SUPPLEMENTARY DOCUMENTS

Clinical and biologic significance of *MYC* genetic mutations in *de novo* diffuse large B-cell lymphoma

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MATERIALS AND METHODS

Detection of *MYC* Mutations

Genomic DNAs of the 750 patients were extracted from formalin-fixed and paraffin-embedded tissues using an Invitrogen PureLink genomic DNA kit. All three exons and four splicing sites of the *MYC* gene were resequenced for each patient using the Sanger sequencing method. Totally 11 short *MYC* amplicons were generated by two-step "nested" polymerase chain reaction (to increases the specificity) and used as templates for sequencing. The amplicon products were separately sequenced using either the forward or reverse inside polymerase chain reaction primer as a sequencing primer and the ABI BigDye 3.1 reagents with an ABI 3730xl DNA sequencer (Applied Biosystems). Generated electropherogram results were aligned and analyzed, and any divergence from the reference sequence (NG_007161.1) in the Genebank (NC_000008.10) was noted and scored with a Quality Score (QS, similar to a Phred value) for variation validation. To account for heterozygous variations, the cutoff of QS was set as 17 (corresponding to a calling confidence of 98%). Using the same methods, we also sequenced the CDS or UTR regions of *AKT1*, *BCL6*, *CD58*, *CDKN2A*, *EZH2*, *PRDM1*, *PTEN*, *RMRP*, and *TP63* for approximately half of the *MYC* study cohort. The analysis was performed by Polymorphic DNA Technologies Inc. CA. The variants discovered were subgrouped into either germline single nucleotide polymorphism (SNP) according to the dbSNP database (Build 132) or putative somatic mutations. Sequencing analyses on matched corresponding normal tissue were also performed in 92 patients for comparison and validation. The data analysis was performed using the Agent Software from Paracel, Inc., a division of Applera Corp.

Functional Prediction of Myc Mutants

Functional effect prediction of missense and nonsense Myc mutations were computed by 10 models, including PhyloP, LRT, PolyPhen2 (including PolyPhen2_HVAR and PolyPhen2_HDIV), FATHMM, SiPhy, MutationTaster, MutationAssessor, SIFT, GERP++. Mutations were considered to affect Myc function only if at least four of these algorithms predicted that they were not neutral.

Assessment of *MYC* Rearrangements and Myc Expression

MYC rearrangements were detected by fluorescence *in situ* hybridization using two probes (a locus-specific identifier *IGH/MYC/CEP8* tri-color dual-fusion probe and a locus-specific identifier *MYC* dual-color, break-apart probe).

Tumor cell-rich areas from the diagnostic formalin-fixed, paraffin-embedded tissue blocks were selected to prepare the tissue microarrays using a tissue microarrayer (Beecher Instruments). Immunohistochemistry for Myc using a monoclonal anti-(c)MYC antibody, clone Y69 (Epitomics, Burlingame, CA, USA) and other biomarkers with respective antibodies were performed on the tissue microarray sections undergoing deparaffinization, heat induced antigen retrieval techniques and primary antibody incubation. Details of technics and scoring processes have been described previously.[1, 2, 3]

Functional Studies of Myc Mutants *in vitro* and *in vivo*

To exam the effect of Myc mutants, we constructed stable cell lines using Rat1A as a parent line. A wild-type human Myc plasmid was purchased from Addgene (pBabe-c-myc-zeo, Plasmid #17758; Cambridge, MA), and different mutants were constructed using PCR-based

mutagenesis and validated by DNA sequencing. To obtain stable lines, Rat1A cells were transduced with viral particles with *MYC* constructs or the control vector (pBabe-zeo). Forty-eight hours after transduction, cells were selected using 200 μg/ml of Zeocin (ThermoFisher, Waltham, MA) for 2 weeks. For proliferation assay, 5×10^4 cells were seeded in 6-well plates and cell numbers were counted 4 days later. For apoptosis assay, cells were incubated in medium without serum for 48 h and apoptosis was determined by flow cytometry with Annexin-V FITC/ PI. For protein expression analysis, lysates of $5x10^6$ cells were used for a Western blotting analysis.[4] For colon formation, 3,000 cells were plated on 60mm soft agar plates and allowed to grow for 2 weeks. For *in vivo* tumorigenesis, $5x10^6$ cells were injected subcutaneously into 8-week old outbred athymic *Foxn1*^{nu}/*Foxn1*^{nu} male mice (The Jackson Laboratory, Bar Harbor, ME).

References:

1. Tzankov A, Xu-Monette ZY, Gerhard M, Visco C, Dirnhofer S, Gisin N, *et al.* Rearrangements of MYC gene facilitate risk stratification in diffuse large B-cell lymphoma patients treated with rituximab-CHOP. *Mod Pathol* 2014, **27:** 958-971.

2. Visco C, Tzankov A, Xu-Monette ZY, Miranda RN, Tai YC, Li Y, *et al.* Patients with diffuse large B-cell lymphoma of germinal center origin with BCL2 translocations have poor outcome, irrespective of MYC status: a report from an International DLBCL rituximab-CHOP Consortium Program Study. *Haematologica* 2013, **98:** 255-263.

3. Hu S, Xu-Monette ZY, Tzankov A, Green T, Wu L, Balasubramanyam A, *et al.* MYC/BCL2 protein coexpression contributes to the inferior survival of activated B-cell subtype of diffuse large B-cell lymphoma and demonstrates high-risk gene expression signatures: a report from The International DLBCL Rituximab-CHOP Consortium Program. *Blood* 2013, **121:** 4021-4031; quiz 4250.

4. Takwi A, Li Y, Buscaglia L, et al. A Statin-regulated MicroRNA Represses Human c-Myc Expression and Function. *EMBO Mol Med*. 2012;4(9):896-909.

Supplementary Table S1. Numbers of *MYC* mutation present in the sequenced 750 patients with diffuse large B-cell lymphoma (DLBCL) and numbers of AGCT sequence motif (a preferred site by activation-induced cytidine deaminase) in the 5′ or 3′ untranslated regions (UTRs) and the coding sequence (CDS) of *MYC*

	5′UTR	**CDS**	**3′UTR**
Mutation numbers	173	254	35
DNA length, bp	569	1317	479
Mutation rate (per 1000 bp)*	0.405	0.257	0.097
AGCT motif numbers	3	14	0

Note: * The mutation rates are the mean number of mutations per 1000 bp over the whole cohort.

Supplementary Table S2. Clinical and molecular characteristics of the 368 patients with diffuse large B-cell lymphoma (DLBCL) in the training set harboring wild-type (WT) or mutated (MUT) Myc, or N11S single nucleotide polymorphism in the coding sequence of *MYC*

	DLBCL			GCB-DLBCL			ABC-DLBCL			DLBCL		
Variables	***MUT-Myc*** ***N* (%)**	***WT-Myc*** ***N* (%)**		***MUT-Myc*** ***N* (%)**	***WT-Myc*** ***N* (%)**		***MUT-Myc*** ***N* (%)**	***WT-Myc*** ***N* (%)**		***WT-Myc-11S*** ***N* (%)**	***WT-Myc-11N*** ***N* (%)**	
Age												
< 60 y	27 (52.9)	129 (40.7)	0.13	17 (56.7)	74 (46.5)	0.33	10 (47.6)	54 (34.6)	0.33	10 (50)	119 (40.1)	0.48
≥ 60 y	24 (47.1)	188 (59.3)		13 (43.3)	85 (53.5)		11 (52.4)	102 (65.4)		10 (50)	178 (59.9)	
Sex												
Female	18 (35.3)	137 (43.2)	0.29	11 (36.7)	73 (45.9)	0.35	7 (33.3)	63 (40.4)	0.54	8 (40)	129 (43.4)	0.76
Male	33 (64.7)	180 (56.8)		19 (63.3)	86 (54.1)		14 (66.7)	93 (59.6)		12 (60)	168 (56.6)	
Stage												
I - II	21 (41.2)	135 (44.4)	0.67	15 (50.0)	77 (50.3)	0.97	6 (28.6)	57 (38.3)	0.39	10 (55.6)	125 (43.7)	0.33
III - IV	30 (58.8)	169 (55.6)		15 (50.0)	76 (49.7)		15 (71.4)	92 (61.7)		8 (44.4)	161 (56.3)	
B-symptoms												
No	31 (63.3)	193 (64.8)	0.84	18 (64.3)	106 (71.6)	0.44	13 (61.9)	85 (57.4)	0.70	12 (63.2)	181 (64.9)	0.88
Yes	18 (36.7)	105 (35.2)		10 (35.7)	42 (28.4)		8 (38.1)	63 (42.6)		7 (36.8)	98 (35.1)	
LDH level												
Normal	20 (41.7)	117 (40.9)	0.92	13 (46.4)	62 (43.7)	0.79	7 (35.0)	54 (38.0)	0.79	10 (52.6)	107 (40.1)	0.28
Elevated	28 (58.3)	169 (59.1)		15 (53.6)	80 (56.3)		13 (65.0)	88 (62.0)		9 (47.4)	160 (59.9)	
No. of extranodal sites												
0 - 1	38 (76.0)	227 (75.9)	0.99	22 (75.9)	117 (79.1)	0.70	16 (76.2)	108 (72.5)	0.72	13 (76.5)	214 (75.9)	0.96
≥ 2	12 (24.0)	72 (24.1)		7 (24.1)	31 (20.9)		5 (23.8)	41 (27.5)		4 (23.5)	68 (24.1)	
ECOG performance status												
0 - 1	39 (83.0)	230 (82.1)	0.89	21 (77.8)	118 (87.4)	0.19	18 (90.0)	110 (76.9)	0.18	14 (82.4)	216 (82.1)	0.97
≥ 2	8 (17.0)	50 (17.9)		6 (22.2)	17 (12.6)		2 (10.0)	33 (23.1)		3 (17.6)	47 (17.9)	
Size of largest tumor												
< 5cm	21 (60.0)	146 (59.8)	0.98	10 (47.6)	76 (60.3)	0.27	11 (78.6)	69 (59.0)	0.15	11 (84.6)	135 (58.4)	**0.06**
≥ 5cm	14 (40.0)	98 (40.2)		11 (52.4)	50 (39.7)		3 (21.4)	48 (41.0)		2 (15.4)	96 (41.6)	
IPI score												
0 - 2	32 (64.0)	184 (60.5)	0.64	18 (62.1)	102 (67.1)	0.60	14 (66.7)	80 (53.3)	0.25	15 (78.9)	169 (59.3)	**0.09**
3 - 5	18 (36.0)	120 (39.5)		11 (37.9)	50 (32.9)		7 (33.3)	70 (46.7)		4 (21.1)	116 (40.7)	
Therapy response												
CR	36 (70.6)	235 (74.1)	0.59	21 (70.0)	118 (74.2)	0.63	15 (71.4)	115 (73.7)	0.82	17 (85.0)	218 (73.4)	0.25
PR	6	47		4	21		2	26		2	45	
SD	0	16		0	8		0	8		0	16	
PD	9	19		5	12		4	7		1	18	
Primary origin												
Nodal	39 (76.5)	193 (62.5)	**0.05**	25 (83.3)	92 (59.4)	**0.013**	14 (66.7)	99 (65.1)	0.89	12 (60)	181 (62.6)	.81
Extranodal	12 (23.5)	116 (37.5)		5 (16.7)	63 (40.6)		7 (33.3)	53 (34.9)		8 (40)	108 (37.4)	

Cell-of-origin												
GCB	30 (58.8)	159 (50.5)	0.29	30 (100)	159 (100)		0 (0)	0 (0)		10 (50)	149 (50.5)	1.00
ABC	21 (41.2)	156 (49.5)		0 (0)	0 (0)		21 (100)	156 (100)		10 (50)	146 (49.5)	
Ki-67 index												
< 70%	17 (33.3)	104 (33.7)	1.00	10 (33.3)	58 (37.7)	0.69	7 (33.3)	46 (29.7)	0.80	7 (35)	97 (33.6)	1.00
≥ 70%	34 (66.7)	205 (66.3)		20 (66.7)	96 (62.3)		14 (66.7)	109 (70.3)		13 (65)	192 (66.4)	
***TP53* mutations**												
No	38 (76.0)	237 (78.5)	0.69	22 (75.9)	113 (73.4)	1.00	16 (76.2)	122 (83.6)	0.37	16 (94.1)	221 (78)	0.13
Yes	12 (24.0)	65 (21.5)		7 (24.1)	41 (26.6)		5 (23.8)	24 (16.4)		1 (5.9)	64 (22)	
***MYC* translocation**												
No	24 (72.7)	206 (90)	**0.0094**	12 (63.2)	98 (88.3)	**0.011**	12 (85.7)	108 (91.5)	0.62	13 (100)	193 (89.4)	0.37
Yes	9 (27.3)	23 (10)		7 (36.8)	13 (11.7)		2 (14.3)	10 (8.5)		0 (0)	23 (10.6)	
***BCL2* translocation**												
No	33 (73.3)	222 (82.5)	0.15	16 (61.5)	90 (68.2)	0.50	17 (89.5)	132 (96.4)	0.20	12 (80)	210 (82.7)	0.73
Yes	12 (26.7)	47 (17.5)		10 (38.5)	42 (31.8)		2 (10.5)	5 (3.6)		3 (20)	44 (17.3)	
***BCL6* translocation**												
No	24 (60.0)	165 (69.9)	0.21	19 (79.2)	91 (76.5)	0.77	5 (31.3)	74 (63.2)	**0.015**	10 (76.9)	155 (69.5)	0.57
Yes	16 (40)	71 (30.1)		5 (20.8)	28 (23.5)		11 (68.8)	43 (36.8)		3 (23.1)	68 (30.5)	
***MYC* 5'UTR mutations**												
Mutations	22 (41.5)	47 (14.9)	**<0.0001**	15 (50)	26 (16.4)	**<0.0001**	7 (33.3)	21 (13.5)	**0.028**	2 (10)	44 (15)	0.54
WT	30 (58.5)	270 (85.1)		15 (50)	133 (83.6)		14 (66.7)	135 (86.5)		18 (90)	251 (85)	
***MYC* 3'UTR mutations**												
Mutations	4 (7.7)	16 (5.1)	0.50	2 (6.7)	9 (5.7)	0.83	2 (9.5)	7 (4.5)	0.32	1 (5)	15 (5.1)	1.00
WT	47 (92.3)	301 (94.9)		28 (93.3)	150 (94.3)		19 (90.5)	149 (95.5)		19 (95)	280 (94.9)	
p53 expression												
< 20%	31 (62.0)	181(62.2)	1.00	18 (62.1)	92 (62.2)	1.00	13 (61.9)	89 (62.2)	1.00	13 (81.3)	166 (61)	0.12
≥ 20%	19 (38.0)	110 (37.8)		11 (37.9)	56 (37.8)		8 (38.1)	54 (37.8)		3 (18.8)	106 (39)	
MDM2 expression												
< 20%	30 (58.8)	174 (56.7)	0.77	20 (66.7)	92 (59.7)	0.48	10 (47.6)	82 (53.6)	0.61	15 (78.9)	159 (54.5)	**0.043**
≥ 20%	21 (41.2)	133 (43.5)		10 (33.3)	62 (40.3)		11 (52.4)	71 (46.4)		4 (21.1)	129 (45.5)	
p21 expression												
Negative	33 (66.0)	209 (71.8)	0.40	21 (72.4)	116 (78.4)	0.47	12 (57.1)	93 (65.0)	0.48	11 (68.8)	198 (72.0)	0.78
Positive	17 (34.0)	82 (28.2)		8 (27.6)	32 (21.6)		9 (42.9)	50 (35.0)		5 (31.3)	77 (28.0)	
p16 expression												
≤ 10%	28 (75.7)	157 (62.5)	0.14	16 (72.7)	67 (52.8)	0.10	12 (80.0)	89 (72.4)	0.76	13 (76.5)	144 (61.5)	0.30
> 10%	9 (24.3)	94 (37.5)		6 (27.3)	60 (47.2)		3 (20.0)	34 (27.6)		4 (23.5)	90 (38.5)	
Myc expression												
< 40%	10 (20.8)	93 (31.6)	0.17	6 (21.4)	55 (38.5)	0.13	4 (20.0)	37 (24.8)	0.79	12 (60.0)	81 (29.6)	**0.01**
≥ 40%	38 (79.2)	201 (68.4)		22 (78.6)	88(61.5)		16 (80.0)	112 (75.2)		8 (40)	193 (70.4)	
Bcl-2 overexpression												
< 70%	32 (62.7)	157 (51.0)	0.13	21 (70.0)	95 (61.3)	0.41	11 (52.4)	62 (40.5)	0.35	12 (60)	145 (50.3)	0.49
≥ 70%	19 (37.3)	151 (49.0)		9 (30.0)	60 (38.7)		10 (47.6)	91 (59.5)		8 (40)	143 (49.7)	
Bcl-6 expression												
≤ 30%	8 (16.0)	70 (22.7)	0.36	1 (3.3)	19 (12.3)	0.21	10 (50.0)	73 (47.4)	1.00	5 (26.3)	65 (22.5)	0.78

> 30%	42 (84.0)	238 (77.3)		29 (96.7)	135 (87.7)		10 (50.0)	81 (52.6)		14 (73.7)	224 (77.5)	
GCET1 expression												
< 50%	32 (64.0)	206 (66.9)	0.75	15 (50.0)	69 (44.8)	0.69	17 (85.0)	137 (89.0)	0.71	10 (55.6)	196 (67.6)	0.31
≥ 50%	18 (36.0)	102 (33.1)		15 (50.0)	85 (55.2)		3 (15.0)	17 (11.0)		8 (44.4)	94 (32.4)	
CD5 expression												
Negative	51(100)	286 (92.6)	0.77	30 (100)	148 (97.4)	1.00	21 (100)	138 (88.5)	0.91	16 (80)	270 (93.4)	**0.05**
Positive	0 (0)	23(7.4)		0 (0)	4 (2.6)		0 (0)	18 (11.5)		4 (20)	19 (6.6)	
CD10 expression												
< 40%	22 (43.1)	201(64.2)	**0.0052**	4 (13.3)	53 (33.8)	**0.03**	18 (85.7)	148 (94.9)	0.13	11 (57.9)	190 (64.6)	0.62
≥ 40%	29 (56.9)	112 (35.8)		26 (86.7)	104 (66.2)		3 (14.3)	8 (5.1)		8 (42.1)	104 (35.4)	
FOXP1 expression												
< 60%	18 (36.0)	124 (39.9)	0.64	13 (44.8)	100 (64.5)	**0.06**	5 (23.8)	24 (15.4)	0.35	8 (44.4)	116 (39.6)	0.80
≥ 60%	32 (64.0)	187 (60.1)		16 (55.2)	55 (35.5)		16 (76.2)	132 (84.6)		10 (55.6)	177 (60.4)	
MUM1 expression												
< 30%	28 (54.9)	151 (48.7)	0.45	20 (66.7)	119 (76.8)	0.25	8 (38.1)	32 (20.6)	**0.095**	10 (50)	141 (48.6)	1.00
≥ 30%	23 (45.1)	159 (51.3)		10 (33.3)	36 (23.2)		13 (61.9)	123 (79.4)		10 (50)	149 (51.4)	
CD30 expression												
Negative	47 (92.2)	258 (82.7)	0.10	29 (96.7)	128 (82.1)	**0.052**	18 (85.7)	129 (83.2)	1.00	16 (80)	242 (82.9)	0.76
Positive	4 (7.8)	54 (17.3)		1 (3.3)	28 (17.9)		3 (14.3)	26 (16.8)		4 (20)	50 (17.1)	
PI3K overexpression												
< 70%	24 (55.8)	204 (72.1)	**0.048**	14 (56.0)	104 (75.4)	**0.055**	10 (55.6)	100 (69.4)	0.29	17 (89.5)	187 (70.8)	0.11
≥ 70%	19 (44.2)	79 (27.9)		11 (44.0)	34 (24.6)		8 (44.4)	44 (30.6)		2 (10.5)	77 (29.2)	
pSTAT3 overexpression												
< 50%	38 (88.4)	214 (81.4)	0.39	23 (95.8)	109 (85.2)	0.20	15 (78.9)	105 (77.8)	1.00	13 (76.5)	201 (81.7)	0.53
≥ 50%	5 (11.6)	49 (18.6)		1 (4.2)	19 (14.8)		4 (21.1)	30 (22.2)		4 (23.5)	45 (18.3)	
pAKT overexpression												
≤ 60%	39 (83.0)	244 (80.5)	0.84	20 (74.1)	121 (79.6)	0.61	19 (95.0)	122 (87.3)	0.23	16 (80)	228 (80.7)	1.00
> 60%	8 (17.0)	59 (19.5)		7 (25.9)	31 (20.4)		1 (5.0)	28 (18.7)		4 (20)	55 (19.3)	
p63 expression												
< 10%	20 (43.5)	177 (58.4)	**0.078**	14 (51.9)	90 (60.4)	0.41	6 (31.6)	86 (56.2)	**0.052**	11 (57.9)	166 (58.5)	1.00
≥ 10%	26 (56.5)	126 (41.6)		13 (48.1)	59 (39.6)		13 (68.4)	67 (43.8)		8 (42.1)	118 (41.5)	
BLIMP-1 expression												
< 10%	34 (69.4)	217 (72.8)	0.61	24 (82.8)	129 (86.6)	0.56	10 (50.0)	88 (59.1)	0.48	15 (75)	202 (72.7)	1.00
≥ 10%	15 (30.6)	81 (27.2)		5 (17.2)	20 (13.4)		10 (50.0)	61 (40.9)		5 (25)	76 (27.3)	
Nuclear p50 expression												
Negative	21 (47.7)	134 (48.6)	1.00	16 (61.5)	82 (60.3)	1.00	5 (27.8)	52 (37.1)	0.60	8 (44.4)	126 (48.4)	1.00
Positive	23 (52.3)	142 (51.4)		10 (38.5)	54 (39.7)		13 (72.2)	88 (62.9)		10 (55.6)	132 (51.6)	
Nuclear p52 expression												
Negative	41 (89.1)	200 (69.2)	**0.0044**	24 (92.3)	97 (67.8)	**0.0092**	17 (85.0)	102 (70.3)	0.20	13 (72.2)	187 (69.0)	1.00
Positive	5 (10.9)	89 (30.8)		2 (7.7)	46 (32.3)		3 (15.0)	43 (29.7)		5 (27.8)	84 (31.0)	
Nuclear p65 expression												
Negative	19 (42.2)	117 (40.9)	0.87	12 (46.2)	49 (35.3)	0.38	7 (36.8)	67 (46.2)	0.47	6 (35.3)	111 (41.3)	0.80
Positive	26 (57.8)	169 (59.1)		14 (53.8)	90 (64.7)		12 (63.2)	78 (53.8)		11 (64.7)	158 (58.7)	
Nuclear RelB expression												

Negative	39 (86.7)	243 (87.4)	0.81	22 (84.6)	123 (88.5)	0.53	14 (77.8)	94 (67.1)	0.43	17 (94.4)	226 (86.9)	0.71
Positive	6 (13.3)	35 (12.6)		4 (15.4)	16 (11.5)		4 (22.2)	46 (32.9)		1 (5.6)	34 (13.1)	
Nuclear c-Rel expression												
Negative	35 (79.5)	181 (66.8)	0.11	21 (80.8)	87 (66.4)	0.17	14 (77.8)	94 (67.1)	0.43	9 (52.9)	172 (67.7)	0.29
Positive	9 (20.5)	90 (33.2)		5 (19.2)	44 (33.6)		4 (22.2)	46 (32.9)		8 (47.1)	82 (32.3)	
CXCR4 expression												
< 20%	34 (73.9)	197 (70.1)	0.73	20 (76.9)	108 (77.1)	1.00	14 (70.0)	88 (62.9)	0.62	14 (82.4)	183 (69.3)	0.41
≥ 20%	12 (26.1)	84 (29.9)		6 (23.1)	32 (22.9)		6 (30.0)	52 (37.1)		3 (17.6)	81 (30.7)	

Abbreviations: GCB, germinal-center B-cell–like; ABC, activated B–cell like; LDH, lactate dehydrogenase; ECOG, Eastern Cooperative Oncology Group; IPI, International Prognostic Index; CR, complete remission; PR, partial response; SD, stable disease; PD, progressive disease. For therapy response, we calculated *P* values as CR *vs.* other responses. Some clinicopathologic data for some cases were not available due to various reasons such as laboratory failure or tissue exhaustion.

Supplementary Table S3. Lists of Myc mutations found in the training and validation sets and corresponding patient survival

(**A**) Training Set

Accession	#Myc^{high}	Myc mutations	AA changes	OS	Status	PFS	Status
N0004	0	MUT	151S>F; 168H>R	111.72	0	111.72	0
N0005	0	MUT	75V>I	83.05	0	83.05	0
N0009	0	MUT	119P>S	94.85	0	94.85	0
N0012	0	MUT	84G>E;288S>N;290P>S	7.66	1	7.66	1
N0031	1	MUT	58T>A; 138F>S; 154A>P; 175S>R; 178L>M	12.43	1	12.43	1
N0070	0	MUT	58T>A	1.28	1	1.28	1
N0081	0	MUT	185A>V	48.07	0	48.07	0
N0089	1	MUT	202S>N	72.59	0	72.59	0
N0111	0	MUT	401A>T	13.02	1	13.02	1
N0114	0	MUT	265V>A; 216F>L	24.2	1	24.2	1
N0121	0	MUT	5V>I; 299R>K	20.25	1	20.25	1
N0124	0	MUT	splicing	103.96	0	103.96	0
N0125	0	MUT	174S>F	103.2	0	103.2	0
N0137	0	MUT	79P>L; 432E>G	71.01	1	71.01	1
N0142	0	MUT	83R>L	100.14	0	100.14	0
N0165	0	MUT	186S>L	186.71	0	78.71	1
N0183	0	MUT	421R>Q	14.24	1	4.31	1
N0186	0	MUT	164P>S; 202S>N	74.47	0	74.47	0
N0193	1	MUT	405S>F	71.05	0	71.05	0
N0194	0	MUT	356R>K	2.17	1	2.17	1
N0211	0	MUT	237P>L	36.03	0	36.03	0
N0214	.	MUT	141A>T	75.39	0	75.39	0
N0226	0	MUT	321A>T	59.97	0	59.97	0
N0228	1	MUT	355K>R; 386N>S	75.75	0	75.75	0
N0230	1	MUT	364R>H; 421R>W	74.76	0	74.76	0
N0240	1	MUT	357R>Q;150A>V	15.19	1	6.54	1
N0246	0	MUT	63P>L	88.87	0	88.87	0
-	0	MUT	135W>X; 239V>M	15.35	0	15.35	0
N0311	0	MUT	39E>K; 250S>N	7.59	1	7.59	1
N0317	0	MUT	173T>I; 213S>F	61.94	0	61.94	0
N0320	0	MUT	106L>P	3.55	0	3.55	0
N0324	1	MUT	141A>T; 243E>K; 282P>L	18.18	0	18.18	0
N0343	1	MUT	240L>F; 302V>I	76.11	1	41.49	1
N0380	1	MUT	11N>S; 376A>T	2.79	1	2.79	1
N0397	1	MUT	135W>X; 413L>P	103.2	0	103.2	0

N0405	0	MUT	379D>N; 429H>R	75.91	0	75.91	0
N0407	0	MUT	185A>T	75.88	0	75.88	1
N0421	0	MUT	256Q>X	18.97	0	18.97	1
N0439	0	MUT	10R>K	38.14	0	38.14	0
N0456	1	MUT	62S>P	44.75	0	44.75	1
N0486	0	MUT	197Y>H; 44A>T; 62S>P	82.88	0	82.88	0
N0518	1	MUT	111V>M; 39E>D;	20.91	0	20.91	0
N0538	0	MUT	383E>K	74.5	0	74.5	0
N0539	1	MUT	150A>T	73.78	0	73.78	0
N0571	0	MUT	435R>W	25.35	1	25.35	1
N0578	0	MUT	132D>N	85.48	0	85.48	0
N0595	0	MUT	153Q>X; 324R>K; 330V>I	62.83	0	62.83	0
N0597	0	MUT	392K>E; 11N>S	53.95	*0*	53.95	1
N0600	1	MUT	58T>N; 138F>C	5.85	1	4.87	1
N0612	1	MUT	77V>I; 85D>N; 154A>T	10.82	1	10.65	1
N0616	0	MUT	insertion at 60^{th} codon	124.04	0	124.04	0
N0015	0	SNP	11N>S	58.78	0	58.78	0
N0055	0	SNP	11N>S	36.53	0	36.53	0
N0094	0	SNP	11N>S	75.29	1	75.29	1
N0101	0	SNP	11N>S	78.9	0	78.9	0
N0141	0	SNP	11N>S	108.69	0	108.69	0
N0188	0	SNP	11N>S	49.81	1	31.56	1
N0222	1	SNP	11N>S	85.18	0	85.18	0
-	1	SNP	11N>S	39.16	0	39.16	0
-	0	SNP	11N>S	5.39	1	0.36	1
-	0	SNP	11N>S	25.02	1	9.99	1
N0263	0	SNP	11N>S	44.58	0	44.58	0
N0417	0	SNP	11N>S	48.79	0	48.79	0
N0422	0	SNP	11N>S	26.83	1	26.83	1
N0433	1	SNP	11N>S	57.8	0	57.8	0
N0435	0	SNP	11N>S	8.61	0	8.61	0
N0452	0	SNP	11N>S	42.44	0	42.44	1
N0479	1	SNP	11N>S	21.6	0	21.6	0
N0499	0	SNP	11N>S	44.35	0	44.35	0
N0581	1	SNP	11N>S	86.53	0	86.53	0
N0585	0	SNP	11N>S	77.42	0	77.42	0
N0022	0	SNP	11N>S; 57P>A	79.2	0	79.2	0
N0118	.	SNP	59P>S	96.43	0	96.43	0
N0171	0	SNP	59P>S	39.29	0	39.29	0
N0204	0	SNP	231P>L	60.82	0	60.82	0
N0191	0	SNP	11N>S	76.83	0	76.83	0

N0393	0	SNP	11N>S	128.25	0	94.03	1

(B) Validation Set

Case #	Myc^{high}	Myc Mutations	AA changes	OS	Status	PFS	Status
1	0	MUT	62S>F;284A>T	12.23	0	12.23	1
2	0	MUT	329S>N	0.82	0	0.82	0
3	0	MUT	150A>T; 263D>N	66.28	0	66.28	0
4	0	MUT	11N>S; 36Q>X; 255E>K	13.94	1	13.74	1
5	1	MUT	159S>R	85.00	0	7	1
6	0	MUT	179Q>X; 383E>K	10.29	0	10.29	0
7	0	MUT	79P>S; 377L>P	41.33	0	14.5	1
8	0	MUT	11N>I; 31F>S; 50W>X; 406V>I	13.64	0	13.64	0
9	0	MUT	84G>R; 185A>T	109.28	0	13.84	1
10	1	MUT	29E>K	121.28	0	121.28	0
11	0	MUT	313P>S	119.51	1	119.51	1
12	0	MUT	205P>L	80.28	0	53.06	1
13	0	MUT	155A>T; 165A>T; 430K>E	63.65	0	63.65	0
14	0	MUT	67S>F	44.09	0	44.09	0
15	1	MUT	296V>I	44.09	1	21.11	1
16	0	MUT	65R>C	40.87	0	40.87	0
17	0	MUT	248T>S	0.95	0	0.95	1
18	0	MUT	175S>R	52.54	0	52.54	0
19	1	MUT	8T>I; 11N>S	52.14	0	52.14	0
20	1	MUT	138F>C	24.53	0	24.53	0
21	0	MUT	191P>S; 245P>S;	45.63	0	11.11	1
22	0	MUT	123T>I	36.46	0	36.46	0
23	0	MUT	64S>N;415S>F;423R>X	45.60	0	45.6	0
24	0	MUT	228E>G;320P>L, and splicing at 2nd exon	88.04	0	88.04	0
25	1	MUT	138F>C	11.00	1	11	1
26	0	MUT	79P>S	67.63	0	67.63	0
27	0	MUT	335Q>X; frameshift	57.70	0	32.02	1
28	0	MUT	92S>N	48.39	0	48.39	0
29	0	MUT	11N>S;286G>S;418D>N	51.35	0	51.35	0
30	1	MUT	184A>V	36.99	0	36.99	0
31	1	MUT	141A>V	2.66	0	2.66	0
32	0	MUT	168H>R;294P>S	52.47	0	52.47	0
33	1	MUT	67S>F	11.18	0	11.18	0
34	.	MUT	83R>Q;320P>S	91.76	0	4.44	1

35	.	MUT	172S>F	72.03	0	72.03	0
36	.	MUT	164P>L	68.52	0	68.52	0
37	.	MUT	11N>S;179Q>X	52.41	0	52.41	0
38	.	MUT	102V>M	29.16	0	29.16	0
39	.	MUT	41Q>X;207S>F;310A>V	88.67	0	88.67	0
40	.	MUT	175S>R;309Y>C	4.44	1	2.17	1
41	.	MUT	48D>N;INDEL	15.09	0	15.09	0
42	.	MUT	274G>S	9.67	1	9.67	1
43	.	MUT	26D>N	0.99	1	0.99	1
44	.	SNP	11N>S	65.13	0	65.13	0
45	1	SNP	170V>I	61.61	0	1.28	1
46	0	SNP	170V>I	60.33	0	60.33	0
47	0	SNP	160G>S	36.95	0	36.95	0
48	0	SNP	11N>S; 33Q>H	16.77	0	16.77	0
49	0	SNP	11N>S	72.53	0	72.53	0
50	0	SNP	11N>S	49.71	0	49.71	0
51	0	SNP	11N>S	12.10	1	11.51	1
52	0	SNP	11N>S	51.91	0	51.91	0
53	0	SNP	11N>S	48.20	0	48.2	0
54	.	SNP	218P>S	68.58	0	68.58	0
55	.	SNP	11N>S	23.67	0	23.67	0
56	.	SNP	11N>S	54.02	0	54.02	0
57	0	SNP	11N>S	29.03	0	29.03	0

Abbreviations: MUT, mutated; SNP, single nucleotide polymorphism; OS, overall survival; PFS, progression-free survival.

Supplementary Table S4. Multivariate survival analysis in the study cohort

(A)

	OS			PFS		
Variables	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***
IPI > 2	2.65	1.82-3.87	**< 0.0001**	2.37	1.66-3.38	**< 0.0001**
Female sex	0.70	0.48-1.02	0.063	0.78	0.55-1.12	0.18
≥5cm tumor	1.24	0.86-1.80	0.25	1.19	0.83-1.69	0.35
B-symptoms	1.59	1.09-2.31	**0.016**	1.51	1.05-2.15	**0.025**
Myc^{high}	1.78	1.22-2.58	**0.003**	1.61	1.13-2.30	**0.009**
Myc mutation	0.61	0.34-1.11	0.11	0.57	0.32-1.02	0.057

(B)

	OS			PFS		
Variables	**HR**	**95% CI**	***P***	**HR**	**95% CI**	***P***
IPI > 2	2.31	1.57-3.39	**< 0.0001**	2.02	1.40-2.91	**< 0.0001**
Female sex	0.77	0.52-1.14	0.18	0.80	0.56-1.16	0.24
≥5cm tumor	1.36	0.93-1.99	0.11	1.30	0.90-1.87	0.16
B-symptoms	1.48	1.00-2.18	**0.048**	1.48	1.02-2.15	**0.038**
***MYC*-3´UTR mutation**	2.23	1.11-4.46	**0.024**	1.85	0.93-3.69	0.079

Abbreviations: OS, overall survival; PFS, progression-free survival; HR, hazard ratio; CI, confidence interval; IPI, International Prognostic Index. IPI includes age, stage, lactate dehydrogenase level, Eastern Cooperative Oncology Groups performance status, and numbers of extranodal sites.

Supplementary Table S5. Molecular characteristics of the 368 patients with diffuse large B-cell lymphoma in the training set with wild-type (WT) or mutated (MUT) *MYC*-UTR

Variables	MUT-3'UTR *N* (%)	WT-3'UTR *N* (%)		MUT-5'UTR *N* (%)	WT-5'UTR *N* (%)	
Ki-67 index						
< 70%	6 (31.6)	115 (33.7)	1.00	19 (28.4)	102 (34.8)	0.39
≥ 70%	13 (68.4)	226 (66.3)		48 (71.6)	191(65.2)	
***TP53* mutations**						
No	14 (73.7)	261 (78.4)	0.63	53 (77.9)	222 (78.2)	0.97
Yes	5 (26.3)	72 (21.6)		15 (22.1)	62 (21.8)	
***MYC* translocation**						
No	9 (90)	221 (87.7)	1.00	35 (83.3)	195 (88.6)	0.31
Yes	1 (10)	31 (12.3)		7 (16.7)	25 (11.4)	
***BCL2* translocation**						
No	13 (81.3)	242 (81.2)	1.00	45 (80.4)	210 (81.4)	0.85
Yes	3 (18.8)	56 (18.8)		11 (19.6)	48 (18.6)	
***MYC* CDS mutations**						
Mutations	4 (20)	47 (13.8)	0.50	22 (31.9)	30 (10.0)	**<0.0001**
WT	16 (80)	301 (86.2)		47 (68.1)	270 (90.0)	
***MYC* 5'UTR mutations**						
Mutations	7 (33.3)	62 (17.8)	**0.077**			
WT	14 (66.7)	286 (82.2)				
***MYC* 3'UTR mutations**						
Mutations				7 (10.1)	13 (4.3)	**0.073**
WT				62 (89.9)	286 (95.7)	
p53 expression						
< 20%	12 (63.2)	200 (62.1)	1.00	42 (62.7)	170 (62)	1.00
≥ 20%	7 (36.8)	122 (37.9)		25 (37.3)	104 (38)	
MDM2 expression						
< 20%	11 (55)	193 (57.1)	1.00	37 (53.6)	167 (57.8)	0.59
≥ 20%	9 (45)	145 (42.9)		32 (46.4)	122 (42.2)	
p21 expression						
Negative	13 (68.4)	229 (71.1)	0.80	46 (68.7)	196 (71.5)	0.65
Positive	6 (31.6)	93 (28.9)		21 (31.3)	78 (28.5)	
p16 expression						
≤ 10%	10 (71.4)	175 (63.9)	0.78	30 (65.2)	155 (64)	1.00
> 10%	4 (28.6)	99 (36.1)		16 (34.8)	87 (36)	
Myc^{high} expression						
< 70%	17 (85)	226 (66.9)	0.13	40 (61.5)	203 (69.3)	0.24
≥ 70%	3 (15)	112 (33.1)		25 (38.5)	90 (30.7)	
Bcl-2^{high} expression						
< 70%	10 (50)	179 (52.8)	0.82	37 (53.6)	152 (52.4)	0.89
≥ 70%	10 (50)	160 (47.2)		32 (46.4)	138 (47.6)	
Bcl-6 expression						

≤ 30%	2 (10)	76 (22.5)	0.27	6 (9)	72 (24.7)	**0.0048**
> 30%	18 (90)	262 (77.5)		61 (91)	219 (75.3)	
GCET1 expression						
< 50%	9 (45)	229 (67.8)	**0.05**	42 (60.9)	196 (67.8)	0.32
≥ 50%	11 (55)	109 (32.2)		27 (39.1)	93 (32.2)	
CD5 expression						
Negative	19 (95)	318 (93.5)	1.00	64 (95.5)	273 (93.2)	0.59
Positive	1 (5)	22 (6.5)		3 (4.5)	20 (6.8)	
CD10 expression						
< 40%	13 (65)	210 (61)	0.82	38 (55.1)	185 (62.7)	0.27
≥ 40%	7 (35)	134 (39)		31 (44.9)	110 (37.3)	
CD30 expression						
Negative	16 (80)	289 (84.3)	0.54	60 (87)	245 (83.3)	0.58
Positive	4 (20)	54 (15.7)		9 (13)	49 (16.7)	
PI3K^{high} expression						
< 70%	13 (68.4)	215 (70)	1.00	38 (70.4)	190 (69.9)	1.00
≥ 70%	6 (31.6)	92 (30)		16 (29.6)	82 (30.1)	
pSTAT3^{high} expression						
< 50%	18 (100)	234 (81.3)	**0.05**	47 (88.7)	205 (81)	0.24
≥ 50%	0 (0)	54 (18.8)		6 (11.3)	48 (19)	
pAKT^{high} expression						
≤ 60%	15 (78.9)	268 (81)	0.23	47 (77)	236 (81.7)	0.47
> 60%	4 (21.1)	63 (19)		14 (23)	53 (18.3)	
p63 expression						
< 10%	9 (45)	188 (57.1)	0.35	34 (54)	163 (57)	0.68
≥ 10%	11 (55)	141 (42.9)		29 (46)	123 (43)	
BLIMP-1 expression						
< 10%	14 (73.7)	237 (72.3)	1.00	50 (75.8)	201 (71.5)	0.54
≥ 10%	5 (26.3)	91 (27.7)		16 (24.2)	80 (28.5)	
Nuclear p50 expression						
Negative	10 (52.6)	145 (48.2)	0.81	28 (50.9)	127 (47.9)	0.77
Positive	9 (47.4)	156 (51.8)		27 (49.1)	138 (52.1)	
Nuclear p52 expression						
Negative	14 (73.7)	227 (71.8)	1.00	44 (75.9)	197 (71.1)	0.52
Positive	5 (26.3)	89 (28.2)		14 (24.1)	80 (28.9)	
Nuclear p65 expression						
Negative	8 (42.1)	128 (41)	1.00	29 (49.2)	107 (38.9)	0.18
Positive	11 (57.9)	184 (59)		30 (50.8)	168 (61.1)	
Nuclear RelB expression						
Negative	15 (88.2)	267 (87.3)	1.00	51 (89.5)	231 (86.8)	0.67
Positive	2 (11.8)	39 (12.7)		6 (10.5)	35 (13.2)	
Nuclear c-Rel expression						
Negative	12 (63.2)	204 (68.9)	0.62	40 (72.7)	176 (67.7)	0.52
Positive	7 (36.8)	92 (31.1)		15 (27.3)	84 (32.3)	

CXCR4 expression						
< 20%	12 (63.2)	219 (71.1)	0.45	39 (70.9)	192 (70.6)	1.00
≥ 20%	7 (36.8)	89 (28.9)		16 (29.1)	80 (29.4)	

Abbreviations: UTR, untranslated region. Some biomarkers' data were missing due to laboratory failure or tissue exhaustion.

Supplementary Table S6. Lists of *MYC* 3´UTR mutations found in the study cohort and corresponding patient survival.

(**A**) Training Set

Accession #	CDS mutations	AA changes	5'UTR mutations	3'UTR mutations	miR-TS	Recurrent	Myc^{high}	OS	Status	PFS	Status
N0111	MUT	401A>T	MUT	MUT			0	13.02	1	13.02	1
N0183	MUT	421R>Q	WT	MUT		*368C	0	14.24	1	4.31	1
N0230	MUT	364R>H; 421R>W	MUT	MUT	mir-24		1	74.76	0	74.76	0
N0311	MUT	39E>K; 250S>N	MUT	MUT	mir-331; mir34a, Let-7adef, mir-135a, 449abc		0	7.59	1	7.59	1
N0141	SNP	11N>S	WT	MUT			0	108.69	0	108.69	0
N0048	WT		WT	MUT			0	51.09	0	51.09	0
N0069	WT		MUT	MUT			0	56.94	0	56.94	0
N0099	WT		WT	MUT	mir-24		0	80.65	1	80.65	1
N0113	WT		WT	MUT			1	73.58	1	73.58	1
N0023	WT		WT	MUT		*2G*	0	16.67	1	16.67	1
N0191	WT		MUT	MUT		*2G*	0	76.83	0	76.83	0
N0196	WT		WT	MUT	mir-331		0	67.59	0	67.59	0
-	WT		MUT	MUT			0	39.58	0	39.58	1
N0325	WT		MUT	MUT	mir-196b	*22C*	0	19.36	1	19.36	1
N0062	WT		WT	MUT	mir-196b	*22C*	0	2.76	1	2.76	1
N0388	WT		WT	MUT			0	4.41	1	4.41	1
N0424	WT		WT	MUT	mir-24		0	29.46	1	29.46	1
N0516	WT		WT	MUT			0	16.83	0	16.83	0
N0541	WT		SNP	MUT	mir-34a;Let7-a,d,e,f; mir-98;mir-449a,b,c;		0	36.16	0	36.16	0
N0570	WT		WT	MUT			1	98.99	1	98.99	1

(**B**) Validation Set

Case #	CDS Mutations	AA changes	5'UTR mutations	3'UTR mutations	miR-TS Recurrent	Myc^{high}	OS	Status	PFS	Status
13	MUT	155A>T; 165A>T; 430K>E.	MUT	MUT	mir-429 *345C*	0	63.65	0	63.7	0
24	MUT	228E>G;320P>L, and splicing at 2nd exon	MUT	MUT		0	88.04	0	88	0
25	MUT	138F>C	MUT	MUT	mir-24	1	11.00	1	11	1
27	MUT	335Q>X; frameshift	WT	MUT	mir-33b	0	57.70	0	32	1
29	MUT	11N>S;286G>S;418D>N	MUT	MUT		0	51.35	0	51.4	0

31	MUT	141A>V	MUT	MUT		1	2.66	0	2.66	0
34	MUT	83R>Q;320P>S	MUT	MUT	mir-148a-5p	.	91.76	0	4.44	1
40	MUT	175S>R;309Y>C	WT	MUT		.	4.44	1	2.17	1
51	SNP	11N>S	MUT	MUT		0	12.10	1	11.5	1
57	SNP	11N>S	WT	MUT		0	29.03	0	29	0
58	WT		SNP	MUT	mir-33b *83G	0	4.11	1	4.11	1
59	WT		WT	MUT	mir-33b *83G	0	48.89	1	10.92	1
60	WT		WT	MUT	mir-331	1	23.08	0	23.1	0
61	WT		SNP	MUT		0	45.30	0	45.3	0
62	WT		WT	MUT	mir-24	.	80.12	0	8.98	1

Abbreviations: UTR, untranslated region; WT, wild type; MUT, mutated; SNP, single nucleotide polymorphism; OS, overall survival; PFS, progression-free survival.

Supplementary Table S7. Gene profiling comparisons between wild-type (WT) and mutated (MUT) *MYC*.

(A) Differentially expressed genes between patients expressing Myc T58 mutants and patients expressing WT-Myc (false discovery rate: 0.01)

Function	Upregulated	Down regulated
Signaling transduction; ion channel	*FGD4, BMP3, GRIK3, MUC13, MUC3A/B, BEST3, MUC17, TRPC4*	
Transcription regulation	*NR1H4, FOXL1, EPS8L3, TFDP2, TCEB2, HNF4G, ZNF73, ZNF717*	*ZMIZ1, ELK3*
Cell adhesion, cytoskeleton, extracellular matrix	*TUBAL3, TINAG, SCIN, SPTA1*	*FNBP1*
Immune response, inflammation	*NCR3LG1/ DKFZp686O24166, MEP1B*	
Apoptosis	*PEG10, ZNF385B*	
Metabolism	*MTTP, FABP6, ALDOB, GCG, AGPAT5, SLC10A2, ENPEP, SCLY, C8orf38*	
Protein degradation, chaperone	*DNAJC21*	
IncRNA genes; Unknown function	*TM4SF20, KIAA2022, LOC284422, LOC100129961, BTNL8, LOC257396, C22orf34, LOC100287163*	

(B) Differentially expressed genes between patients with 3´UTR mutations recurrent in the DLBCL cohort and patients with WT-3´UTR (false discovery rate: 0.10)

Function	Upregulated	Downregulated
miRNA-mediated gene suppression		*TNRC6B*
Signaling transduction; ion channel	*KCNK6, MARK1, FGFR1OP2, DACT1, ITPR3, WISP2, RABL2A/B, OR10A5, RHOBTB3, ARHGAP28, OPRM1*	
Mitosis, cell cycle, DNA replication and DNA repair	*ENDOG, PARD6B, CEP70, ESCO2*	*RAD1, ADSL, BOLA2*
Cell adhesion, migration, cytoskeleton and extracellular matrix remodeling	*RAPH1, SPTAN1, LOXL4, DNAH1, HPSE, MYLK, PSG6*	
Transcription regulation	*ZNF667, MAGEA2B, MAGEA2, HDAC9, ZNF415, SOHLH2*	
Protein synthesis, processing, trafficking, transporters	*DNAJC5, ENOX2, PPP1R3F, MUDENG,*	*SRP68*

Metabolism	*HPRT1, APOF, EXT1,WBSCR27*	
Immune responses, antibacterial activity	*C17orf87, DEFB106A/B*	
Neuroendocrine differentiation	*DLK1*	
IncRNA genes	*DKFZp434H1419, DKFZP434H168*	*HERC2P2/3*
Unknown function	*KLHL18 , C9orf114 , CCDC78 , NT5DC2, LOC554203 , TMEM39A, N4BP2L1, GRAMD2, LOC152274, C18orf8, LRTM1, KRTAP9-2/KRTAP9-8*	

(C) Differentially expressed genes between patients with nonsense Myc mutants and patients expressing WT-Myc (false discovery rate: 0.05)

Function	Upregulated	Down regulated
Immune response, antibacterial and antifungal activities, detoxification	*CCL28, SMR3A, SMR3B, HTN3, HTN1, MUC7, STATH , DEFB1, SAA1/2, TGFB2*	
Signaling transduction; ion channel	*LPAR3, PROM1, MCOLN3, ANXA3, FGFR2*	*FOXH1*
Transcription regulation	*ELF5, MEIS1, OVOL2*	
Cell adhesion, cytoskeleton, extracellular matrix	*NEBL, CLDN8, SORBS2, ODAM*	
Apoptosis	*PHLDA1*	
Metabolism, transporters, trafficking, degradation, chaperone	*TF, AZGP1, SLC34A2, ZFYVE16, AP4S1, PASK, CSN3, CNKSR3*	
Differentiation	*FOXQ1, GULP1*	
Neural function	*ENPP5, NTNG1*	
IncRNA genes; Unknown function	*C21orf37, ZG16B, PIP, LOC100129410*	

(D) Differentially expressed genes between patients expressing Myc-11S variant and patients expressing WT-Myc-11N (false discovery rate: 0.05)

Function	Upregulated	Down regulated
Signaling transduction; ion channel	*GNG13, HTR2C, RGS5, NLK, RAPGEF4, RASGEF1B, RAPGEF5, RGS4, TAAR2, EPHA5*	
Mitosis, DNA repair, RNA helicases	*INSC, PMS1, DDX3Y, CENPI*	

Transcription regulation	*SOX9, HIPK1, KLF15, ESRRG, ZNF876P, FOXN2, ZNF625, ZNF20, GATA4, ZNF350, SALL3*
Metabolism	*CYP39A1, TMEM135, GAD2*
Cell adhesion, migration, cytoskeletal organization, microtubules, extracellular matrix	*MKLN1, PPFIA1, THSD7A, COL6A6, NCAM1, GIT2, TLL1, ADAMTS19*
Degradation, trafficking	*WDR20, FBXO30, USP9Y*
Immune response, inflammation, apoptosis	*RAGE, HMCN1, S100A12, IGLL1, IFNA5*
Potential tumor suppressor	*CSMD1*
Neural function	*NLGN4X,bNLGN4Y, SLITRK5, SEZ6L, FAT3*
IncRNA genes; Unknown function	*C8orf12, C6orf99, YIPF6, LOC100144602, ZNF321, PHF20L1, SLMO1, C20orf197, C9orf93, LOC731157, LOC284950*

Supplementary Table S8. Brief summary of the major findings by this study

***MYC* Mutations (in 33% DLBCL)**					
Classification by mutation positions	**Associated prognostic effect**	**Expression levels (*vs.* wild-type Myc)**	**Prognostic effect of Myc overexpression (Myc^{high})**	**Functional impact by functional and GEP study**	**Other associated clinicopathologic factors in this study cohort**
(I) Nonsynonymous CDS mutations (in 16% DLBCL)					
	Variable (specified as below)	Similar	No effect in either GCB- or ABC-DLBCL after excluding T58/F138 mutations (compared to the adverse effect of wild-type Myc overexpression in both GCB and ABC). (In contrast, *MYC*-R^+ correlated with poor survival in GCB-DLBCL independent of *MYC* mutation status)	Most were loss-of-function mutations (reduced proliferation and apoptosis), and probably had different tumor microenvironment compared with DLBCL with wild-type *MYC*	in overall DLBCL, associated with *MYC*-5´UTR mutations, $CD10^+$, $PI3K^{high}$, and less $p52^+$; in GCB-DLBCL, associated with nodal DLBCL, *MYC*-R^+, and less $CD30^+$; in ABC-DLBCL, associated with *BCL6*-R^+ and $p63^+$
(I.1) Mutations associated with poor survival (in ~1.1% of DLBCL)					
T58/F138 mutations		Higher	N/A (all overexpressed)	see GEP signatures in Table S7	*MYC*-R^+
(I.2) Mutations associated with better survival (in ~6.3% of LBCL)		Similar			
Some recurrent (≥2 occurrence in our cohort) non-T58/F138 mutations		Higher	No effect	No GEP signatures	
Nonsense, frame-shift, and splicing mutations		Lower	No effect	see GEP signatures in Table S7	
(I.3) Other mutations (in ~8.5% of DLBCL) (probably mix of beneficial, neutral, and harmful mutations)		Similar	No effect	No GEP signatures	
(II) 5´UTR mutations (in 20% patients)					
	No effect (possible adverse effect in *MYC*-R^- cases)	No association		No GEP signatures	*MYC*-CDS mutations, and $Bcl\text{-}6^+$
(III) 3´UTR mutations (in 5.8% patients)					
	Only recurrent mutations showed adverse effect (in 1.3% of DLBCL)	No association		Might have differences in microRNA function, apoptosis and DNA repair	Men
Single Nucleotide Polymorphisms					
	Associated prognostic effect	**Expression levels (*vs.* the canonical Myc-11N)**	**Prognostic effect of Myc overexpression (Myc^{high})**	**Functional impact by functional and GEP study**	**Other associated clinicopathologic factors in this study cohort**
N11S (in 6.5% of DLBCL)	Better survival	Lower	No effect (Myc^{high} was associated with a trend towards better survival P=0.22)	Alterations in immunity, apoptosis and DNA repair	Less $MDM2^+$, $CD5^+$, and trend of smaller tumor size

Abbreviations: DLBCL, diffuse large B-cell lymphoma ; GCB, germinal center B-cell–like; ABC, activated B-cell–like; CDS, coding sequence; UTR, untranslated regions; *MYC*-R, *MYC* rearrangement; Myc^{high}**,** high levels of Myc expression, GEP, gene expression profiling.

LEGENDS FOR SUPPLEMENTARY FIGURES

Supplementary Figure S1. Survival analysis for *MYC* mutations in diffuse large B-cell lymphoma (DLBCL). **(A)** In germinal center B-cell–like DLBCL (GCB-DLBCL), both somatic mutations and single nucleotide polymorphisms in the *MYC* coding sequence leading to a mutated Myc (MUT-MYC) or polymorphic Myc (SNP-MYC) were associated with strong trends toward better progression-free survival (PFS) than wild type Myc (WT-MYC). (**B**) In activated B-cell–like DLBCL (ABC-DLBCL), patients with SNP-MYC had significant better PFS than those with WT-MYC; there was no significant difference in PFS between patients with MUT-MYC and those with WT-MYC although MUT-MYC patients had a slight better PFS rate. (**C-D**) Between homozygous (homo) and heterozygous (hetero) mutations (MUT), and between homozygous and heterozygous SNPs in the *MYC* coding sequence, there were no significant differences in overall survival (OS) or PFS. **(E-F)** In both GCB-DLBCL and ABC-DLBCL with non-T58/F138 MUT-MYC, MUT-Myc expression levels did not show adverse prognostic impact. **(G-H)** In the training set, the favorable prognosis associated with *MYC* variants (MUT or SNP) compared with wild-type Myc (WT-MYC) was only shown in patients without *MYC* rearrangements (*MYC*-R). **(I-J)** In the validation set, the favorable prognosis associated with *MYC* variants compared with wild-type Myc was only shown in patients without *MYC* rearrangements. **(K)** Among W*T-Myc* GCB-DLBCL patients in the training set, *MYC* rearrangement was associated with significantly poorer PFS. **(L**) In *MUT-Myc* GCB-DLBCL patients, *MYC* rearrangement was also associated with significantly poorer PFS.

Supplementary Figure S2. Comparison of Myc levels and gene expression profiles between DLBCL groups. **(A)** In the DLBCL subset without *MYC* rearrangements (*MYC*-R^{-}), there was no significant difference between the levels of wild-type (WT) or mutated Myc (MUT-Myc) (though *MUT-Myc*/*MYC*-R^{-} compared with the *WT-Myc*/*MYC*-R^{-} group showed a slight trend of lower Myc expression levels), but the expression of germline variants (SNP-Myc) was significantly lower than both *MUT-Myc*/*MYC*-R^{-} and *WT-Myc*/*MYC*-R^{-} groups. **(B)** In the DLBCL subset with *MYC* rearrangements (*MYC*-R^{+}), the *MUT-Myc*/*MYC*-R^{+} group had a trend of higher mean level of Myc expression compared with the *WT-Myc*/*MYC*-R^{+} group. **(C)** In the *MYC*-R^{+} DLBCL subset, after excluding T58/F138-*MUT-Myc* cases from the *MUT-Myc*/*MYC*-R^{+} group, there was no significant difference in Myc levels between the *MUT-Myc*/*MYC*-R^{+} and *WT-Myc*/*MYC*-R^{+} groups. **(D)** Heatmap of GEP comparison between Myc T58 mutants and DLBCL expressing WT-Myc. Differentially expressed genes are listed in Supplementary Table S7A. **(E)** Heatmap of GEP comparison between DLBCL with *MYC* 3'UTR mutations recurrent in the DLBCL cohort and DLBCL with WT-*MYC*-3'UTR. Differentially expressed genes are listed in Supplementary Table S7B. **(F)** Heatmap of GEP comparison between DLBCL with nonsense Myc mutants and DLBCL expressing WT-Myc. Differentially expressed genes are listed in Supplementary Table S7C. **(G)** Heatmap of GEP comparison between DLBCL expressing WT-Myc-11S variant and DLBCL expressing WT-Myc-11N. Differentially expressed genes are listed in Supplementary Table S7D.

Supplementary Figure S3. Differential expression of genes between the *WT-Myc* and *MUT-Myc* groups. (**A-J**) The *MUT-Myc* group compared with the *WT-Myc* group had significantly increased *MYC, MDM2, TP63, CD10,* and *CD22* mRNA, and decreased *STAT3*, *STAT5A, CD44*,

ICAM1, and *TNFSF13B*/*BAFF* mRNA levels. **(K)** *FBXW9* mRNA expression was significantly lower in the *MUT-Myc* group compared with WT-Myc^{low} and WT-Myc^{high}. **(L-T)** *MDM2*, *TP63*, *CD10*, *CD22*, *STAT3*, *STAT5A*, *CD44*, *ICAM1*, and *TNFSF13B*/*BAFF* did not show significant up- or downregulation in the WT-Myc^{high} compared with the WT-Myc^{low} group, whereas appeared to "gain" higher or lower expression in the *MUT-Myc* group.

Note: Y-axes represent Log2 values of microarray expression data. Red asterisks * indicate significant ($P \leq 0.05$) upregulation and green asterisks * indicate significant downregulation.

Supplementary Figure S4. Comparison of gene expression between the *WT-Myc* and *MUT-Myc* groups. (**A-B**) The *MUT-Myc* group compared with the *WT-Myc* group had significantly decreased *CTLA4* and *ICOS* mRNA levels. (**C**) Both the *MUT-Myc* group and the WT-Myc^{high} group had lower levels of *CTLA4* mRNA than the WT-Myc^{low} group. (**D**) The *MUT-Myc* group had lower level of *ICOS* mRNA than both the WT-Myc^{low} and WT-Myc^{high} groups (significant *P* and marginal *P* values respectively). (**E**) The *MUT-Myc* group compared with the *WT-Myc* group had decreased *HLA* mRNA levels (here shows the mean values of *HLA-A*, *-B*, *-C*, *-E*, and *–F*) with a border-line *P* value. (**F**) The *MUT-Myc* group showed decreased *HLA* mRNA levels compared with the group with canonical *WT-Myc*. (**G**) Both the *MUT-Myc* group and the WT-Myc^{high} group had lower levels of *HLA* mRNA than the WT-Myc^{low} group. (**H**) The *MUT-Myc* group (but not the WT-Myc^{high} group) had significantly higher level of *CDKN2A* mRNA compared with the WT-Myc^{low} group. (**I**) The *MUT-Myc* group (but not the WT-Myc^{high} group) showed trends of decreased level of *BCL2* mRNA compared with the WT-Myc^{low} and WT-Myc^{high} groups. **(J)** The *MUT-Myc* group had significantly lower levels of *PMAIP1*/*NOXA* mRNA compared with the WT-Myc^{high} group; *PMAIP1*/*NOXA* upregulation correlated with high

level of WT-Myc expression. (**K)** The WT-Myc^{high} but not *MUT-Myc* group had significantly higher level of *TP5*3 mRNA compared with the WT-Myc^{low} group. (**L**) The WT-Myc^{high} but not *MUT-Myc* group had significantly higher level of *BCL2L11*/*BIM* mRNA compared with the WT-Myc^{low} group. **(M)** *BID* was significantly upregulated in the WT-Myc^{high}, but not in the *MUT-Myc*, compared with the WT-Myc^{low} group. **(N-P)** *MIR17HG*, *E2F1* and *EZH2* were upregulated in Myc^{high} compared with Myc^{low} groups irrespectively to the Myc mutation status. **(Q)** The WT-Myc^{high} but not *MUT-Myc* group had significantly higher level of *CHUK*/*IKK1* mRNA compared with the WT-Myc^{low} group. (**R-T**) The *IKBKB*, *NFKBIZ*, and *NFKBIA* mRNA levels appeared decreased in the *MUT-Myc* groups.

Note: Y-axes represent Log2 values of microarray expression data. Red asterisks * indicate significant ($P \leq 0.05$) upregulation and green asterisks * indicate significant downregulation.

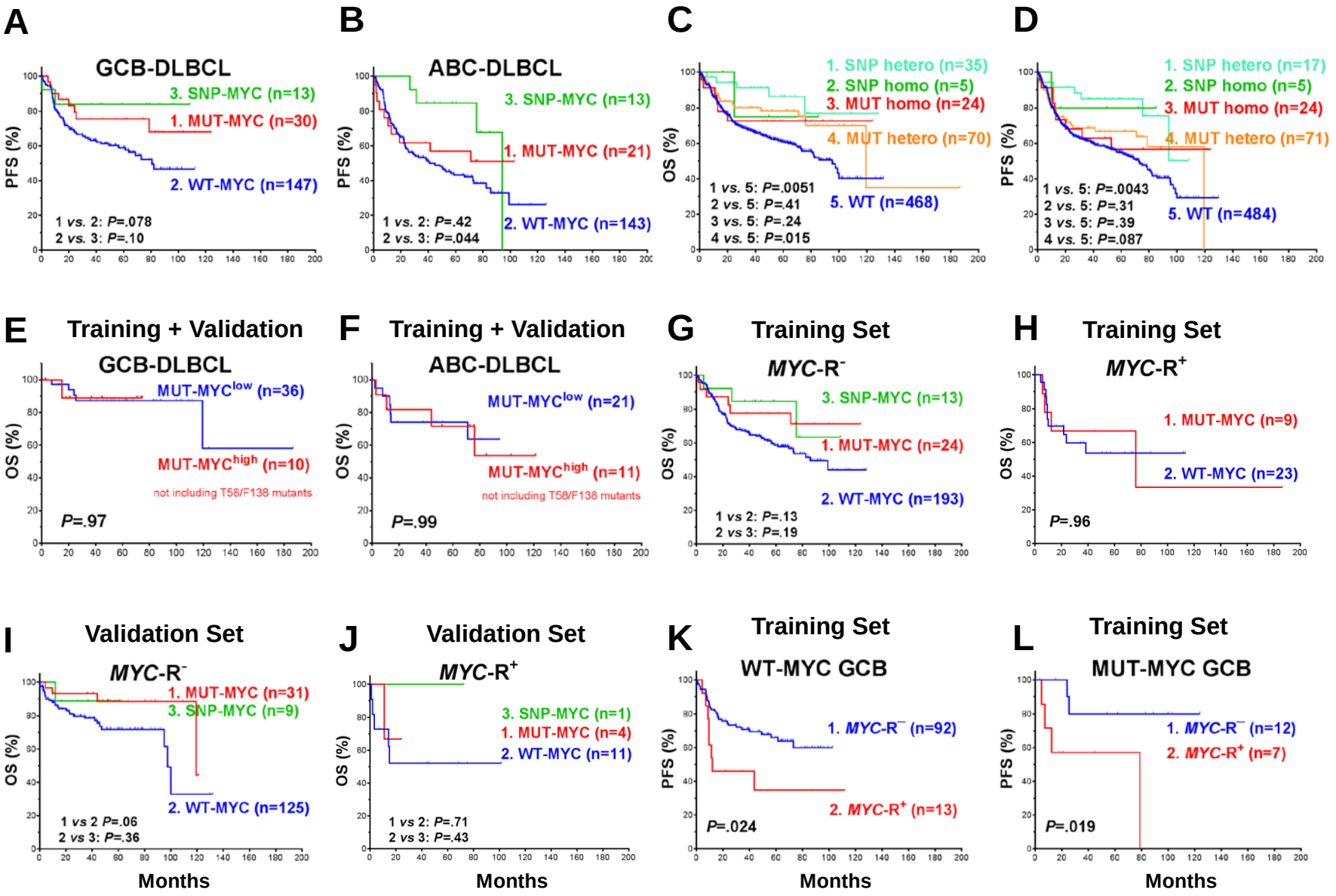
Between the MUT-Myc^{high} and MUT-Myc^{low} groups, there were no significant differences in *ICOS*, *CDKN2A*, *BCL2*, *PMAIP1*, *TP53*, *BCL2L11*, *BID IKK1*, *IKBKB*, and *NFKBIZ* mRNA levels in Figures S4D, H-M and Q-S. In Figures S4C, *CTLA4* levels were lower in the MUT-Myc^{high} than the MUT-Myc^{low} group ($P = 0.12$), but the MUT-Myc^{low} group still had significant lower *CTLA4* levels than the WT-Myc^{low} group ($P = 0.026$).

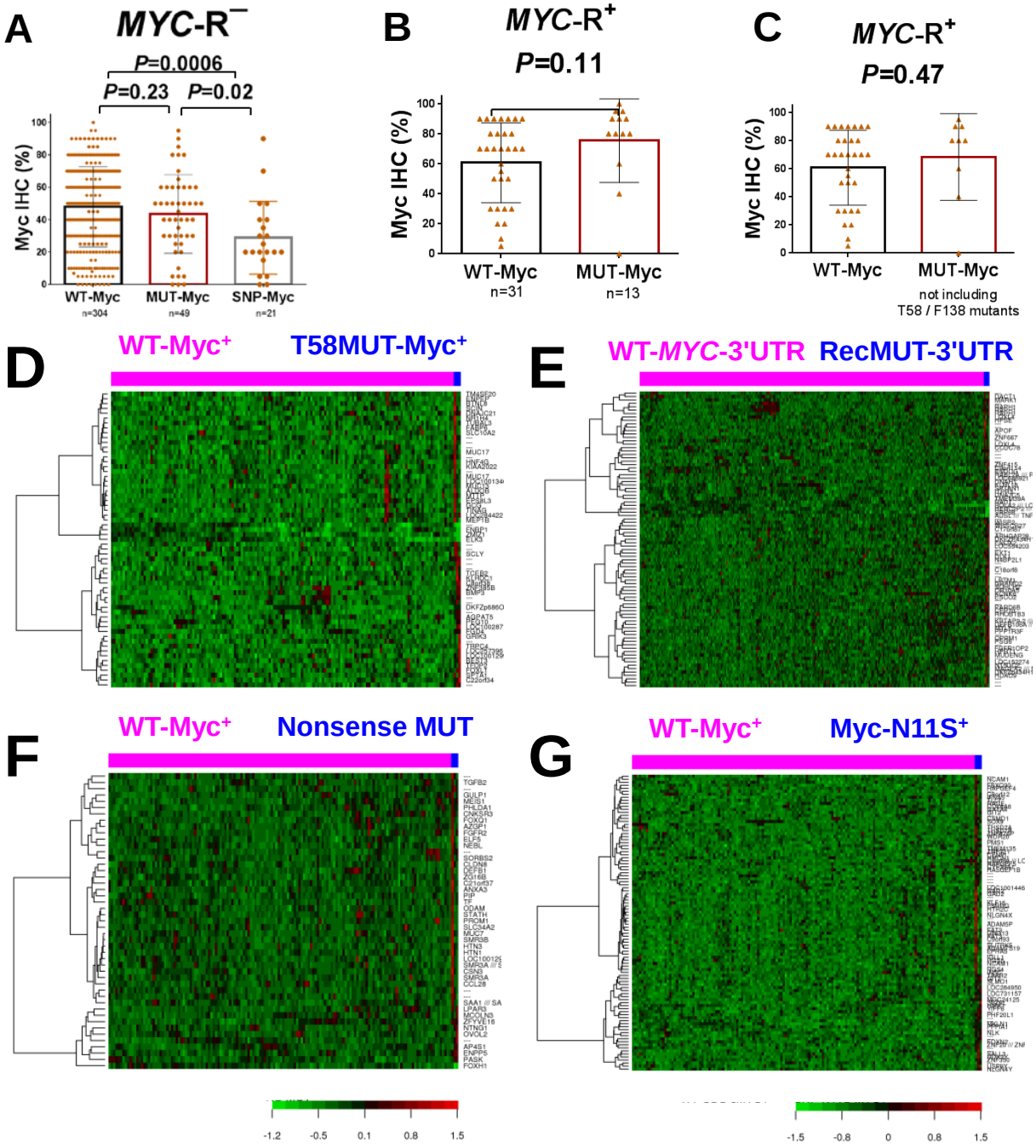
Supplementary Figure S5. Differential expression of proteins (assessed by immunohistochemistry) between the *WT-Myc* and *MUT-Myc* groups. (**A-B**) The *MUT-Myc* group compared with the *WT-Myc* group had significantly increased CD10 and decreased nuclear p52/NF-κB2 expression levels. (**C-D**) The *MUT-Myc* compared with the WT-Myc^{high} group had significantly lower levels of p53 and Ki-67. (**E**) Between the *MUT-Myc* group and the WT-Myc$^{low/high}$ groups, there were no significant difference in MDM2 levels. (**F**) Nuclear

expression of NF-κB subunit c-Rel was significantly lower in the *MUT-Myc* group compared with the WT-Myc^{low} group. (**G-I**) Myc expression levels affect the levels of p53, Ki-67, and pAkt regardless Myc mutation status (the differences in Figure C-D may result from alteration in expression status). (**J**) Bcl-2 levels appeared to be decreased in the MUT-Myc^{high} compared with the WT-Myc^{high} group. (**K-L**) The *MUT-Myc* compared with *WT-Myc* group appeared to have increased p63 expression levels.

Note: Red asterisks * indicate significant ($P \leq 0.05$) upregulation and green asterisks * indicate significant downregulation.

Supplementary Fig. S1

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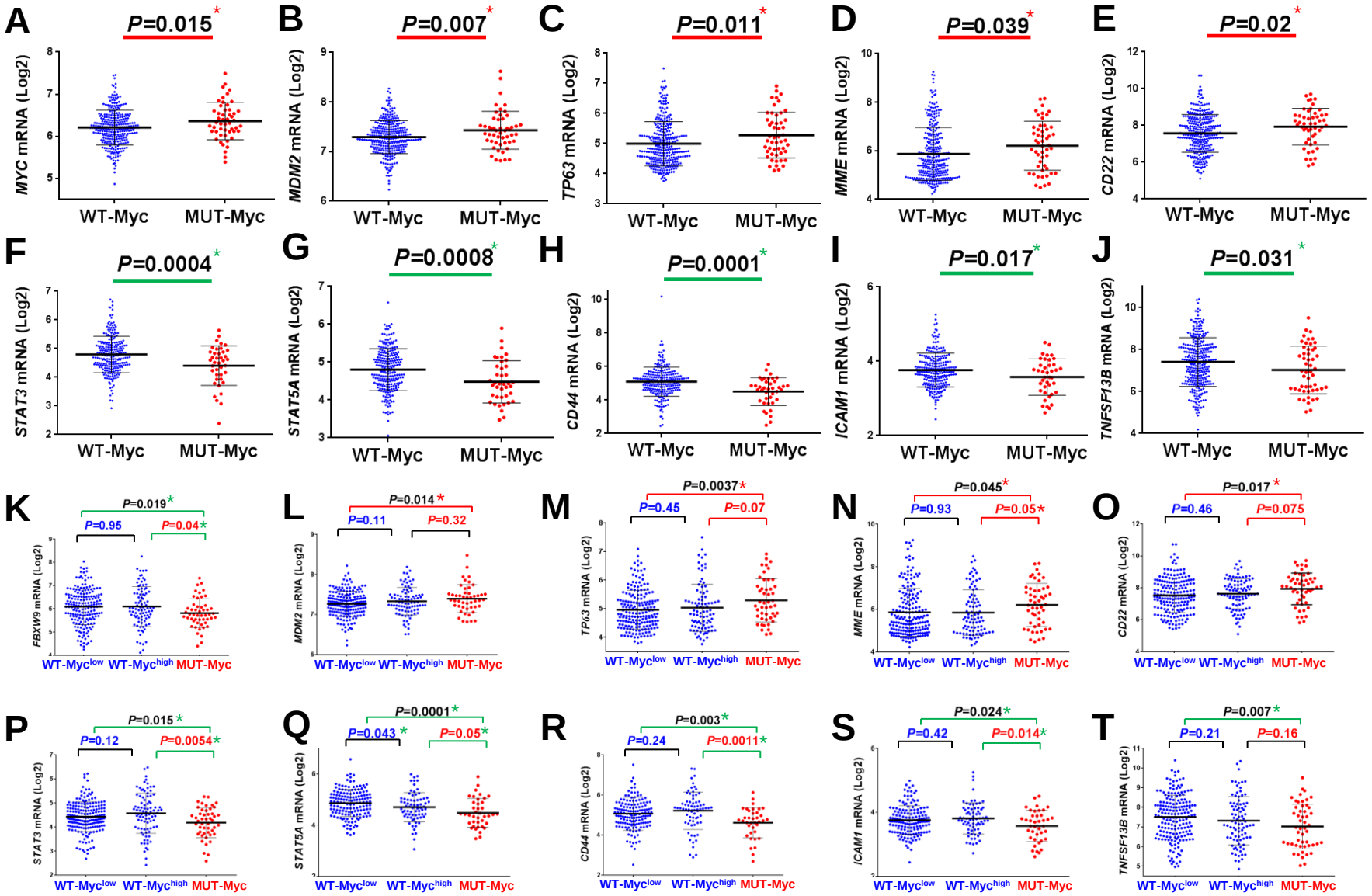
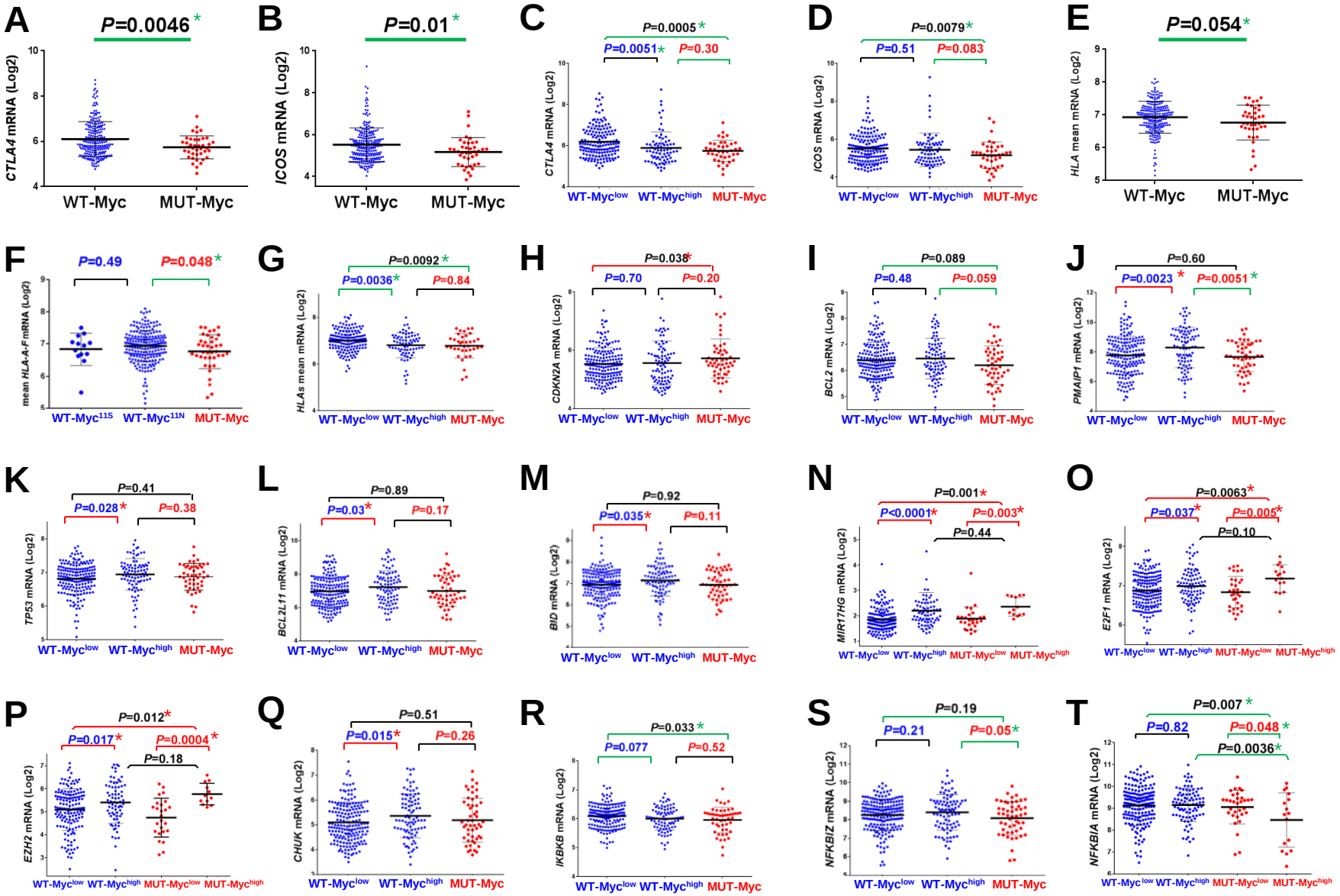
Supplementary Fig. S3



Supplementary Fig. S4



Supplementary Fig. S5