours. Bliss score, n=4.

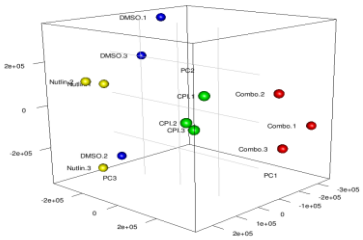
S. EOB plot between RG7388 and ARV-825 in MV411 cells. Cell viability by CellTiter-Glo after 24 hours. Bliss score, n=4.

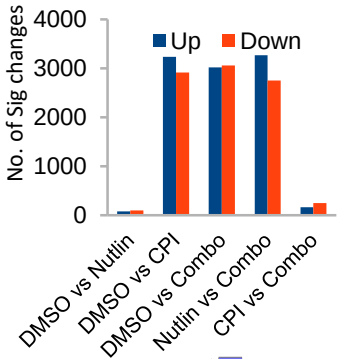
Supp Figure 2

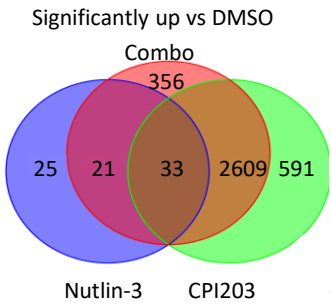


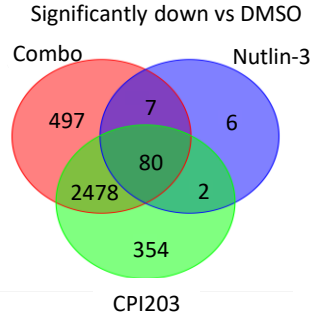
Supp Figure 2. MDM2 and BET inhibitors cooperate to eradicate AML in *in vivo* mouse models.
A. Normal non-leukemic mouse weights at indicated daily drug doses (mg/kg) at day 0 and on completion of drug treatment (day 21). For combo, low dose = 30mg/kg CPI0610 and 100mg/kg RG7112; high dose = 45mg/kg CPI0610 and 150mg/kg RG7112 (Means +/- SD are shown, n = 4 for all except for combo high day 0 and combo high day 21, where n = 3).
B. Percent of indicated cell types in indicated tissue in normal non-leukemic mice treated with vehicle, low, medium or high dose of CPI0610 (15, 30 or 45mg/kg), RG7112 (50, 100, 150mg/kg) or combination (M+M = 30mg/kg CPI0610 and 100mg/kg RG7112; H+H = 45mg/kg CPI0610 and 150mg/kg RG7112).
C. Disease burden (percent GFP+ blasts in thymus or spleen) in Trib2 mice after 21 days of drug treatment (****=p≤0.0001, ***=p≤0.001, *=p≤0.05, by two tailed unpaired t-test, Means +/- SD, n = 6 for vehicle, n=7 for single drug and combo conditions).
D. Disease burden (percent GFP+ blasts in peripheral blood) in Trib2 mice pre-drug treatment (Error bars are SD, n for each group labeled)
E. Disease burden (percent GFP+ blasts in peripheral blood) in Trib2 treated mice after 21 days treatment in peripheral blood according to treatment condition. Right panel shows representative FACS data (****=p≤0.0001, ***=p≤0.001, **=p≤0.01, two tailed unpaired t-test, Means +/- SD, n=14, gating strategy for sorting GFP Positive cells was live cells>cell profile>single cells>GFP positive cells).
F. MLL-AF9 disease burden (percent GFP+ blasts in peripheral blood) pre-drug treatment. Top shows representative FACS data (Gating strategy for sorting GFP Positive cells was live cells>cell profile>single cells>GFP positive cells.).
G. Representative MLL-AF9 disease burden in peripheral blood, based on percent $CD11b^{low}$ Gr1-expressing immature blasts known to expand in AML (Keeshan et al. 2006), after 7 days of drug treatment. Also shown are the more normal, mature myeloid cells ($CD11b^{high}$ Gr1+) (Gating strategy for sorting GFP Positive cells was live cells>cell profile>single cells>GFP positive cells).
H. % MLL-AF9 GFP+ blasts at time of cull in indicated tissues after indicated drug treatments.

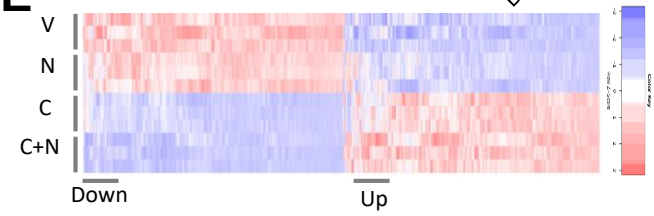
Supp Figure 3

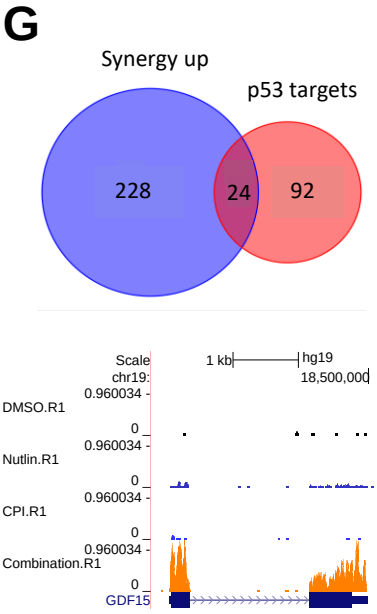


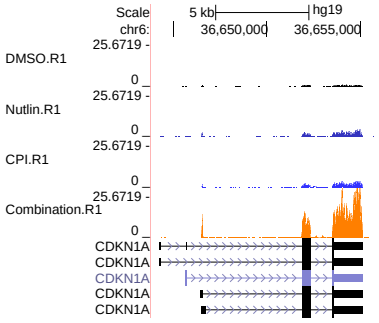


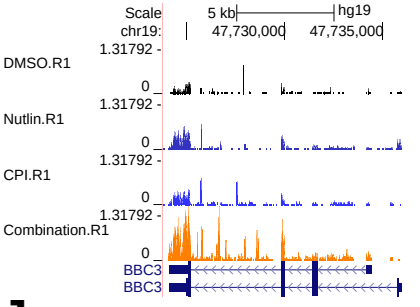




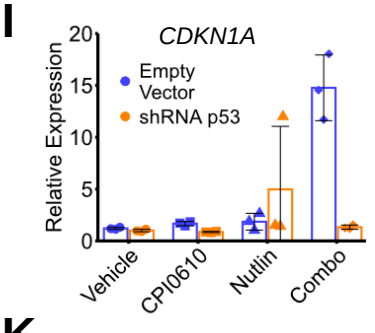


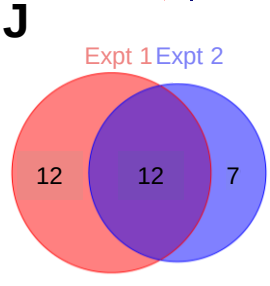


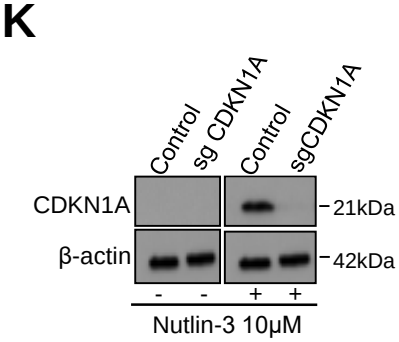


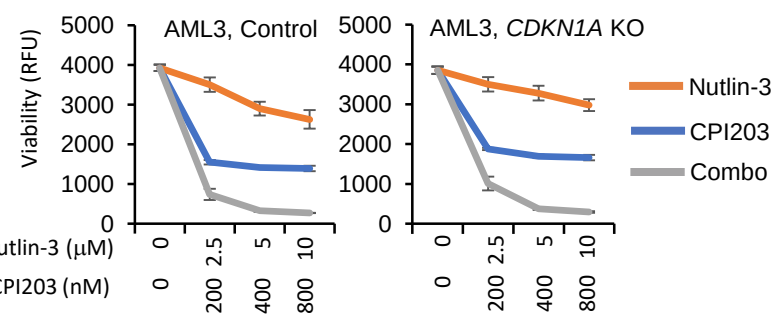


Synergy Down genes	
Ingenuity Canonical Pathways	-log(p-value)
Mitotic Roles of Polo-Like Kinase	3.74
Cell Cycle: G2/M DNA Damage Checkpoint Regulation	2.92
Hereditary Breast Cancer Signaling	2.36
Myc Mediated Apoptosis Signaling	2.32
DNA Methylation and Transcriptional Repression Signaling	2.04
3-phosphoinositide Biosynthesis	1.94
5-aminoimidazole Ribonucleotide Biosynthesis I	1.9
Coenzyme A Biosynthesis	1.9
Diphthamide Biosynthesis	1.9
Pyrimidine Ribonucleotides Interconversion	1.82
Synergy Up genes	
Ingenuity Canonical Pathways	-log(p-value)
p53 Signaling	8.33
SAPK/JNK Signaling	3.53
Autophagy	3.25
IL-8 Signaling	3.17
Agrin Interactions at Neuromuscular Junction	2.76
Molecular Mechanisms of Cancer	2.74
NRF2-mediated Oxidative Stress Response	2.67
Pancreatic Adenocarcinoma Signaling	2.59
HMGB1 Signaling	2.39
TNFR2 Signaling	2.36



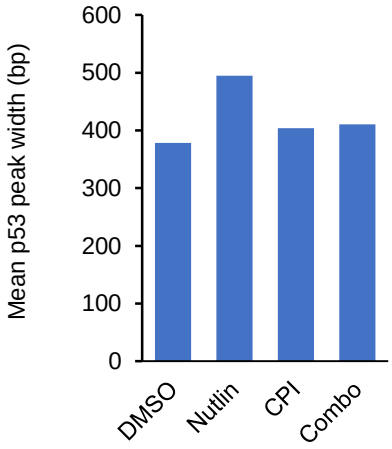


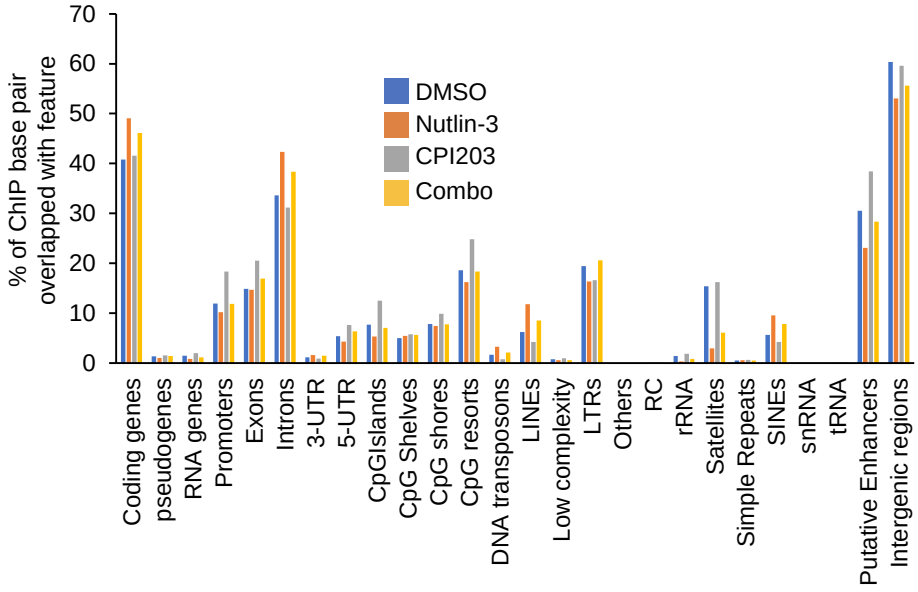


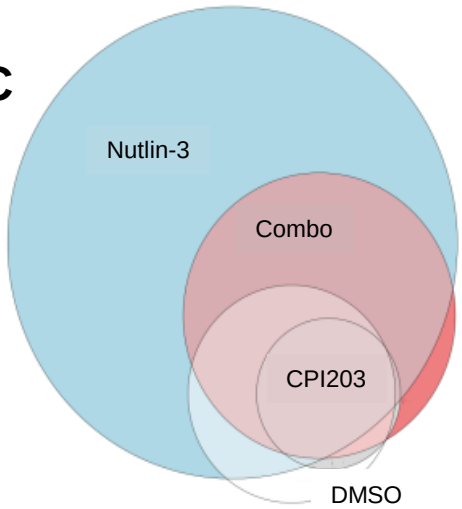


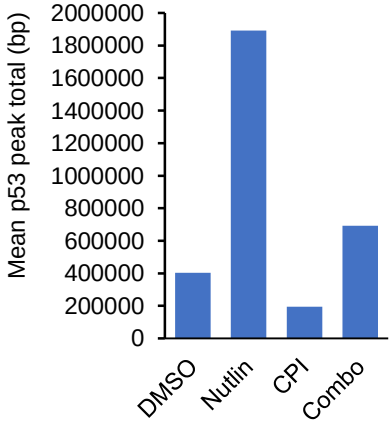
Supp Figure 3. BET inhibitors potentiate activation of p53 target genes by p53.
A. A principal component analysis of RNA-seq data of the OCI-AML3 cell line (from Figure 3E), according to treatment condition.
B. Number of significant gene expression changes between treatment conditions in RNA-seq data of treated OCI-AML3 cells .
C. Venn diagram of genes significantly up-regulated in RNA-seq data, according to treatment condition of OCI-AML3 cells.
D. Venn diagram of genes significantly down-regulated in RNA-seq data, according to treatment condition of OCI-AML3 cells.
E. Heat map of all significant changes in gene expression in RNA-seq data of OCI-AML3 cells according to treatment condition.
F. An IPA analysis of synergistically up-regulated and down-regulated genes (synergy as defined in Figure 3E, p-values from fisher's exact test).
G. Venn diagram showing the number of high-confidence p53 target genes synergistically up-regulated by the drug combination**.**
H. Representative examples of RNA-seq gene tracks for the p53 target genes *CDKN1A*, *GDF15* and *BBC3*, according to treatment condition.
I. qPCR assessment of expression of CDKN1A in control (empty-vector) OCI-AML3 cells and shRNA p53 OCI-AML3 cells, according to treatment condition (Means +/- SD are shown, n=3)**.**
J. Venn diagram showing number of expression changes in p53 target genes in OCI-AML3 cells expressing wild-type p53 in the first and second RNA-seq experiments (i.e. Figures 3F and 3N, respectively).
K. Western blot analysis of control (empty-vector) OCI-AML3 cells and CRISPR/CAS9 CDKN1A knock out OCI-AML3 cells, treated with vehicle or 10μM nutlin-3 (left); and a resazurin analysis of control (empty-vector) OCI-AML3 cells and CDKN1A knock out OCI-AML3 cells according to treatment condition, following 72 hours of drug treatment (right) (Means +/- SD are shown, n=3).

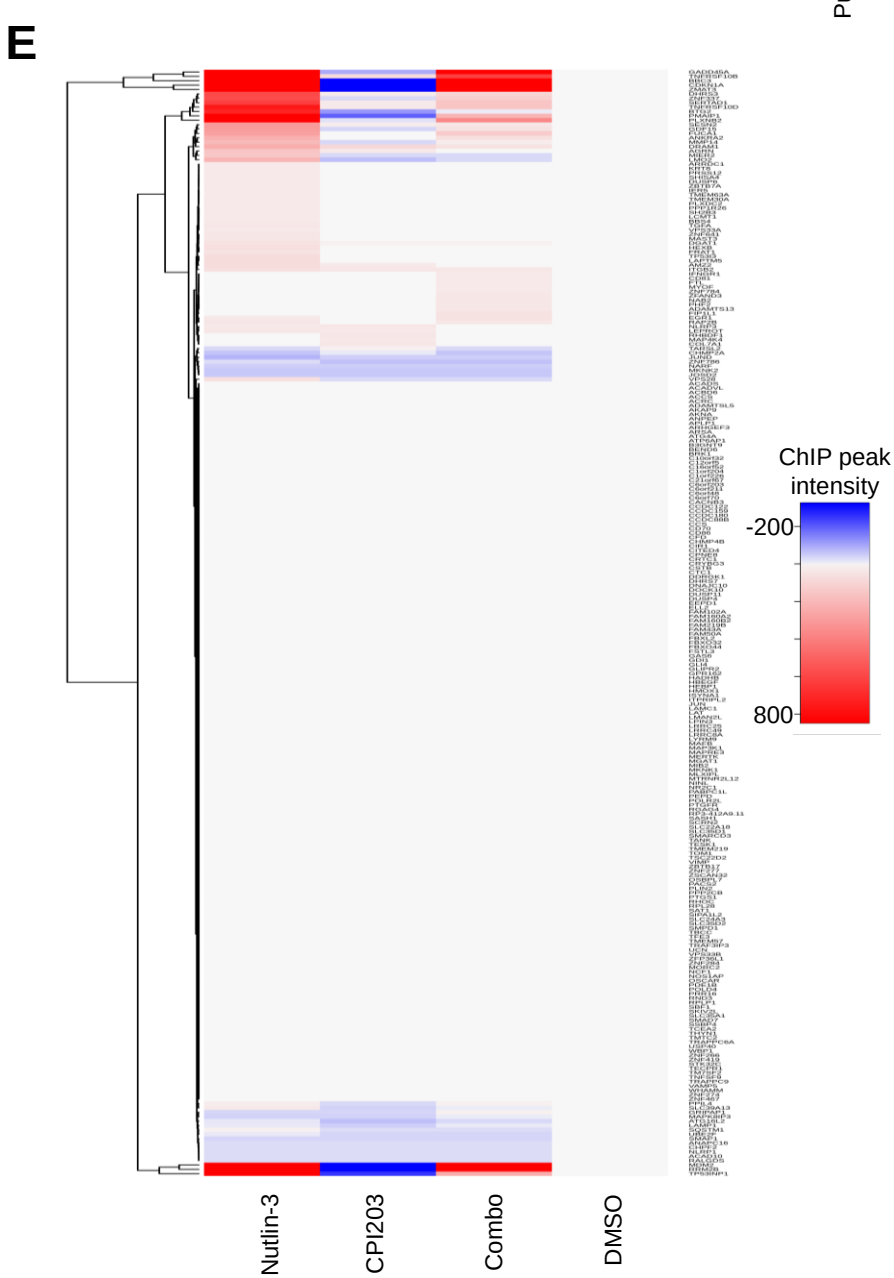
Supp Figure 4











Supp Figure 4. BET inhibitors do not stabilize p53 target mRNAs nor increase binding of p53 to target genes.

A. Mean p53 peak width in base pairs (peaks present in at least 2 out of 3 ChIP-seq replicates; mean of 2-3 replicates) after the indicated drug treatments of OCI-AML3 cells.

B. Genomic distribution of p53 binding sites identified by ChIP-seq (peaks present in at least 2 out of 3 replicates) after the indicated drug treatments of OCI-AML3 cells.

C. Venn diagram showing relative overlap of p53 ChIP-seq peaks (peaks present in at least 2 out of 3 replicates) after the indicated drug treatments of OCI-AML3 cells.

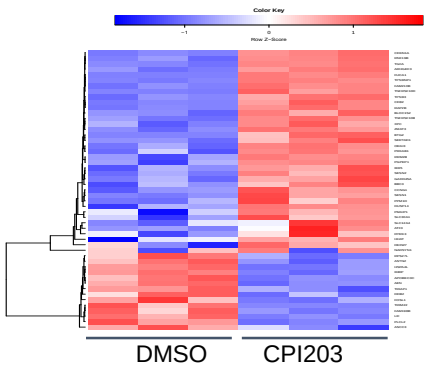
D. Mean total base pairs covered by p53 ChIP-seq peaks (peaks present in at least 2 out of 3 replicates; mean of 2-3 replicates) after the indicated drug treatments of OCI-AML3 cells.

E. Heat map of p53 ChIP-seq peaks (peaks present in at least 2 out of 3 replicates) at 252 synergy up genes (rows) after the indicated drug treatments (columns) of OCI-AML3 cells.

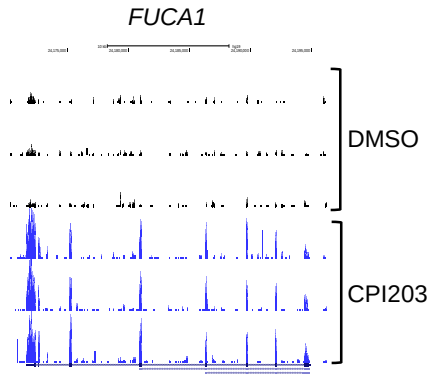
A

Rank	Upstream Regulator	P-value of overlap	Rank	Upstream Regulator	P-value of overlap
1	E2F4	1.13E-24	6	FOXM1	2.02E-12
2	NUPR1	1.17E-23	7	CCND1	6.38E-11
3	MYC	4.16E-21	8	SPI1	7.13E-10
4	p53	9.60E-19	9	TCF4	9.01E-10
5	E2F1	2.20E-17	10	TP63	4.36E-09

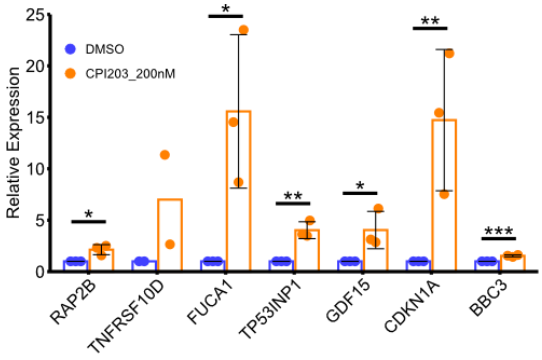
B



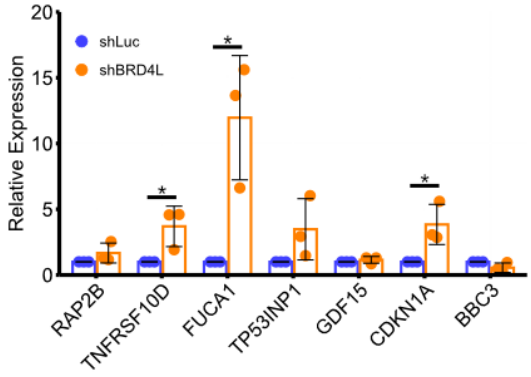
C



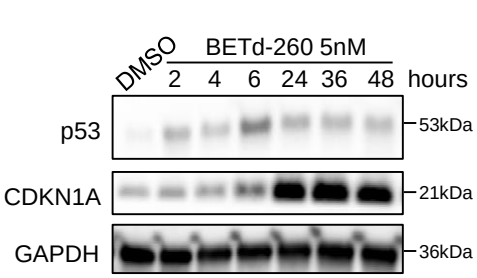
D



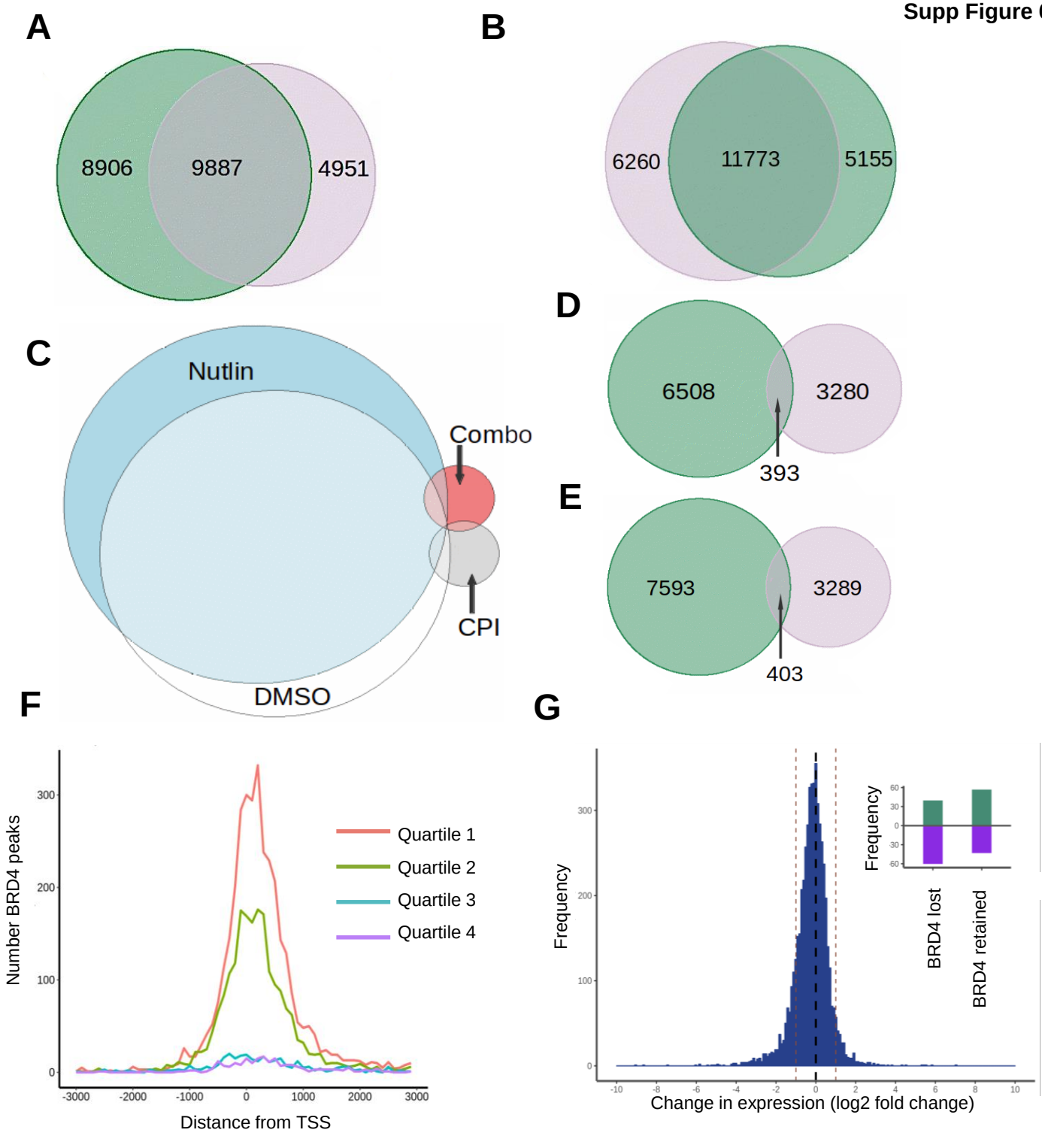
E



F



Supp Figure 5. Inhibition of BET family proteins activates p53.
A. Ingenuity pathway analysis (IPA) identifies p53 as a candidate regulator of genes differentially expressed between control and CPI203-treated AML3 cells. p53 is predicted to be activated, with an activation z-score 5.802 (fisher's exact test).
B. Heat map of all significant changes in gene expression of known p53 target genes (from Ref. 34) in RNA-seq data of OCI-AML3 cells, control (DMSO) vs CPI203 treated AML3 cells (3 replicates of each).
C. RNA-seq sequence tags from control (DMSO) and CPI203-treated AML3 cells aligned to *FUCA1* gene (3 replicates of each).
D. qPCR analysis of indicated p53 target genes in samples from Figure 3E (***=p≤0.001, **=p≤0.01, *=p≤0.05, two tailed unpaired t-test, Means +/- SD are shown, n=3).
E. qPCR analysis of indicated p53 target genes in OCI-AML3 cells expressing small hairpin RNA(shRNA) specific for luciferase gene (shLuc, control) or BRD4L (shBRD4L) (*=p≤0.05, two tailed unpaired t-test, Means +/- SD are shown, n=3).
F. Western blot of p53 and CDKN1A in OCI-AML3 cells treated with BETd-260 at 5nM for different times.


A
8906
9887
4951
B
6260
11773
5155
C
Nutlin
Combo
CPI
DMSO
D
6508
3280
393
E
7593
3289
403
F
Number BRD4 peaks
Quartile 1
Quartile 2
Quartile 3
Quartile 4
Distance from TSS
G
Frequency
Change in expression (log2 fold change)
BRD4 lost
BRD4 retained
H
DMSO
CPI203
ChIP - P53 Target Genes

Supp Figure 6. BRD4 ChIP-seq data

A. Venn diagram showing overlap of 2 replicates of BRD4 ChIP-seq peaks in OCI-AML3 cells treated with DMSO

B. Venn diagram showing overlap of 2 replicates of BRD4 ChIP-seq peaks in OCI-AML3 cells treated with nutlin-3

C. Venn diagram showing overlap of BRD4 ChIP-seq peaks (peaks present in 2 out of 2 replicates) in OCI-AML3 cells treated with DMSO, nutlin-3, CPI203 or combo.

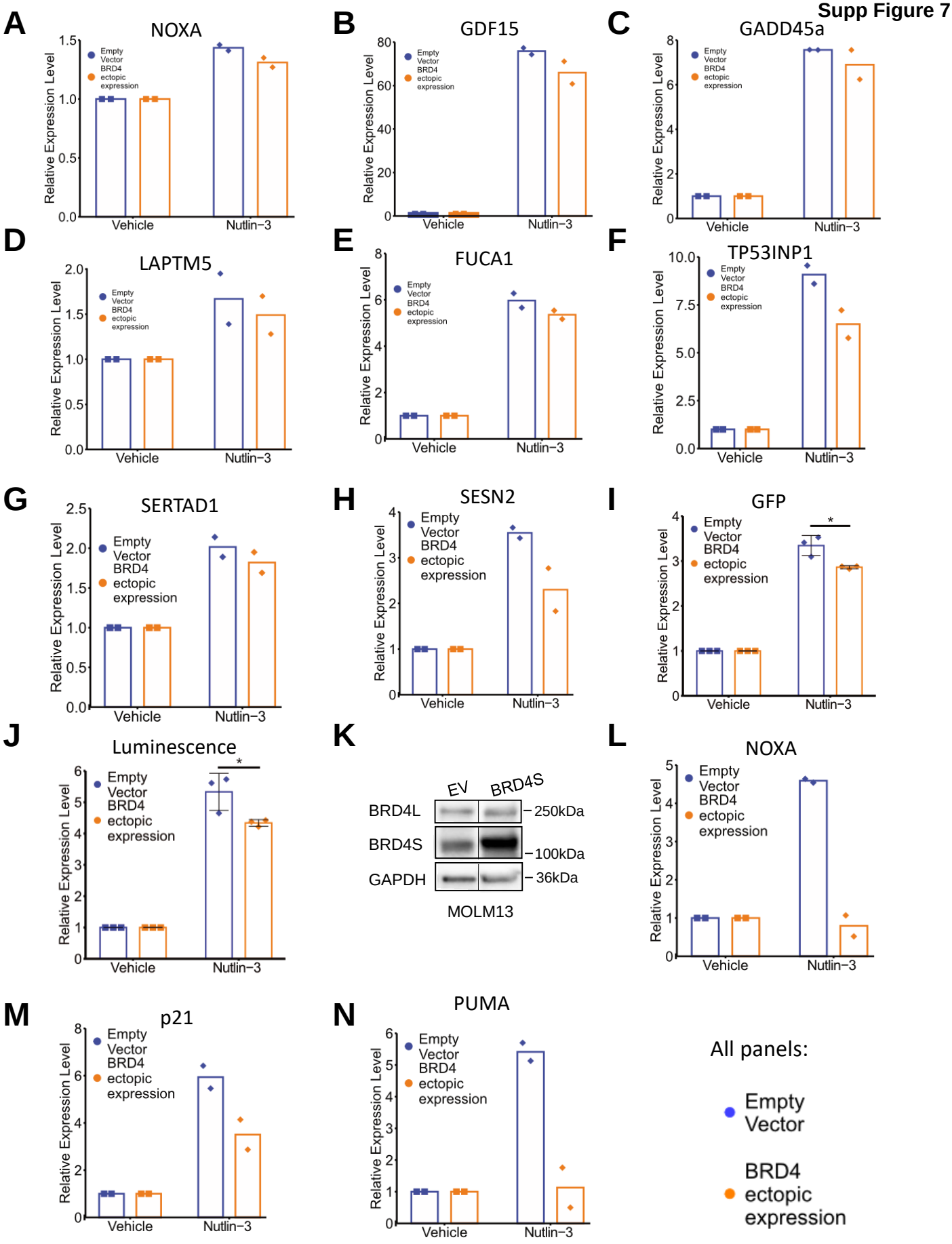
D. Venn diagram showing overlap of 2 replicates of BRD4 ChIP-seq peaks in OCI-AML3 cells treated with CPI203

E. Venn diagram showing overlap of 2 replicates of BRD4 ChIP-seq peaks in OCI-AML3 cells treated with CPI203 and nutlin-3.

F. Histogram of number of BRD4 ChIP-seqs centered around gene TSS (0) for genes in the highest (quartile 1) to lowest (quartile 4) quartiles of expression in DMSO treated OCI-AML3 cells.

G. The histogram shows the spread of change in expression for genes losing BRD4 binding after treatment with CPI203. Loss of BRD4 on CPI203 treatment was associated with a decrease in gene expression ($p<0.05$). Inset figure: BRD4 peaks overlapping TSS were divided into two groups, those losing BRD4 on CPI203 treatment and those retaining BRD4. Plotted is the % change in expression after CPI203 treatment for the genes within each group.

H. Heat map of BRD4 ChIP-seq peaks in known p53 target genes from Control (DMSO) and CPI203 treatment. Values represent the aggregated read counts associated with each gene across samples in log scale.


A
NOXA
B
GDF15
C
GADD45a
D
LAPTM5
E
FUCA1
F
TP53INP1
G
SERTAD1
H
SESN2
I
GFP
J
Luminescence
K
EV
BRD4S
BRD4L
250kDa
BRD4S
100kDa
GAPDH
36kDa
MOLM13
L
NOXA
M
p21
N
PUMA
Relative Expression Level
Empty Vector
BRD4 ectopic expression
Vehicle
Nutlin-3
All panels:
Empty Vector
BRD4 ectopic expression

Supp Figure 7. BRD4 represses p53 target genes.
A. qPCR analysis of *NOXA* in OCI-AML3 cells ectopically expressing BRD4S, in absence or presence of nutlin-3 (2 replicates of each).
B. qPCR analysis of *GDF15* in OCI-AML3 cells ectopically expressing BRD4S, in absence or presence of nutlin-3 (2 replicates of each).
C. qPCR analysis of *GADD45a* in OCI-AML3 cells ectopically expressing BRD4S, in absence or presence of nutlin-3 (2 replicates of each).
D. qPCR analysis of *LAPTM5* in OCI-AML3 cells ectopically expressing BRD4S, in absence or presence of nutlin-3 (2 replicates of each).
E. qPCR analysis of *FUCA1* in OCI-AML3 cells ectopically expressing BRD4S, in absence or presence of nutlin-3 (2 replicates of each).
F. qPCR analysis of *TP53INP1* in OCI-AML3 cells ectopically expressing BRD4S, in absence or presence of nutlin-3 (2 replicates of each).
G. qPCR analysis of *SERTAD1* in OCI-AML3 cells ectopically expressing BRD4S, in absence or presence of nutlin-3 (2 replicates of each).
H. qPCR analysis of *SESN2* in OCI-AML3 cells ectopically expressing BRD4S, in absence or presence of nutlin-3 (2 replicates of each).
I. GFP measurement in OCI-AML3 cells ectopically expressing BRD4S, in absence or presence of nutlin-3(*=p≤0.05 by a two-tailed unpaired t-test, n=3).
J. Luminescence measurement in OCI-AML3 cells ectopically expressing BRD4S, in absence or presence of nutlin-3(*=p≤0.05 by a two-tailed unpaired t-test, n=3).
K. Western blot for BRD4 in MOLM13 cells ectopically expressing the short isoform of BRD4 (BRD4S). BRD4L is the long isoform.
L. qPCR analysis of *NOXA* in OCI-AML3 cells ectopically expressing BRD4S, in absence or presence of nutlin-3 (**=p≤0.01) (2 replicates of each).
M. qPCR analysis of *CDKN1A* in OCI-AML3 cells ectopically expressing BRD4S, in absence or presence of nutlin-3 (2 replicates of each).
N. qPCR analysis of *PUMA* in OCI-AML3 cells ectopically expressing BRD4S, in absence or presence of nutlin-3 (*=p≤0.05) (2 replicates of each).