Immunohistochemical detection of inhibitor of DNA binding 3 mutational variants in mature aggressive B-cell lymphoma

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Supplemental Data

Letter to the Editor

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III Supplemental Materials and Methods

Lymphoma sample selection, pathology review and description of the cohort

Eighty nine primary formalin-fixed and paraffin-embedded (FFPE) biopsies of 38 molecular Burkitt lymphomas (mBL), 14 intermediate lymphomas, 36 non-mBL with molecular diagnoses according to [1] and 1 nodal manifestation of BL leukemia without molecular diagnosis due to lacking molecular classification algorithms were extensively characterized in terms of histopathological diagnosis, immunophenotype, molecular diagnosis, molecular subtype and genomic aberrations (refer to Additional File 5).
Forty one cases were diagnosed and molecularly characterized within the framework of the MMML joint project by expert reference pathology, standard immunohistochemistry (CD20, CD10, BCL2, BCL6, Mum-1, Ki-67), standard clonality IGH-PCR, fluorescence in situ hybridisation (FISH), array comparative genome hybridisation (array-CGH), single nucleotide polymorphism (SNP) array hybridisation and gene expression profiling by mRNA hybridisation as described [1].

Forty three cases were diagnosed and characterized within the German ICGC MMML-Seq subproject by expert reference pathology, standard immunohistochemistry (CD20, CD10, BCL2, BCL6, Mum-1, Ki-67), FISH, whole genome, exome and transcriptome sequencing [2,3].

Five additional cases (ID 15, 31, 50, 81, and 88) were characterized and analysed in both joint projects.

Ethics, consent and permissions

The protocols of the MMML network have been approved by central (University of Göttingen) and local ethic review boards (Institutional Review Board of the Medical Faculty of the University of Kiel, D403/05).
The ICGC MMML-Seq study has been approved by Ethics Committee of the Medical Faculty of the University of Kiel (A150/10) and of the recruiting centres.
All cases within the frameworks of the projects were studied with informed consent of the respective parents and patients (if above 14 years old).

Cell culture

The dimethyl sulfoxide (DMSO)-preserved cell lines BL-2, BL-41, BL-70, BLUE-1, DAUDI, EB-1, RAJI, SU-DHL-10, CA-46, DG-75, NAMALWA, RAMOS, U-689-M, MC-116, HT, SU-DHL-5 and SU-DHL-6 were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany) and controlled for authenticity by STR analysis using the StemElite ID System (Promega, Mannheim, Germany) [2].
Cell lines were thawed and cultured in RPMI 1640 medium with 2 mM L-glutamine (Life Technologies, Germany), supplemented with 10-20% FCS and 1x penicillin/streptomycin (Life Technologies, Germany) in 5,5% CO_2 at 37°C. Cells were grown until nearly reaching the log phase and harvested for protein extraction ($5x10^6$ cells per extraction) and FFPE block production ($5\text{-}10x10^6$ cells).

Cell line FFPE blocks

Freshly grown $5\text{-}10x10^6$ cells were centrifuged at 800 rpm for 10 min and washed once in 1,5 ml 1x PBS buffer. After centrifugation at 800 rpm for 5 min cells were re-suspended in 4,5% PBS-buffered formalin and incubated for 20 min at room temperature. After centrifugation at 800 rpm for 5 min the cell pellet was washed in 3-5 ml 1x PBS, centrifuged again and re-suspended in 2 ml 96% isopropyl

and 3 droplets of premade chicken egg white/glycerol solution (Waldeck GmbH & Co. KG, Muenster, Germany). After 5 min centrifugation at 800 rpm the pellet was wrapped into a filter paper, placed in a metal capsule and incubated overnight in 96% isopropyl. The fixed cell pellet was further routinely processed for paraffin embedding.

Immunohistochemistry

FFPE blocks of cell lines and patient tumor biopsies were cut (3-5 µm) onto glass slides, dried over night at 37°C and attributed to immunohistochemical stainings according to standard protocols described previously [1]. The anti-ID3 antibody test and experimental stainings were performed as described in Additional File 1. ID3 IHC scoring was performed by WK according to the percentage of positive tumor cells within the lymphoma biopsy or cell line FFPE section from 0 to 4 (0=0%; 1=1-25%; 2=26-50%; 3=51-75%; 4=76-100%). ID3 staining results for cell lines and cases are shown in Figure 1, Additional File 2, 4, and 5.

Protein extraction

Freshly grown 5-10x10^6 cells were harvested by centrifugation as described above. The supernatant was completely removed. The cell pellet was immediately snap-frozen in liquid nitrogen, transferred to -80°C freezer and stored until extraction.
For protein extraction from BL-2, BL-41, BL-70, BLUE-1, DAUDI, EB-1, RAJI, and SU-DHL-10, frozen pellets of 5x10^6 cells were re-suspended in 500 µl ice-cold lysis buffer (1x PBS, 2% Triton, 1 mM EDTA), freshly supplemented with 75 µl 25x protease blocker (Roche, Germany) and incubated for 30 min on ice. For protein extraction from CA-46, DG-75, NAMALWA, RAMOS, U-698-M, MC-116, HT, SU-DHL-5 and SU-DHL-6 1 ml DMSO-stabilized 5x10^6 cells were rapidly thawed in a water bath at 37°C, briefly mixed with 10 ml warmed RPMI 1640 medium and pelleted at 800 rpm for 10 min at room temperature. After removal of supernatant, cells were lysed as described above. After 10 min centrifugation at 14 000 rpm at 4°C the protein extract was aliquoted, snap-frozen in liquid nitrogen and stored at -80°C until usage. 5 µl aliquots were taken prior to freezing to measure the protein concentration using the Qubit 2.0 Fluorometer (Life Technologies, Darmstadt, Germany).

Western blots

An equivalent volume of protein extract containing 30 µg of protein was supplemented with an appropriate volume of 6x loading buffer (0.35 M Tris-HCl, 10.28% SDS, 36% glycerol, 5% 2-mercaptoethanol, 0.012% bromophenol blue), mixed and incubated at 95°C for 5 min to denature proteins. The protein extracts were loaded onto the precast NuPAGE 4-12% Bis-Tris gels (Life Technologies, Darmstadt, Germany) which were pre-run for 20 min at 40V and run with the 1x NuPAGE MES-SDS Running Buffer (Life Technologies, Darmstadt, Germany) at 100V for 1.5 h. Protein transfer was done in 1,44 % glycine, 0,3% TRIZMA, and 20% methanol according to manufacturer's instructions using the Hybond-P PVDF membrane (Amersham Biosciences / GE Healthcare, Buckinghamshire, UK). After blocking the membrane in 1x TBST buffer (0.242 % TRIZMA, 0.8 % NaCl, 0.01% Tween-20, pH 7,6) with 5% non-fat dry-milk for 1 h (BioRad Laboratories GmbH, Germany) and washing it for 5 min in 1x TBST, the rabbit monoclonal anti-mouse/human ID3 antibody clone 17-3 (BioCheck Inc., USA) in blocking buffer was applied to the membrane at 1:2500 dilution for 2 h at room temperature. After washing 3x for 15 min in 1x TBST a HRP-conjugated goat anti-rabbit antibody (DAKO Deutschland GmbH, Hamburg, Germany) at 1:10000 dilution was incubated with the membrane for 45 min, the latter washed again thrice in 1x TBST and developed with the ECL Western Blotting Detection Reagent (Amersham Biosciences / GE Healthcare, UK). The chemiluminescence was recorded using the ChemiDocMP System (BioRad Laboratories GmbH, München, Germany).

Blot stripping was performed by incubating the membrane in 1x stripping buffer according to manufacturer's recommendation for 30 min at 50°C (ECL Western Blotting Detection Reagent booklet, Amersham Biosciences / GE Healthcare, UK). Loading control Western blots were performed analogously to the anti-ID3 procedure using the monoclonal mouse anti-human β-tubulin antibody (#T-4026, Sigma-Aldrich Chemie, Taufkirchen, Germany) at 1:5000 dilution in TBST for 1 h at room temperature. The secondary antibody was a HRP linked sheep ECL anti-mouse IgG (#NA931V, Amersham Biosciences).

***ID3* mutational analysis**

DNA and RNA were isolated as previously described [1,2]. *ID3* mutational analyses for both the MMML cohort and the cell lines used in this study were performed previously as described [2]. The *ID3* mutational status for the ICGC MMML-Seq cohort was obtained from whole-genome sequencing data by using the following methods: (i) an in-house analysis pipeline based on SAMtools mpileup and bcftools was used to detect single nucleotide variants (SNVs) [4] (ii) Indels were called with Platypus [5] on the tumor BAM file and control BAM file and custom scripts were used to extract high-confidence somatic variants. SNVs and Indels were annotated using Annovar [6] and in-house-developed scripts. The *ID3* mutational status was verified by manual inspection using the Integrative Genomics Viewer [7].

Molecular diagnosis

Molecular diagnoses of the MMML cases were assigned as previously described [1]. DLBCL subtypes of the MMML cohort were assigned according to Wright et al. [8] with adaptations for the HGU133A Affymetrix GeneChip, which were previously described [1] and according to Hans et al., [9] based on immunohistochemistry.
Further algorithm adaptation was performed to obtain molecular diagnoses and DLBCL subtypes from RNAseq data for the ICGC MMML-Seq cohort In short: RNAseq data of exemplar regions of the Affymetrix probesets included in the classifiers was analysed. Initial classification was derived by pathological review for BL/DLBCL classification and by unsupervised hierarchical clustering for DLBCL subtypes. For each probeset the distribution of the subtype-specific expression was estimated using initial classification. The final classification was determined by comparison of the expression of all probesets of a sample with the respective estimated probability distributions (Kreuz et al., manuscript in preparation).

Availability of data sets

MMML data sets are available at the GEO database (http://www.ncbi.nlm.nih.gov/geo/) under GEO accessions: GSE4475, GSE10172, GSE22470, GSE48184, GSE57612 [1,10-13]. For review purposes of the ICGC MMML-Seq cohort, please contact the data access committee (DAC; http://www.icgc.org/daco) of the International Cancer Genome Consortium (ICGC, http://www.icgc.org/) to get access to the data, which will be made public upon publication of submitted and/or revised current manuscripts for the ICGC MMML-Seq.

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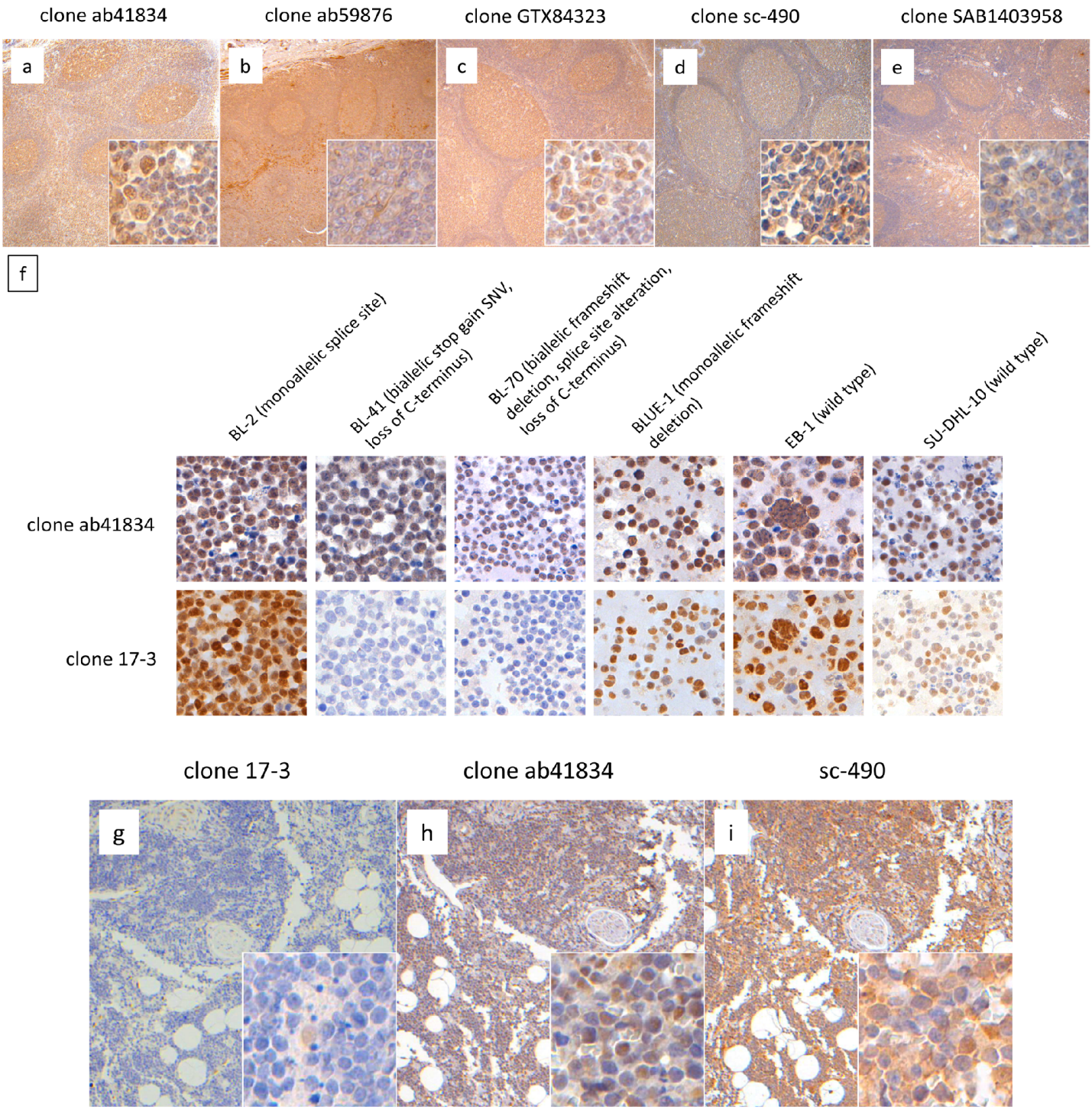
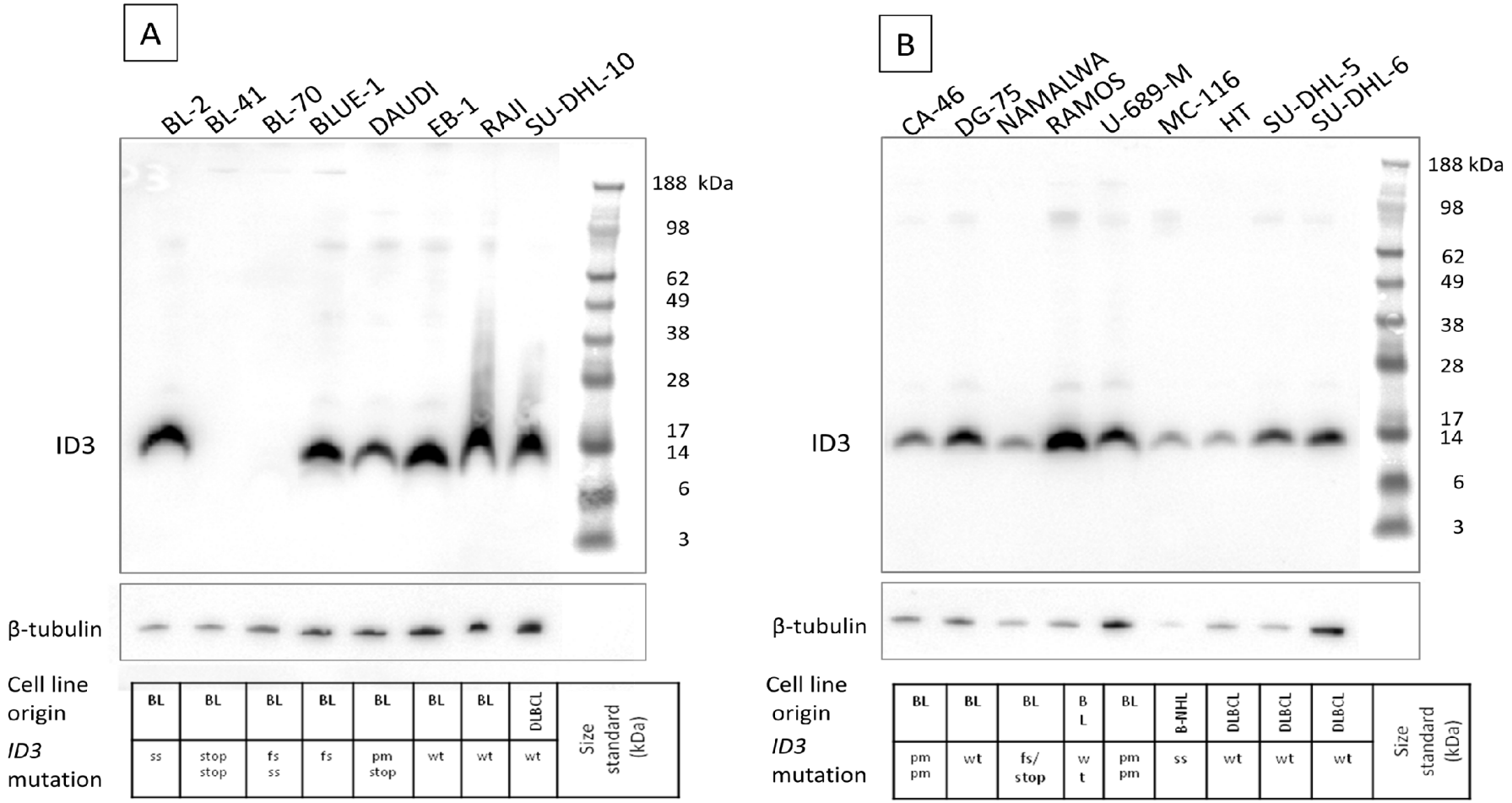
V Supplemental Figures



Supplemental Figure 1. ID3 immunohistochemistry of tonsils, FFPE cell lines, and FFPE mBL. Upper panel, **a-e.** FFPE sections of tonsils were stained with various antibodies and protocols to obtain best possible results. Representative examples are shown (original magnification 50x). Inlets represent high magnifications (400x) from the boundary between the germinal center and the mantle zone; **f.** Immunohistochemical staining of FFPE cell line sections with clone 17-3 and ab41834. Cell lines and their *ID3* mutational status are indicated. Clone ab41834 showed an artificially positive staining for ID3 in BL-41 and BL-70 with both cell lines harbouring biallelic losses of the ID3 C-terminus.; **g-i.** Case 6, mBL, homozygous deletion in 1p.36 (including the *ID3* locus); **g.** Clone 17-3, expectedly negative ID3 staining; **h.** ab41834 and **i.** sc-490, non-specific positive ID3 staining. For details on the mutations and the immunohistochemical score refer to Supplemental Table 3.



Supplemental Figure 2. ID3 expression in BL and DLBCL cell lines. Western blots were performed using the anti-ID3 antibody clone 17-3. 20 µg protein were used per cell line. (A) Cell lines BL-41 and BL-70 with homozygous loss of *ID3* C-terminal domains expectedly show no ID3 expression indicating specificity of the antibody for ID3. In (A) and (B) further cell lines with wt and monoallelic mutations of *ID3* stain positively for ID3. For details regarding the *ID3* mutational status of cell lines refer to [3] and to the Supplementary Table 2. Size standard: SeeBlue Plus 2 (Novex #LC5925). Abbreviations: BL, Burkitt lymphoma; BNHL, B-cell non-Hodgkin lymphoma; DLBCL, diffuse large B-cell lymphoma; fs, frameshift mutation; pm, point mutation; stop, nonsense mutation; ss, splice site mutation; wt, wild type.

VI Supplemental Tables

Supplemental Table 1. Antibodies and staining protocols.

Antibody catalogue no.	Clone	Manufacturer	Epitope	Species	Dilution	Antigene Retrival
ab41834	n.a.	abcam	C-terminal	rabbit poly	1:25, 1:50, 1:100	3 min citrate buffer at pH 6 or 8
ab50876	n.a.	abcam	N-terminal	rabbit poly	1:5, 1:25 and o.n., 1:50, 1:100	3 min citrate buffer at pH 6
sc-490	C20	Santa Cruz Technology	C-terminal	rabbit poly	1:25, 1:50, 1:100	3 min citrate buffer at pH 6 or 8 / 15 min proteinase K at 37° / BOND ER2
GTX84323	8B3	Gene Tex	not specified	mouse mono	1:50, 1:100	3 min citrate buffer at pH 6 or 8
SAB1403958	3E10	Sigma Aldrich	not specified	mouse mono	1:50, 1:100	3 min citrate at pH 6
BCH-4/17-3	17-3	BioCheck Inc.	not specified	rabbit mono	1:500	3 min citrate buffer at pH 9 (manually) / BOND ER2

Supplemental Table 2. Cell line data. Sections of 3 µm were cut from cell line FFPE blocks and stained for ID3 with clone 17-3 (BioCheck Inc., Foster City, USA) and ab41834 (abcam, Cambridge, UK), the latter showing the second best immunohistochemistry staining pattern in tonsils (refer to Supplemental Figure 1). Clone ab41834 showed an artificially positive staining for ID3 in BL-41 and BL-70 with both cell lines harbouring biallelic losses of the ID3 C-terminus. Thus, only clone 17-3 was further used to detect ID3 protein expression in protein extracts from snap-frozen B-cell lymphoma cell lines (refer to Supplemental Figure 2). ID3 immunohistochemistry scoring: 0 = 0% ID3+ tumor cells, 1 = 1-25% ID3+ tumor cells, 2 = 26-50% ID3+ tumor cells, 3 = 51-75% ID3+ tumor cells, 4 = 76-100% ID3+ tumor cells. ID3 Western blot scoring: +++ strong expression, ++ slightly reduced expression, + reduced expression, - no expression. # mutational data according to [3].

Cell line	Entity	*ID3* status#	*ID3* mutation#	ID3 IHC score clone 17-3	Western blot clone 17-3	ID3 IHC score ab41834
BL-2	BL	splice site	c.300G>A (sm), c.300+1G>C	4	+++	4
BL-41	BL	stop gain (biallelic)	c.202G>C; 202G>C, p.Q68*; Q68*	0	-	4
BL-70	BL	frameshift deletion + splice site (biallelic)	c.139_264del, p.C47P*32; c.300+1G>A	0	-	4
BLUE-1	BL	frameshift deletion	c.236_240delACCTG, p.N79Afs*13	4	++	4
DAUDI	BL	nonsyn SNV + stop gain	c.160C>G, p.L54V; c.241C>T, p.Q81*	4	++	4
EB-1	BL	wt	wt	4	+++	4
RAJI	BL	wt	wt	4	+++	n.d.
SU-DHL-10	DLBCL	wt	wt	4	+++	4
CA-46	BL	nonsyn SNV	c.160C>G, p.L54V; c.190C>T, p.L64F	4	+	n.d.
DG-75	BL	wt	wt	4	++	n.d.
NAMALWA	BL	frameshift deletion with stop gain	c.220_360+66del, p.I74V*26	3	+	n.d.
RAMOS	BL	wt	wt	4	+++	n.d.
U-689-M	BL	nonsynSNV	c.166C>T, p.P56S; c.233T>C, p.L78P	4	++	4
MC-116	B-cell lymphoma	splice site	c.300G>A (sm), c.300+1G>A	4	+	n.d.
HT	DLBCL	wt	wt	3	+	4
SU-DHL-5	B-NHL	wt	wt	4	++	n.d.
SU-DHL-6	B-NHL	wt	wt	4	++	n.d.

Abbreviations: wt, wild type; nonsyn SNV, nonsynonymous single nucleotide variant; B-NHL, B-cell non-Hodgkin lymphoma; sm, silent mutation; n.d., not determined

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Supplemental Table 3: Case data. All cases were diagnosed by a panel of 5 expert hematopathologists and characterized by means of standard immunohistochemistry as published previously [11]. *MYC, IG, BCL2, and BCL6* translocation status was analysed as published previously [3,11] (and hitherto unpublished data). ID3 immunohistochemistry by clone 17-3 was analysed according to the scoring criteria related to the percentage of ID3+ tumor cells: 0=0%, 1=1-25%, 2=26-50%, 3=51-75%, 4=76-100%. *ID3* mutational status was partly published [2,3] (and hitherto unpublished data). Molecular diagnosis according to * MMML gene expression profiling [11]; $ according to [8]; & according to [9]. # *ID3* mutation status according to [2,3] and hitherto unpublished data (§).

Case no.	Molecular data by	Age	Gender	Reference Panel Diagnosis	Molecular Diagnosis*	Subtype$	Subtype &	*MYC* (df and bap FISH)	*IGH* (bap FISH)	*BCL2* (bap FISH)	*BCL6* (bap FISH)	*ID3* mutation type	*ID3* mutation	ID3 ihc score
1	ICGC MMML-Seq	13	male	BL	mBL	GCB	GCB	*IGH-MYC*	pos	neg	neg	frameshift insertion; deletion, biallelic	c.119_120insT,p.L40fs; LOH 1p from pter to 50 MB = ca. del1p36.33-1p33 (chr1: 1-50700000) (§)	0
2	ICGC MMML-Seq	4	male	BL	mBL	GCB	GCB	*IGH-MYC*	pos	neg	neg	frameshift deletion; deletion, biallelic	c.205delA, p.I69fs; del 1p36.11-1p36.12 (chr1:23216697-23905176) (§)	0
3	MMML	12	male	BL, a	mBL	GCB	GCB	*IGH-MYC*	pos	neg	neg	frameshift deletion; splice site, biallelic	c.137_151del, p.H46Lfs*80; c.220A>T, Q71L; c.300+12delAGTCGC (#)	0
4	MMML	4	male	BL, a	mBL	GCB	GCB	*IGH-MYC*	pos	neg	neg	nonsyn SNV; biallelic splice site	c.190C>T, L64F; c.300+1G>A,T (#)	0
5	MMML	17	male	BL, a	mBL	Type III	GCB	*IGH-MYC*	pos	neg	neg	biallelic splice site	c.300+1G>A; c.300+1G>A (#)	0
6	MMML	63	female	BL	mBL	GCB	GCB	*IGH-MYC*	pos	neg	neg	homozygous deletion	homozygous deletion in 1p36 (#)	0
7	MMML	10	female	BL	mBL	GCB	GCB	*IGH-MYC*	pos	neg	neg	homozygous deletion	homozygous deletion in 1p36 (#)	0
8	MMML	7	male	BL	mBL	GCB	GCB	*IGH-MYC*	pos	neg	neg	inframe insertion; splice site	c.222_227dupCGACTA, p.D75_Y76insDY; c.300+1G>T (#)	0
9	MMML	4	male	DLBCL, cb	mBL	GCB	GCB	*IGH-MYC*	pos	neg	neg	in-frame duplication	c.207_236dup, p.R72_Q81dup (#)	0
10	ICGC MMML-Seq	4	male	BL leukaemia	[BL leukaemia]	n.a.	GCB	*IGH-MYC*	pos	neg	neg	homozygous deletion	del 1p36.11-p36.12 (§)	0
11	MMML	13	female	DLBCL, cb	mBL	GCB	non-GCB	*IGH-MYC*	pos	neg	neg	syn +nonsyn SNV stop gain SNV	c.189G>A, Q63Q; c.166C>A; P56T; c.202G>T, E68X (#)	1
12	MMML	5	male	BL, a	mBL	GCB	GCB	*IGH-MYC*	pos	neg	neg	frameshift deletion	c.116delG, p.S39Tfs*6 (#)	3

13	MMML	57	male	BL	mBL	GCB	GCB	*IGH-MYC*	pos	neg	neg	frameshift deletion	c.120_138del, p.L41Cfs*84 (#)	3
14	MMML	10	male	FL grade 3b	mBL	Type III	GCB	*IGH-MYC*	pos	neg	neg	nonsyn SNV	c.239T>G, L80R (#)	3
15	MMML and ICGC MMML-Seq	10	male	BL	mBL	GCB	GCB	*IGH-MYC*	pos	neg	neg	stop gain SNV, splice site	c.C241T:p.Q81X; c.301-1G>A (no structural consequence) (#)(§)	4
16	ICGC MMML-Seq	17	male	BL	mBL	GCB	GCB	*IGK-MYC*	neg	neg	neg	syn SNV	c.G300A:p.Q100Q (§)	4
17	ICGC MMML-Seq	4	male	BL	mBL	Type III	GCB	*IGH-MYC*	pos	neg	neg	nonsyn SNV	c.C166T:p.P56S; c.A130G:p.M44V (§)	4
18	ICGC MMML-Seq	14	male	BL	mBL	GCB	GCB	*IGH-MYC*	pos	neg	neg	nonsyn SNV	c.C190T:p.L64F (§)	4
19	ICGC MMML-Seq	8	male	BL	mBL	Type III	GCB	*IGH-MYC*	pos	neg	neg	nonsyn SNV	c.C208G:p.L70V; c.C190T:p.L64F (§)	4
20	MMML	9	male	BL	mBL	GCB	GCB	*IGH-MYC*	pos	neg	neg	nonsyn SNV	c.164T>C, V55A; c.191T>G, L64R (#)	n.a.
21	MMML	13	male	BL, a	mBL	GCB	GCB	*IGH-MYC*	pos	neg	neg	nonsyn SNV	c.166C>T; P56S (#)	4
22	MMML	4	male	BL, a	mBL	GCB	GCB	*IGH-MYC*	pos	neg	neg	stop gain SNV	c.241C>T, Q81X; c.33C>G, Y11X (#)	4
23	MMML	24	male	BL, a	mBL	GCB	GCB	*IGH-MYC*	pos	neg	neg	frameshift deletion	c.145_169del, p.Ser 49Profs*27 (#)	4
24	MMML	40	male	BL, a	mBL	GCB	GCB	*IGH-MYC*	pos	neg	neg	stop gain SNV, frameshift deletion	c.298C>T, Q100X; c.189del G, p.Q63Hfs*20 (#)	4
25	MMML	5	male	BL, a	mBL	GCB	GCB	*IGH-MYC*	pos	neg	neg	nonsyn SNV, stop gain SNV, syn SNV	c.190C>T, L64F; c.33C>G, Y11X; c.300G>A, Q100Q (#)	4
26	MMML	5	male	BL, a	mBL	GCB	GCB	*IGH-MYC*	pos	neg	neg	nonsyn SNV	c.152T>A, L51H; c.190C>G, L64V; c.248T>G, V83G (#)	n.a.
27	MMML	12	male	BL	mBL	GCB	GCB	*IGH-MYC*	pos	neg	neg	nonsyn SNV, syn SNV	c.164T>A, V55E; c220A>G, I74V; c.267T>C, P89P, c.287A>G, H96R (#)	4
28	MMML	2	female	BL	mBL	GCB	GCB	*IGH-MYC*	pos	neg	neg	nonsyn SNV	c.190C>G, L64V; c.195C>G, S65R (#)	4
29	MMML	28	male	B-NHL, high	mBL	Type III	GCB	*IGH-MYC*	pos	neg	neg	nonsyn SNV	c.166C>T, P56S; c.166C>G, P56A (#)	4
30	MMML	9	male	B-NHL, high	mBL	GCB	GCB	*IGH-MYC*	pos	neg	neg	nonsyn SNV	c.167C>G; P56R (#)	4
31	MMML and ICGC MMML-Seq	12	male	BL	mBL	GCB	GCB	*IGH-MYC*	pos	neg	neg	wt	wt (#)(§)	4

32	ICGC MMML-Seq	18	female	BL	mBL	GCB	GCB	*IGH-MYC*	pos	neg	neg	wt	wt (§)	4
33	ICGC MMML-Seq	5	male	BL	mBL	GCB	GCB	*IGH-MYC*	pos	neg	neg	wt	wt (§)	4
34	ICGC MMML-Seq	2	male	BL	mBL	GCB	GCB	*IGH-MYC*	pos	neg	neg	wt	wt (§)	4
35	MMML	9	male	BL	mBL	GCB	GCB	*IGL-MYC*	pos t(8;14) neg	neg	neg	wt	wt (#)	4
36	MMML	5	female	BL	mBL	GCB	GCB	*IGH-MYC*	pos	neg	neg	wt	wt (#)	4
37	ICGC MMML-Seq	6	male	Intermediate BL/DLBCL	mBL	GCB	GCB	*IGL-MYC*	neg	neg	neg	wt	wt (§)	4
38	MMML	74	female	B-NHL, high	mBL	GCB	GCB	*IGH-MYC*	pos	neg	pos	wt	wt (#)	4
39	MMML	74	male	DLBCL, cb-ib	mBL	Type III	GCB	*IGH-MYC*	pos	pos	neg	wt	wt (#)	4
40	MMML	15	male	BL	intermediate	GCB	GCB	*IGH-MYC*	pos	neg	neg	frameshift insertion; splice site	c.143_161dupACTCCCGCCTGCGGGAACT, p.V55Lfs*17; c.300+1G>A (#)	0
41	MMML	49	male	DLBCL, cb	intermediate	GCB	GCB	*IGH-MYC*	pos	neg	neg	frameshift mutation	c.202_250del, p.E68Wfs*37 (#)	0
42	MMML	5	female	BL, a	intermediate	GCB	GCB	*IGH-MYC*	pos	neg	neg	frameshift deletion, stop gain	c.190_191del, p. L64X (#)	1
43	MMML	68	female	DLBCL, ib	intermediate	GCB	GCB	*IGH-MYC*	pos	pos	neg	wt	wt (#)	1
44	MMML	70	male	DLBCL, ib	intermediate	GCB	GCB	*IGH-MYC*	pos	pos	pos	wt	wt (#)	2
45	MMML	51	male	DLBCL, cb	intermediate	GCB	GCB	*IGH-MYC*	pos	pos	neg	wt	wt (#)	3
46	MMML	15	male	BL, a	intermediate	GCB	GCB	*IGH-MYC*	pos	neg	neg	nonsyn SNV	c.194G>A; S65N (#)	4
47	MMML	6	female	BL, a	intermediate	GCB	GCB	*IGK-MYC*	neg	neg	neg	syn SNV	c.174C>T, V58V (#)	4
48	MMML	10	male	BL	intermediate	GCB	GCB	*IGH-MYC*	pos	neg	neg	nonsyn SNV	c.160C>G, L54V (#)	4
49	MMML	42	female	DLBCL, cb	intermediate	Type III	GCB	*IGH-MYC*	pos	neg	neg	stop gain, nonsyn SNV	c.169G>T, G57X; c.236A>G, D79G (#)	4
50	MMML and ICGC MMML-Seq	8	male	BL	intermediate	GCB	GCB	neg	neg	neg	neg	wt	wt (#)(§)	4

51	MMML	73	male	DLBCL, ib	intermediate	ABC	non-GCB	*IGH-MYC*	pos	neg	neg	wt	wt (#)	4
52	MMML	n.a.	n.a.	DLBCL, ib	intermediate	GCB	GCB	*IGH-MYC*	pos	neg	neg	wt	wt (#)	4
53	ICGC MMML-Seq	16	female	BL, a	intermediate	Type III	GCB	*IGH-MYC*	pos	neg	neg	wt	wt (§)	4
54	ICGC MMML-Seq	72	male	DLBCL, cb	non-mBL	ABC	non-GCB	neg	neg	neg	pos	deletion, monoallelic	copy-neutral LOH in 1p (§)	0
55	ICGC MMML-Seq	46	male	DLBCL, cb	non-mBL	GCB	GCB	neg	pos	neg	neg	deletion, monoallelic	LOH (4 Mbp deletion); might be subclonal (§)	0
56	MMML	64	female	DLBCL, pb	non-mBL	Type III	GCB	*IGH-MYC*	pos	neg	neg	wt	wt (#)	0
57	ICGC MMML-Seq	75	female	DLBCL cb	non-mBL	GCB	GCB	neg	neg	neg	neg	wt	wt (§)	0
58	ICGC MMML-Seq	57	female	DLBCL, cb	non-mBL	GCB	GCB	neg	pos	pos	neg	wt	wt (§)	0
59	ICGC MMML-Seq	74	female	FL grade 1/2	non-mBL	Type III	GCB	neg	pos	pos	pos	wt	wt (§)	0
60	ICGC MMML-Seq	74	female	FL grade 1	non-mBL	GCB	GCB	neg	neg	neg	neg	wt	wt (§)	0
61	ICGC MMML-Seq	70	male	PTLD, CNS DLBCL	non-mBL	Type III	non-GCB	neg	neg	neg	neg	wt	wt (§)	0
62	MMML	48	male	DLBCL, ana	non-mBL	GCB	n.a.	*IGH-MYC*	pos	neg	pos	wt	wt (#)	1
63	ICGC MMML-Seq	84	male	B-NHL, high	non-mBL	GCB	GCB	neg	pos	pos	pos	wt	wt (§)	1
664	ICGC MMML-Seq	49	male	DLBCL, cb	non-mBL	ABC	non-GCB	neg	pos	neg	pos	wt	wt (§)	1
65	ICGC MMML-Seq	16	male	DLBCL, cb	non-mBL	Type III	GCB	neg	pos	neg	neg	wt	wt (§)	1
66	ICGC MMML-Seq	64	male	DLBCL, cb	non-mBL	ABC	non-GCB	neg	neg	neg	neg	wt	wt (§)	1
67	ICGC MMML-Seq	47	male	FL grade 3a; DLBCL	non-mBL	GCB	GCB	neg	pos	pos	neg	wt	wt (§)	1
68	ICGC MMML-Seq	85	female	DLBCL, cb	non-mBL	GCB	GCB	neg	pos	pos	neg	wt	wt (§)	1

69	ICGC MMML-Seq	40	female	FL grade 1	non-mBL	GCB	GCB	neg	pos	pos	neg	wt	wt (§)	1
70	ICGC MMML-Seq	48	male	FL grade 1	non-mBL	GCB	GCB	neg	pos	pos	neg	wt	wt (§)	1
71	ICGC MMML-Seq	76	male	FL grade 2; FL grade 3a	non-mBL	Type III	GCB	neg	neg	pos	neg	wt	wt (§)	1
72	ICGC MMML-Seq	52	female	FL grade 2; FL grade 3a	non-mBL	GCB	GCB	neg	pos	pos	neg	wt	wt (§)	1
73	ICGC MMML-Seq	67	female	FL grade 1/2	non-mBL	Type III	GCB	neg	pos	pos	neg	wt	wt (§)	1
74	ICGC MMML-Seq	46	male	FL grade 1	non-mBL	GCB	GCB	neg	pos	pos	pos	wt	wt (§)	1
75	ICGC MMML-Seq	62	male	PMBCL	non-mBL	GCB	GCB	neg	pos	neg	pos	wt	wt (§)	1
76	ICGC MMML-Seq	16	male	PMBCL	non-mBL	GCB	GCB	neg	neg	neg	neg	wt	wt (§)	1
77	MMML	10	female	DLBCL, cb	non-mBL	GCB	GCB	*IGK-MYC*	neg	neg	neg	wt	wt (#)	2
78	ICGC MMML-Seq	66	male	DLBCL, cb	non-mBL	ABC	non-GCB	neg	neg	neg	neg	wt	wt (§)	2
79	ICGC MMML-Seq	73	female	FL grade 3a; DLBCL	non-mBL	Type III	non-GCB	neg	pos	pos	neg	wt	wt (§)	2
80	ICGC MMML-Seq	74	female	DLBCL, cb	non-mBL	ABC	non-GCB	neg	neg	neg	neg	wt	wt (§)	2
81	MMML and ICGC MMML-Seq	41	female	PMBCL	non-mBL	ABC	non-GCB	neg	neg	neg	neg	wt	wt (§)	2
82	ICGC MMML-Seq	70	female	FL grade 3b; DLBCL	non-mBL	Type III	GCB	neg	pos	neg	pos	wt	wt (§)	2
83	ICGC MMML-Seq	68	female	FL grade 3a	non-mBL	ABC	non-GCB	neg	neg	neg	neg	wt	wt (§)	2
84	ICGC MMML-Seq	59	male	DLBCL, cb	non-mBL	GCB	GCB	neg	pos	neg	pos	wt	wt (§)	3
85	ICGC MMML-Seq	70	male	FL grade 3a; DLBCL	non-mBL	Type III	GCB	neg	pos	neg	pos	wt	wt (§)	3
86	ICGC MMML-Seq	61	female	DLBCL, cb	non-mBL	ABC	non-GCB	neg	pos	neg	pos	wt	wt (§)	3
87	ICGC MMML-Seq	52	male	DLBCL, ib	non-mBL	ABC	non-GCB	*IGH-MYC*	pos	neg	neg	wt	wt (§)	3

88	MMML and ICGC MMML-Seq	15	male	DLBCL, ib	non-mBL	Type III	GCB	neg	neg	neg	pos	wt	wt (§)	4
89	ICGC MMML-Seq	75	female	prim. CNS DLBCL	non-mBL	ABC	non-GCB	neg	neg	neg	neg	wt	wt (§)	4

Abbreviations: ana, anaplastic; BL, Burkitt lymphoma; BL a, atypical Burkitt lymphoma; B-NHL, high, high-grade B cell non-Hodgkin lymphoma; bap, break-apart probe; cb, centroblastic; CNS, central nervous system; DLBCL, diffuse large B cell lymphoma; df, dual fusion probe; FISH, fluorescence in situ hybridization; FL, follicular lymphoma; ib, immunoblastic; ihc, immunohistochemistry; mBL, molecular Burkitt lymphoma; n.a., not available; non-mBL, molecularly defined non-Burkitt lymphoma; nonsyn SNV, non-synonymous single nucleotide variant; PTLD, post-transplant lymphoproliferative disease; syn SNV, synonymous single nucleotide variant; wt, wild type.