Supplementary Information for

Mutations of multiple genes cause deregulation of NF-κB in diffuse large B-cell lymphoma

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SUPPLEMENTARY METHODS

Cell lines. The following DLBCL cell lines were used in the study: OCI-Ly3, OCI-Ly10, HBL1, SUDHL-2, U2932, RIVA, RC-K8, OCI-Ly2, OCI-Ly4, OCI-Ly7, SUDHL-4, SUDHL-5, SUDHL-6, SUDHL-7, SUDHL-8, SUDHL-10, DB, FARAGE, VAL and WSU. Cells were maintained in Iscove's Modified Dulbecco Medium (IMDM) (Invitrogen) supplemented with 10% fetal calf serum, 100 U/ml penicillin, 100 U/ml streptomycin and 2mM L-glutamine. The OCI-Ly10 line was cultured in IMDM with 20% heparinized human plasma and 55μM β-mercaptoethanol.

Tumor samples. Frozen specimens from 155 newly diagnosed, previously untreated DLBCL patients were obtained from the archives of the Departments of Pathology at Columbia University and Weill Cornell Medical College, after approval by the respective Institutional Review Boards. The fraction of tumor cells, assessed by Southern blot analysis of the rearranged immunoglobulin heavy chain locus and/or by analysis of frozen sections isolated before and after obtaining tissue for molecular studies, corresponded to >80% in most of the cases and to >50% in all cases. Adequate material for simultaneous extraction of DNA and RNA was available from 77 samples, which were characterized by gene expression profile analysis; the remaining samples were classified using immunohistochemical stains as described below. Genomic DNA from 15 additional DLBCL patients was included in the *A20* mutation analysis.

High-Density SNP Array analysis for *A20* copy number determination. Genome-wide DNA profiles were obtained from high molecular weight genomic DNA of DLBCL patients using the GeneChip Human Mapping 250K-Nsp arrays and the Affymetrix Genome-Wide Human SNP Array 6.0 (Affymetrix, Santa Clara, CA, USA) following the manufacturer's instructions. A diploid reference set of 46 Caucasian normal female samples from the HapMap Project was used as a reference set for copy number estimation. For the 250K-Nsp arrays, data acquisition was performed using the Affymetrix GCOS 1.4 and GTYPE 4.1 software as described[36]. Genotype calls were calculated using the BRLMM algorithm within a data set of over 100 B-cell tumors analyzed in house to improve the genotype call. The Affymetrix CNAT version 4.0.1 software and the Hidden Markov Model were used to calculate copy numbers (CN) starting from the BRLMM-CHP files. Analyses were performed using CN Gaussian bandwidth of 100 Kb and LOH transition decay of 10Mb. Gains and losses were defined in the presence of CN above 2.1 and CN below 1.9, respectively. For the SNP 6.0 arrays, image data analysis and quality control for the hybridized samples were performed using the Affymetrix Genotyping Console 3.0.1 software. Only samples passing the Affymetrix recommended contrast QC and SNP call rates threshold (in the Birdseed v2.0 algorithm) were considered for analysis. Copy number determination was performed using the Partek Genomics Suite 6.08 software (http://www.partek.com/). To identify regions of amplification and deletion, the genomic segmentation algorithm of Partek was implemented with the following parameters: minimum of ten genomic markers at a p-value of 0.001 and a signal to noise ratio of 0.3. A p-value of 0.01 was used to filter for

regions of interest from the segmentation results.

Expression constructs. The pHII-Luc NF-κB reporter construct, containing two NF-κB canonical binding sites from the MHCII promoter, was obtained from David Baltimore. Plasmids encoding HA-tagged wild-type A20, CARD11, TRAF2, TRAF5 and TAK1 proteins were constructed by introducing the corresponding Pfu-PCR amplified human cDNAs into the pCMV-HA vector (Clontech), in frame with HA. These plasmids served as templates to generate various DLBCL-associated mutant constructs, using the QuickChange site-directed mutagenesis kit (Stratagene). The lentiviral expression constructs FUGW-HA-TRAF2 and FUGW-HA-TRAF2-P186R were obtained by subcloning the corresponding full-length cDNA sequences into the HpaI restriction site of the FUGW vector[35], in front of an IRES-GFP sequence. All final constructs were verified by enzymatic digestion and confirmed by DNA sequencing.

Transient transfections/reporter gene assays. Transfection of 293T cells was performed by the calcium-phosphate precipitation method as previously described[28], using 0.1 μg of the pHII-Luc reporter construct and the indicated doses of wild-type and mutant expression vectors. The total amount of transfected DNA was kept constant in each experiment by adding pCMV-HA vector sequences to a final amount of 2.1μg/35mm plate. All experiments were performed in duplicate and luciferase activities were measured forty-eight hours after transfection using the Dual-Luciferase Reporter Assay Kit (Promega), according to the manufacturer's instructions.

Protein extraction and western blot analysis. Whole cell extracts were prepared in RIPA buffer as described[28] and analyzed by Western blot according to standard methods,

with the following primary antibodies: anti-NF-kappaB2 p100/p52(18D10) (Cell Signaling Technology), anti-HA(3F10) (Roche), anti-TRAF2(H10) (Santa Cruz Biotechnology), anti-GFP (JL-8) (Clontech), anti-β-actin (A5441) and anti-β-tubulin (clone B-5-1-2)(Sigma). The endogenous A20 protein was detected using a mouse monoclonal anti-A20 antibody (clone 59A426; eBioscience). Proteins were visualized using a chemiluminescence detection kit (Pierce) as recommended by the manufacturer.

LEGENDS TO SUPPLEMENTARY FIGURES

Supplementary Figure 1. Experimental Strategy used for the NF-κB Mutations Discovery Screen and Validation Screen.

Supplementary Figure 2. Lack of A20 protein expression in DLBCL samples carrying genetic inactivation of the *A20* gene. a, Diagram of the wild-type A20 protein, with its relevant functional domains; the Serine 381 residue reported to be phosphorylated by IKKβ is also indicated[37]. The predicted A20 polypeptides in two cell lines carrying biallelic truncating mutations of the *A20* gene are aligned below, and their molecular weight is given on the right. In the RC-K8 derived N720 allele, a frameshift deletion T at position +2227 leads to an aberrant cDNA sequence utilizing a novel 3' stop codon and encoding for a longer chimeric protein that retains the N-terminal 720 amino acids of the original A20 polypeptide, fused to 94 unrelated residues (grey segment). **b,** Western blot analysis of A20 expression in representative ABC- and GCB-DLBCL cell lines (+/+, *A20* wild-type; +/-, *A20* monoallelic deletion; m/m, *A20* biallelic mutation). β-tubulin is used as protein loading control. The arrow points to the correct size of the full-length A20 protein, while asterisks indicate a non specific band of approximately 60kD, occasionally detected in cell lines (see also Coornaert et al., 2008)[38]; RT-PCR amplification and sequencing of the A20 full length cDNA from the same samples did not show any evidence of additional alterations that may suggest the presence of alternative, smaller transcripts (not shown). Note the expression of the endogenous truncated A20 mutant proteins N720 and S459X in RC-K8 and SUDHL2, respectively.

As expected, the inducible A20 protein, which is itself a target of NF-κB, is not expressed in most GCB-DLBCL samples, where NF-κB is not active. **c,** Western blot analysis of A20 expression in primary DLBCL biopsies carrying wild-type (+/+) or abnormal *A20* alleles (m/-, inativating mutation of one allele with deletion of the second copy; -/-, homozygous deletion). The cell line Ly7 is used as positive control. β-actin monitors for protein loading.

Supplementary Figure 3. *A20* mutations abrogate its ability to negatively regulate NF-κB. a, Schematic representation of the A20 expression constructs used in transient transfection assays. Mutants carrying frameshift deletions are denoted by the letter N, followed by the amino acid length of the residual A20 protein. **b,** Western blot analysis of exogenous A20 expression in 293T cells, transfected with equimolar amounts (0.2μg) of the constructs shown in (a). Short and long exposures are shown for the anti-HA western blot. The anti-A20 antibody used recognizes an epitope in the middle portion of the A20 protein; thus, truncated mutants lacking the C-terminal amino acids 450-790 cannot be detected. Western blot analysis of GFP expression, co-transfected as control, documents comparable transfection efficiencies; β-actin controls that equal amounts of protein were loaded. Note the significantly lower levels of the N720 protein with respect to wild-type (WT) A20, despite expression of comparable mRNA levels, measured by semi-quantitative RT-PCR on increasing number of cycles (bottom two panels). These results suggest that, despite retaining most of its coding domains, the N720 mutant is functionally defective, due to protein instability. **c,** NF-κB inhibitory activity of wild-type and mutant A20 proteins in 293T cells co-transfected with the NF-κB luciferase reporter

vector pHII-Luc, a MYC-tagged TRAF2 expression vector, and the A20 mutant constructs M2-M8, the amounts of which had been titrated to obtain comparable protein expression levels. Data are reported as relative fold induction compared to the activity of the reporter only, arbitrarily set as 1, and are representative of three independent experiments performed in duplicate, with error bars indicating standard deviations. In the bottom panels, western blot analysis using anti-HA antibodies documents that comparable levels of exogenous A20 protein were expressed for each mutant. β-actin controls for loading. Note that all A20 mutants tested were significantly impaired in their ability to negatively regulate TRAF2-induced NF-κB activation.

Supplementary Figure 4. *A20* deletions in DLBCL primary biopsies. a, Distribution of SNP markers represented in the Affymetrix GeneChip Human Mapping 250K-Nsp array and the Affymetrix Genome-Wide Human SNP array 6.0. **b,** Copy number heatmap showing deletions encompassing the *A20* gene in 7 representative DLBCL primary cases, as compared to two control normal samples (N). The boxed area defines the minimal deleted region, corresponding to a focal homozygous deletion identified in sample 2110 and spanning two genes: *A20* and *OLIG3* (arrows). Since *OLIG3* is not expressed in B cells (our own data on Affymetrix U133Plus_2 oligonucleotide arrays, and EST profile at http://www.ncbi.nlm.nih.gov), these findings point to *A20* as the target of the deletion. * region shown in panel (c). **c,** Higher magnification of the copy number heatmap from DLBCL samples 2110 and 2170, and two normal DNAs (top panel). The total copy number intensity ratio is illustrated in the middle panel, and the *A20* and *OLIG3* genes are aligned below. A black line in the copy number plot indicates the

normal (baseline) intensity of 2. **d,** Total copy number plots of four DLBCL samples harboring small 6q23 deletions encompassing the *A20* gene (boxed area). The ideogram of the long arm of chromosome 6 is aligned below the plots.

Supplementary Figure 5. Lentiviral transduction of A20 expressing constructs in cell lines carrying functionally inactive *A20* alleles. Western blot analysis of A20 and β-actin expression in three DLBCL cell lines, untransduced (UT) and transduced with lentiviral vectors expressing GFP alone (pWPI) or HA-tagged A20 linked to GFP via an IRES element (pWPI-HA-A20). SUDHL4 and SUDHL7 are GCB-DLBCL cell lines that carry an intact *A20* locus. The Burkitt lymphoma cell line P3HR1, stimulated with CD40 to induce NF-κB activation (and expression of its target gene *A20*), was used as control for physiologic levels of endogenous A20. The arrow points to the endogenous, truncated A20 protein expressed in the A20 functionally deficient SUDHL2 cell line.

Supplementary Figure 6. Missense mutations of *CARD11* and *TRAF2* lead to enhanced NF-κB activation. a, Schematic representation of the constructs used in transient transfection/luciferase reporter assays (R, zinc finger RING-type domain; TZ, zinc finger TRAF-type domain; MATH, meprin and TRAF homology domain; CARD, caspase-associated recruitment domain; SH3, Src homology domain; GUK, [membrane-associated] guanylate kinase homology domain). **b,** Enhanced NF-κB activity of DLBCL-derived CARD11 mutant proteins. The reporter construct indicated in (a) was transfected into 293T cells, alone or in the presence of equal amounts of wild-type (WT) and mutant (M1-M5) CARD11-expressing plasmids. The M1 (L251P) construct harbors

a missense mutation previously reported to confer enhanced NF-κB activity[3], and was included as internal control. Luciferase activities, measured forty-eight hours after transfection, showed ≥2 fold increase of the reporter gene in extracts obtained from all five CARD11 mutants, as compared to CARD11-WT (mean ± standard deviation; n=3)(top panel). Western blot analysis of HA expression documents comparable levels of protein in all samples except M5 (D357V), which cannot be detected likely due to protein instability (see comparable cDNA expression levels by semi-quantitative RT-PCR in the bottom panels). GFP shows comparable transfection efficiencies, and β-actin controls for loading. **c,** Mutations of TRAF2 potentiate its ability to transactivate NF-κB in 293T cells. Significantly enhanced, dose-dependent induction of the reporter gene by the ABC-DLBCL associated TRAF2-P186R mutant protein (black bars, lanes 8-10), as compared to TRAF2-WT (grey bars, lanes 5-7). In the bottom panels, western blot analysis using anti-HA antibodies, which detect both WT and mutant TRAF2, controls that equivalent amounts of proteins were expressed. Comparable NF-κB activity could be achieved by expressing approximately 5-fold higher amounts of the WT protein (lanes 2-4). β-Actin was used as protein loading control. Data are representative of three independent experiments performed in duplicate. **d,** Increased NF-κB activity of the TRAF2-P186R mutant *in vivo*. The DLBCL cell line SUDHL6 was engineered to express HA-tagged WT and mutant TRAF2 proteins via lentiviral transduction, and analyzed for the presence of active nuclear NF-κB complexes by immunofluorescence staining of NFKB1/p50 (red, left panels), in the absence of further stimuli. Nuclei were counter stained with DAPI (blue, middle panels) and the merged images are shown on the right. Nuclear p50 localization, indicative of active NF-κB signaling, was detected in the majority (~60%) of

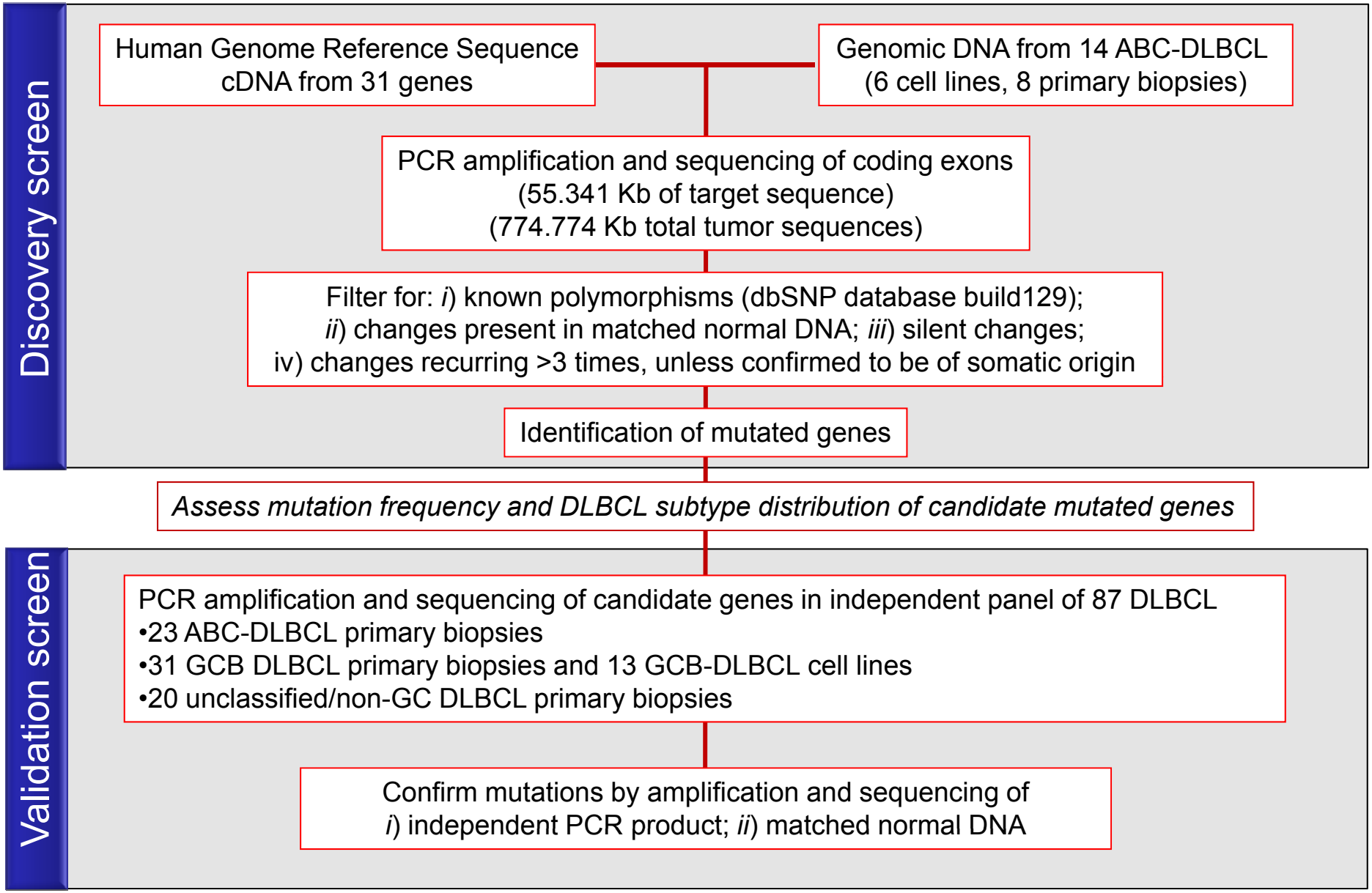
TRAF2-P186R-transduced cells (bottom right panel), but not in cells expressing TRAF2-WT, where p50 is restricted to the cytoplasm (top right panel).

REFERENCES TO SUPPLEMENTARY MATERIAL

36. Forconi, F. *et al.* High density genome-wide DNA profiling reveals a remarkably stable profile in hairy cell leukaemia. *Br. J. Haematol.* **141**, 622-630 (2008).

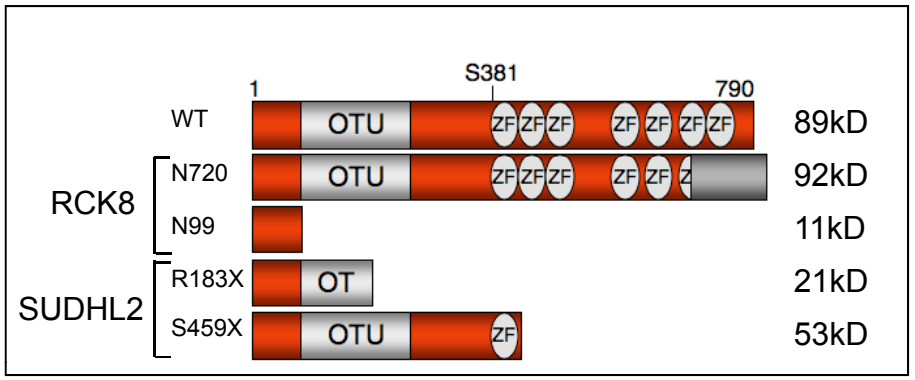
37. Hutti, J.E. *et al.* IκB Kinase β phosphorylates the K63 deubiquitinase A20 to cause feedback inhibition of the NF-κB pathway. *Mol. Cell. Biol.* **27**, 7451-7461 (2007).

38. Coornaert, B. *et al.* T cell antigen receptor stimulation induces MALT1 paracaspase-mediated cleavage of the NF-kappaB inhibitor A20. *Nature Immunol* **9**, 263-271 (2008).



Supplementary Figure 1. Experimental Strategy used for NF-κB Mutations Discovery Screen and Validation Screen

a.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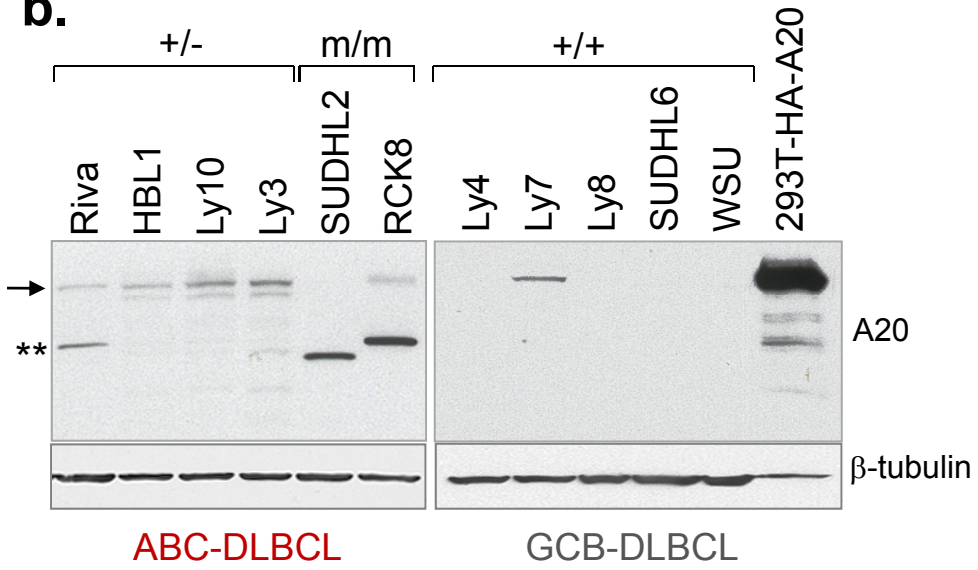
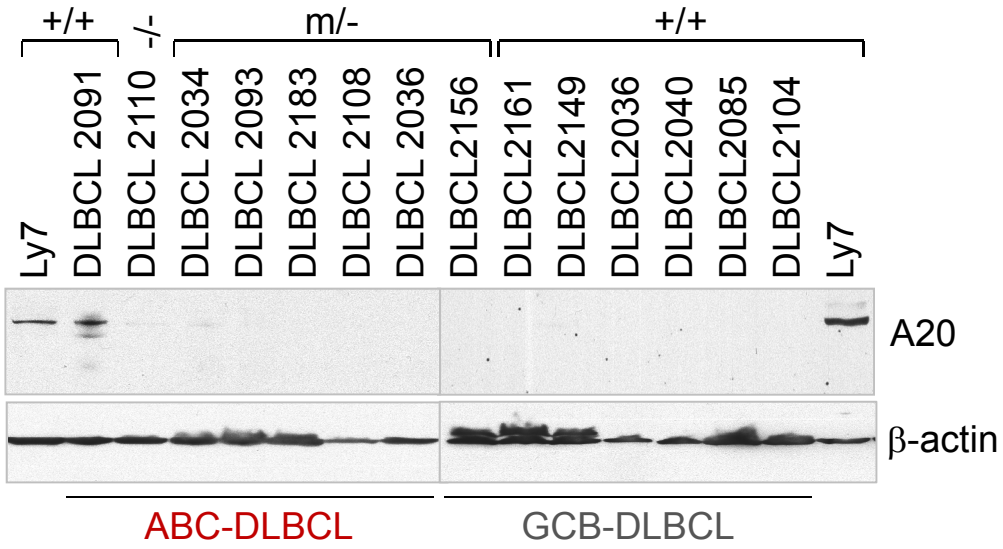
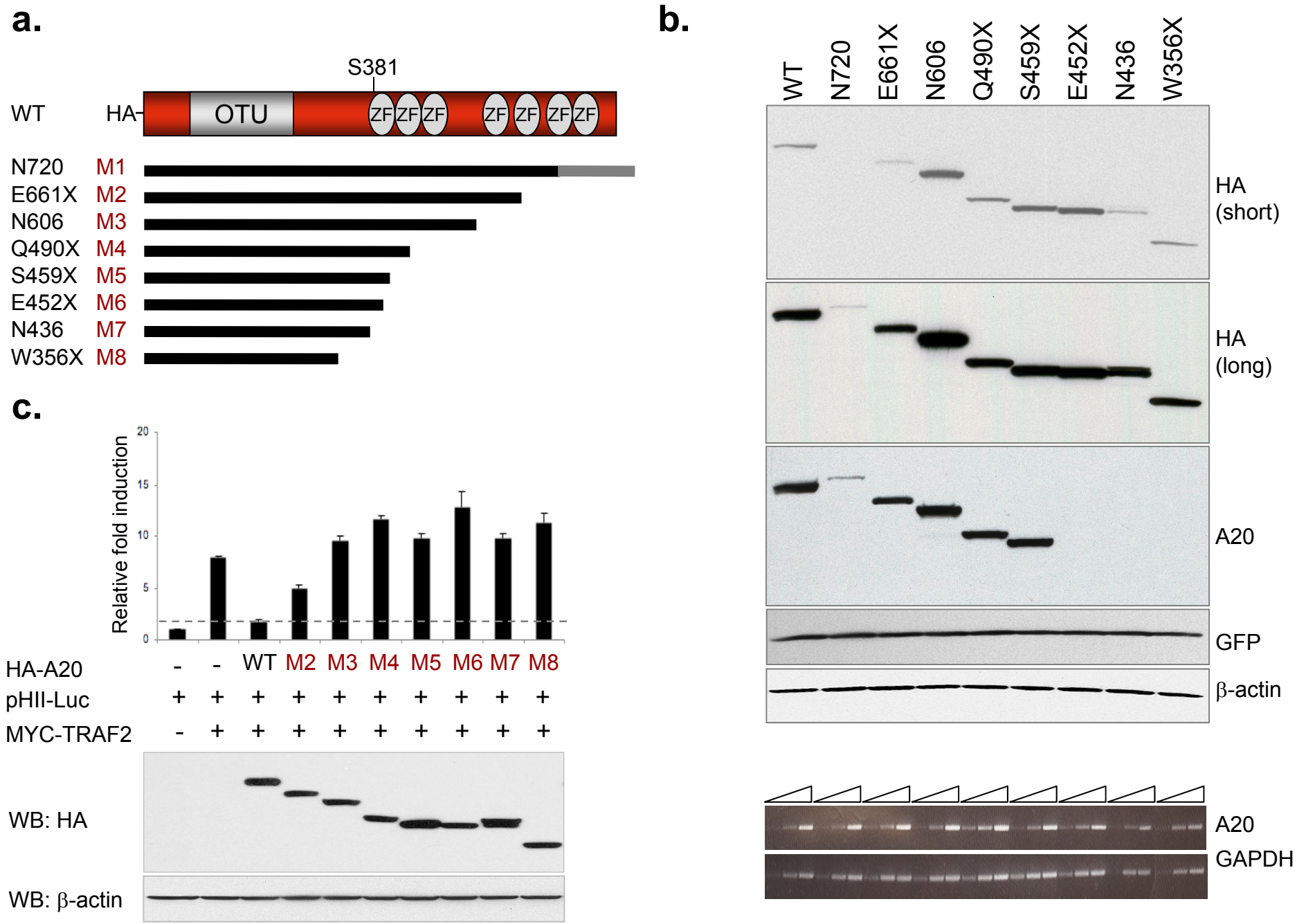
S381
790
WT
OTU
ZF
89kD
RCK8
N720
92kD
N99
11kD
SUDHL2
R183X
OT
21kD
S459X
53kD

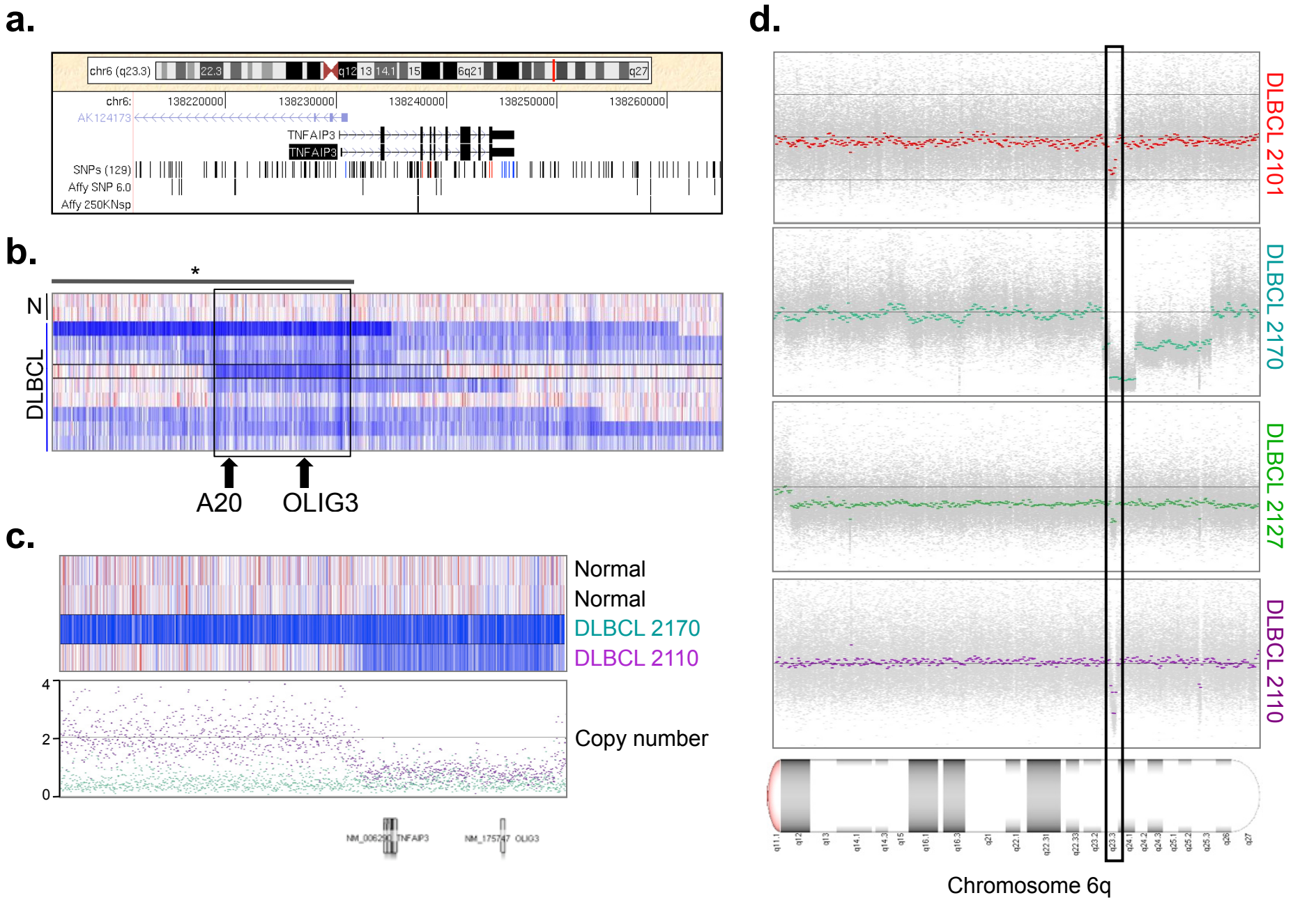
b.


+/-
m/m
+/+
Riva
HBL1
Ly10
Ly3
SUDHL2
RCK8
Ly4
Ly7
Ly8
SUDHL6
WSU
293T-HA-A20
**
A20
β-tubulin
ABC-DLBCL
GCB-DLBCL

c.


+/+
-/-
m/-
+/+
Ly7
DLBCL 2091
DLBCL 2110
DLBCL 2034
DLBCL 2093
DLBCL 2183
DLBCL 2108
DLBCL 2036
DLBCL2156
DLBCL2161
DLBCL2149
DLBCL2036
DLBCL2040
DLBCL2085
DLBCL2104
Ly7
A20
β-actin
ABC-DLBCL
GCB-DLBCL


a.
S381
WT
HA-
OTU
ZF ZF ZF ZF ZF ZF ZF
N720 M1
E661X M2
N606 M3
Q490X M4
S459X M5
E452X M6
N436 M7
W356X M8
b.
WT
N720
E661X
N606
Q490X
S459X
E452X
N436
W356X
HA (short)
HA (long)
A20
GFP
β-actin
A20
GAPDH
c.
Relative fold induction
20
15
10
5
0
HA-A20 - - WT M2 M3 M4 M5 M6 M7 M8
pHII-Luc + + + + + + + + + +
MYC-TRAF2 - + + + + + + + + +
WB: HA
WB: β-actin


a.
chr6 (q23.3)
chr6:
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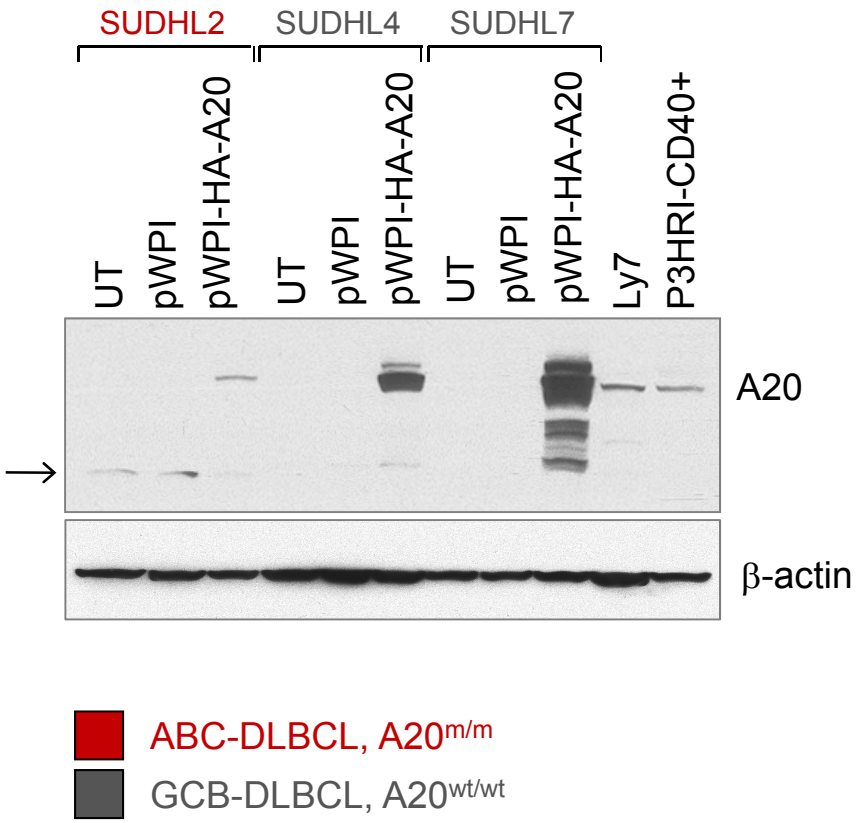
138230000
138240000
138250000
138260000
AK124173
TNFAIP3
TNFAIP3
SNPs (129)
Affy SNP 6.0
Affy 250KNsp
b.
*
N
DLBCL
A20
OLIG3
c.
Normal
Normal
DLBCL 2170
DLBCL 2110
4
2
0
Copy number
NM_006290 TNFAIP3
NM_175747 OLIG3
d.
DLBCL 2101
DLBCL 2170
DLBCL 2127
DLBCL 2110
Chromosome 6q


SUDHL2
SUDHL4
SUDHL7
UT
pWPI
pWPI-HA-A20
UT
pWPI
pWPI-HA-A20
UT
pWPI
pWPI-HA-A20
Ly7
P3HRI-CD40+
A20
β-actin
ABC-DLBCL, A20m/m
GCB-DLBCL, A20wt/wt

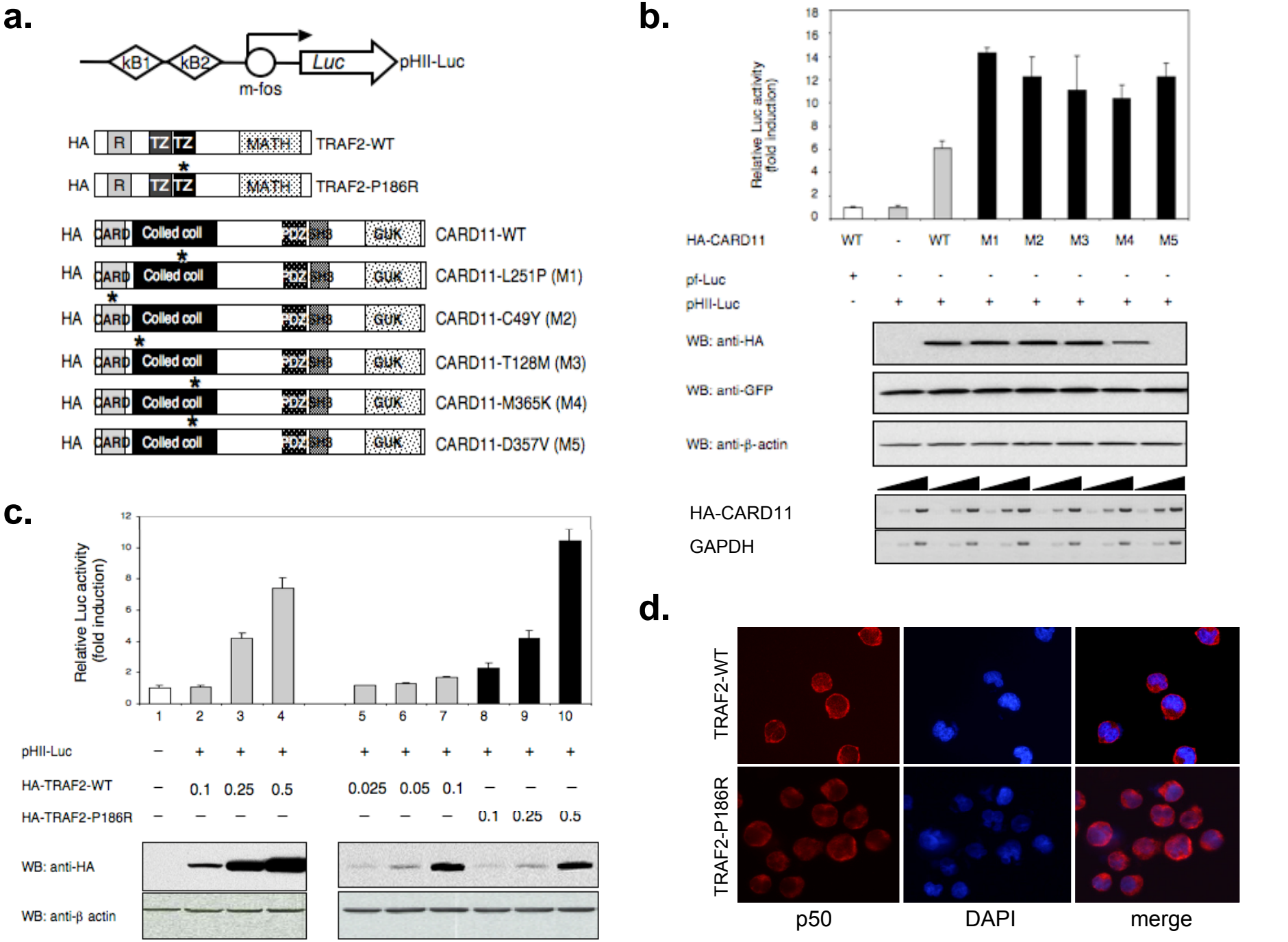

a.
kB1
kB2
Luc
pHII-Luc
m-fos
HA
R
TZ
TZ
MATH
TRAF2-WT
TRAF2-P186R
CARD
Coiled coil
PDZ
SH3
GUK
CARD11-WT
CARD11-L251P (M1)
CARD11-C49Y (M2)
CARD11-T128M (M3)
CARD11-M365K (M4)
CARD11-D357V (M5)
b.
Relative Luc activity (fold induction)
HA-CARD11
WT
-
WT
M1
M2
M3
M4
M5
pf-Luc
pHII-Luc
WB: anti-HA
WB: anti-GFP
WB: anti-β-actin
HA-CARD11
GAPDH
c.
Relative Luc activity (fold induction)
pHII-Luc
HA-TRAF2-WT
HA-TRAF2-P186R
0.1
0.25
0.5
0.025
0.05
0.1
WB: anti-HA
WB: anti-β actin
d.
TRAF2-WT
TRAF2-P186R
p50
DAPI
merge

Table S1. NF-kB target Gene Set used for GSEA

#	Gene Symbol	Reference	#	Gene Symbol	Reference
1	AHR	5	61	IL4RA	6
2	BANK1	5	62	IL4RB	5
3	BATF	3,5	63	IL6	1,3,5
4	BCL2	1,2	64	IL8	1,5
5	BCL2A1	1,4,5	65	IRF1	1,5
6	BCL2L1	1,5	66	IRF4	1,2,3,4,5
7	BIRC2	1,6	67	JUNB	1,3,5
8	BIRC3	1,3,4,5	68	KLF10	6
9	BUB1B	5	69	LITAF	5
10	C6ORF32	3,4,5	70	LSP1	6
11	CCL2	6	71	LTA	4,5
12	CCL22	4	72	LYN	3,5
13	CCL3	1	73	MAP3K1	5
14	CCL4	1,5	74	MAP3K8	1,5
15	CCND2	1,2,5	75	MIRN155	3,5
16	CCR4	5	76	MYB	6
17	CCR7	2,3,5	77	NCF2	5
18	CD36	5	78	NCL	5
19	CD40	4,5	79	NFKB1	4,5
20	CD40LG	5	80	NFKB2	1,4,5
21	CD44	1,3,4,5	81	NFKBIA	1,2,3,4,5
22	CD69	1,6	82	NFKBIE	1,5
23	CD82	1	83	PASK	6
24	CD83	1,4,5	84	PBEF1	5
25	CEP110	5	85	PECAM1	1,6
26	CFLAR	1,2,3,5	86	PIM1	4,5
27	CISH	5	87	PIM2	5
28	CSF2	1	88	PLEK	5
29	CX3CL1	6	89	PRKCD	6
30	CXCL1	6	90	PRPF4B	5
31	CXCL10	3,4,5	91	PTGS2	6
32	CXCL13	3,5	92	PTPN1	1
33	CXCL2	1	93	PTPN3	5
34	CXCL9	3,4,5	94	RASGRP1	3,5
35	CXCR7	5	95	REL	1,6
36	DUSP1	6	96	RELB	1,5
37	DUSP2	1,4	97	RET	5
38	EBI2	3,5	98	RFTN1	4
39	EBI3	4,5	99	RGS1	1,5
40	EGR1	6	100	RRAS2	6
41	ELL2	5	101	SDC4	1,6
42	EMR1	6	102	SELL	3,5
43	FAS	6	103	SLAMF7	5
44	FCER	1	104	SLC2A5	6
45	FGF12	5	105	SMAD7	6
46	FNDC3A	5	106	SMARCA2	4,5
47	GADD45B	3,4	107	SOCS2	3,5
48	HLA-F	6	108	SOD2	6
49	HSPA1L	5	109	SPI1	1,4,5
50	ICAM1	1,4,5	110	STAT1	5
51	ID2	3,4,5	111	STAT5A	6
52	IER2	6	112	STX4	6
53	IER3	1,6	113	TNF	1,4,5
54	IL10	5	114	TNFAIP3	1,3,4,5
55	IL12 B	5	115	TNIP2	6
56	IL15RA	1,6	116	TPMT	6
57	IL1B	1	117	TRAF1	1,4,5
58	IL2	5	118	VIM	1,6
59	IL2RA	1,6	119	WTAP	6
60	IL32	6	120	ZFP36L1	5

Ref 1: Shaffer et al., Immunity 2001
Ref 2: Davis et al., J Exp Med 2001
Ref 3: Ngo et al., Nature 2006
Ref 4: Lam et al., Clin Cancer Res 2005
Ref 5: Lam et al., Blood 2008
Ref 6: Feuerhake et al., Blood 2005

Table S2. List of NF-kB related genes analyzed

Gene Symbol (alias)	Chromosomal location	Reference mRNA	N. of exons	Coding exons	CDS Length	Notes
BCL10	1p22	NM_003921.3	4	Exon 2-4	702 bp	
BIRC2 (cIAP1)	11q22	NM_001166.3	9	Exon 2-9	1857 bp	
BIRC3 (cIAP2)	11q22	NM_001165.3	9	Exon 2-9	1815 bp	variant 1
CARD11	7p22	NM_032415.3	25	Exon 2-25	3444 bp	
CD40	20q12-q13.2	NM_001250.4	9	Exon 1-9	834 bp	variant 1
CHUK (IKKα)	10q24-q25	NM_001278.3	21	Exon 1-21	2238 bp	
CSNK1A1	5q32	NM_001025105.1	11	Exon 1-11	1098 bp	isoform 1
CYLD	16q12.1	NM_015247.2	20	Exon 4-20	2871 bp	
IKBKB (IKKβ)	8p11.2	NM_001556.1	22	Exon 2-22	2271 bp	
IKBKG (NEMO)	Xq28	NM_003639.3	10	Exon 2-10	1260 bp	variant 3
MALT1	18q21	NM_006785.2	17	Exon 1-17	2475 bp	variant 1
MAP3K14 (NIK)	17q21	NM_003954.2	16	Exon 2-16	2844 bp	
MAP3K7 (TAK1)	6q16.1-q16.3	NM_145331.1	17	Exon 1-17	1821 bp	isoform B
MAP3K7IP2 (TAB2)	6q25.1-q25.3	NM_015093.3	7	Exon 2-7	2082 bp	
MAP3K7IP3 (TAB3)	Xp21.2	NM_152787.3	11	Exon 5-11	2139 bp	
NFKB1 (p105/p50)	4q24	NM_003998.2	24	Exon 2-24	2910 bp	
NFKBIA (IκBα)	14q13	NM_020529.2	6	Exon 1-6	954 bp	
NFKBIB (IκBβ)	19q13.1	NM_002503.3	6	Exon 1-6	1071 bp	isoform alpha
PRKCB1	16p11.2	NM_002738.5	17	Exon 1-17	2022 bp	variant 2
REL (cREL)	2p13-p12	NM_002908.2	11	Exon 1-11	1860 bp	
RIPK1	6p25.2	NM_003804.3	10	Exon 1-10	2016 bp	
TNFAIP3 (A20)	6q23.3	NM_006290.2	9	Exon 2-9	2373 bp	
TNFRSF11A (RANK)	18q22.1	NM_003839.2	10	Exon 1-10	1851 bp	
TNFRSF13C (BAFFR)	22q13.1-q13.31	NM_052945.2	3	Exon 1-3	555 bp	
TNFSF13B (BAFF)	13q32-34	NM_006573.3	6	Exon 1-6	858 bp	
TRAF1	9q33-q34	NM_005658.3	8	Exon 2-8	1251 bp	
TRAF2	9q34	NM_021138.3	11	Exon 2-11	1506 bp	
TRAF3	14q32.32	NM_145725.1	12	Exon 3-12	1707 bp	variant 1
TRAF4	17q11-q12	NM_004295.3	7	Exon 1-7	1413 bp	variant 1
TRAF5	1q32	NM_004619.3	11	Exon 2-11	1674 bp	variant 1
TRAF6	11p12	NM_145803.1	8	Exon 3-8	1569 bp	variant 1

Table S3. Unmutated NF-kB pathway genes

Gene Symbol (alias)	N of mutated cases/tested (%)
BCL10	0/22 (0%)
BIRC2 (cIAP1)	0/14 (0%)
BIRC3 (cIAP2)	0/14 (0%)
CD40	0/20 (0%)
CHUK (IKKα)	0/14 (0%)
CSNK1A1	0/24 (0%)
CYLD	0/14 (0%)
IKBKB (IKKβ)	0/14 (0%)
IKBKG (NEMO)	0/14 (0%)
MALT1	0/14 (0%)
MAP3K14 (NIK)	0/14 (0%)
MAP3K7IP2 (TAB2)	0/14 (0%)
MAP3K7IP3 (TAB3)	0/24 (0%)
NFKB1 (p105/p50)	0/14 (0%)
NFKBIA (IκBα)	0/14 (0%)
NFKBIB (IκBβ)	0/22 (0%)
PRKCB1	0/14 (0%)
REL (cREL)	0/14 (0%)
RIPK1	0/23 (0%)
TNFSF13B (BAFF)	0/14 (0%)
TNFRSF13C (BAFFR)	0/23 (0%)
TRAF1	0/24 (0%)
TRAF3	0/14 (0%)
TRAF4	0/14 (0%)
TRAF6	0/21 (0%)

Table S4. DLBCL samples found mutated in the discovery/validation screening

Sample ID	Subtype	Status	N of Mutations	Mutated gene
Ly3	ABC	M	2	CARD11, RANK
SUDHL2	ABC	M	2	TNFAIP3
RC-K8*	ABC	M	4	TNFAIP3
U2932	ABC	M	1	TAK1
2024	ABC	M	1	CARD11
2025	ABC	M	2	TRAF2, RANK
2026	ABC	M	1	RANK
2033	ABC	M	1	TNFAIP3
2043	ABC	M	1	TRAF5
2044	ABC	M	2	TRAF5
2063	ABC	M	1	TNFAIP3
2072	ABC	M	1	RANK
2091	ABC	M	1	CARD11
2093	ABC	M	1	TNFAIP3
2108	ABC	M	1	TNFAIP3
2152	ABC	M	1	CARD11
2160	ABC	M	1	TNFAIP3
2183	ABC	M	2	TNFAIP3, TAK1
2195	ABC	M	1	TNFAIP3
2027	GCB	M	1	TRAF5
2032	GCB	M	1	RANK
2073	GCB	M	1	CARD11
2082	GCB	M	1	TRAF2
2089	GCB	M	1	TNFAIP3
2144	GCB	M	1	TRAF5
SUDHL5	GCB	M	1	TRAF2
SUDHL7	GCB	M	1	CARD11
WSU	GCB	M	2	CARD11, TRAF2
FARAGE	GCB	M	1	TRAF2
2015	NC	M	2	TNFAIP3, RANK
2112	NC	M	1	CARD11
2014	non-GC	M	1	TNFAIP3
2131	non-GC	M	3	TNFAIP3, TRAF2
2151	non-GC	M	2	CARD11, RANK
2186	non-GC	M	1	TNFAIP3
2189	non-GC	M	1	TRAF5

*This cell line also carries biallelic inactivating mutations in the NFKBIA gene

Table S5. Biallelic inactivation of the *A20* gene in DLBCL

Sample	DLBCL subtype	Structural alterations		Mutation^	Location	Predicted functional consequences
		Allele	Status			
SUDHL2	ABC	A	Mutated	C613T (R183X)	Exon 4	truncated protein of 182 aa
		B	Mutated	C1442A (S459X)	Exon 7	truncated protein of 458 aa
RCK8	ABC	A	Mutated	G(+1)A	Intron2	frameshift; truncated protein of 99 aa
		B	Mutated	ΔT2227	Exon 9	frameshift; truncated protein of 720 aa
2186	non-GC	A	Mutated	C613T (183R>X)	Exon 4	truncated protein of 182 aa
		B	Mutated	+A (351-352)	Exon 2	frameshift; truncated protein of 95 aa
2131	non-GC	A	Mutated	ΔC340	Exon 2	frameshift; truncated protein of 91 aa
		B	Mutated	+ GTAAA [1883_1884]	Exon 7	frameshift; truncated protein of 606 aa
2093	ABC	A	Mutated	+A [557_558]	Exon 4	frameshift; truncated protein of 163 aa
		B	Deleted			
2033	ABC	A	Mutated	C1534T (Q490X)	Exon 7	truncated protein of 489 aa
		B	Deleted			
2063	ABC	A	Mutated	C228A (C54X)	Exon 2	truncated protein of 53 aa
		B	Deleted			
2108	ABC	A	Mutated	+G [550_551]	Exon 3	frameshift; truncated protein of 162 aa
		B	Deleted			
2015	NC	A	Mutated	G1060T (E332X)	Exon 7	truncated protein of 331 aa
		B	Deleted			
I07-216	GC	A	Mutated	ΔAT [586-587]	Exon 4	frameshift; truncated protein of 173 aa
		B	Deleted			
2195	ABC	A	Mutated	ΔAG465_466	Exon 2	frameshift; truncated protein of 133 aa
		B	Deleted**			
2036	non-GC*	A	Mutated	G1053A (splice site)	Exon 7	aberrant splicing
		B	Deleted			
I07-212	non-GC*	A	Mutated	Δ10bp [1376-1385]	Exon 7	frameshift; truncated protein of 436 aa
		B	Deleted			
2176	non-GC	A	Mutated	G1133A (W356X)	Exon 7	truncated protein of 355 aa
			Deleted**			
2183	ABC	A	Mutated	Δ16bp [814-829]	Exon 5	truncated protein of 250 aa
		B	Deleted			

2089	GCB	A B	Mutated Deleted	G73T (E3X); G118C (A18P)	Exon 2	truncated protein of 2 aa
2014	non-GC	A B	Mutated Normal	G1420T (E452X) -	Exon 7	truncated protein of 451 aa
2156	non-GC*	A B	Mutated Normal	G2047T (E661X) -	Exon 8	truncated protein of 660 aa
2160	ABC	A B	Mutated nd	C613T (183R>X)	Exon 4	truncated protein of 182 aa
2149	non-GC	A B	Mutated nd	+C [2340_2341]	Exon 9	frameshift; truncated protein of 758 aa
2188	non-GC	A B	Mutated nd	ΔAG [465_466]	Exon 2	frameshift; truncated protein of 133 aa
2161	non-GC	A B	Mutated nd	G321A (W85X)	Exon 2	truncated protein of 84 aa

^Numbering according to GenBank accession No. NM_006290.2 (nucleotide changes) and NP_006281.1 (amino acid changes, in brackets)
** as assessed by sequencing analysis of genomic DNA
Abbreviations: aa, amino acid; Δ, deletion; +, insertion; nd, not determined
ABC, activated B cell type; GCB, germinal center B cell type; NC, unclassified; non-GC, non germinal center type (IHC-based classification)
non-GC* denotes cases coexpressing CD10 and MUM1

Table S6. SNP array analysis of DLBCL cases carrying deletions (<6Mb) involving *TNFAIP3 (A20)*

Sample ID	Aberration	Cytoband	Start position^	End position^	Length (Mb)	Annotated genes in region^^	Array
2030	Hemizygous Deletion	6q23.3	137114313	138543999	1.43	TNFAIP3 and 9 other genes	SNP 6.0
2093	Hemizygous Deletion	6q23.3	137475615	138388944	0.91	TNFAIP3, OLIG3, IL22RA2, IFNGR1	SNP 6.0
2101	Hemizygous Deletion	6q23.3	136761774	138406359	1.64	TNFAIP3 and 8 other genes	SNP 6.0
2110*	Homozygous Deletion	6q23.3	137651612	138335112	0.68	TNFAIP3, OLIG3	SNP 6.0
2127*	Homozygous Deletion	6q23.3	137444914	138522109	1.08	TNFAIP3, OLIG3, PERP, IL22RA2, IFNGR1	SNP 6.0
2165	Hemizygous Deletion	6q23.2-6q24.1	135195324	141099925	5.90	TNFAIP3 and 26 other genes	SNP 6.0
2170*	Homozygous Deletion	6q23.3-6q24.1	137398912	143038752	5.64	TNFAIP3 and 18 other genes	SNP 6.0
05-292*	Homozygous Deletion	6q23.3	138140569	138501502	0.36	TNFAIP3, PERP	250K Nsp
07-208	Hemizygous Deletion	6q23.2 - 6q24.1	134406736	139940965	5.53	TNFAIP3 and 28 other genes	250K Nsp

*In these cases, the interval refers to the homozygously deleted region

^ Numbering according to NCBI Build 36.1

^^ According to the NCBI RefSeq Database

Table S7. Copy number analysis of *A20* in DLBCL

Sample ID	Mutation Status	*TNFAIP3*(*A20*) locus	% cells with deletion	% tumor cells in the biopsy^	Method
2015	M	Hemizygous Deletion	30-40%	40%	FISH; Affymetrix 6.0 SNP array
2033	M	Hemizygous Deletion	80%	60-80%	FISH; Affymetrix 6.0 SNP array
2036	M	Hemizygous Deletion	30%	40%	FISH; Affymetrix 6.0 SNP array
2063	M	Hemizygous Deletion	80%	80%	FISH; Affymetrix 6.0 SNP array
2089	M	Hemizygous Deletion	70%	80%	FISH; Affymetrix 6.0 SNP array
2093	M	Hemizygous Deletion	40%	50%	FISH; Affymetrix 6.0 SNP array
2108	M	Hemizygous Deletion	50%	50%	FISH
2176	M	Hemizygous Deletion	>95%	>95%	PCR amplification and direct sequencing
2183	M	Hemizygous Deletion	30-50%	60-80%	FISH
2195	M	Hemizygous Deletion	>95%	>95%	PCR amplification and direct sequencing
07-212	M	Hemizygous Deletion	na	>80%	250K Nsp SNP array
07-216	M	Hemizygous Deletion	na	>80%	250K Nsp SNP array
2014	M	Normal	0	30%	FISH
2156	M	Normal	0	nd	FISH
2053	na	Homozygous Deletion	95%	90%	FISH
2069	na	Homozygous Deletion	80%	80%	FISH
2110	na	Homozygous Deletion	na	40%	Affymetrix 6.0 SNP array
2170	na	Homozygous Deletion	na	>80%	Affymetrix 6.0 SNP array
2127	na	Homozygous Deletion	na	>80%	Affymetrix 6.0 SNP array
05-292	na	Homozygous Deletion	na	>80%	250K Nsp SNP array
07-215	na	Homozygous Deletion	na	>80%	250K Nsp SNP array
2026	WT	Hemizygous Deletion	65%	70%	FISH
2035	WT	Hemizygous Deletion	50%	40%	FISH
2040	WT	Hemizygous Deletion	30-50%	60%	FISH
2059	WT	Hemizygous Deletion	50-70%	60%	FISH
2064	WT	Hemizygous Deletion	23%	20%	FISH
2088	WT	Hemizygous Deletion	50%	50%	FISH
2102	WT	Hemizygous Deletion	25%	30%	FISH
2112	WT	Hemizygous Deletion	30-40%	50%	FISH
2116	WT	Hemizygous Deletion	20%	20%	FISH
2185	WT	Hemizygous Deletion	50%	60%	FISH
2019	WT	Hemizygous Deletion	50%	50%	FISH; Affymetrix 6.0 SNP array
2034	WT	Hemizygous Deletion	40%	90%	FISH; Affymetrix 6.0 SNP array
2046	WT	Hemizygous Deletion	30-50%	30-80%	FISH; Affymetrix 6.0 SNP array
2062	WT	Hemizygous Deletion	50%	70-80%	FISH; Affymetrix 6.0 SNP array
2101	WT	Hemizygous Deletion	50%	50%	FISH; Affymetrix 6.0 SNP array
2017	WT	Hemizygous Deletion	na	>80%	Affymetrix 6.0 SNP array
2161	WT	Hemizygous Deletion	na	>80%	Affymetrix 6.0 SNP array
2165	WT	Hemizygous Deletion	na	>80%	Affymetrix 6.0 SNP array
2171	WT	Hemizygous Deletion	na	>80%	Affymetrix 6.0 SNP array
2179	WT	Hemizygous Deletion	na	>80%	Affymetrix 6.0 SNP array
2182	WT	Hemizygous Deletion	na	>80%	Affymetrix 6.0 SNP array
Ly10	WT	Hemizygous Deletion	100%	100%	FISH
Ly3	WT	Hemizygous Deletion	100%	100%	FISH
RIVA	WT	Hemizygous Deletion	100%	100%	FISH
U9232	WT	Hemizygous Deletion	100%	100%	FISH
2096	WT	Monosomy 6	70%	80%	FISH
2131	M/M	Normal	na	<50%	RT-PCR amplification and sequencing of cloned products
2186	M/M	Normal	na	>90%	RT-PCR amplification and sequencing of cloned products
RCK8	M/M	Normal	na	100%	RT-PCR amplification and sequencing of cloned products
SUDHL2	M/M	Normal	na	100%	RT-PCR amplification and sequencing of cloned products

M, mutated; M/M, biallelically mutated; WT, wild-type

na, not applicable (cases studied by SNP array analysis); nd, not determined

^ as assessed by morphologic and histologic analysis of serial tissue sections

Table S8. Missense mutations affecting NF-kB pathway components in DLBCL

Gene (alias)	Role in NF-kB pathway	Sample ID	Exon	Nucleotide change*	AA change**	Affected protein domain
CARD11	adaptor protein/signaling intermediate required for IKK complex activation	2091	3	G550A	Cys 49 Tyr	CARD domain
		2058	5	G771A	Gly123 Ser	proximity of coiled-coil domain 1
		2112^	5	C787T	Thr 128 Met	proximity of coiled-coil domain 1
		SUDHL7	5	C787T	Thr 128 Met	proximity of coiled-coil domain 1
		2024	6	G1092A	Asp 230 Asn	coiled-coil domain 1
		2151	6	A1135C	Lys 244 Thr	coiled-coil domain 1
		OCI-Ly3	6	T1156 (+/+)	Leu 251 Pro	coiled-coil domain 1
		WSU	8	A1474T	Asp 357 Val	coiled-coil domain 4
		2073^	8	T1498A	Met 365 Lys	coiled-coil domain 4
		2152	9	C1671T	Arg 423 Trp	coiled-coil domain 4
TRAF2	adaptor protein/signal transducer for CD40 and TNF-R	2025^	6	C614G	Pro 186 Arg	Zinc finger-TRAF domain
		2082	3	C269T	Ala 71 Val	RING domain (protein-protein interaction)
		2131	5	C527T	Pro 157 Leu	-
		SUDHL5	6	A640G	Lys 195 Glu	Zinc finger-TRAF domain (receptor association)
		FARAGE	8	G895T	Ala 280 Ser	TRAF-n domain (self association)
		WSU	9	C1150T	Arg 365 Cys	MATH domain (adaptor domain)
TRAF5	adaptor protein/signal transducer for CD40, CD30 and LT-beta	2044^	7	G717 T	Glu 219 Asp	Zinc finger-TRAF domain
		2044^	8	ΔA 846	Δ AA299-557	MATH domain (adaptor domain)
		2027	2	C247T	Arg 63 Cys	RING domain (protein-protein interaction)
		2144	7	C659T	Ala 200 Val	Zinc finger-TRAF domain
		2043	11	C1214A	Ala 385 Glu	MATH domain (interaction domain)
		2189	11	G1666A	Ala 536 Thr	MATH domain (interaction domain)
MAP3K7 (TAK1)	serine-threonine kinase required for IKKB phosphorylation/activation	2183	1	T184G	Ser 8 Ala	Tyrosine protein kinase domain
		U2932	12	T1411G	Ser 417 Ala	-
TNFRSF11A (RANK)	cell surface receptor	2025^	10	C1810T	Pro 591 Leu	-
		2032	4	G400T	Ser 121 Ile	Extracellular domain
		2026	5	C515A	Phe 159 Leu	TNFR_c6 (extracellular domain)
		2015	7	A756G	Lys 240 Glu	Intracellular domain
		2072	7	A756G	Lys 240 Glu	Intracellular domain
		2151	7	A756G	Lys 240 Glu	Intracellular domain
		OCI-Ly10	4	G388A^^	Gly 117 Glu	Extracellular domain

*, ** Numbering according to the corresponding mRNA and protein Reference Sequence, respectively

^Paired normal DNA was available for these samples and confirmed the somatic origin of the mutation

^^Mutation detected in a minority of the tumor population

+/+, homozygous or hemizygous change; Δ, deletion; AA, aminoacid

Table S9. Oligonucleotides used for genomic amplification of the NF-kB-related genes found mutated in DLBCL

Gene Symbol (alias)	Genomic Region	Primer name	Position^	Oligonucleotide Sequence	Reference mRNA
TNFAIP3(A20)	Exon 2	A20-E2F	-145	5'-ccgggagtagaggtgctaa-3'	NM_006290.2
		A20-E2R	+167	5'-gtctgctattatcacatacccc-3'	NM_006290.2
	Exon 3	A20-E3F	-149	5'-tcagtttgcccttgactagga-3'	NM_006290.2
		A20-E3R	+159	5'-tgagtcccactggaggtttc-3'	NM_006290.2
	Exon 4/Exon 5	A20-E4F	-216	5'-ctccccaacttttgagtttgc-3'	NM_006290.2
		A20-E5R	+136	5'-aaccaagcaagtcacagaacaa-3'	NM_006290.2
	Exon 6	A20-E6F	-220	5'-cacctccaggctggttaatg-3'	NM_006290.2
		A20-E6R	+200	5'- tgttttgatttgaaacccaagtt-3'	NM_006290.2
	Exon 7	A20-E7F	-168	5'-gttgcgtgaaaagtgtgagc-3'	NM_006290.2
		A20-E7R	+190	5'-cagtgccttttgcctccat-3'	NM_006290.2
	Exon 8	A20-E8F	-206	5'-gattggtaaagccaaagatgtt-3'	NM_006290.2
		A20-E8R	+191	5'-ggaggtagcatttcggacc-3'	NM_006290.2
	Exon 9	A20-E9F	-237	5'-gcttggcggttttcctcag-3'	NM_006290.2
		A20-E9R	2645*	5'-ctttgctttctaaggccacct-3'	NM_006290.2
TNFRSF11A (RANK)	Exon 1	RANK_E1F	-141	5'-gagcttgggcaccacctg-3'	NM_003839.2
		RANK_E1R	+316	5'-ggtgctcaagaagcatttgg-3'	NM_003839.2
	Exon 2	RANK_E2F	-182	5'-gaggtagaggttgcggtgaa-3'	NM_003839.2
		RANK_E2R	+121	5'-cacctgccactaccataacg-3'	NM_003839.2
	Exon 3	RANK_E3F	-176	5'-actgcattgtggcctcttct-3'	NM_003839.2
		RANK_E3R	+147	5'-aacgcatctttcttgggatg-3'	NM_003839.2
	Exon 4	RANK_E4F	-171	5'-gaagtgcgaggaggaacttg-3'	NM_003839.2
		RANK_E4R	+206	5'-tgaagaacctaagccgagga-3'	NM_003839.2
	Exon 5	RANK_E5F	-176	5'-cctgtgggagctgaagaagt-3'	NM_003839.2
		RANK_E5R	+252	5'-agctgcaaccgtaagtcaca-3'	NM_003839.2
	Exon 6	RANK_E6F	-211	5'-agagaacacaggcagcgttt-3'	NM_003839.2
		RANK_E6R	+206	5'-cccccaatccagtgtagaaa-3'	NM_003839.2
	Exon 7	RANK_E7F	-175	5'-acccttggaatgtgagtgc-3'	NM_003839.2
		RANK_E7R	+244	5'-caagtcatccgtttcctgaac-3'	NM_003839.2
	Exon 8	RANK_E8F	-203	5'-ggatcttgccacacacacat-3'	NM_003839.2
		RANK_E8R1	+65	5'-ggatggctctaccagcctaa-3'	NM_003839.2
	Exon 9	RANK_E9F	-121	5'-ccatctgtacttgttatggtgga-3'	NM_003839.2
		RANK_E9R	+101	5'-cccattagcttcctccttcc-3'	NM_003839.2
	Exon 10	RANK_E10F	-75	5'-gaaccttcctctcggcagac-3'	NM_003839.2
		RANK_E10R	2058*	5'-cacttcctgaaaagcccatt-3'	NM_003839.2
CARD11	Exon 3	CARMA1_E3F	-73	5'-cactccagaggagttgatagagc-3'	NM_032415.3
		CARMA1_E3R	+120	5'-ccccagtttaaaaatccctga-3'	NM_032415.3
	Exon 4	CARMA1_E4F	-157	5'-catctgggctccaagatacaa-3'	NM_032415.3
		CARMA1_E4R	+157	5'-gtggttgacagaccccagtt-3'	NM_032415.3
	Exon 5	CARMA1_E5F	-90	5'-tgagtgaatgaatggcacct-3'	NM_032415.3
		CARMA1_E5R	+86	5'-gcacctgctttatgggagaa-3'	NM_032415.3
	Exon 6	CARMA1_E6F	-153	5'-ggtttcttggagcccttctc-3'	NM_032415.3
		CARMA1_E6R	+128	5'-atgcctgtgatcctgcagtc-3'	NM_032415.3
	Exon 7/Exon 8	CARMA1_E7F	-100	5'-tgtccgtatctgttgctggt-3'	NM_032415.3
		CARMA1_E8R	+141	5'-tgggaaagatggattcagga-3'	NM_032415.3
	Exon 9	CARMA1_E9F2	-181	5'-ggctctgtgatcgcgttaatg-3'	NM_032415.3
		CARMA1_E9R2	+105	5'-cttcaggtgtgggtctcca-3'	NM_032415.3
	Exon 10	CARMA1_E10F	-97	5'-gccgagggaatgatgatg-3'	NM_032415.3
		CARMA1_E10R	+180	5'-taagaagcgagtcgcaggat-3'	NM_032415.3
	Exon 11	CARMA1_E11F	-123	5'-cgtatgacgagggacacgat-3'	NM_032415.3
		CARMA1_E11R	+283	5'-cagttcagctaacgcaccag-3'	NM_032415.3
	Exon 12	CARMA1_E12F	-261	5'-ggaggtttttgagggctctt-3'	NM_032415.3
		CARMA1_E12R	+110	5'-tgagctctggagggagagtg-3'	NM_032415.3
	Exon 13	CARMA1_E13F	-157	5'-gctcagcacaagcctcctc-3'	NM_032415.3
		CARMA1_E13R	+204	5'-acaggcagctgggtgaac-3'	NM_032415.3
	Exon 14	CARMA1_E14F	-158	5'-cttccttgtcccaacctcct-3'	NM_032415.3
		CARMA1_E14R	+246	5'-agacctagctcgcaccctct-3'	NM_032415.3
	Exon 15	CARMA1_E15F	-160	5'-gggaacctcagagacccatc-3'	NM_032415.3
		CARMA1_E15R	+161	5'-ccagccaggaagtgatttct-3'	NM_032415.3
	Exon 16/Exon 17	CARMA1_E16F	-67	5'-tgtgtcccagtagggctttt-3'	NM_032415.3
		CARMA1_E17R	+184	5'-ctgttgccgtccctgtgt-3'	NM_032415.3
	Exon 18/Exon 19	CARMA1_E18F	-113	5'-agagcagcatattgcacagg-3'	NM_032415.3
		CARMA1_E19R	+74	5'-agacccgggagttaggtttg-3'	NM_032415.3
	Exon 20	CARMA1_E20F	-189	5'-caggactcctctcccctacc-3'	NM_032415.3
		CARMA1_E20R	+167	5'-gagaggataaaacgggacagg-3'	NM_032415.3
	Exon 21	CARMA1_E21F	-155	5'-cctgcttttataggggaaatga-3'	NM_032415.3
		CARMA1_E21R	+120	5'-tcctctccctttggaggact-3'	NM_032415.3
	Exon 22	CARMA1_E22F	-105	5'-tcacactgacggtggcttc-3'	NM_032415.3
		CARMA1_E22R	+82	5'-ggaggagggaagagaagagg-3'	NM_032415.3
	Exon 23	CARMA1_E23F	-175	5'-ccctcaaggagcttcacagt-3'	NM_032415.3
		CARMA1_E23R	+50	5'-atcatcacggagggtcctg-3'	NM_032415.3

	Exon 24	CARMA1_E24F	-208	5'-tctctgcccatgaacatcac-3'	NM_032415.3
		CARMA1_E24R	+150	5'-catcagctctgcaaggtcag-3'	NM_032415.3
	Exon 25	CARMA1_E25F	-141	5'-ttaagaggcacggacgctat-3'	NM_032415.3
		CARMA1_E25R	+80	5'-ctaagctcactgggcatggt-3'	NM_032415.3
TRAF5	Exon 2/Exon 3	TRAF5_E2/3F	-69	5'-catctgatggctgtggttgt-3'	NM_004619.3
		TRAF5_E2/3R	+115	5'-ggactccaaggaaggtctcc-3'	NM_004619.3
	Exon 4	TRAF5_E4F	-131	5'-tccattccagctgatgtctg-3'	NM_004619.3
		TRAF5_E4R	+171	5'-cgtaggtgggctacagcact-3'	NM_004619.3
	Exon 5/Exon 7	TRAF5_E5-7F	-73	5'-taggccttccagctgacttc-3'	NM_004619.3
		TRAF5_E5-7R	+89	5'-ccatcctcgatggaacagag-3'	NM_004619.3
	Exon 8	TRAF5_E8F	-148	5'-ttcctgcaacccacctagag-3'	NM_004619.3
		TRAF5_E8R	+187	5'-tcctaagggcaccttccaat-3'	NM_004619.3
	Exon 9	TRAF5_E9F1	-195	5'-atgtggctttttggaaccac-3'	NM_004619.3
		TRAF5_E9R1	+164	5'-catgagtgaatcagggtgga-3'	NM_004619.3
	Exon 10/Exon 11	TRAF5_E10/11F	-130	5'-tgggcttgtatttttcattctg-3'	NM_004619.3
		TRAF5_E10/11R	1359*	5'-gaaggactggctgaagatgg-3'	NM_004619.3
	Exon 11-I	TRAF5_E11F	1244*	5'-aactgctggagggtacttgc-3'	NM_004619.3
		TRAF5_E11R	2454*	5'-gggttgaacctaacaaaatctga-3'	NM_004619.3
TRAF2	Exon 2/Exon 3	TRAF2_E2-3F	-88	5'-atcgcctgctactgatcacc-3'	NM_021138.3
		TRAF2_E2-3R	+140	5'-actcaagcaatccccctacc-3'	NM_021138.3
	Exon 4	TRAF2_E4F2	-109	5'-aaaggcgatgtgacgcagtattg-3'	NM_021138.3
		TRAF2_E4R2	+132	5'-ctccacagacatcagacaggct-3'	NM_021138.3
	Exon 5	TRAF2_E5F	-156	5'-ctgtgggagccctgagag-3'	NM_021138.3
		TRAF2_E5R	+109	5'-ggtggaacgagtgcaaagg-3'	NM_021138.3
	Exon 6	TRAF2_E6F	-190	5'-taagatctccgaggctcctg-3'	NM_021138.3
		TRAF2_E6R	+145	5'-tccatgacccaacgttacag-3'	NM_021138.3
	Exon 7	TRAF2_E7F	-145	5'-ggctgttgggtttcattcag-3'	NM_021138.3
		TRAF2_E7R	+161	5'-agaccaagctgggaacactc-3'	NM_021138.3
	Exon 8/Exon 9	TRAF2_E8/9F	-115	5'-tctatgaccctggcctgtct-3'	NM_021138.3
		TRAF2_E8/9R	+92	5'-cttgggagccatcctatgtg-3'	NM_021138.3
	Exon 10	TRAF2_E10F	-134	5'-ttttctttgcaccccagaac-3'	NM_021138.3
		TRAF2_E10R	+209	5'-acggaccagtgctcagaaat-3'	NM_021138.3
	Exon 11	TRAF2_E11F	-81	5'-cctgccagtgtccagacc-3'	NM_021138.3
		TRAF2_E11R	+96	5'-gaggggtctgtgagaggaga-3'	NM_021138.3
MAP3K7 (TAK1)	Exon 1	TAK1-F2	-86	5'-ctgaggccctgtaataaagg-3'	NM_145331.1
		TAK1-R2	+839	5'-gtgcggtatcggagaaattg-3'	NM_145331.1
	Exon 2	TAK1-E2F2	-159	5'-tgttcagtggcaaatccaga-3'	NM_145331.1
		TAK1-E2R2	+258	5'-tgaattccatggatgaaactgt-3'	NM_145331.1
	Exon 3	TAK1-E3F	-394	5'-tagcatgacgtctggtttgg-3'	NM_145331.1
		TAK1-E3R	+268	5'-atgccacggtgctgtattct-3'	NM_145331.1
	Exon 4	TAK1-E4F	-59	5'-caaaggccctaagatgatgg-3'	NM_145331.1
		TAK1-E4R	+618	5'-atgatgaacacatgggcagt-3'	NM_145331.1
	Exon 5	TAK1-E5F	-131	5'-aagcaggactgatggagcat-3'	NM_145331.1
		TAK1-E5R	+349	5'-gcaagaaaaacgtcagaccta-3'	NM_145331.1
	Exon 6	TAK1-E6F	-357	5'-actcccatttcaggataccg-3'	NM_145331.1
		TAK1-E6R	+218	5'-tgaggatcaactgtacatatgcttaga-3'	NM_145331.1
	Exon 7	TAK1-E7F	-281	5'-tcaagtcctgtgcctcttgat-3'	NM_145331.1
		TAK1-E7R	+233	5'-agccagtgaacatcctcagc-3'	NM_145331.1
	Exon 8	TAK1-E8F	-267	5'-tgtggagactgttcagcattg-3'	NM_145331.1
		TAK1-E8R	+176	5'-ttccctctcgaaatgtttcctt-3'	NM_145331.1
	Exon 9	TAK1-E9F	-237	5'-gagaaatatttgctgttttcttcaa-3'	NM_145331.1
		TAK1-E9R	+207	5'-gacttggaagcaggtcttcaat-3'	NM_145331.1
	Exon 10	TAK1-E10F	-203	5'-ttaggcatcttggttccttttt-3'	NM_145331.1
		TAK1-E10R	+225	5'-gggatttagaattggcaaagg-3'	NM_145331.1
	Exon 11	TAK1-E11F	-157	5'-ttacccctgtagctccctctc-3'	NM_145331.1
		TAK1-E11R	+318	5'-ctggttttggcagaatttcaa-3'	NM_145331.1
	Exon 12	TAK1-E12F	-262	5'-cctgcagctttgtctctgttc-3'	NM_145331.1
		TAK1-E12R	+247	5'-ggtgttctcccttctccaaaa-3'	NM_145331.1
	Exon 13	TAK1-E13F	-231	5'-tgttgtaggccagcaactttt-3'	NM_145331.1
		TAK1-E13R	+248	5'-cagcgctaatgaacaatttactt-3'	NM_145331.1
	Exon 14	TAK1-E14F	-204	5'-tcttgcaagctcatcagttttt-3'	NM_145331.1
		TAK1-E14R	+278	5'-gaattcaatacgcttcgcaac-3'	NM_145331.1
	Exon 15	TAK1-E15F	-254	5'-ttgtgttctattcagtttatagccaac-3'	NM_145331.1
		TAK1-E15R	+227	5'-tggaggattttaagatgactgaa-3'	NM_145331.1
	Exon 16	TAK1-E16F	-269	5'-tgcagcctgaaatgtcatacc-3'	NM_145331.1
		TAK1-E16R	+175	5'-tcaaaaggattgagttttgcat-3'	NM_145331.1
	Exon 17	TAK1-E17F	-76	5'-aattgagcttccatcatcagc-3'	NM_145331.1
		TAK1-E17R	+36	5'-tccattttccattatttttcttca-3'	NM_145331.1

^ Numbering indicates the distance from the corresponding exon, except where otherwise indicated

* Numbering refers to the corresponding reference mRNA sequence