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PRDM1/BLIMP1 is commonly inactivated in anaplastic large T-cell lymphoma.

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Supplemental Materials

Supplementary Figure 1. Frequency of DNA gains (up) and losses (down) observed in seven ALCL cell lines [five derived from ALK(+), one from ALK(-) and one from cutaneous ALCL]. Red (up) represents gains and blue (down) represents losses. X-axis, chromosome localization and physical mapping; Y-axis, proportion of cases showing the aberrations.

Supplementary Figure 2. Validation of BLIMP1 deletion. FISH analysis has been performed for *PRDM1* gene deletion on all ALCL cell lines and three clinical specimens previously analyzed through the Affymetrix SNP6.0 platforms. Karpas299, SUDHL1 and ALK(-) clinical specimen 2475/05 pictures are reported as example. Orange: *PRDM1* gene locus (134 E15); Green: Cep 6 (RP11-164C22).

Figure S3. *PRDM1* is a tumor suppressor gene in other *in vitro* models of ALCL. (A) BLIMP1 protein level after infection with empty vector (pWPI) or vector for BLIMP1 re-expression (pWPI-HA-BLIMP1) in JB6 and SUDHL1 ALK(+) cell lines. (B) Growth curve after infection for JB6 and SUDHL1 cell lines, cells counted at day 2, 3, 4 after infection. (C) Percentage of GFP positive cells after infection at day 2, 3 and 4 in JB6 and SUDHL1 cell lines. (D) Percentage of dead cells after infection at day 2, 3 and 4 in JB6 and SUDHL1 cell lines.

Supplementary Figure 4. Kaplan-Meier graph showing OS in systemic ALCL according to the presence of *PRDM1* inactivation and/or *TP53* locus loss. X-axis, months; Y-axis, percentage of alive patients.

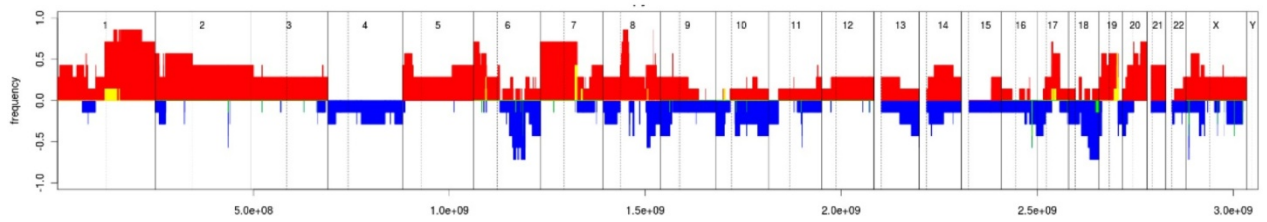
Supplementary Table 1. Primers used for DNA sequencing or real-time PCR.

Supplementary Table 2. Clinical characteristics of the ALCL series.

Supplementary Table 3. Significant regions affected by DNA gains and losses in ALCL, as estimated using the GISTIC algorithm.

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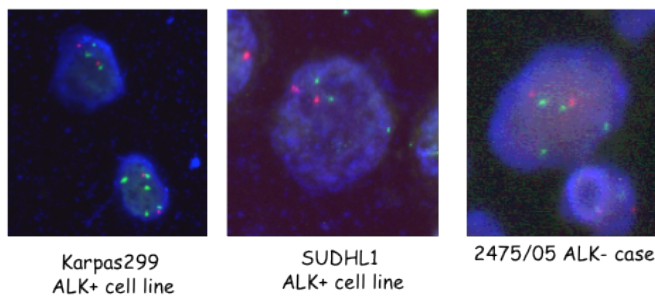
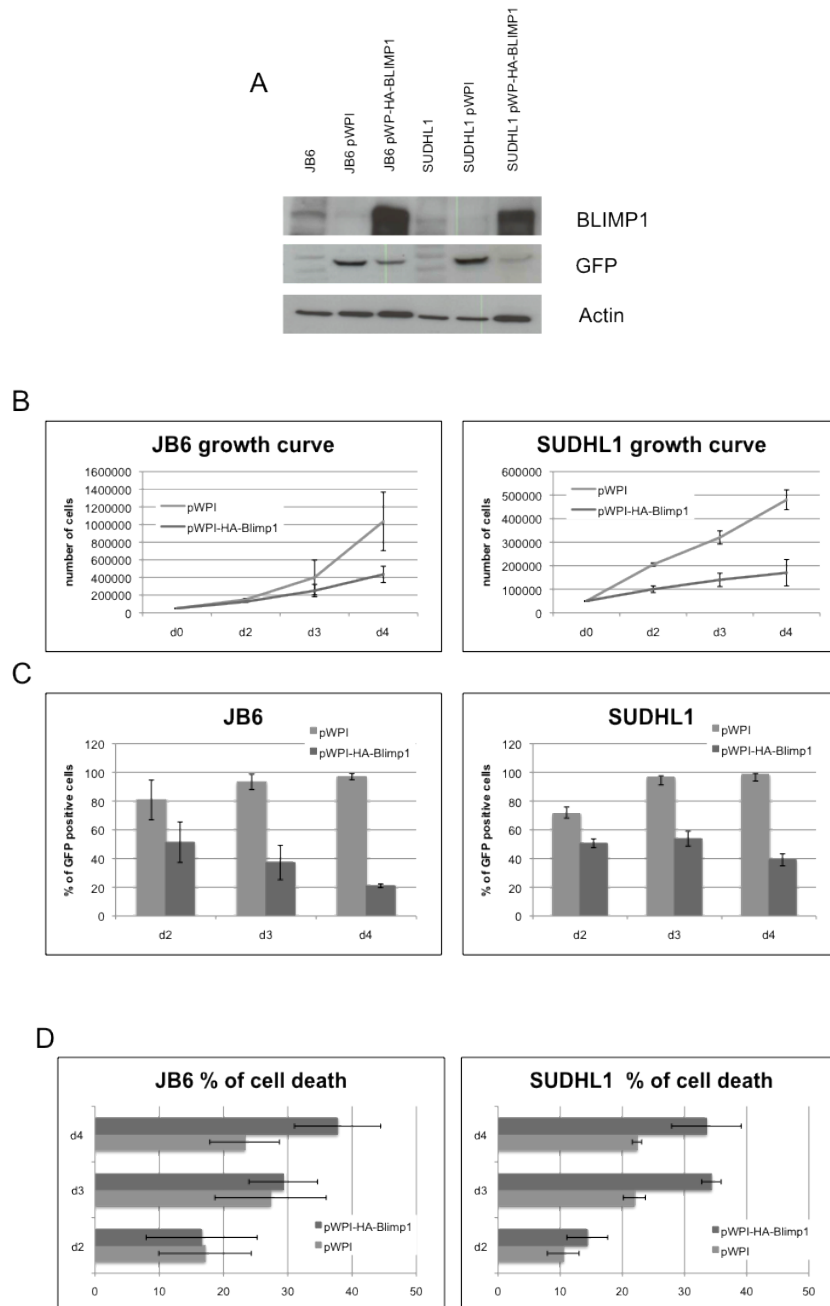
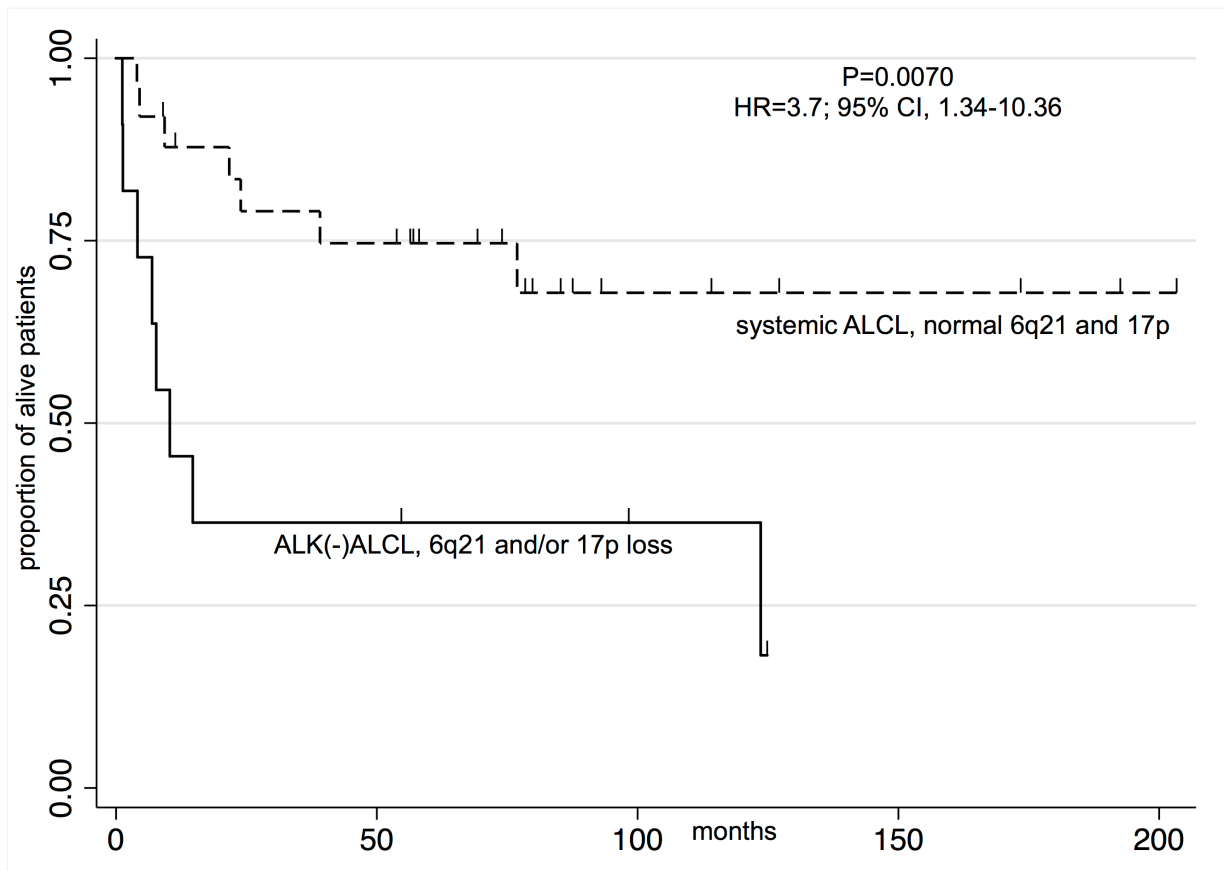


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Supplementary Figure 4. Kaplan-Meier graph showing OS in systemic ALCL according to the presence of *PRDM1* inactivation and/or *TP53* locus loss. X-axis, months; Y-axis, percentage of alive patients.



Supplementary Table 1. Primers used for DNA sequencing or real-time PCR.

| Application | Target | Primer FW (5'-...-3') | Primer REV (5'-...-3') |
|----------------------|------------------------|---------------------------|---------------------------|
| Mutational analysis | PRDM1-ex1 | TGACGCCAAACACATGTTAAA | GTTCCAGCTCACACTCGTCA |
| Mutational analysis | PRDM1-ex2 ¹ | TATACGGCTTCTTGGCTCTT | AGGAACAGTTGAAGGCTGG |
| Mutational analysis | PRDM1-ex3 ¹ | AGATGGTCTCCCCCTATGGT | AAGCAAGCAACAACTGTTTC |
| Mutational analysis | PRDM1-ex4 ¹ | GCCCTGATTTCTGCTGATTC | GTCCCTAGCTTAAGCCACCT |
| Mutational analysis | PRDM1-ex1b | TAGATGTTTCATCCCGTTCTGA | ACTTGAGAATGACCAAAATG |
| Mutational analysis | PRDM1-ex5 ¹ | TTGAGTGAGTGGCCAGAG | AGGGAAGTCACTTGTCCAAA |
| Mutational analysis | PRDM1-ex6 ¹ | AAACTCCCTGCTAGCCTGTG | GCCATCTCAAGTCATCAGCA |
| Mutational analysis | PRDM1-ex7 ¹ | CACAAGGAGGCTTCTCACCT | GATTTTCAGTAACCTTGGAGTT |
| Mutational analysis | TP53-ex4 ² | TCCTCTGACTGCTCTTTTCAC | TGAAGTCTCATGGAAGCCAG |
| Mutational analysis | TP53-ex5 ² | GTTTCTTTGCTGCCGTCTTC | AGCAATCAGTGAGGAATCAG |
| Mutational analysis | TP53-ex6 ² | TCTGATTCCTCACTGATTGCTC | CCACTGACAACCACCCTTAAC |
| Mutational analysis | TP53-ex7 ² | TCATCTTGGGCCTGTGTTATC | AGTGTGCAGGGTGGCAAG |
| Mutational analysis | TP53-ex8 ² | AGGACCTGATTTCTTACTGCC | ATAACTGCACCCTTGGTCTCC |
| Methylation analysis | PRDM1-meth | TTAGTAAATTTGGGGGAAAGTTTTG | TTAGTAAATTTGGGGGAAAGTTTTG |
| Real time PCR | PRDM1 | ACATGACCGGCTACAAGACC | GGCATTTCATGTGGCTTTTCT |
| Real time PCR | FGG | TGCATTAAGAGTGGAAGTGGAA | TGTTAGGCGTCACTTGTGTCAG |
| Real time PCR | SERPINA3 | GTTTCAGAGAGATAGGTGAGC | CTGGTGAAGGCTTCTCAAT |
| Real time PCR | SHIP1 | CCCTGCAAGAAATCACCAGT | ATCCGGTTCTCGTGCTCAG |
| Real time PCR | PMAIP1 | GAGATGCCTGGGAAGAAGG | TTCTGCCGGAAGTTCAGTTT |
| Real time PCR | GAPDH | CGACCACTTTGTCAAGCTCA | CCCTGTTGCTGTAGCCAAAT |

1. Pasqualucci L, Compagno M, Houldsworth J, et al. Inactivation of the PRDM1/BLIMP1 gene in diffuse large B cell lymphoma. *J Exp Med.* 2006;203(2):311-317.
2. Rassidakis GZ, Thomaidis A, Wang S, et al. p53 gene mutations are uncommon but p53 is commonly expressed in anaplastic large-cell lymphoma. *Leukemia.* 2005;19(9):1663-1669.

Supplementary Table 2. Clinical characteristics of the ALCL series ^a.

| | ALK- | | | ALK+ | | |
|---------------------------|---------------|-------|------------|----------------|-------|------------|
| | n | Valid | Percentage | n | Valid | Percentage |
| median age (range) | 60 (13-83) | 28 | | 21.5 (8-68) | 30 | |
| Male/Female | 17/11 | 28 | | 16/14 | 30 | |
| Stage | | | | | | |
| I | 3 | 14 | 21.4% | 3 | 18 | 16.7% |
| II | 6 | 14 | 42.8% | 3 | 18 | 16.7% |
| III | 1 | 14 | 7.2% | 4 | 18 | 22.2% |
| IV | 4 | 14 | 28.6% | 8 | 18 | 44.4% |
| B symptoms | | | | | | |
| Yes | 6 | 9 | 66.7% | 9 | 15 | 60% |
| No | 3 | 9 | 33.3% | 6 | 15 | 40% |
| Elevated LDH | | | | | | |
| Yes | 6 | 10 | 60% | 10 | 18 | 55.6% |
| No | 4 | 10 | 40% | 8 | 18 | 44.4% |
| BM involvement | | | | | | |
| Yes | 0 | 13 | 0% | 5 | 18 | 27.8% |
| No | 13 | 13 | 100% | 13 | 18 | 72.2% |
| Performance status | | | | | | |
| 0 | 3 | 8 | 37.5% | 7 | 12 | 58.3% |
| 1 | 4 | 8 | 50% | 1 | 12 | 8.3% |
| 2 | 1 | 8 | 12.5% | 4 | 12 | 33.4% |
| Therapy | | | | | | |
| CHOP regimen | 7 | 13 | 53.8% | 13 | 22 | 59.1% |
| no CHOP regimen | 6 | 13 | 46.2% | 9 | 22 | 40.9% |

^a LDH, lactate dehydrogenase; BM, bone marrow; CHOP, cyclophosphamide, doxorubicin, vincristine, prednisone.

Supplementary Table 3. Significant regions affected by DNA gains and losses in ALCL, as estimated using the GISTIC algorithm.

| Lesions | Cytoband | Frequency | Start* | Size* | q-value | Residual q-value | Candidate gene |
|---------|----------|-----------|-----------|----------|-----------|------------------|---------------------------------|
| Gains | | | | | | | |
| | 9p24.1 | 11% | 5,51E+06 | 2,64E+06 | 0,23624 | 0,23624 | <i>NCAM2</i> |
| | 3p26.3 | 6% | 1,67E+06 | 1,14E+05 | 0,23624 | 0,23624 | |
| | 15q11.2 | 7% | 2,53E+07 | 3,74E+04 | 0,23624 | 0,23624 | <i>IPW</i> |
| | 20q11.22 | 6% | 3,28E+07 | 2,69E+05 | 0,23624 | 0,23624 | <i>ASIP, AHCY, ITCH, MIR644</i> |
| | 11p13 | 5% | 3,48E+07 | 6,87E+05 | 0,23624 | 0,23624 | <i>APIP, PDHX, CD44</i> |
| | 13q31.3 | 3% | 8,90E+07 | 4,22E+06 | 0,23624 | 0,23624 | <i>MIR17HG</i> |
| | 15q25.2 | 3% | 8,45E+07 | 2,85E+04 | 0,23624 | 0,23624 | <i>ADAMTSL3</i> |
| Losses | | | | | | | |
| | 17p13.1 | 25% | 6094336 | 1,62E+06 | 0,081305 | 0,0099598 | <i>TP53</i> |
| | 6q21 | 17% | 94817995 | 2,12E+07 | 4,68E-11 | 2,81E-10 | <i>ATG5, PRDM1</i> |
| | 13q32.3 | 16% | 93231989 | 1,52E+07 | 0,0025428 | 0,011625 | |
| | 14q11.2 | 16% | 22185014 | 1,29E+06 | 6,57E-08 | 1,88E-07 | <i>TCRA, TRAC</i> |
| | 16q23.2 | 16% | 78750889 | 4,46E+03 | 1 | 0,1503 | <i>WWOX</i> |
| | 12q24.31 | 13% | 121569998 | 5,68E+05 | 0,052865 | 0,08445 | |
| | 13q21.31 | 13% | 62409809 | 3,97E+06 | 0,0092452 | 0,040311 | |
| | 13q31.1 | 13% | 82365728 | 6,66E+06 | 0,052865 | 0,098264 | <i>SLITRK1, SLITRK6</i> |
| | 1p13.2 | 13% | 108240651 | 1,09E+07 | 0,026836 | 0,014931 | |
| | 13q21.2 | 11% | 60450644 | 2,02E+05 | 1 | 0,18826 | <i>DIAPH3</i> |
| | 12q12 | 9% | 44431029 | 3,90E+05 | 0,57913 | 0,11838 | <i>TMEM117</i> |
| | 15q26.1 | 9% | 89159161 | 4,58E+04 | 0,052865 | 0,08445 | <i>AEN, ISG20</i> |
| | 2q37.3 | 8% | 240125114 | 1,50E+05 | 0,026836 | 0,041884 | <i>HDAC4, MIR4269</i> |
| | 18q22.1 | 5% | 65059883 | 3,95E+05 | 0,0067821 | 0,013671 | <i>DSEL</i> |
| | 20q13.13 | 5% | 48907930 | 7,76E+04 | 0,048594 | 0,069286 | |
| | 7q31.1 | 5% | 110681611 | 8,13E+04 | 0,14064 | 0,18826 | <i>IMMP2L</i> |
| | 1q32.1 | 3% | 198580256 | 3,31E+05 | 0,026836 | 0,041884 | <i>PTPRC</i> |
| | 14q23.1 | 3% | 61926178 | 1,41E+03 | 1 | 0,18826 | <i>PRKCH</i> |
| | 1p31.3 | 3% | 66726940 | 9,79E+04 | 0,081305 | 0,1503 | <i>PDE4B</i> |
| | 21q21.1 | 2% | 22619036 | 3,63E+03 | 1 | 0,11838 | <i>NCAM2</i> |

* Numbering according to Genome Reference Consortium Human Build 37 (GRCh37) (hg19).

Supplementary Table 4. Significant regions affected by DNA gains and losses in ALK-ALCL, as estimated using the GISTIC algorithm.

| Lesions | Cytoband | Frequency | Start* | Size* | q-value | Residual q-value | Candidate Gene |
|---------|----------|-----------|----------|----------|-----------|------------------|---------------------------------|
| Gains | | | | | | | |
| | 20q11.22 | 12% | 3,28E+07 | 2,69E+05 | 0,22719 | 0,22719 | <i>ASIP, AHCY, ITCH, MIR644</i> |
| | 11p13 | 6% | 3,48E+07 | 6,87E+05 | 0,22719 | 0,22719 | <i>APIP, PDHX, CD44</i> |
| | 13q31.3 | 6% | 8,90E+07 | 4,22E+06 | 0,22719 | 0,22719 | <i>MIR17HG</i> |
| Losses | | | | | | | |
| | 17p13.1 | 42% | 7,47E+06 | 1,06E+04 | 0,99208 | 0,1585 | <i>TP53</i> |
| | 14q11.2 | 39% | 2,22E+07 | 1,29E+06 | 6,74E-06 | 3,35E-05 | <i>TCRA, TRAC</i> |
| | 6q21 | 33% | 9,48E+07 | 1,86E+07 | 1,43E-08 | 8,94E-08 | <i>ATG5, PRDM1</i> |
| | 12q24.31 | 27% | 1,22E+08 | 5,68E+05 | 0,021862 | 0,039734 | |
| | 1p13.2 | 24% | 9,85E+07 | 2,19E+07 | 0,0095216 | 0,0063173 | |
| | 13q21.31 | 24% | 6,24E+07 | 3,97E+06 | 0,003499 | 0,066829 | |
| | 13q21.33 | 24% | 6,64E+07 | 7,05E+06 | 0,0010479 | 0,25555 | <i>PCDH9, KHLH1</i> |
| | 13q33.1 | 24% | 9,32E+07 | 1,52E+07 | 0,003499 | 0,047195 | <i>SLITRK1, SLITRK6</i> |
| | 13q21.2 | 21% | 6,05E+07 | 2,02E+05 | 0,99208 | 0,14511 | <i>DIAPH3</i> |
| | 13q31.1 | 21% | 8,24E+07 | 6,59E+06 | 0,021862 | 0,091229 | |
| | 20p13 | 18% | 7,35E+04 | 1,93E+03 | 0,1217 | 0,21329 | |
| | 20q13.13 | 18% | 4,89E+07 | 4,00E+08 | 0,019672 | 0,039734 | |
| | 4q22.2 | 15% | 9,40E+07 | 1,39E+04 | 1 | 0,21329 | <i>GRID2</i> |
| | 18q23 | 15% | 6,51E+07 | 7,06E+07 | 0,021862 | 0,039734 | |
| | 21q21.1 | 6% | 2,26E+07 | 3,63E+03 | 1 | 0,047195 | <i>NCAM2</i> |

* Numbering according to Genome Reference Consortium Human Build 37 (GRCh37) (hg19).