Supplemental Table 1

Antibody Name	Dilution	Company
For FACS/ImageStreamX		
PE Mouse Anti-Human CD19	1:2	BD Pharmingen
PE Mouse Anti-Human CD20	1:2	Beckman Coulter
FITC Mouse Anti-Human CD20	1:20	BD Pharmingen
FITC Mouse Anti-Human CD79b	1:2	BD Pharmingen
FITC Mouse Anti-Human CD15	1:2	BD Pharmingen
FITC Mouse Anti-Human CD30	1:2	BD Pharmingen
For Western Blot		
Rabbit Monoclonal Antibody PAX5	1:1000	EPITOMICS
Rabbit Polyclonal to CD20 (C-term)	1:1000	Acris Antibodies
Phospho-Akt (Ser473)	1:1000	Cell Signaling Technology
Akt Rabbit mAb	1:1000	Cell Signaling Technology
Phospho-BTK (Tyr223)	1:1000	Cell Signaling Technology
BTK (D3H5) Rabbit mAb	1:500	Cell Signaling Technology
α-Tubulin	1:2000	Sigma
For Immunohistochemistry		
Mouse Anti-Human CD20 (L26)	1:750	Dako
Mouse Anti-Human CD30 (BerH2)	1:10	Dako

Secondary Antibody	1:2000	Amersham
Anti-mouse HRP Linked Antibodies	1:2000	Amersham
Anti-rabbit HRP Linked Antibodies		
For ChIP		
Tri-Methyl-Histone H3 (Lys9)	1:33	Abcam
Tri-Methyl-Histone H3 (Lys27)	1:33	Cell Signaling Technology
Trimethyl-Histone H3(Lys4)	1:33	Cell Signaling Technology
IgG, normal Rabbit	1:33	Cell Signaling Technology

Supplemental Table 2

TaqMan Assays

Target gene	Cat. no.
CD19	Hs01047410_g1
CD20 (MS4A1)	Hs00544818_m1
CD79A	Hs00233566_m1
CD79B	Hs00236881_m1
Bob1	Hs01573371_m1
Oct2	Hs00231269_m1
PAX5	Hs00172003_m1
CD30	Hs00174277_m1
TCF7	Hs01009044_m1
SDHA	Hs00188166_m1
EHMT2	Hs001198710_m1

All Taqman assays and TaqMan® Gene Expression Master Mix were ordered from Applied Biosystems.

Oligonucleotide	Sequence (5'-3')
For ChIP	
CD19 P1 For	AGAGGCACATAGAGAGTGAC
CD19 P1 Rev	GCAACACAACAAGACCCTG
CD19 P2 For	CTGGGACCATAGGCGTGTA
CD19 P2 Rev	CATCATAAGCCCTCAGTTAA

CD19 P3 For	CTTGCAATTAGTGGTGAAC
CD19 P3 Rev	CATCCCAGCTCTCACTACCA
CD19 P4 For	TCTGGAGGGTTCCTGGAGA
CD19 P4 Rev	CTGGAGGGCACTCAACCAT
CD19 P5 For	GAGAGTCTGACCACCATGC
CD19 P5 Rev	TGCCCTTTGGACATACCTTC
For PCR	
pGL3 Forward	GGAAGACGCCAAAAACATAAAG
pGL3 Reverse	CATCGGTCGACGGATCCTTATC
For RNA interference	
shEHMT2-668 (TRCN0000115668, Dharmacon)	GGAGAAGCTGAATCTGTTGTT
shEHMT2-669 (TRCN0000115669, Dharmacon)	CGAGGTCCTTAAATTGTTCTA

All oligonucleotides (if not otherwise specified) were synthesized by Biotez. SYBR® Green PCR Master Mix was purchased from Applied Biosystems.

Supplemental Table 3

Drug Name	Final/working concentration	Solvents*	Company
5-aza-2'-deoxycytidine (5-Aza)	3 µM	DMSO	Sigma-Aldrich
Trichostatin A (TSA)	625 nM	DMSO	Sigma-Aldrich
All-trans retinoic acid (ATRA)	As indicated	DMSO	Sigma-Aldrich
Arsenic trioxide (ATO)	As indicated	PBS	Sigma-Aldrich
BIX-01294	4.2 µM	DMSO	Sigma-Aldrich
Rituximab	20 µg/ml	Stock solution	Roche
F(ab')2 crosslinker	50 µg/ml	H_2O	Rockland
Tositumomab	10 µg/ml	Stock solution	GlaxoSmithKline
Ibrutinib	2.5 nM	DMSO	Selleckchem
Idelalisib	5 µM	DMSO	Selleckchem
G418	As indicated	PBS	Sigma-Aldrich
Miferpristone	10 nM	Ethanol	Invitrogen
Blasticidin	As indicated	PBS	InvivoGen

*) Note that untreated (UT) comparisons reflect volume-matched solvent-only exposure throughout the study.

5-Aza/TSA treatment

Cells were treated with 5-aza-2'-deoxycytidine (5-Aza) and Trichostatin A (TSA) as published before. Cells were split one day prior to treatment and seeded at a density of 1×10^5/ml on day 0, followed by 6 days of 5-Aza treatment (3 µM). Fresh drug was added on day 2 and day 5. On day 5, TSA was supplied at a final concentration of 625 nM.

ATO/ATRA treatment

cHL cell lines were treated with ATRA or ATO at indicated concentration for 4 days, with fresh drugs supplied every second day. Starting one day after ATRA or ATO incubation, Rituximab and the F(ab')2 crosslinker or Tositumomab were added to the culture medium daily. Cell viability was measured on day 4.

BIX-01294 treatment

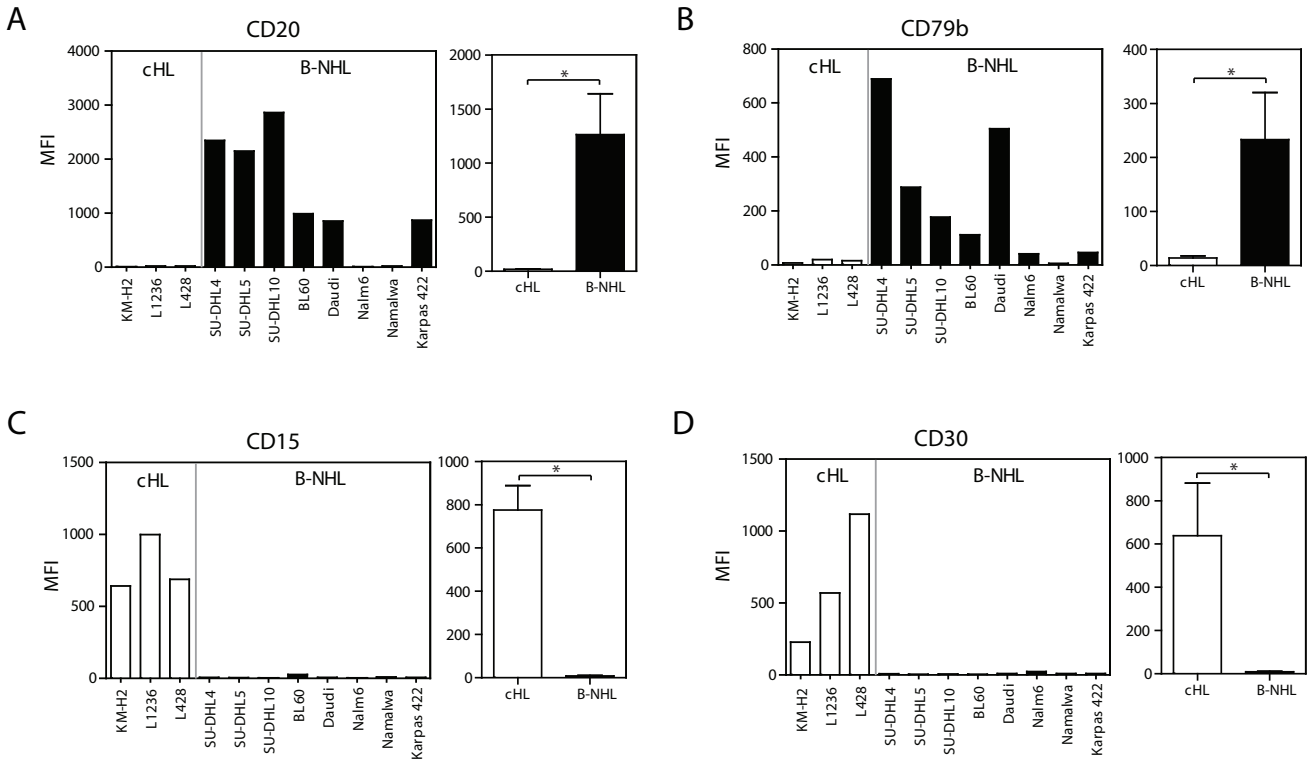
cHL cells were treated with BIX-01294 for 7 days with refresh on day 2 and day 6 at 4.2 µM. RNA was purified on day 7.

Ibrutinib and Idelalisib treatment

Cells were incubated with ATO, the drug screen-identified compound 40, or both for 2 days, followed by transfer to new media with freshly added Ibrutinib or Idelalisib. Viability was measured after one more day.

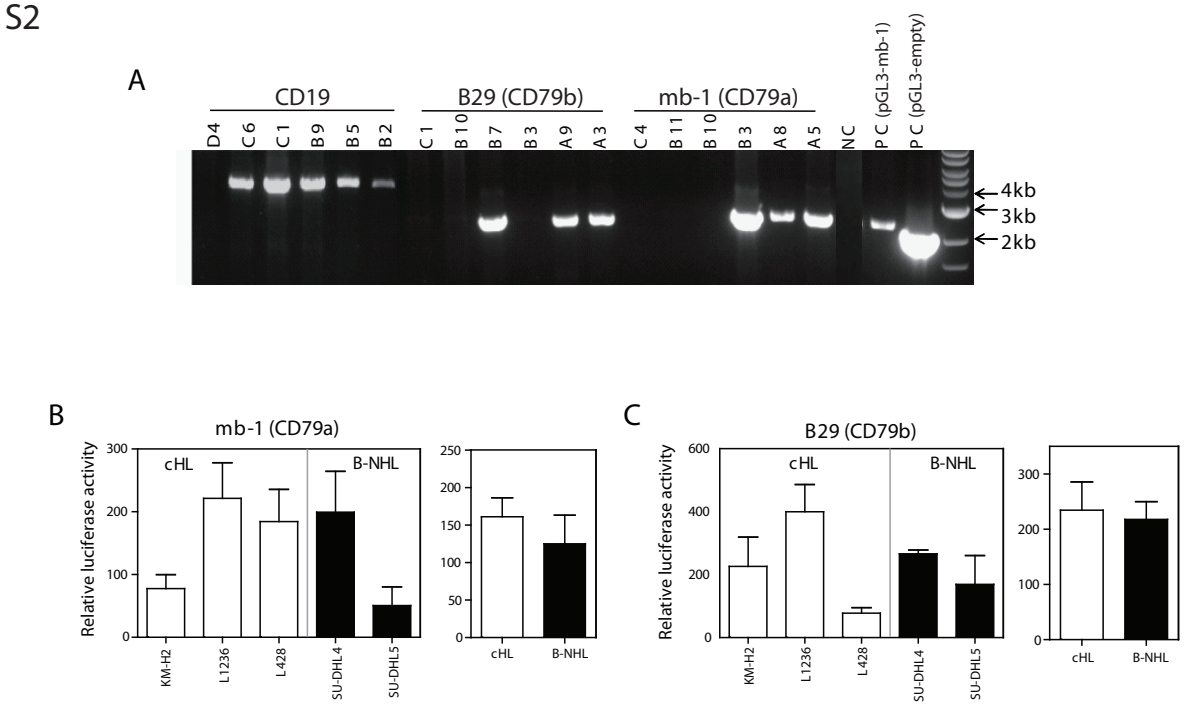
Supplemental Figures

S1



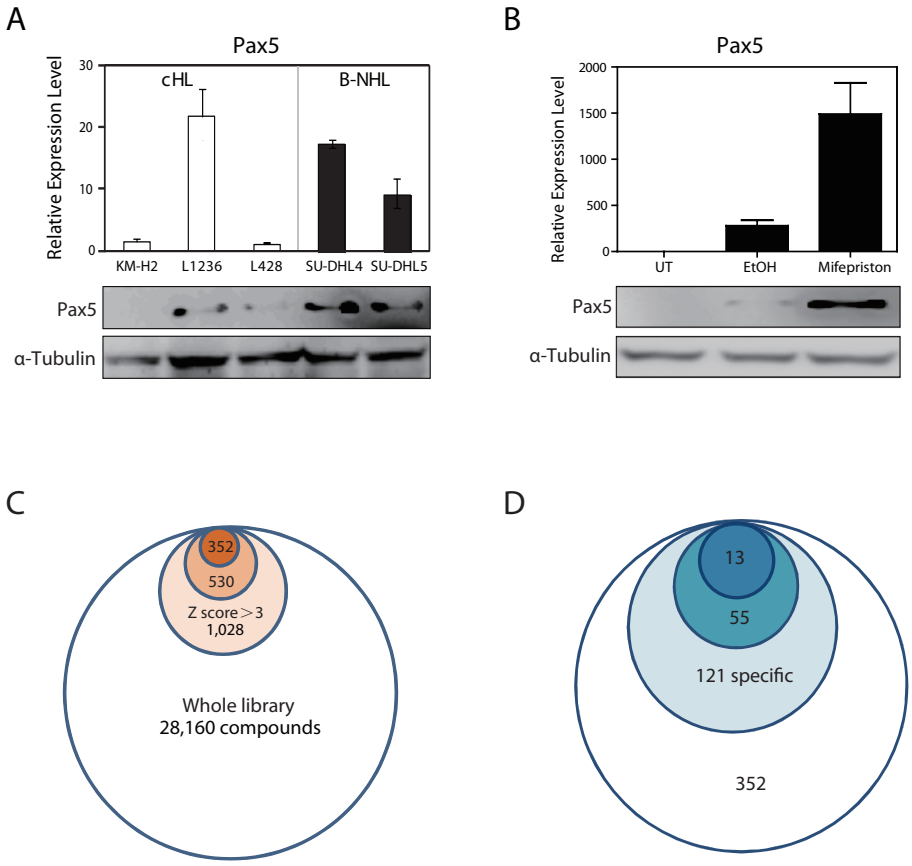
Supplemental Figure 1. B-cell-specific and Hodgkin-typical surface marker expression status in cHL and B-NHL cell lines. (A) CD20 surface antigen expression on individual cHL (white bars) and B-NHL (black bars) cell lines detected by flow cytometry and presented as mean fluorescence intensity (MFI; left). Average CD20 MFI of cHL and B-NHL cell line groups (right). (B) CD79b surface antigen expression as in A. (C) CD15 surface antigen expression as in A. (D) CD30 surface antigen expression as in A. Data are presented as mean ± SEM, with "*" denoting $P < .05$.

S2



Supplemental Figure 2. Stable integration and functional assessment of B-cell-specific promoter-driven luciferase constructs in cHL cell lines. (A) PCR amplification of integrated luciferase reporter constructs specific for the indicated B-cell promoters. Single clones (reflected by single letter/number codes) derived from the L428 cell line are shown here as a representative example (for all cHL cell lines used in the study). Mini-preparations of the pGL3-empty vector backbone at a 10^{-4} dilution and of the pGL3-mb-1 plasmid at a 10^{-6} dilution were included as positive control (PC). As negative control (NC), genomic DNA from the untransfected parental L428 cell line was used. (B) *mb-1 (CD79a)* promoter activity in individual cHL and B-NHL cell lines assessed by firefly luciferase reporter activity (left), and as average activity in the respective groups (right). (C) *B29 (CD79b)* promoter activity assessed as in B. The reporter assays were conducted in triplicate, presented as mean ± SD, with "*" denoting $P < .05$.

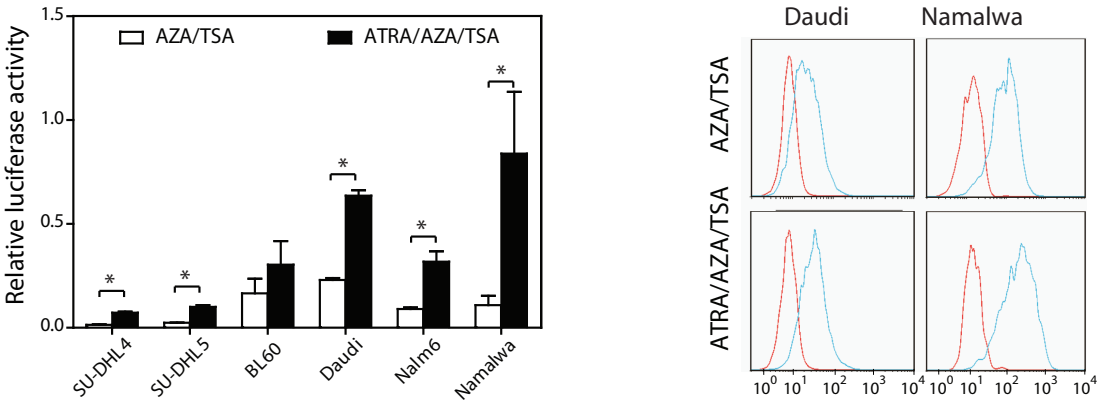
S3



Supplemental Figure 3. Pharmacological library screening in L428 reporter cells. (A) *Pax5* expression level in the indicated cHL and B-NHL cell lines, detected by RQ-PCR and normalized to KM-H2 (upper panel), and by immunoblot analysis, with α-Tubulin serving as a loading control (lower panel). (B) Enforced Pax5 expression in L428 cells stably transduced with both a Mifeprostine-inducible Pax5 and a CD19-luciferase reporter construct. Cells were treated with Mifepristone at 10 nM or ethanol (EtOH) as a solvent control for 48 hours prior to detection by RQ-PCR and normalized to untreated control (upper panel), and by immunoblot analysis, with α-Tubulin serving as a loading control (lower panel). Experiments were conducted at least in triplicate; data are presented as mean ± SD. (C) Luciferase-based pharmacological screening of a 28,160-compound library enriched for potentially bioactive compounds. Selection strategy of the primary screen conducted in stably pGL3-hCD19-Luc-transfected L428 cells. After excluding those with high auto-fluorescence, compounds for subsequent re-screenings were selected based on their Z score (*i.e.* the number of standard deviations a measured value is above the mean). Out of the 28,160 compounds initially tested, 1,028 agents prompted increased luminescence signals with a Z score above 3. For 530 drugs within this list, there was at least one other molecule with a

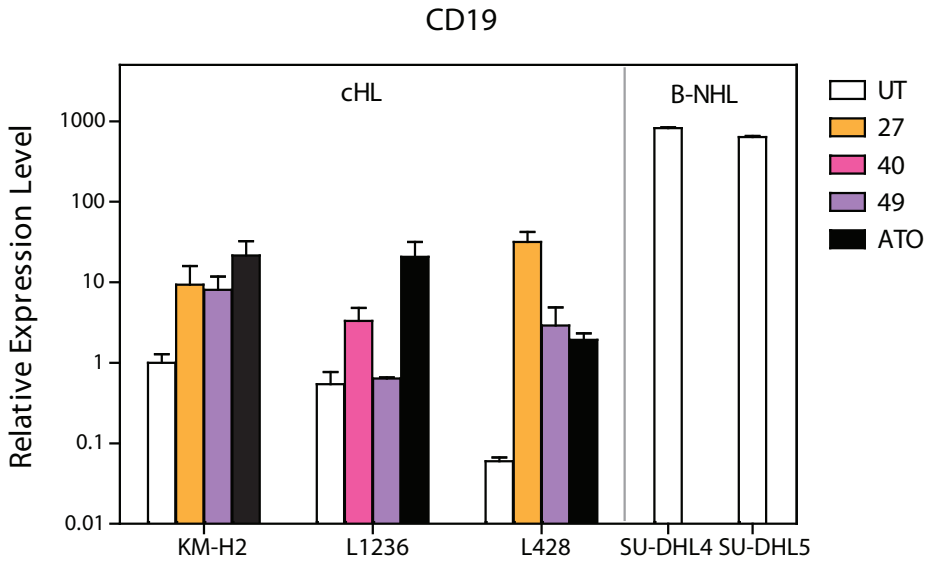
similar structure (with a Tanimoto score > 0.6 [see Materials and Methods for details]). Ranked by their Z score, the top 352 agents were subjected to a secondary screening (see D). (D) Counter-screening to exclude false-positive hits, *i.e.* drugs that may enhance luciferase enzyme activity non-specifically. To this end, we used L428 cells carrying an SV40 promoter-driven luciferase reporter, and excluded another 231 agents. Within the remaining 121 compounds, 66 had again at least one other compound similar in structure (with a Tanimoto score > 0.6). After discarding drugs with purity below 90% by mass spectrometry, we finally came up with a list of 55 compounds, out of which we selected 13 chemicals (reflecting six structurally distinct groups) that were effective in all three cHL cell lines (see also blue-boxed compounds in Figure 2A).

S4



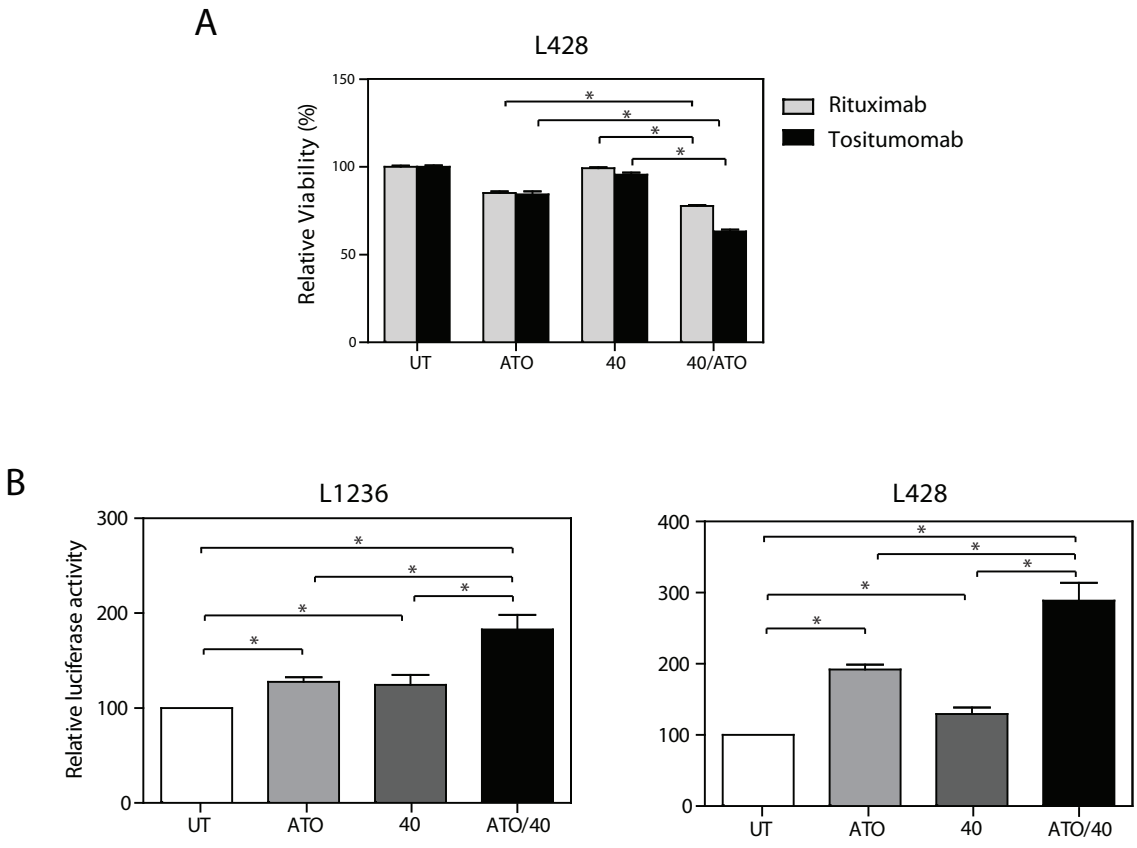
Supplemental Figure 4. All-trans retinoic acid (ATRA) protects from 5-Aza/TSA-enforced CD19 suppression in B-NHL cells. *CD19* expression in the indicated B-NHL reporter cell lines exposed to 5-Aza/TSA with and without ATRA detected by firefly luciferase reporter activity (left) and flow cytometry (right). Red curve: control staining; blue curve: anti-CD19 staining. Cells were treated with ATRA at 10 µM. Experiments were conducted in triplicate; data are presented as mean ± SD, with "*" denoting $P < .05$.

S5



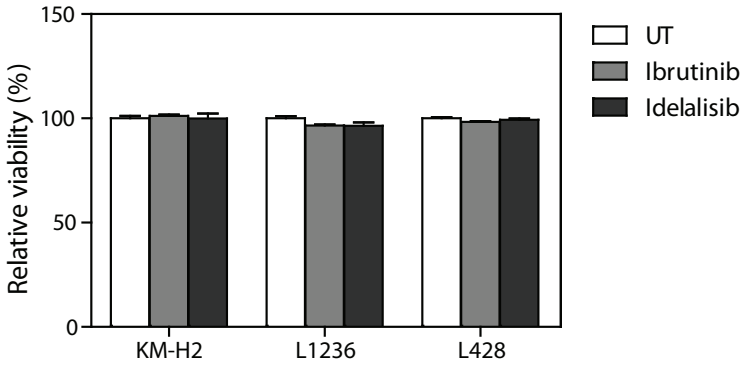
Supplemental Figure 5. Absolute *CD19* transcript levels in B-NHL cells as compared to cHL cells after "restoration" therapy. Absolute *CD19* transcript levels detected by RQ-PCR in the indicated cHL cell lines in response to the specified single-agent exposure (all at 10 µM for 48 hours) *vs.* untreated (UT), as compared to endogenous expression levels in the untreated B-NHL cell lines SU-DHL4 and SU-DHL5. Experiments were conducted at least in triplicate; data are presented as mean ± SD.

S6



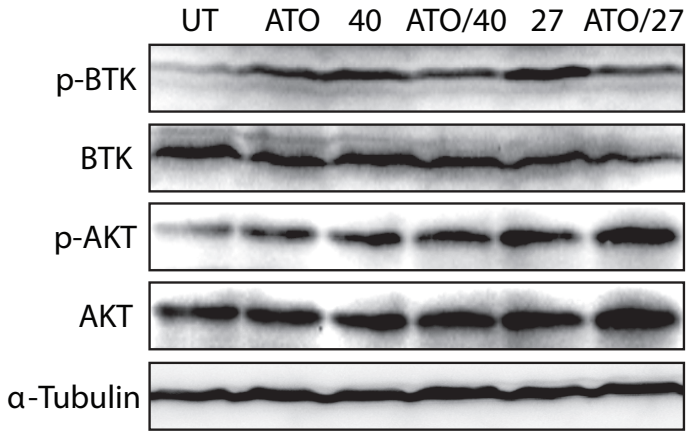
Supplemental Figure 6. Direct cell death and antibody-dependent cellular cytotoxicity in L428 cells after exposure to CD20-restoring agents. (A) Relative viability of L428 cells exposed to the CD20 antibodies Rituximab (20 µg/ml) or Tositumomab (10 µg/ml) after the indicated pre-treatments (*i.e.* ATO, 5 µM; Compound 40, 10 µM). (B) Tositumomab-mediated ADCC values in L1236 (left) or L428 cHL cells (right) pre-exposed to the indicated drugs or combinations as in A, 6 hours after the addition of the NFAT-luciferase-engineered effector T-cells, relative to no pre-exposure (untreated [UT]). Experiments were conducted in triplicate; data are presented as mean ± SD, with "*" denoting $P < .05$.

S7



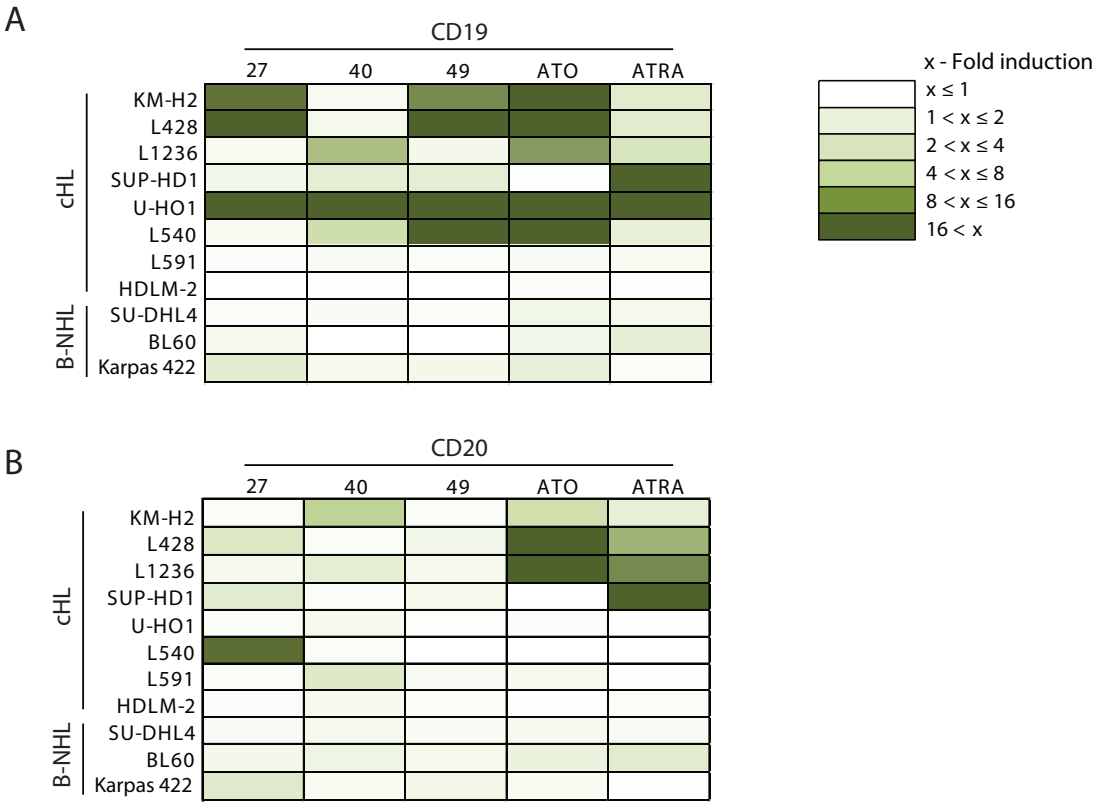
Supplemental Figure 7. Cytotoxicity in response to Ibrutinib or Idelalisib in otherwise treatment-naïve cHL cells. Viability of the indicated cHL cell lines after exposure to single-agent Ibrutinib or Idelalisib or left untreated (UT). As in Figure 6A,B, but pre-exposed to the solvent control for ATO and Compound 40 only, *i.e.* without any "B-cell phenotype-restoring" pre-treatment). Experiments were conducted at least in triplicate; data are presented as mean ± SD.

S8



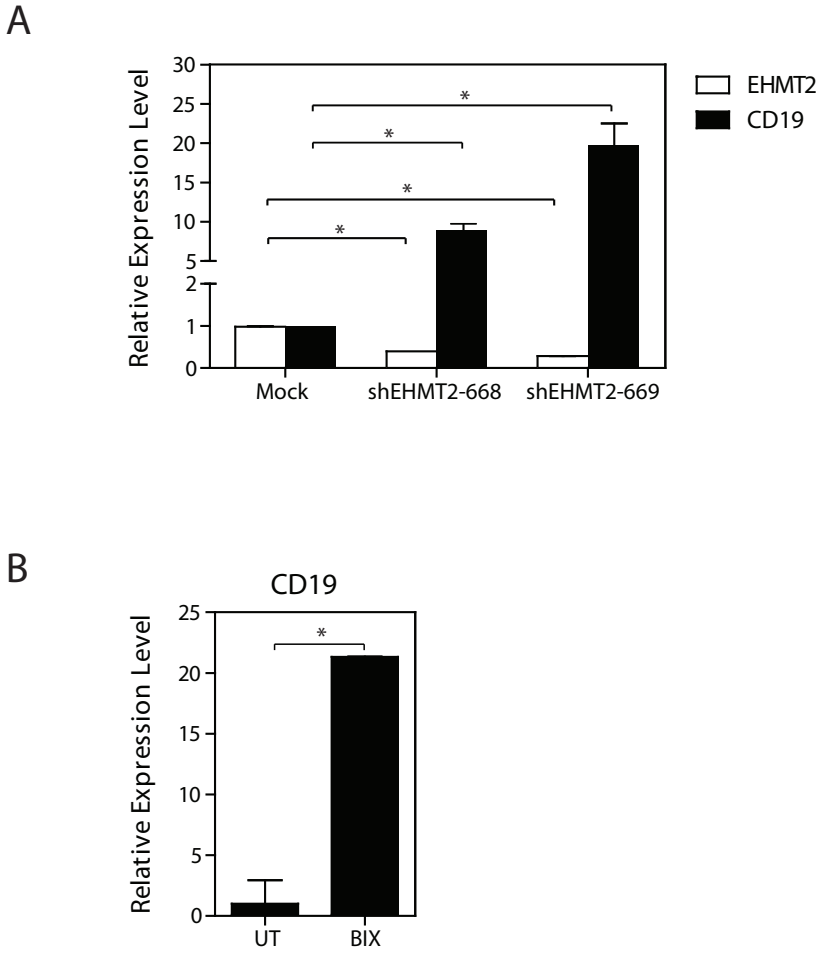
Supplemental Figure 8. Reactivation of BCR signaling components in the cHL cell line L428. Immunoblot analysis to detect p-BTK/BTK and p-AKT/AKT levels (as in Figure 6C) in L428 cells after single-agent or combination treatments or left untreated (UT) as indicated. α-Tubulin as a loading control.

S9



Supplemental Figure 9. Induction of B-cell-specific transcripts in an expanded panel of cHL cell lines. (A) Relative induction of *CD19* transcript levels by RQ-PCR after single-agent exposure to the indicated compounds (all at 10 µM for 48 hours) in an expanded panel of Hodgkin's cell lines (see color coding for fold-induction at the right). (B) As in A, but for *CD20* transcript levels.

S10



Supplemental Figure 10. CD19 induction following genetic or pharmacological inhibition of EHMT2. (A) Relative induction of *CD19* transcript levels and relative reduction of EHMT2 transcript levels in L428 cells by RQ-PCR after EHMT2 knockdown by two different small hairpins (shEHMT2-668 and shEHMT2-669; pLKO-empty as a control). (B) Relative induction of *CD19* transcript levels in L428 cells by RQ-PCR after exposure to the pharmacological EHMT2 inhibitor BIX-01294 (at 4.2 µM for 7 days) or left untreated (UT). Experiments were conducted at least in triplicate; data are presented as mean ± SD.