BCL6 repression of *EP300* provides a basis for rational combinatorial therapy in human diffuse large B cell lymphoma cells

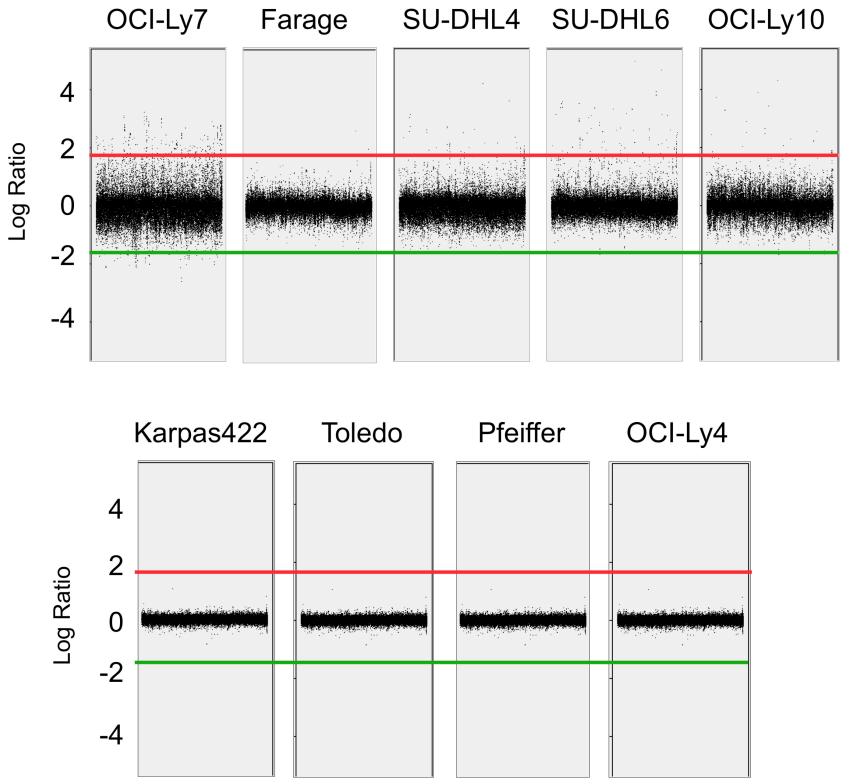
Leandro C. Cerchietti[1,2], Katerina Hatzi[1,2], Eloisi Caldas-Lopes[3], Shao Ning Yang[1,2], Maria E. Figueroa[1,2], Ryan D. Morin[4], Martin Hirst[4], Lourdes Mendez[1], Rita Shaknovich[1,2], Philip A. Cole[5], Kapil Bhalla[6], Randy D. Gascoyne[7], Marco Marra[4], Gabriela Chiosis[3], Ari Melnick[1,2].

1. Hematology and Oncology Division. Weill Cornell Medical College. New York, NY.
2. Department of Pharmacology. Weill Cornell Medical College. Cornell University. New York, NY.
3. Department of Molecular Pharmacology and Chemistry, Sloan-Kettering Institute, New York, NY.
4. Genome Sciences Centre, British Columbia Cancer Agency, Vancouver, BC.
5. Department of Pharmacology and Molecular Sciences. Johns Hopkins University School of Medicine. Baltimore, MD.
6. The University of Kansas Cancer Center, Kansas University Medical Center, Kansas City, KS.
7. Centre for Lymphoid Cancers and the Departments of Pathology and Experimental Therapeutics, British Columbia Cancer Agency, British Columbia Cancer Research Centre, Vancouver, BC.

Supplementary Figures

Figure S1

A



B

UP-REGULATED

ANTRX1
BC000845
C16ORF72
CD274
CD55
CRLF2
CSAG2
CYSLTR2
DNAJA4
DUB3
DUSP5
ETV3
FOSB
GAGE1
GAGE3
GAGE5
HN1
HSPA6
IL1RL1
KIR2DL3
KRTAP21-1
LOC284861
LOC349196
LOC65122
MT-ATP6
MT-CYB
MT-ND2
MT-ND3
MT-ND6
MUC11
MUC3
MUC4
OR2M5
OR2M7
OR4F16
OR4F29
OR4K2
PSG1
REXO1L1
S100A7
SEMG1
SPRR2A
SPRYD5
TRIM51

DOWN-REGULATED

ABCD1
C13ORF18
CYTH4
NRLC3
NUP210
OPRL1
SIPA1L3
SLC16A13

Figure S1. A core RI-BPI response signature in BCL6 dependent DLBCL cells. A: Dot-plots of log-ratio intensity (Y-axis) vs. probe (X-axis) in a panel of five BCL6- dependent DLBCL cell lines (OCI-Ly7, Farage, SU-DHL4, SU-DHL6, and OCI-Ly10) and four BCL6-independent DLBCL cell lines (Karpas422, Toledo, Pfeiffer and OCI-Ly4). Log-ratios were obtained by comparison of the genes mobilized in RI-BPI 10 μM treated cells (Cy3 channel) over the genes mobilized in CP 10 μM treated cells (Cy5 channel) in each cell line. Cells were exposed to treatments for 24 h. Experiments were carried out in duplicates. Up- and down-regulated probes were defined by a cut-off of log-ratio ≥ 1.8 (shown by the red and green lines respectively). The up- and down-regulated probes common to all RI-BPI-responsive cell lines were considered the core gene expression signature. **B:** The list of genes in the RI-BPI core signature, which was used to query the connectivity map. Previously known BCL6-target genes are shown in bold.

Figure S2

A

Rank	Batch	Name	Dose
1	506	Radicicol	100 nM
2	504	Geldanamycin	1 μM
5	63	VA	1 mM
9	33	VA	500 μM
12	506	17-AAG	1 μM
14	513	VA	1 mM
18	506	Geldanamycin	1 μM
25	506	TSA	100 nM
26	55	17-AAG	1 μM
28	40	17-AAG	1 μM
34	33	VA	2 mM
41	502	17-AAG	1 μM
47	45	TSA	100 nM
56	513	17-AAG	1 μM
61	506	VA	200 μM
64	504	TSA	1 μM
65	506	SAHA	10 μM
68	74	17-AAG	1 μM
83	513	TSA	100 nM
86	502	Geldanamycin	1 μM
93	506	17-AAG	1 μM
97	513	SAHA	10 μM
101	54	17-AAG	1 μM
103	513	17-AAG	1 μM
112	513	Geldanamycin	1 μM
113	513	TSA	1 μM
119	506	TSA	1 μM
121	61	17-AAG	1 μM
122	33	VA	50 μM
129	101	Geldanamycin	1 μM
133	28	TSA	100 nM
136	513	17-AAG	1 μM
143	506	17-AAG	1 μM
148	513	17-AAG	1 μM
159	506	17-AAG	1 μM
161	506	VA	1 mM
184	504	17-AAG	1 μM
185	502	TSA	1 μM
196	506	VA	50 μM
199	33	VA	10 mM
204	514	TSA	100 nM
214	505	17-AAG	1 μM
219	44	VA	1 mM
224	44	VA	10 mM
229	60	TSA	100 nM
238	28	TSA	100 nM
245	35	TSA	100 nM
272	109	VA	1 mM
275	17	17-AAG	100 nM
306	70	VA	1 mM
329	506	VA	500 μM
335	95	Geldanamycin	1 μM
336	513	VA	500 μM
350	2	VA	1 mM
360	56	VA	1 mM
369	513	VA	50 μM
382	73	17-AAG	1 μM

B

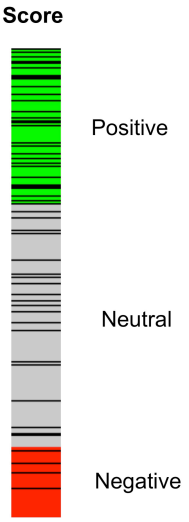


Figure S2. Connectivity map analysis of RI-BPI reveals potential functional relationship with Hsp90 inhibitors and HDI. A: Connectivity-map query result for the RI-BPI genomic signature. The list shows ranking of Hsp90 inhibitors (Radicicol, Geldanamycin, 17-AAG) and HDAC inhibitors (TSA, SAHA and VA), with the associated batch and dose used to generate the database. **B:** Connectivity score (positive, negative and neutral) for the RI-BPI genomic signature and the Hsp90 inhibitors and HDI.

Figure S3

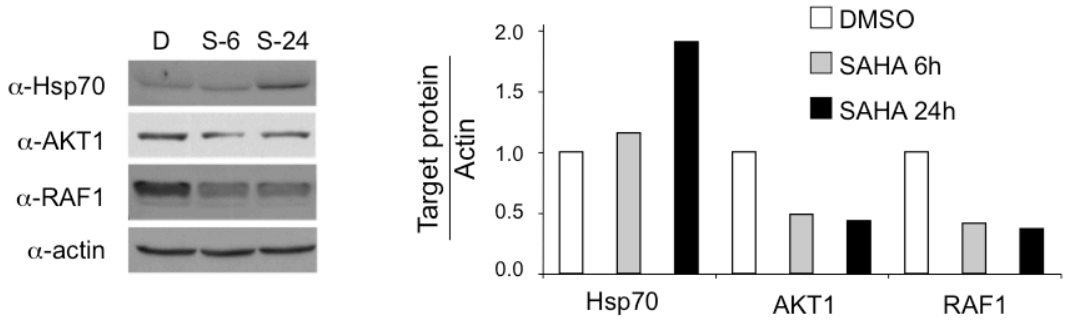


Figure S3. SAHA induces degradation of Hsp90 client proteins and induction of Hsp70. Immunoblotting was performed for Hsp70, AKT1, RAF1 and actin (as control) in cell extracts of OCI-Ly7 cells treated with SAHA 1 μM for 6 and 24 h. Densitometry analysis is shown on the right.

Figure S4

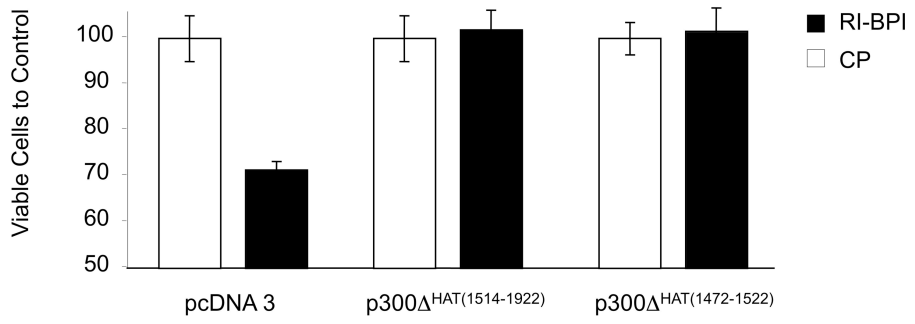


Figure S4. RI-BPI-induced cell death is rescued by dominant negative p300ΔKAT. SU-DHL6 cells were transfected with two different p300-KAT dominant-negative constructs (P300$\Delta^{KAT(1514-1922)}$ and p300$\Delta^{KAT(1472-1522)}$) or control (pcDNA3) followed by treatment with RI-BPI 10 μM (black bars) or CP (white bars). After 48 h the viability was determined using a metabolic assay. Experiments were done in duplicates. Bars represent S.E.M. for duplicates.

Figure S5

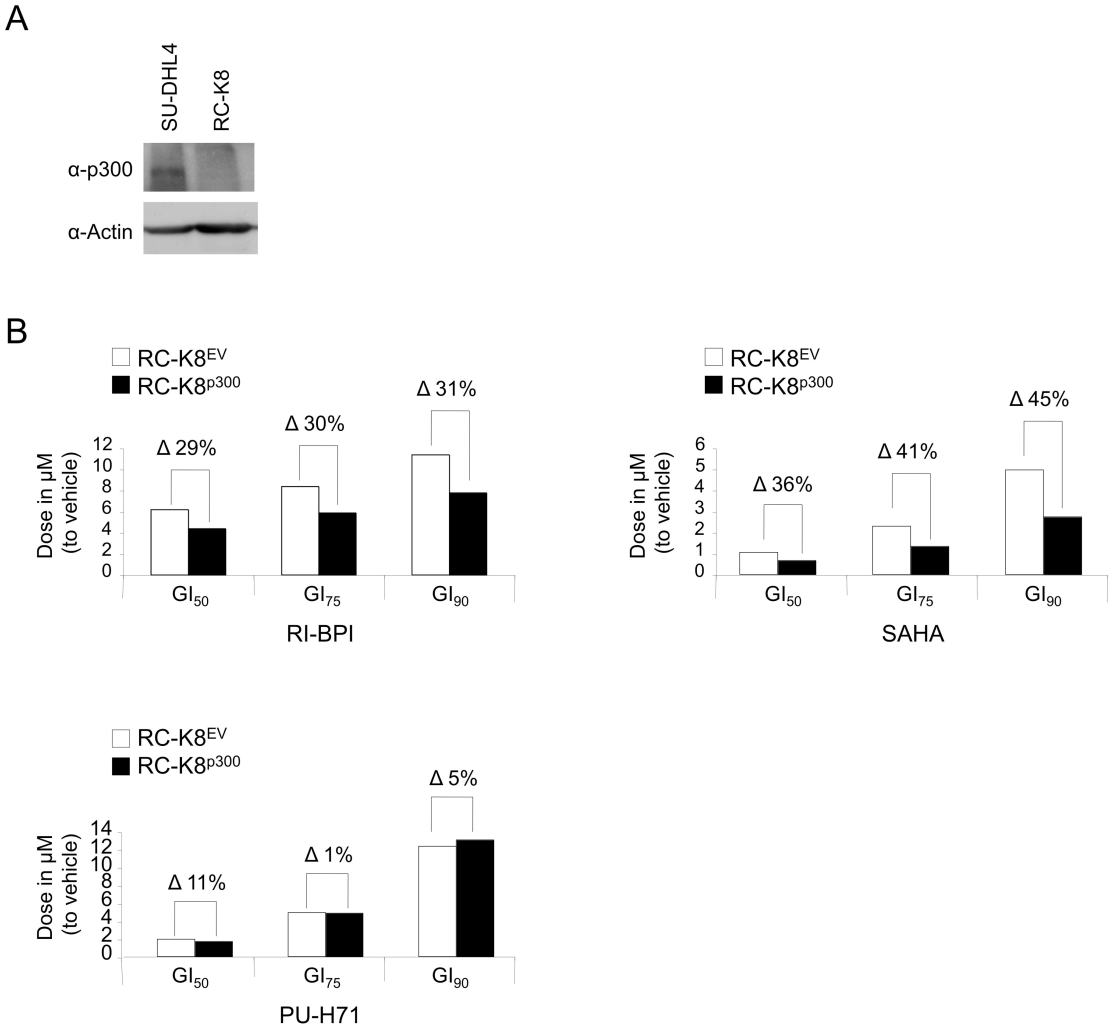


Figure S5. Expression of wild type p300 expression enhances response to RI-BPI and SAHA in *EP300* null DLBCL cells. A: Immunoblot for p300 (N-15) and actin (as control) in the SU-DHL4 and RC-K8 DLBCL cell lines. **B:** Drug doses (Y-axis) correspondent to the Growth Inhibition $(GI)_{50}$, GI_{75} and GI_{90} of RI-BPI, SAHA and PU-H71 in RC-K8 cells transfected with a p300 plasmid (RC-K8^{p300}, Black bars) or its correspondent empty vector (RC-K8^{EV}, white bars). The difference in the dose required to achieve the respective growth inhibition concentrations between RC-K8^{EV} and RC-K8^{p300} are indicated as percentage.

Figure S6

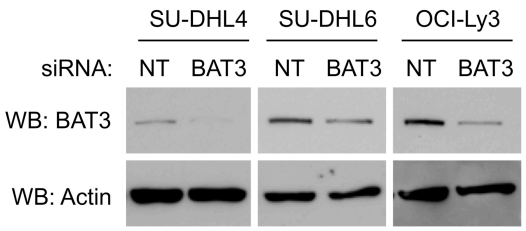


Figure S6. Confirmation of the efficacy of *BAT3* siRNA. SU-DHL4, SU-DHL6 and OCI-Ly3 cells were electroporated with siRNA targeting *BAT3* or with non-targeting siRNA (NT). Protein abundance was determined by immunoblot. Actin was used as loading control.

Figure S7

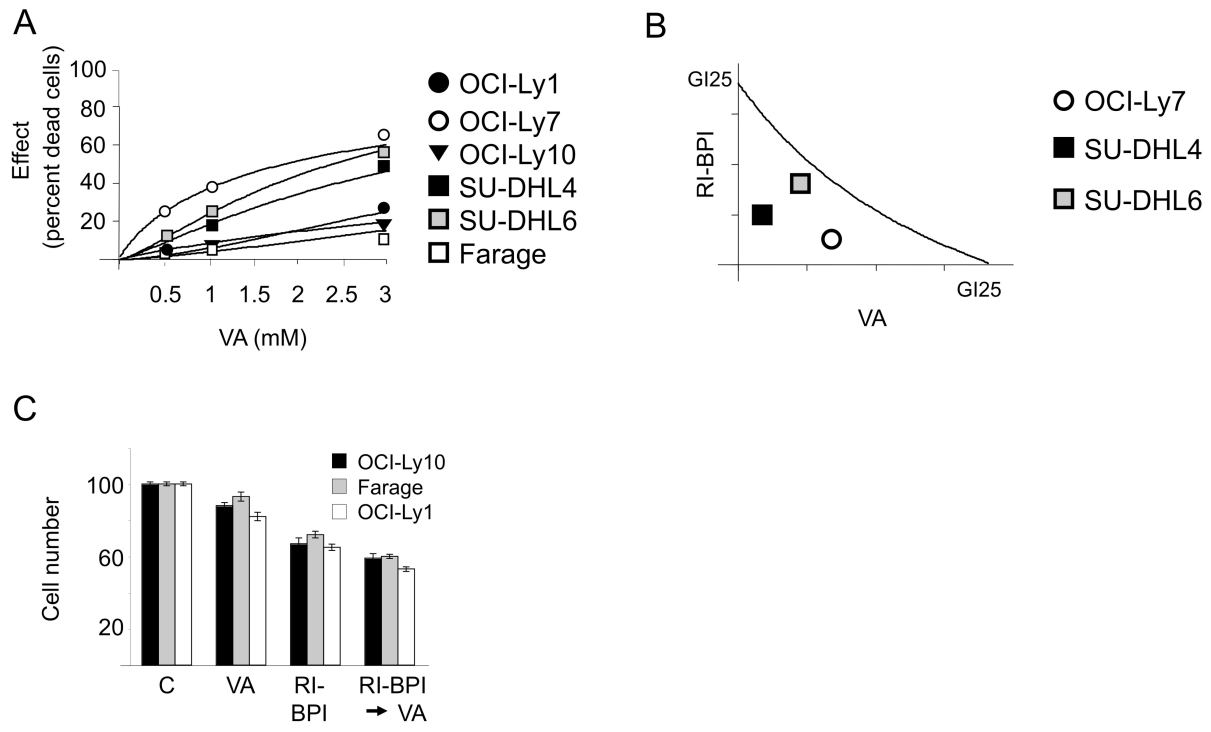


Figure S7. RI-BPI exhibits additive to synergistic effects with the HDAC inhibitor valproic acid (VA). A: A panel of six BCL6-dependent DLBCL cell lines (OCI-Ly7, SU-DHL6, OCI-Ly1, Farage, SU-DHL4 and OCI-Ly10) was exposed in triplicate to five concentrations of VA (from 0.125 to 3 mM) or vehicle control (water) for 48 h and analyzed for cell viability. Dose-effect (percent dead cells) curves were plotted. The X-axis shows the dose of VA. The Y-axis shows the fractional effect of VA as compared to control on cell viability. **B:** The cell lines for which a VA GI_{25} was obtained (OCI-Ly7, SUDHL-4 and SU-DHL6), were treated with five concentrations of VA, RI-BPI and the combination in a constant ratio (concurrent schedule). A conservative GI_{25} isobologram for the combination of VA with RI-BPI was plotted for each particular cell line. The dose values for each GI_{25} for each cell line are shown in **Table S1. C:** For the cell lines that were resistant to VA (i.e.: the GI_{25} was higher than the upper dose limit), a potentiation effect with RI-BPI was calculated. OCI-Ly10, OCI-Ly1 and Farage cells were treated with 10 μM RI-BPI, 1 mM VA or the combination for 48 h (sequential schedule BPI→drug). Cell viability was determined and compared to control treated cells (water).

Figure S8

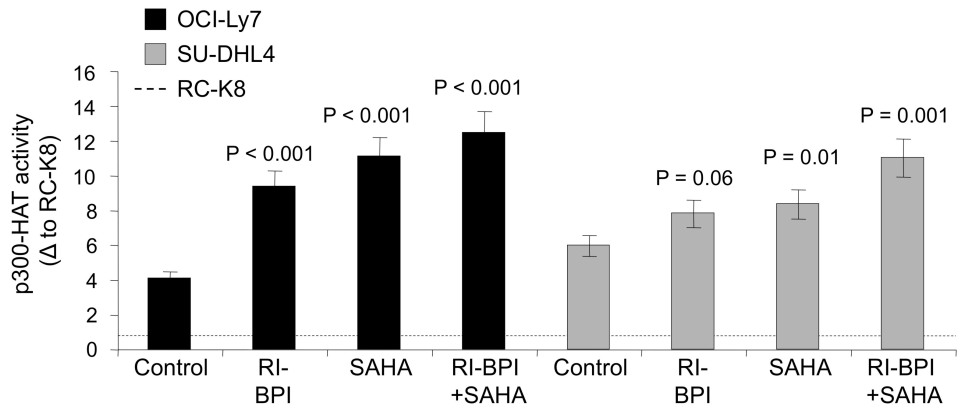


Figure S8. The combination of RI-BPI and SAHA increases p300 KAT activity to a greater level than either drug alone. P300-HAT activity was measured in OCI-Ly7 (black bars) and SU-DHL4 (grey bars) cells before (control) and after treatment with 10 μM RI-BPI, 1 μM SAHA or SAHA + BPI for 24 h normalized to the P300-HAT activity in RC-K8 cells (dotted line). The HAT-activity associated with p300 was determined by p300 IP vs. IgG followed by incubation of the immunoprecipitates with specific HAT substrates and cofactors. The resulting acetylated product was measured by spectrophotometry (OD_{440nm}). Results expressed in fold induction vs. control.

Figure S9

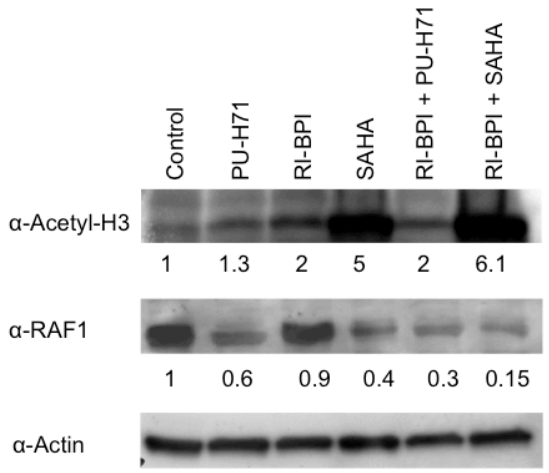


Figure S9. Effect of the combined drug treatment on the abundance of RAF1 and acetyl-H3. Immunoblotting for acetyl-H3, RAF1 and actin (as control) for OCI-Ly7 cells treated with 1 μM PU-H71, 10 μM BPI, 1 μM SAHA and the combination of PU-H71 + BPI and SAHA + BPI for 24 h. The numeric values represent the ratio between the densitometry for acetyl-H3 and RAF1 vs. actin for each lane.

Figure S10

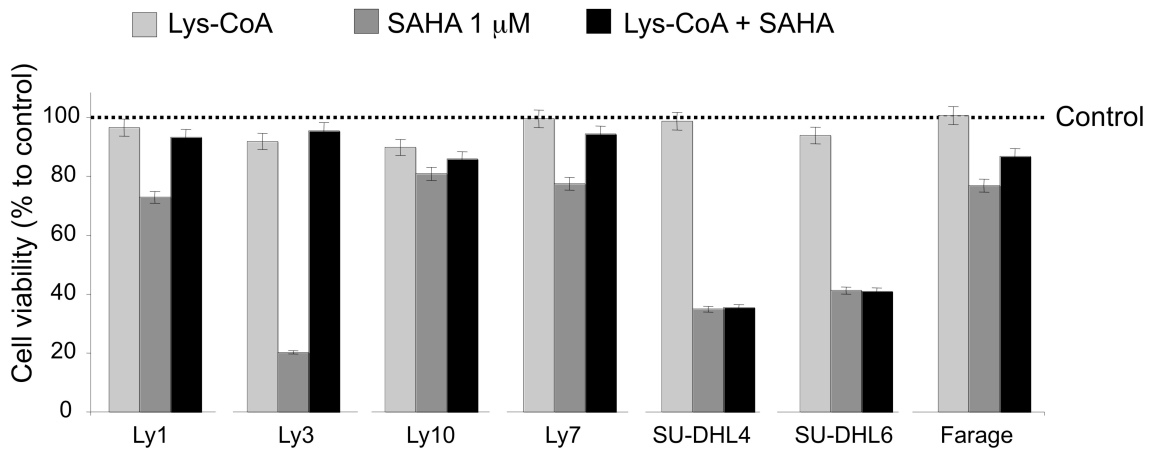


Figure S10. SAHA-induced cell death is partially rescued by p300 inhibition. A panel of seven BCL6-dependent DLBCL cell lines (OCI-Ly7, SU-DHL6, OCI-Ly1, Farage, OCI-Ly3, SU-DHL4 and OCI-Ly10) was exposed in triplicate to 1 μM SAHA (dark grey bars), the p300-HAT inhibitor Lys-CoA-TAT (light grey bars) and the combination of both (black bars) for 48 h. Cell viability (as percent to control) is shown on the Y-Axis.

Figure S11

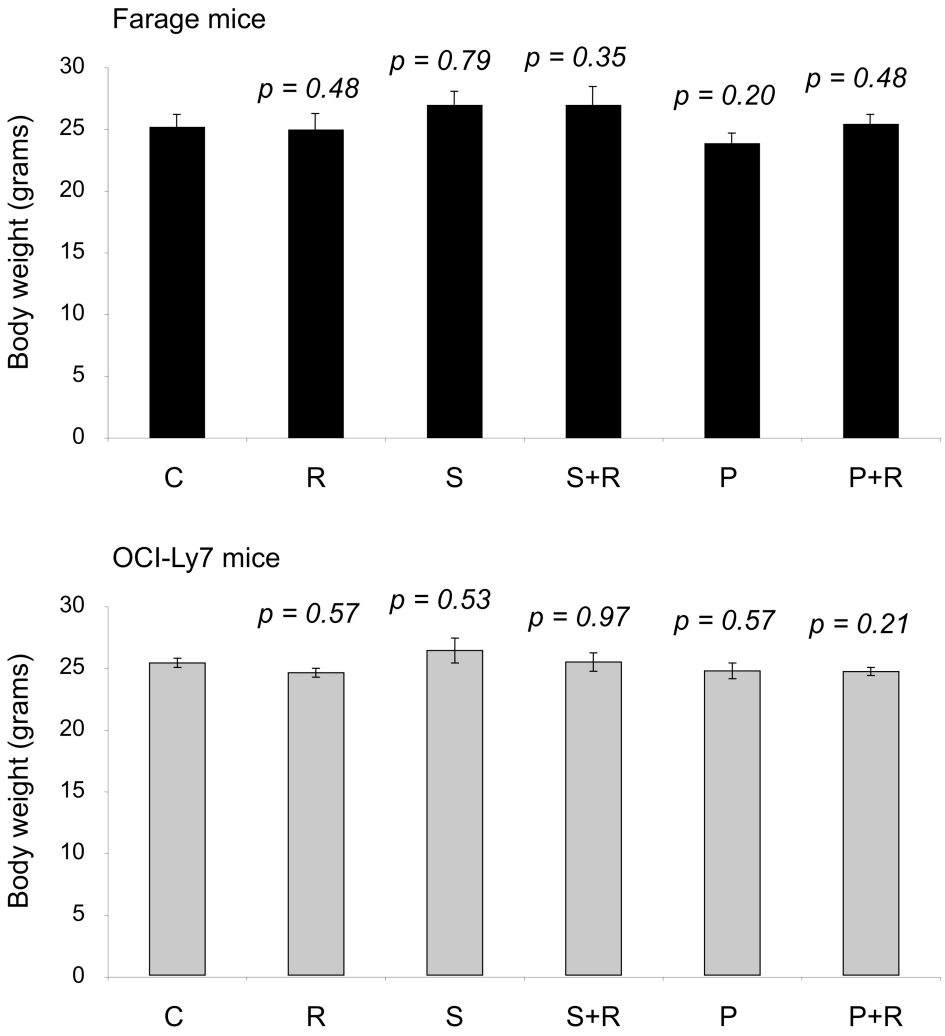


Figure S11. Body weight of mice after treatment with anti-lymphoma drugs. The columns represent the average of the body weight (with the SE) for the mice bearing Farage (top) and OCI-Ly7 (bottom) xenografts treated with vehicle control (C), RI-BPI (R), SAHA (S), PU-H71 (P), SAHA + RI-BPI (S+R) and PU-H71 + RI-BPI (P+R). The body weight was adjusted to tumor weight. The p values correspond to the two-tailed T-test comparison with the vehicle control mice.

Figure S12

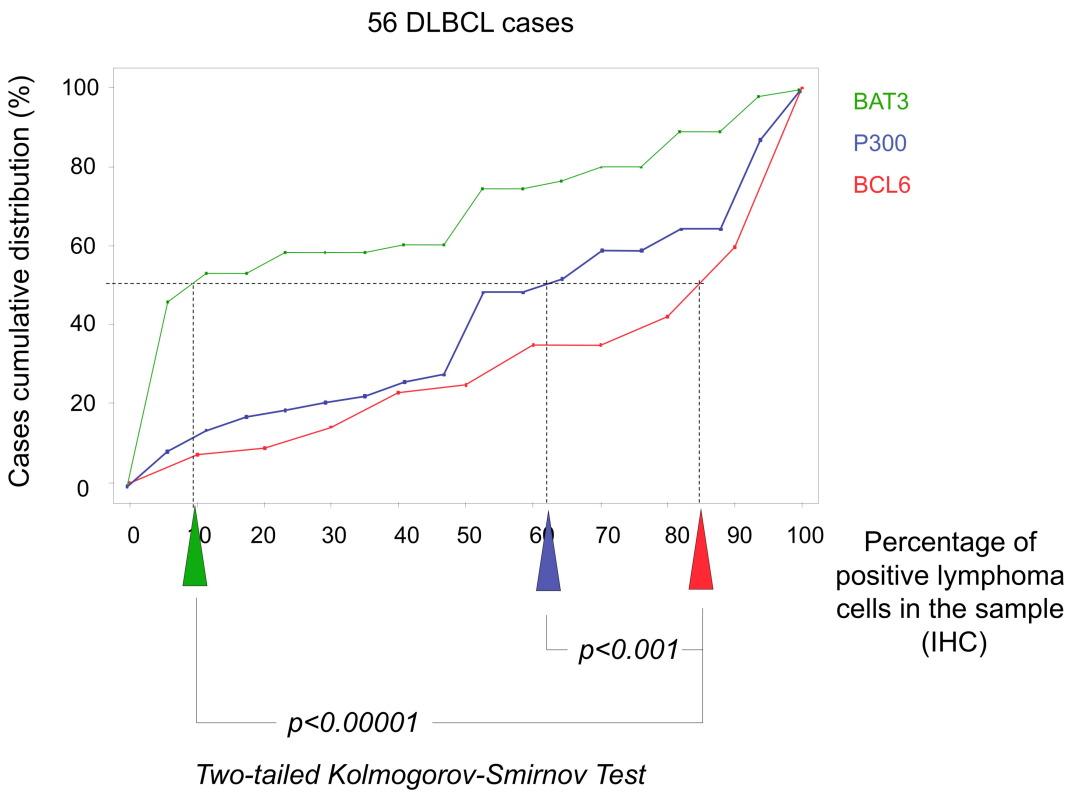


Figure S12. BCL6, p300 and BAT3 expression in DLBCL cases. The presence of BCL6, p300 and BAT3 was determined by immunohistochemistry in a TMA including 57 DLBCL cases. The percentage of positive lymphoma cells for each protein per sample (X-axis) was plotted against the cumulative distribution of cases (Y-axis). The arrows indicate the median percentage of positive cells corresponding to 50% of the cases (BAT3: 38%, P300: 62%, and BCL6: 85%). The closer a curve is to upper left corner, the lower the number of positive cells. The closer a curve is to the lower right corner, the higher the number of positive cells.

Supplementary Tables

Table S1

	GI_{50} RI-BPI (μM)	GI_{50} PUH71 (μM)	GI_{50} 17-DMAG (μM)
Farage	7.9 (5.6-11.2)	0.72 (0.68-0.77)	0.49 (0.30-0.81)
OCI-Ly1	13.3 (8.5-20)	0.92 (0.27-1.80)	0.68 (0.35-1.34)
OCI-Ly7	16.9 (12.6-22)	0.31 (0.18-0.55)	0.33 (0.13-0.92)
OCI-Ly10	13 (8-21)	3.53 (1.11-5.61)	1.77 (1.47-2.14)
SU-DHL6	7.1 (4.6-11)	0.35 (0.22-0.57)	0.32 (0.13-0.83)
SU-DHL4	4.3 (3.5-5.4)	3.29 (2.39-7.69)	0.64 (0.60-0.67)
OCI-Ly3	19 (18-21)	1.37 (0.85-2.58)	4.56 (0.83-8.22)

	GI_{50} SAHA (μM)	GI_{50} TSA (nM)	GI_{25} VA (mM)
Farage	1.68 (1.31-2.16)	110.5 (91-133)	>3
OCI-Ly1	2.05 (1.78-2.3)	207.3 (178-240)	>3
OCI-Ly7	0.75 (0.37-1.52)	71 (33-150)	0.35 (0.27-0.46)
OCI-Ly10	>10	1695 (1050-2735)*	>3
SU-DHL6	1.4 (1.23-1.6)	72 (57-91)	0.72 (0.68-0.76)
SU-DHL4	4.28 (3.58-5.13)	1010 (794-1283)*	1.5 (1.1-1.9)
OCI-Ly3	0.73 (0.35-1.49)	54.4 (34.1-86.8)	NA

**Calculated, NA: not available*

Table S1. GI_{50} or GI_{25} concentrations of RI-BPI, PU-H71, 17-DMAG, SAHA, TSA and VA obtained in the seven DLBCL cell lines used in the experiments. Values higher than the upper dose used in the experiments appear as calculated.

Table S2

ChIP Primers	
BAT3-Upstream	*5'-GGAGAAGCCTGTGGTCTTTG* *5'-CTGGGACTTGGGATTCAAAA*
BAT3	*5'-AAGCGCCTCTTTTCTTGACA* *5'-GTCACATTCGTCGGTGTGTG*
EP300-Upstream	*5'-ATTCGGCAGAGGGAAGAAAC* *5'-CCAGCCTAGCACACAAACAA*
EP300	*5'-ACAAGCGAGGAAAACCAGAA* *5'-TTTCTATCGAGTCCGCATCC*
Q-PCR primers	
BAT3	*5'-GCACCAGAGGTTCAGGAGAG* *5'-GGGGACTGTAGTTGGGGTCT*
EP300	*5'-GACCCTCAGCTTTTAGGAATCC* *5'-TGCCGTAGCAACACAGTGTCT*
Microarray primer	
Oligo-dT	*5'-GGCCAGTGAATTGTAATACGACTCACTATAGGGAGGCGGTTTTTTTTTTTTTTTTTTTTTTTT*

Table S2. Sequence of the ChIP and q-PCR primers used to detect *BAT3* and *EP300*.