Histone acetylation and DNA demethylation of T cells result in an anaplastic large cell lymphoma-like phenotype

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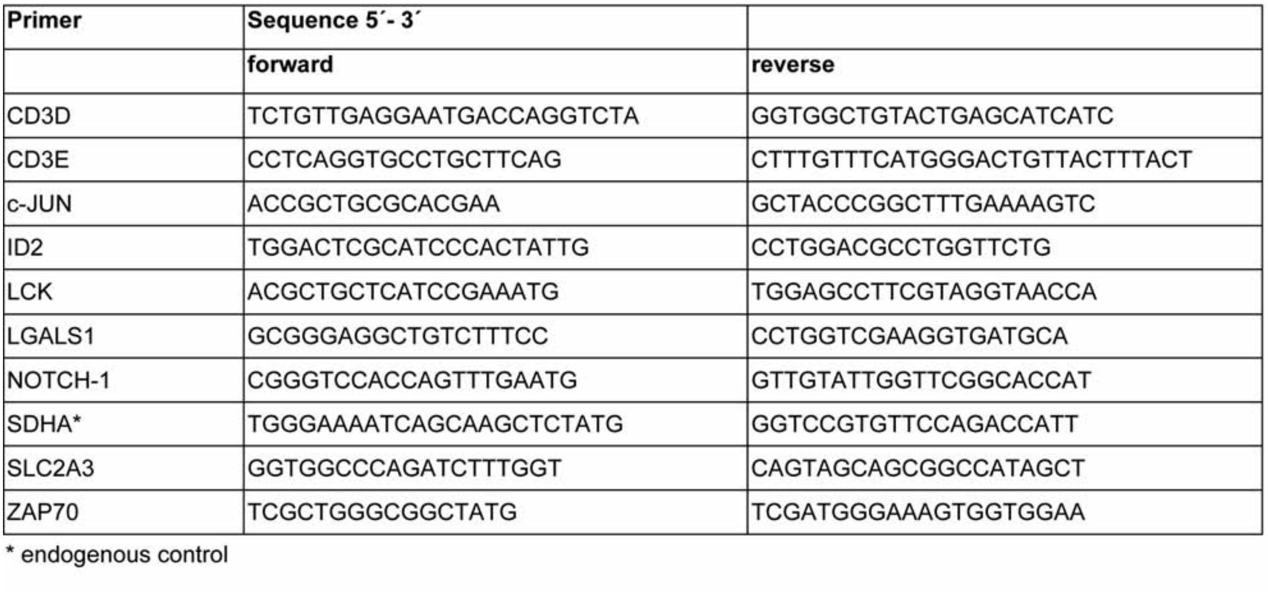
Online Supplementary Table S1. Primers used for real-time RT-PCR.



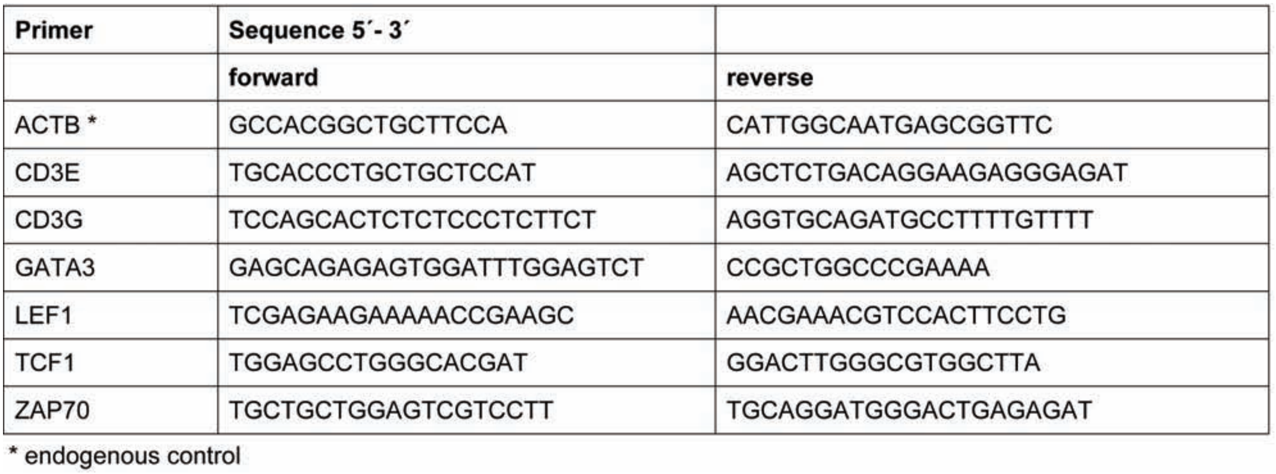
Primer	Sequence 5´- 3´	
	forward	reverse
CD3D	TCTGTTGAGGAATGACCAGGTCTA	GGTGGCTGTACTGAGCATCATC
CD3E	CCTCAGGTGCCTGCTTCAG	CTTTGTTTCATGGGACTGTTACTTTACT
c-JUN	ACCGCTGCGCACGAA	GCTACCCGGCTTTGAAAAGTC
ID2	TGGACTCGCATCCCACTATTG	CCTGGACGCCTGGTTCTG
LCK	ACGCTGCTCATCCGAAATG	TGGAGCCTTCGTAGGTAACCA
LGALS1	GCGGGAGGCTGTCTTTCC	CCTGGTCGAAGGTGATGCA
NOTCH-1	CGGGTCCACCAGTTTGAATG	GTTGTATTGGTTCGGCACCAT
SDHA*	TGGGAAAATCAGCAAGCTCTATG	GGTCCGTGTTCCAGACCATT
SLC2A3	GGTGGCCCAGATCTTTGGT	CAGTAGCAGCGGCCATAGCT
ZAP70	TCGCTGGGCGGCTATG	TCGATGGGAAAGTGGTGGAA

* endogenous control

Online Supplementary Table S2. Primary antibodies used for western blot analysis and immunohistochemistry.

Proteine	Catalog # / clone	Company
5-Methylcytidine	GTX75897/ 33D3	Gene Tex, Irvine, CA, USA
CD3	NCL-L-CD3-565/ UCHT1	Leica Biosystems Newcastle upon Tyne, UK
GATA3	SC-268/ HG3-31	Santa Cruz, Santa Cruz, CA, USA
Histone H3 (Acetyl Lys 9)	NB110-57045/ Y28	Novus Biologicals, Littleton, CO, USA
LEF1	2230/ C12A5	Cell SignalingTechnology, Danvers, MA, USA
RYBP	ab5976	abcam, Cambridge, UK
TCF1	WH0006932M1/ 1D2	Sigma Aldrich, Munich, Germany
ZAP70	05-253 / 2F3.2	Upstate Lake Placid, NY, USA

Online Supplementary Table S3. Primers used for real-time DNA-PCR after ChIP with an H3K27 trimethylation antibody.



Primer	**Sequence 5´- 3´**	
	forward	**reverse**
ACTB *	GCCACGGCTGCTTCCA	CATTGGCAATGAGCGGTTC
CD3E	TGCACCCTGCTGCTCCAT	AGCTCTGACAGGAAGAGGGAGAT
CD3G	TCCAGCACTCTCTCCCTCTTCT	AGGTGCAGATGCCTTTTGTTTT
GATA3	GAGCAGAGAGTGGATTTGGAGTCT	CCGCTGGCCCGAAAA
LEF1	TCGAGAAGAAAAACCGAAGC	AACGAAACGTCCACTTCCTG
TCF1	TGGAGCCTGGGCACGAT	GGACTTGGGCGTGGCTTA
ZAP70	TGCTGCTGGAGTCGTCCTT	TGCAGGATGGGACTGAGAGAT

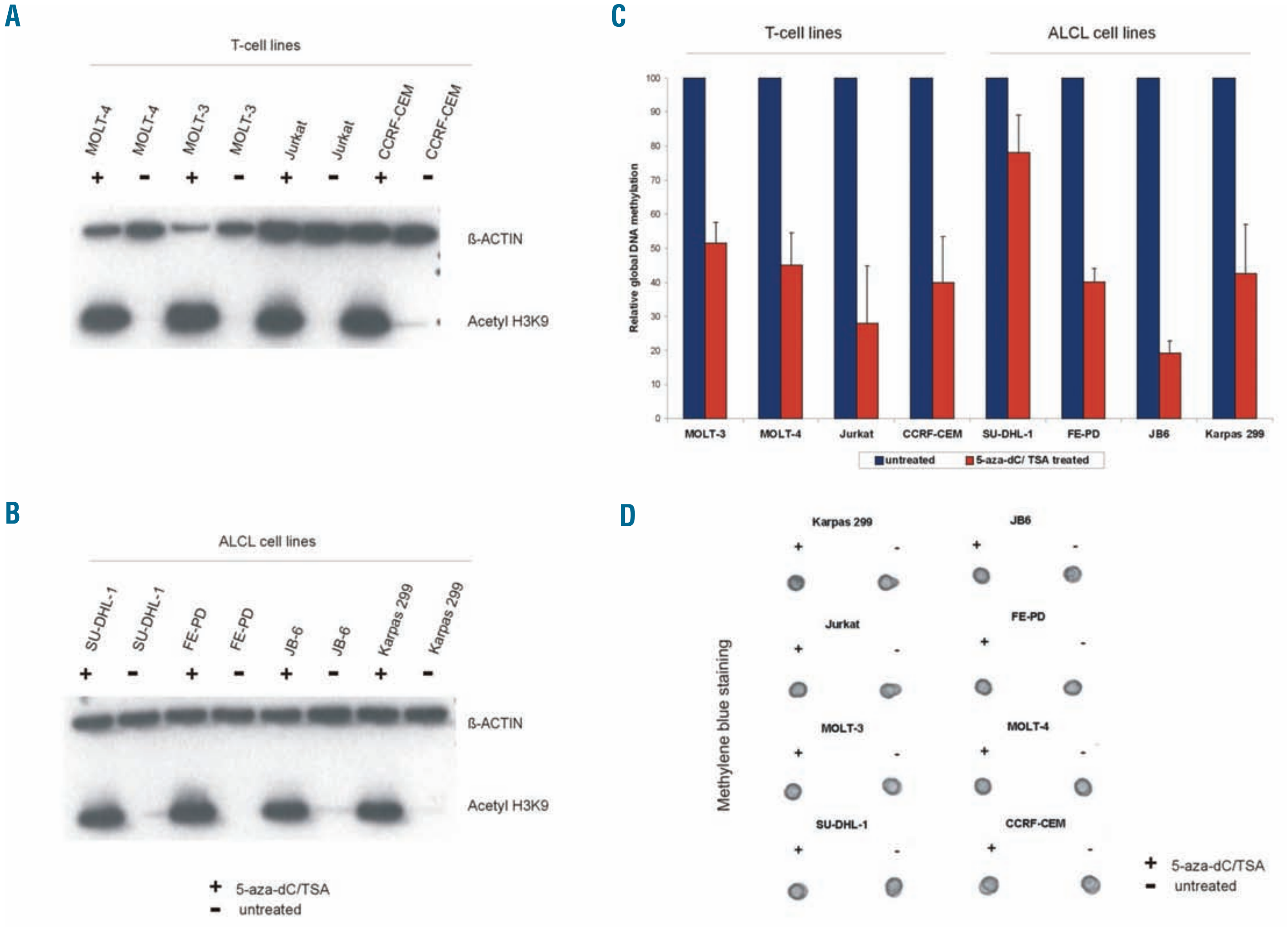
* endogenous control

Online Supplementary Table S4. Specifically 5-aza-dC/TSA-regulated genes in ALCL/T-cell lines. (A) Significantly down-regulated genes after 5-aza-dC/TSA treatment in ALCL cell lines: the gene expression profiles of four ALCL cell lines (FE-PD, JB6, Karpas 299, SU-DHL-1) were analyzed by Affymetrix GeneChip technology. (B) Significantly up-regulated genes after 5-aza-dC/TSA treatment in ALCL cell lines: the gene expression profiles of four ALCL cell lines (FE-PD, JB6, Karpas 299, SUDHL- 1) were analyzed by Affymetrix GeneChip technology. (C) Significantly down-regulated genes after 5-aza-dC/TSA treatment in T-cell lines: the gene expression profiles of four T-cell lines (CCRF-CEM, Jurkat, MOLT-3, MOLT-4) were analyzed by Affymetrix GeneChip technology. (D) Significantly up-regulated genes after 5-aza-dC/TSA treatment in T-cell lines: the gene expression profiles of four T-cell lines (CCRF-CEM, Jurkat, MOLT-3, MOLT-4) were analyzed by Affymetrix GeneChip technology. (SEE PDF FILE)

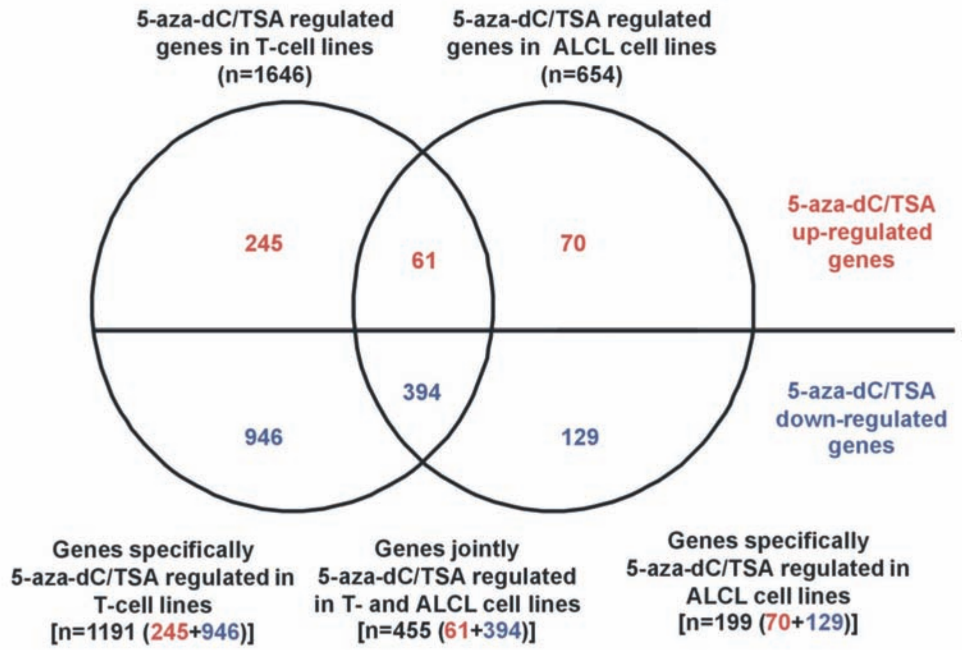
Online Supplementary Table S5. ALCL/T-cell characteristic genes. (A) ALCL characteristic genes: the gene expression profiles of four T-cell lymphoma cell lines (CCRF-CEM, Jurkat, MOLT-3, MOLT-4) and four ALCL cell lines (FE-PD, JB6, Karpas 299, SU-DHL-1) were analyzed by Affymetrix GeneChip technology. Significantly up-regulated genes in ALCL cell lines compared to T-cell lymphoma cell lines were considered as "ALCL characteristic genes". (B) T-cell characteristic genes: the gene expression profiles of four T-cell lines (CCRF-CEM, Jurkat, MOLT-3, MOLT-4) and four ALCL cell lines (FE-PD, JB6, Karpas 299, SU-DHL-1) were analyzed by Affymetrix GeneChip technology. Significantly up-regulated genes in T-cell lines compared to ALCL cell lines were considered as "T-cell characteristic genes". (SEE PDF FILE)

Online Supplementary Table S6. Significantly up-/down-regulated ALCL and T-cell characteristic genes in 5-aza-dC/TSA-treated T-cell lines. (A) Twenty-nine ALCL characteristic genes statistically significant up-regulated in 5-aza-dC/TSA-treated T-cell lines: the 29 genes were identified when the 277 ALCL characteristic genes were compared to the 245 genes up-regulated by 5-aza-dC/TSA treatment of T-cell lines (Figure 1A). (B) One hundred and seventy-six T-cell characteristic genes statistically significant down-regulated in 5-aza-dC/TSA-treated T-cell lines: the 176 genes were identified when the 406 T-cell characteristic genes were compared with the 964 genes down-regulated by 5-aza-dC/TSA treatment of T-cell lines (Figure 1B). (SEE PDF FILE)

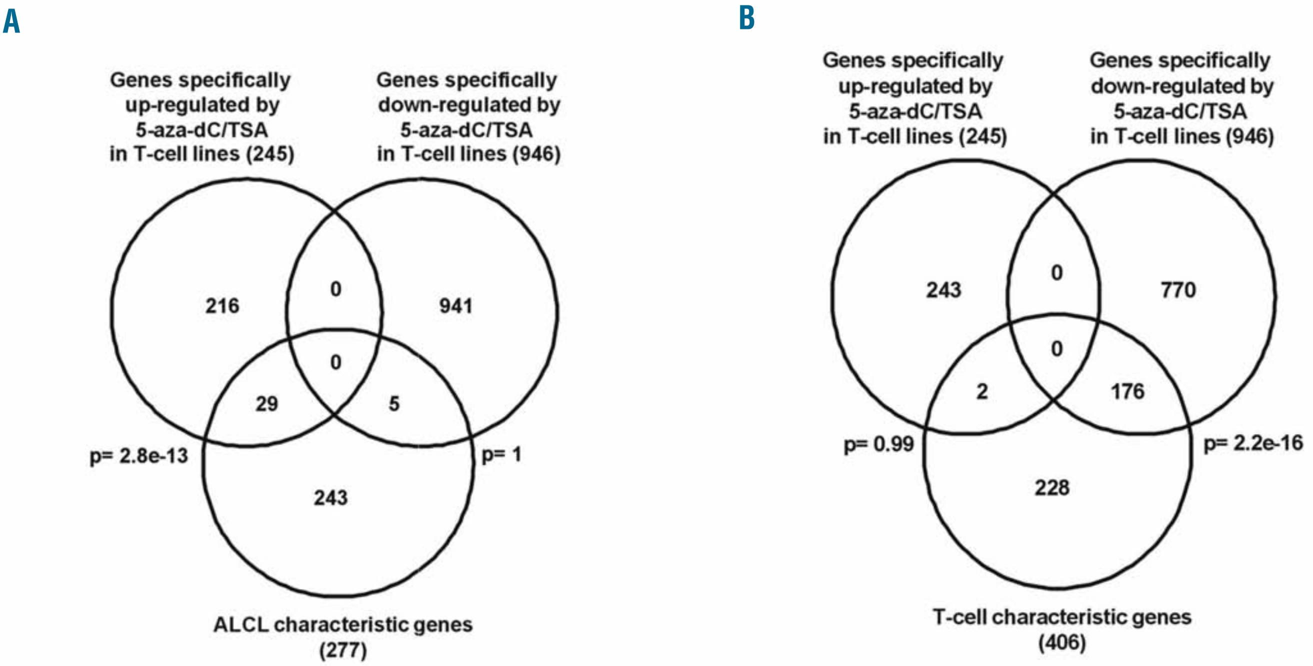
Online Supplementary Table S7. T-cell genes in 5-aza-dC/TSA-treated ALCL cells are not significantly reactivated. (A) ANOVA analysis of all four ALCL cell lines (FE-PD, JB6, Karpas 299, SU-DHL-1) (5-aza-dC/TSA-treated *vs.* untreated). Changes in expression of the listed T-cell characteristic genes are not statistically significant. (A) Normalized U133A hybridization data of several T-cell characteristic genes for each ALCL cell line (both 5-aza-dC/TSA-treated and untreated). Note that the expression value on U133A chips is never zero since there is always a hybridization background. (SEE PDF FILE)



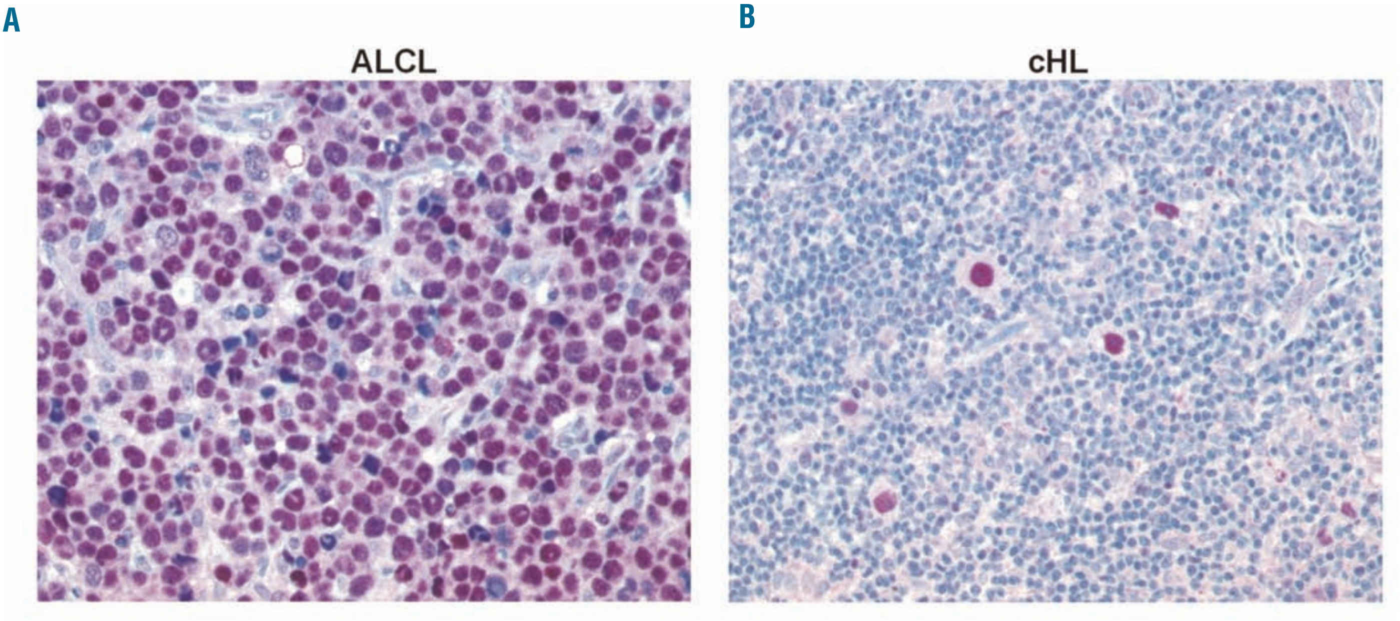
Online Supplementary Figure S1. Global histone H3K9 acetylation and DNA methylation levels after 5-aza-dC/TSA treatment A/B: Western blot analysis of global histone H3K9 acetylation in 5-aza-dC/TSA (+) and untreated (-) T-cell lines (A) and ALCL cell lines (B). Both 5-aza/TSA treated T- and ALCL cell lines show a very strong global increase of H3K9 acetylation. (C) Relative global DNA methylation intensity in 5-aza-dC/TSA T- and ALCL cell lines. The average relative global DNA methylation intensity after 5-aza-dC/TSA treatment was calculated from five independent immuno-dot blots. Both 5-aza/TSA treated T- and ALCL cell lines show a global decrease of DNA methylation. (D) Immuno-dot blots were stained with 0.02% methylene blue in 0.3M sodium acetate to ensure equal loading of total DNA (200 ng).



Online Supplementary Figure S2. Specific 5-aza-dC/TSA regulated transcripts in T-cell lines and ALCL cell lines. To detect specific 5-aza-dC/TSA regulated transcripts the differentially expressed genes in T-cell lines and ALCL cell lines were compared using a Venn diagram. The intersection represents 5-aza-dC/TSA jointly regulated genes in T-cell lines and ALCL cell lines.



Online Supplementary Figure S3. Venn diagrams comparing ALCL and T-cell characteristic genes with up- and down-regulated genes in T-cell lines after epigenetic treatment with 5-aza-dC/TSA. (A) An overlap of 29 genes was identified when the 277 ALCL characteristic genes were compared to the 245 genes up-regulated by 5-aza-dC/TSA treatment of T-cell lines (P=2.8x10^{-13}). In contrast a statistically not significant number of five ALCL characteristic genes were down-regulated in the 5-aza-dC/TSA-treated T-cell lines. (B) An overlap of 176 genes was identified when the 406 T-cell characteristic genes were compared with the 964 genes down-regulated by 5-aza-dC/TSA treatment of T-cell lines (P=2.2x10^{-16}). Only two T-cell characteristic genes were up-regulated in the 5-aza-dC/TSA-treated T-cell lines, which was not statistically significant.



Online Supplementary Figure S4. Immunohistochemistry for RYBP in (A) a primary ALCL and (B) a primary cHL. RYBP was strongly expressed in the nuclei of the tumor cells of ALCL and cHL whereas bystander cells displayed no or only faint expression (original magnification, 200x).