Critical role of SHP2 (PTPN11) signaling in germinal center-derived lymphoma

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Supplementary Methods

Cell culture

The GC lymphoma cell lines used were Raji, OCI-LY8, SU-DHL5, Pfeiffer. The non-GC lymphoma cell lines used were OCI-Ly3, OCI-Ly10. These cells have performed short tandem repeat (STR) DNA analysis at China Center for Type Culture Collection (CCTCC). All cells except OCI-LY10 were cultured in Isocove's modified essential medium (IMDM), supplemented with 10% fetal bovine serum. OCI-Ly10 was cultured in IMDM plus 20% human plasma.

Antibodies, chemical reagents and shRNAs

The following antibodies were used: α/β-Tubulin (2148), AKT (9272), Bcl6 (4242), c-Jun (9165), c-Fos (2250), CD19 (3574), cleaved Caspase3(9664), cyclin D2 (2924), Cytochrome C (4272), ERK1/2 (4695), GAB2 (3239), GRB2 (3972), IκBα (9242), JunB (3753), $p27^{kip1}$ (3686), p38 (8690), PCNA (2586), phospho-Ser32-IκBα (2859), phospho-Ser473-AKT (4060), phospho-Thr58/Ser62-c-Myc(9401), phospho-Thr180/Tyr182-p38 (4511), phospho-Thr202/Tyr204-ERK1/2 (4370), phospho-Tyr416-SRC (6943), phospho-Tyr452-GAB2 (3884), phospho-Tyr531-CD19 (3571), phospho-Tyr542-SHP2 (3751), phospho-Tyr705-STAT3 (9145), pro-Caspase3 (9665), RAS (8955), SHP2 (3397), SRC (2123) and STAT3 (4904, all from Cell Signaling Technology).

Other reagents included c-Myc (764), phospho-Thr58-c-Myc (135647), JNK (7345), phospho-Ser63/73-c-Jun (16312), phospho-Thr183/Tyr185-JNK (6254), phospho-Tyr542-SHP2 (101798, all from Santa Cruz), phospho-Ser374-c-Fos (C0030-01L, USBiologicals), CD19 (HIB19, 302214, Bio-Legend), phospho-Tyr531-CD19 (ABIN683623, anti-bodies-online), phospho-Ser389- GSK3β (361527), GSK3β (PK1111, all from Millipore), and phospho-Ser62-c-Myc (ab11156, Abcam).

Two small hairpin RNAs (shRs) were used to target SHP2, SHP2 shR1 starting at the nucleotide 879 (5'-GTGACCCATGTTATGATTCGC-3'), which targets the consensus coding DNA sequence (CCDS) region, and SHP2 shR2 starting at the nucleotide 6060 (5'-CCACGTATATTAT GTAGTCTA-3'), which targets the 3' untranslated region. CD19 targeting shR was started at the nucleotide 984 (5'-GTGGGCATTCTTC-ATCTTCAA-3') and c-Myc targeting shR was started at the nucleotide 560 (5'-CTCCCGCGACGATGCCCCTCA-3'). All these shRs were sequence-confirmed and cloned into the pLL3.7-GFP vector (Clontech). Rescue experiments were performed with pLV-YFP vector (Addgene) expressing wild type SHP2, CD19 and c-Myc. BAY 11-7082, Dasatinib and LY294002 were purchased from Sigma. PD0325901 and U0126 were purchased from Selleck.

Immunohistochemistry

Paraffin-embedded B-lymphoma tissues were obtained from the files of the Department of Pathology of Zhejiang University School of Medicine the First Affiliated Hospital and Department of Pathology, Basic Medical College, Capital Medical University (Table S6) after necessary informed consent and/or exemption were obtained. The cases were fully anonymized and coded as per Zhejiang University School of Medicine institutional review board regulations and federal provisions. All B-lymphoma diagnoses were made according to the World Health Organization classification system[1]. Paraffin-embedded tissue sections were stained with phospho-Tyr531-CD19 (1:100, antibodies-online), phospho- Thr202/Tyr204-ERK1/2 (1:400, Cell Signaling Technology), phos- pho-Tyr542-SHP2 (1:100, Santa Cruz). Grayscale and color images were taken with an E600-Nikon Microscope (Nikon, Melville, NY), a SPOT-2 CCD camera and software (Diagnostic Instruments, Sterling Heights, MI), and edited for optimal color contrast with Adobe Photoshop 10 (San Jose, CA). Immunoreactive scores for each antibody staining were quantitatively evaluated as in a double- blinded manner. The percentage and intensity of staining of malignant B cells were independently scored in the TMA, each using a 10-tiered scale (0-9). The result of both was used as a case score and a value of 10 or greater was considered positive for χ^2 calculations. The phospho-Tyr542-SHP2

positive cases were further divided into 2 groups: moderate = score 10-29, strong = score 30 and greater (Table S7). The phospho-Tyr531-CD19 positive cases were divided as: moderate = score 10-39, strong = score 40 and above. The phospho- Thr202/Tyr204-ERK1/2 cases were divided as: moderate = score 10-20, strong = score 21 and above.

Chromatin immunoprecipitation assay

Chromatin immunoprecipitation (ChIP) assays were performed following the protocol of EZ-ChIP kit (Upstate, Waltham, MA, USA). Raji and OCI-LY8 cells were crosslinked with formaldehyde (1% final concentration) and incubated for 20 min at room temperature. 4μg of antibody for c-Myc (sc-764, Santa Cruz) was applied to precipitate chromatin from 2×10^6 cells. Immunoprecipitated DNA and input samples were analyzed with a SYBR Green RT-PCR kit (Applied Biosystems), and percentage enrichment relative to the amount of input chromatin was determined as $2^{(Ct\ input-Ct\ Ab)}$. Primers specific for c-Myc recognized E-box1 of CD19 gene were: E-box1 sense (5'-CTAGAAATCA-GCCTCCAGTCAG-3') and E-box1 antisense (5'-GCAATTCAACAG-CCTCTCTCCT-3'). Primers specific for c-Myc recognized E-box2 of CD19 gene were: E-box2 sense (5'-AGAACCAGTCCAGCAAAG-AACC-3') and E-box2 antisense (5'-AAACTCCAACCCAAG-GCCTAG-3').

Luciferase activity assay

The luciferase reporter plasmid pGL3 (Promega) was applied to clone the reporter gene. Two portions spanning the two conversed E-boxes of CD19 gene respectively were amplified using the E-box1 primers (sense: 5'-CGAGCTCCTAGAAATCAGCCTCCAGTCAG-3'; antisense: 5'-C-CGCTCGAGGCAATTCAACAGCCTCTCTCCT-3') and the E-box2 primers (sense: 5'-CGAGCTCAGAACCAGTCCAGCAAAGAACC-3'; antisense: 5'-CCGCTCGAGAAACTCCAACCCAAGGCCTAG-3'), and inserted into the SacI/XhoI site of the pGL3 vector upstream of the luciferase gene to generate the vector pGL3-E-box1 or pGL3- E-box2. All of the constructs were sequenced. For luciferase assays, Raji and OCI-LY8 were cultured in 24-well plates respectively, and transfected with 500ng pGL3-E-box1, pGL3-E-box2 or pGL3-promoter vector using DMRIE-C with or without c-Myc shR. *Renilla* luciferase activity was used for normalization. At 48 h after transfection, *firefly* and *Renilla* luciferase activities were measured using the Dual-Luciferase Reporter Assay (Promega).

MTT assay

To monitor cell survival as a function of ERK phosphorylation, Cells in 96-well plates were treated with PD0325901, or U0126 for 4 days with replacement of fresh medium supplemented with 1×concentration of

drugs every 2 days. At harvesting, cells were treated with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma) for 0.5-1 hour and the produced colored substrate was solubilized in isopropanol and 1% hydrochloric acid. The absorbance of the colored supernatant at 570-nm wavelength was measured by a spectrophotometer with subtraction of background absorbance at 630-nm wavelength.

Quantitative Real-time PCR

Total B cell RNA was prepared with the RNeasy kit (Qiagen). For real-time PCR analysis, total RNA (2 μg) was converted to cDNA with the SuperScript First-Strand Synthesis System (Invitrogen). Real-time PCR was done in triplicate with the appropriate primers using SYBR Green (Roche) and an ABI Prism 7900 Sequence Detector (Applied Biosystems). GAPDH was used as loading control. All the primers were designed to specifically amplify human transcripts (Table S4).

Supplementary Tables

Table S1. prime sequences used for real time PCR.

Gene name	Forward primer sequence	Reverse primer sequence
AICDA	AGAACTTTCAAAGCCTGGGA	CAGAGAAGACTTGAAGGACTG
BCL6	AATCTTGTGGCTTTGAGGGC	CCATTTTGTCTTCACCAATGCC
BPNT1	CTGGGTATTGTGGAGAAG	TTCCCACTGACTGTCTTC
CCND2	GCTGGAGGTCTGTGAGGAAC	AGTTGCAGATGGGACTTCGG
CD38	AATGGATCCCGCAGTAAAAT	AGAAACTTGTCAGGTCTGTAG
CDKN1B	ACCTGCAACCGACGATTCTT	TTCTGAGGCCAGGCTTCTTG
DNAJC10	ATGTAGGATGGATGGACTGT	CCAAACGATCCTCTAGTGTG
DNMT1	CTGGCTAAAGTCAAATCCCT	TAGGTGAAGGTTCAGGCTTA
TCF3	CGGATCACTCAAGCAATAAC	TTCTATCTTACTCTGCAGGC
FGD6	GCTGCTCTCAAAGAAGTATCA	CTAATAAAGGCTGACTCTCCA

FOS	GGGGCAAGGTGGAACAGTTA	AGTTGGTCTGTCTCCGCTTG
GAPDH	GTCAAGGCTGAGAACGGGAA	AAATGAGCCCCAGCCTTCTC
GCET2	AGCAGAACACTCAAGAGATAC	GTCAACATTCTCCTCCTGGAA
HDAC7	TCGTGAGCTAAAGAATGGTT	GTCTTGGTAGAAGGTTTGCT
HMGN1	TCCCAATCCGGTTCCATC	GATCTCCTCTTGGGCTCTT
ITPKB	ATCATTACAGACATGGGCAC	TGATTTGCTCACTCTAGGTT
JUN	GCCTCCAAGTGCCGAAAAAG	TAAGCTGTGCCACCTGTTCC
KATNAL1	GGGAAGTGGAGGTCTCTGAA	TAGCTGGATCTCTGACTGAC
LMO2	ACTATCTCAGGCTTTTTGGG	GGGCCTATATCATCCCATTG
LRMP	GGAAACTGAAGAAAGCAGTGT	TCCAAAGCACCATCCTTTAC
MAN2C1	CGTAGTGCAAGAGCCCCTA	GGGACATCATCAAATAGCAC
MAP4K2	CACAGAGACATCAAGGGAG	TCACATAGCTCATTGTAGCC
MAPK10	CCTTACAGCATCCCTACATC	ACCTGTGCTGAAGGAGAA

MARCKSL1	GGAGAATGGCCACGTGAA	AAGGACAGGCCGCTCAAT
MAST2	TAGAGCTGATCCTGAAGAGT	GAGCTCCTTTTTCTGCTTTC
MME	CTGAGGGGTCACGATTTTAG	CCATCATCGTAGGTTGCATA
MYBL1	GCTGCAAACAAGAGAATACC	TCAAGTTGCACCTATTGTCA
MYC	TAGTGGAAAACCAGCAGC	TTTCTTCCAGATATCCTCGC
NEK6	CTACAACTTCAAGTCCGACA	TGACCAGTTCTCGTAACTTC
PLEKHF2	CGTCGGGGCTATTAGTGAAA	TTCCTGCACAACTTAGTCAA
PRPSAP2	GAGGAATCAATTTTGGAGGA	AGTTTGCTGAAAACAACACC
RUNDC2B	AAGGAGTCAACGCAGAATG	TGTTGGTCACTTGCTTTTTC
SERPINA9	AGAGCTACAACCAAGTAAGC	GGACACACAGTAGATTGGAG
STAT4	TGGAATTGGAGCCCAGTAAG	GGACTTGATTCCACTGAGAC
STK17A	GGATGGAAAAGGCACTAGAA	TCTTGTAGCAAAGGTTCCTC
TMEM123	TGCCTCAGACTCCAGTAATA	TGTCACTGAACTATTGTGGG

TOX	TAGTCTCCCCAGGAACATAG	CTGATAGTCGGGTTGAAGAG
TTC9	CCATCGAGATCGACTGTTAC	AATCACGTTGGTGTCTGTT
VEZT	AAACAGCCGAGCTTTTACTA	GGGGGTAGTTTTTCAGCATA
VPREB3	CTTCCTGTCAGTTTCCCAGAC	GAATCGATCGGGGATGTCA
VNN2	AGAAACACCAGTTTCTCAGG	GGTGTGTGACCAAATCTGT
ZNF608	ATCGAAAGACTCCTGTGAAC	GAGATATGCCCCAGGATAAC

Table S2. Sequence of EMSA probes.

Name	Sequence (5'→ 3')
c-Myc/Max	5'-GGAAGCAGACCACGTGGTCTGCTTCC-3'
E-box1	5'-CAGGGGAAGCACATGACTAACATC-3'
E-box2	5'-GCAGGGAAGCACTTGGCAAGGAGA-3'

Table S3. The COO status of lymphoma cell lines.

Cell line name	Lymphoma subtype	COO status	*MYC/IGH* translocation	Source
Raji	BL	GC lymphoma	+	ATCC
OCI-LY8	FL[2,3]	GC lymphoma	+	Ontario Cancer Institue
SU-DHL5	GCB-DLBCL	GC lymphoma	−	ATCC
Pfeiffer	GCB-DLBCL	GC lymphoma	−	ATCC
OCI-LY3	ABC-DLBCL	non-GC lymphoma	−	Ontario Cancer Institue
OCI-LY10	ABC-DLBCL	non-GC lymphoma	−	Ontario Cancer Institue

Table S4. Proportion of down-regulated proliferative signatures from *MYC/IGH+* GC lymphoma lines after inhibitor treatment.

Gene name	SHP2 shRs, *P* value		c-Myc shR, *P* value		CD19 shR, *P* value		PD0325901, *P* value		U0126, *P* value	
	Raji	OCI-LY8	Raji	OCI-LY8	Raji	OCI-LY8	Raji	OCI-LY8	Raji	OCI-LY8
AICDA	0.0002	0.0122	0.0003	0.0093	0.0003	0.0092	0.0039	0.0209	0.0038	0.0236
BCL6	5.19E-08	0.0009	0.0016	0.0005	1.28E-06	0.0031	0.011	0.0005	0.009	0.0007
BPNT1	0.1072	0.3203	0.4864	0.157546	0.0681	0.2134	0.2566	0.0665	0.6193	0.2845
CCND2	0.0001	0.001	3.42E-05	0.0003	0.0001	0.003	8.43E-06	3.84E-05	0.0002	1.84E-05
CD38	0.0017	0.0181	0.0049	0.0018	0.0057	0.0095	0.0137	0.0017	0.0082	0.0048
DNMT1	0.0128	0.037	0.0062	0.0128	0.1212	0.0363	0.1411	0.2057	0.4355	0.5157
TCF3	0.0027	2.14E-05	0.0038	0.0002	0.0023	2.26E-05	0.034	0.0183	0.0007	0.0291
FGD6	0.0028	0.0015	0.0059	0.0021	0.0085	0.0104	0.0032	0.0009	0.0028	0.0015
FOS	2.43E-05	2.18E-08	0.0535	0.155	0.0001	0.0016	0.0004	2.44E-06	0.0007	2.07E-05
GCET2	0.0001	9.88E-06	0.0017	0.0002	0.0016	0.0018	0.0602	0.0116	0.0227	0.0068
HDAC7	0.9905	0.3514	0.6111	0.1541	0.9121	0.4688	0.7726	0.9356	0.9905	0.3514
HMGN1	0.6923	0.1482	0.3099	0.3976	0.5932	0.1492	0.3629	0.7676	0.6923	0.1482
ITPKB	0.648	0.7235	0.8494	0.9671	0.0023	0.0002	0.5286	0.0357	0.648	0.7235
JUN	2.55E-06	1.1E-07	0.127	0.0579	3.18E-06	7.01E-05	1.76E-05	0.0003	1.36E-05	6.11E-05
KATNAL1	0.947	0.0175	0.8136	0.1384	0.9428	0.3966	0.7536	0.2458	0.947	0.0175
MKI67	0.0002	0.0139	0.0005	0.0384	0.0054	0.0128	0.0046	9.09E-05	0.0048	5.13E-05
LMO2	0.0003	0.0005	1.7E-05	3.99E-08	0.0048	0.0006	0.0078	0.1148	0.0223	0.0256
LRMP	0.0007	0.0012	0.0001	1.58E-07	0.0052	0.0015	0.0002	0.004	0.0007	0.0012
MAN2C1	0.059	0.0442	0.0047	0.0025	0.2573	0.2683	0.6684	0.0839	0.5623	0.5309

MAP4K2	0.0569	0.893	0.2705	0.6116	0.0938	0.4528	0.2019	0.5013	0.0569	0.893
MAPK10	0.8967	0.7572	0.1755	0.5832	0.7117	0.5687	0.3054	0.3824	0.8967	0.7572
MARCKSL1	0.0803	0.0737	0.0086	0.0087	0.2539	0.0416	0.9384	0.096	0.0803	0.0737
MAST2	0.7808	0.7139	0.3099	0.1394	0.3419	0.3877	0.2372	0.643	0.7808	0.7139
MME	0.0006	3.36E-05	0.0016	0.0009	0.0053	0.0011	0.0002	0.0076	0.0015	0.0055
MYBL1	6.8E-05	0.0014	0.0004	0.0006	0.0011	0.0187	0.0005	0.0042	0.0002	0.0021
MYC	0.0008	0.0025	2.6E-05	0.0002	0.0044	0.0025	0.0006	0.0004	0.0005	0.0004
NEK6	0.094	0.7536	0.0267	0.3045	0.0193	0.9825	0.5003	0.6346	0.0117	0.7809
PCNA	0.0021	0.0005	0.0007	0.0018	0.0053	0.0025	0.0071	5.48E-05	0.0036	9.92E-05
PLEKHF2	4.32E-05	3.29E-05	0.0017	1.24E-07	0.074	0.213	0.0416	0.0022	0.2631	0.0278
PRPSAP2	4.71E-05	0.0006	1.65E-05	5.07E-05	0.0013	0.0049	2.99E-05	0.0072	8.73E-05	0.0107
RUNDC2B	0.0029	6.27E-05	5.67E-05	1.7E-06	0.0023	0.0003	0.0008	0.0002	0.0024	0.0062
SERPINA9	5.8E-05	1.14E-05	0.0009	6.29E-05	0.0006	0.0002	0.003	0.0013	0.0003	0.0061
STAT4	0.0782	0.0011	0.0078	0.0659	0.1071	0.1013	0.1503	0.0073	0.3653	0.0021
STK17A	0.0998	0.6682	0.2059	0.8379	0.0588	0.3792	0.249	0.9658	0.161	0.773
TMEM123	0.0026	0.0018	0.0002	6.26E-07	0.0025	0.0002	0.2267	0.002	0.0017	0.0028
TOX	0.3362	0.632	0.9324	0.9605	0.7803	0.8406	0.4895	0.5943	0.2581	0.3999
TTC9	0.0043	0.0001	0.6702	0.1322	0.8085	0.7684	0.0029	0.0003	0.0028	0.0021
VEZT	0.0059	0.007	0.0002	0.0019	0.2021	0.0034	0.0363	0.0028	0.0094	0.0074
VPREB3	0.0134	2.28E-05	0.0033	0.0043	0.0362	0.0127	0.0013	0.0006	0.0174	0.0013
VNN2	0.0034	3.5E-05	0.0005	0.0004	0.1842	0.1463	0.0052	0.0018	0.0049	0.0138
ZNF608	8.77E-06	1.59E-05	1.98E-05	3.2E-06	0.0025	0.0023	0.0008	0.0019	1.85E-06	0.0105
Proportion of down-regulated genes	0.6585	0.7317	0.6829	0.6341	0.5854	0.6341	0.5854	0.6585	0.6341	0.6829

P values for the down-regulation of each individual GC lymphoma proliferative signature shown in Fig. S2A,B are listed. Numbers at the bottom indicate the overall proportion of down-regulated genes ($p < 0.05$) by each inhibitor treatment.

Table S5. Proportion of down-regulated proliferative signatures from *MYC/IGH*− GC lymphoma lines after inhibitor treatment.

Gene name	SHP2 shRs, *P* value		c-Myc shR, *P* value		PD0325901, *P* value		U0126, *P* value	
	Raji	OCI-LY8	Raji	OCI-LY8	Raji	OCI-LY8	Raji	OCI-LY8
AICDA	0.001	0.0003	0.0027	0.0001	0.0019	0.0006	0.0015	0.001
BCL6	0.0002	1.22E-07	3.95E-05	3.9E-07	0.0004	0.0006	0.0002	0.0013
BPNT1	0.6167	0.7092	0.0703	0.5284	0.8222	0.2584	0.4998	0.4775
CCND2	5.43E-05	0.0004	0.0003	0.0019	1.4E-05	0.0002	4.49E-06	0.0008
CD38	0.0004	8.65E-05	0.0021	0.0001	9.65E-05	0.0004	0.001	0.0005
DNMT1	0.479	0.0401	0.1228	0.0026	0.2955	0.0511	0.194	0.0313
TCF3	0.0045	0.0011	0.0004	0.0009	0.0003	0.0007	0.0004	0.0143
FGD6	0.0011	0.0064	0.0002	0.0032	0.0013	0.0058	0.0011	0.0064
FOS	2.81E-05	0.0005	0.1669	0.1052	0.0017	0.0003	0.0002	0.0004
GCET2	2.45E-06	7.49E-06	5.35E-06	0.0015	0.0345	0.0191	0.0105	0.0455
HDAC7	0.1456	0.3862	0.4724	0.271	0.2041	0.8858	0.1456	0.3862
HMGN1	0.8514	0.1271	0.8347	0.2594	0.8351	0.6779	0.8514	0.1271
ITPKB	0.0799	0.5742	0.0607	0.9109	0.1563	0.2965	0.0799	0.5742
JUN	0.0009	0.0038	0.0626	0.3143	0.0009	0.002	0.0007	0.0059
KATNAL1	0.3106	0.7984	0.4586	0.5893	0.9111	0.977	0.3106	0.7984
MKI67	0.0018	6.03E-05	0.0018	0.0011	0.0027	2.1E-05	0.0018	0.0002
LMO2	0.0008	0.0005	0.0003	0.0003	0.0009	0.0011	0.0008	0.0005
LRMP	0.0012	0.0004	1.95E-05	0.0002	0.0004	0.0007	0.1098	0.0004
MAN2C1	0.0688	0.4874	0.0025	0.0041	0.1462	0.2465	0.0688	0.4874
MAP4K2	0.0127	0.049	0.0108	0.4151	0.0909	0.1305	0.0127	0.049

MAPK10	0.5589	0.3043	0.9984	0.7019	0.7024	0.1407	0.5589	0.3043
MARCKSL1	0.8898	0.6558	0.8286	0.8807	0.8377	0.3198	0.8898	0.6558
MAST2	0.854	0.7644	0.7991	0.5813	0.9943	0.4631	0.854	0.7644
MME	0.0009	0.0017	0.0004	0.0017	0.0004	0.002	0.0014	0.0027
MYBL1	0.0014	1.65E-06	0.0005	3.74E-05	0.0008	0.0033	0.0021	0.0058
MYC	0.024	0.0022	2.46E-06	3.85E-05	0.0007	0.0029	0.0007	0.0024
NEK6	0.2358	0.0702	0.7889	0.0208	0.7891	0.005	0.1478	0.0652
PCNA	0.0001	9.22E-05	3.4E-05	0.0015	6.09E-05	0.0007	2.49E-05	4.48E-05
PLEKHF2	2.24E-08	3.75E-05	2.45E-05	3.89E-07	0.0008	0.0025	0.0027	0.0057
PRPSAP2	4.24E-06	3.89E-05	0.0001	2.49E-05	0.0642	0.0048	0.0006	0.0053
RUNDC2B	0.0057	0.0066	4.59E-06	2.89E-05	0.0017	0.0047	0.0105	0.013
SERPINA9	9.96E-06	3.36E-05	0.0003	0.0036	0.0083	0.0005	0.0388	0.0064
STAT4	0.524	0.2018	0.1407	0.1286	0.6539	0.1482	0.3511	0.3401
STK17A	0.6528	0.2979	0.2697	0.0998	0.7444	0.4345	0.336	0.1456
TMEM123	0.001	0.0075	1.91E-05	3.13E-05	0.0021	0.0057	0.0015	0.0109
TOX	0.916	0.2724	0.9038	0.4855	0.2628	0.0795	0.6493	0.5347
TTC9	0.0016	0.0031	0.3261	0.2917	0.0024	0.0012	0.0023	0.0077
VEZT	0.0004	0.0007	5.87E-05	2.39E-06	0.0023	0.0582	0.0008	0.0016
VPREB3	5.3E-06	0.0002	0.0003	0.0024	0.0014	0.0004	0.0085	0.0002
VNN2	0.0004	4.96E-06	0.0022	0.0002	0.0043	0.0044	0.0003	0.0149
ZNF608	3.68E-05	2.64E-05	5.04E-06	1.8E-07	0.0001	0.0032	0.0003	0.0002
Proportion of down-regulated genes	0.6585	0.7073	0.6098	0.6341	0.6098	0.6341	0.6341	0.6829

P values for the down-regulation of each individual GC lymphoma proliferative signature shown in Fig. S2A,B are listed. Numbers at the bottom indicate the overall proportion of down-regulated genes ($p < 0.05$) by each inhibitor treatment.

Table S6. The laboratory characteristics of.56 B-lymphoma patients.

Patient	phosphor-SHP2	Phosphor-ERK1/2	phosphor-CD19	BCL2	BCL6	CD10	CD20	MUM1	EBER	IGH/MYC translocation
GCB-DLBCL-1	-	-	-	+	++	+	++	+	-	N/A
GCB-DLBCL-2	++	++	++	-	++	+	++	-	+	N/A
GCB-DLBCL-3	++	++	+	+	+	+	++	-	-	N/A
GCB-DLBCL-4	++	++	++	+	++	+	++	-	-	-
GCB-DLBCL-5	++	++	++	++	++	++	++	-	-	N/A
GCB-DLBCL-6	++	+	++	-	++	++	++	-	-	N/A
GCB-DLBCL-7	+	+	-	+	++	++	+	-	-	+
GCB-DLBCL-8	+	+	++	-	++	++	++	-	-	-
GCB-DLBCL-9	++	++	++	+	+	++	++	-	+	N/A
GCB-DLBCL-10	+	-	++	+	++	++	++	-	-	-
GCB-DLBCL-11	+	+	-	-	-	++	++	-	-	N/A
GCB-DLBCL-12	-	-	+	+	+	+	++	-	-	N/A
GCB-DLBCL-13	+	+	++	-	++	-	++	-	-	N/A
GCB-DLBCL-14	++	++	++	+	+	+	++	+	+	N/A
GCB-DLBCL-15	++	++	+	-	++	+	++	-	-	N/A
GCB-DLBCL-16	++	+	++	+	+	++	++	-	+	N/A
BL-1	++	++	+	-	++	++	++	N/A	++	+
BL-2	++	++	++	-	++	+	++	N/A	++	+
FL-1	+	-	++	+	++	+	++	-	-	N/A
FL-2	++	+	+	-	+	++	++	+	-	-
FL-3	++	+	++	++	++	+	++	-	+	+
FL-4	+	++	+	++	++	-	++	+	-	-
FL-5	++	++	+	+	++	+	++	-	+	-

FL-6	+	++	+	+	+	+	++	-	-	N/A
FL-7	+	++	++	+	+	-	++	-	+	N/A
FL-8	++	+	++	++	++	+	+	+	-	-
FL-9	+	++	+	++	++	+	+	+	-	-
ABC-DLBCL-1	+	+	+	+	-	-	++	++	-	N/A
ABC-DLBCL-2	+	+	-	-	+	-	++	+	-	N/A
ABC-DLBCL-3	+	+	++	+	+	-	++	++	-	N/A
ABC-DLBCL-4	++	+	+	+	++	-	++	++	-	N/A
ABC-DLBCL-5	++	+	++	+	-	-	+	+	+	N/A
ABC-DLBCL-6	+	+	++	++	-	-	++	++	-	-
ABC-DLBCL-7	-	+	+	-	+	-	++	+	+	N/A
ABC-DLBCL-8	++	++	++	++	-	-	++	+	-	N/A
ABC-DLBCL-9	+	+	+	-	-	-	+	++	+	N/A
ABC-DLBCL-10	-	-	+	++	+	-	++	+	-	N/A
ABC-DLBCL-11	-	+	++	+	-	-	++	++	-	-
ABC-DLBCL-12	+	++	-	-	++	-	++	++	-	N/A
ABC-DLBCL-13	-	-	++	+	-	-	++	++	-	N/A
ABC-DLBCL-14	-	-	++	-	+	-	++	++	+	N/A
ABC-DLBCL-15	-	+	+	+	-	-	++	+	N/A	N/A
ABC-DLBCL-16	-	-	++	+	-	-	++	++	-	N/A
ABC-DLBCL-17	-	++	+	+	+	-	++	+	N/A	N/A
ABC-DLBCL-18	-	-	+	-	-	-	++	+	-	N/A
ABC-DLBCL-19	-	-	+	+	-	-	++	+	N/A	-
ABC-DLBCL-20	-	-	+	-	-	-	++	+	-	N/A
ABC-DLBCL-21	-	-	-	+	-	-	++	++	-	N/A
ABC-DLBCL-22	++	+	+	-	+	-	++	++	-	N/A

ABC-DLBCL-23	-	+	+	+	-	-	++	+	-	N/A
ABC-DLBCL-24	-	-	+	++	-	-	++	++	-	N/A
ABC-DLBCL-25	++	++	++	+	+	-	++	++	-	N/A
ABC-DLBCL-26	-	++	-	-	-	-	+	++	-	N/A
ABC-DLBCL-27	+	+	++	+	-	-	++	++	-	N/A
ABC-DLBCL-28	-	-	++	+	+	-	++	++	-	N/A
ABC-DLBCL-29	-	-	++	-	-	-	++	+	+	N/A

+, Moderate.

++, Strong.

Table S7. Distribution of p-Tyr531-CD19, p-Thr202/Tyr204-ERK1/2 and p-Tyr542-SHP2 staining in GC lymphoma and non-GC lymphoma subgroups.

p-Tyr542 SHP2				
COO subtype	Negative	Moderate	Strong	Total
GC lymphoma	2	10	15	27
non-GC lymphoma	17	7	5	29
Total	19	17	20	56

p-Tyr531 CD19				
COO subtype	Negative	Moderate	Strong	Total
GC lymphoma	3	9	15	27
non-GC lymphoma	4	13	12	29
Total	11	23	22	56

p-Thr202/Tyr204-ERK1/2				
COO subtype	Negative	Moderate	Strong	Total
GC lymphoma	4	9	14	27
non-GC lymphoma	11	13	5	29
Total	15	28	13	56

p-, phosphorylated.

Supplementary Figure Legends

Figure S1. High-level SHP2 activation is preferentially associated with GC lymphoma.

A. Expression of p-Tyr531-CD19, p-Thr202/Tyr204-ERK1/2 and p-Tyr542-SHP2 in the B-lymphoma biopsies by immunohisto- chemistry. p-, phosphorylated. H&E, Hematoxylin and eosin stain.

B. Distribution of p-Tyr531-CD19, p-Thr202/Tyr204-ERK1/2 and p-Tyr542-SHP2 staining in GC lymphoma and non-GC lymphoma subgroups. The staining was scored as described in the supplementary methods.

Figure S2. Effects of SHP2 knockdown or ERK inhibition on GC lymphoma and non-GC lymphoma cells.

A. GC lymphoma and non-GC lymphoma lines transfected with CTRL shR or SHP2 shR2 were immunoblotted with antibodies against SHP2, BCL6, Tubulin, ERK1/2 and p-Thr202/Tyr204-ERK1/2. p-, phosphory-lated. Tubulin and total form of ERK1/2 were used as loading control. Data are representative of three independent experiments.

B. Viability of GC lymphoma and non-GC lymphoma lines lines assessed by MTT assay after 5 days of treatment with varying doses of MEK inhibitors PD0325901, U0126; PI3K inhibitor LY294002 and IκBα inhibitor BAY 11-7082.

C. GC lymphoma and non-GC lymphoma lines were treated with U0126 (10μM) or PD0325901 (0.2μM) for 6 hours. Cell lysates were analyzed by antibodies to c-Myc and Tubulin (loading control). Data are representative of three independent experiments.

Figure S3. SHP2/ERK signaling is required for proliferative signatures expression in GC lymphoma cells.

A. Proliferative gene expression profiling of the GC lymphoma lines after treatment with SHP2 shRs, c-Myc shR or CD19 shR. Gene expression changes were assessed using real time PCR and depicted according to the color scale shown. Gene expression measurements for the GC lymphoma proliferative signatures in the GC lymphoma lines expressing SHP2 shR1 or SHP2 shR2 were averaged for each sample.

B. Proliferative gene expression profiling of the GC lymphoma lines after treatment with MEK inhibitor PD0325901 or U0126 for 24 hours. Gene expression changes were assessed using real time PCR and depicted according to the color scale shown.

Figure S4. Effects of SHP2 rescue on GC lymphoma cells.

A. GC lymphoma transfected with CTRL + CTRL shR (CTRL shR), SHP2 shR2 + CTRL (SHP2 shR2) and SHP2 shR2 + SHP2 rescue (SHP2 rescue) were immunoblotted with antibodies against SHP2 and

Tubulin (loading control). SHP2 shR2 targets the 3' untranslated region of SHP2 gene. Data are representative of three independent experiments.

B. GC lymphoma cells expressing CTRL, CTRL shR, SHP2 shR2 or SHP2 rescue were immunoblotted with antibodies against cyclin D2, $p27^{kip1}$, PCNA, c-Myc and Tubulin (loading control). Data are representative of three independent experiments.

C. GC lymphoma cells expressing CTRL, CTRL shR, SHP2 shR2 or SHP2 rescue were immunoblotted with antibodies against SHP2, p-Tyr542 SHP2, Tubulin, ERK1/2 and p-Thr202/Tyr204-ERK1/2. p-, phosphorylated. Tubulin and total form of ERK1/2 were used as loading control. Data are representative of three independent experiments.

Figure S5. Effects of CD19 on the phosphorylation and stabilization of c-Myc in GC lymphoma cells.

A. MYC/IGH+GC lymphoma lines expressing CTRL shR or CD19 shR were immunoblotted with antibodies against c-Myc, p-Ser62 c-Myc, p-Thr58 c-Myc, p-Ser389 GSK3β, GSK3β and Tubulin. p-, phosphorylated. Tubulin and total form of GSK3β were used as loading control. Data are representative of three independent experiments.

B. GC lymphoma cells expressing CTRL, SHP2 shR2 or SHP2 shR2 + CD19 rescue (CD19 rescue) were immunoblotted with antibodies against CD19, p-Tyr531 CD19, c-Myc, p-Ser62 c-Myc, Tubulin, ERK1/2

and p-Thr202/Tyr204-ERK1/2. p-, phosphorylated. Tubulin and total form of ERK1/2 were used as loading control. Data are representative of three independent experiments.

Figure S6. CD19 ligation resulted in the recruitment of GAB2 and SHP2 to CD19 in *MYC/IGH+*GC lymphoma cells, but not in *MYC/IGH−*GC lymphoma and non-GC lymphoma cells.

A. GAB2 immunoprecipitated (IP) from wild-type B lymphoma cells before and after CD19 crosslinking (10μg/ml). Data are representative of three independent experiments.

B. SHP2 immunoprecipitated (IP) from wild-type B lymphoma cells before and after CD19 crosslinking (10μg/ml). Data are representative of three independent experiments.

Figure S7. Effects of ERK inhibition on the expression of transcripts encoding AP-1, cyclin D2 and p27^{kip1} in *MYC/IGH+*GC lymphoma cells.

A. Real-time PCR of transcript expression by *MYC/IGH+*GC lymphoma cells left unstimulated or stimulated with U0126 (10μM), PD0325901 (0.2μM) or Dasatinib (50nM) for 6 hours. *CCND2* encodes cyclin D2, *CDKN1B* encodes p27^{kip1}, *FOS* encodes c-Fos and *JUN* encodes c-Jun. RA, relative amount (compared with GAPDH). *$p < 0.05$. Data represent mean ± s.e.m. of three independent experiments.

B. Immunoblot analysis of $^{MYC/IGH+}$GC lymphoma cells left unstimulated or stimulated for 6 hours with U0126 (10μM), PD0325901 (0.2μM) or Dasatinib (50nM). Cell lysates were analyzed by antibodies to cyclin D2, $p27^{kip1}$, c-Jun, c-Fos and Tubulin (loading control). Data are representative of three independent experiments.

Figure S8. c-Myc transcriptionally regulates CD19 gene.

A. The two E-boxes (E-box1 and E-box2) and the transcription start site on human CD19 gene.

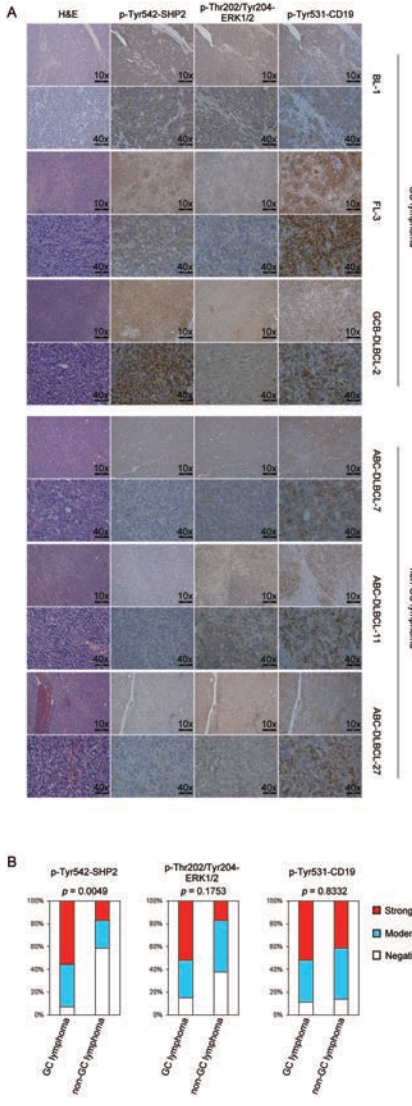
B. c-Myc dependent activation of CD19 in GC lymphoma cells in transient transfection assays. *$p < 0.05$. Data represent mean ± s.e.m. of three independent experiments.

C. ChIP assay in GC lymphoma cells. PCR was performed with primers specific for CD19 E-box1 and E-box2, respectively. Immunoprecipitation was performed with anti-c-Myc Antibidy or Control IgG. *$p < 0.05$. Data represent mean ± s.e.m. of three independent experiments.

Supplementary References

1. Campo E, Swerdlow SH, Harris NL, Pileri S, Stein H, Jaffe ES. The 2008 WHO classification of lymphoid neoplasms and beyond: evolving concepts and practical applications. *Blood*. 2011;117(19): 5019-32.
2. Fruchon S, Kheirallah S, Al Saati T, Ysebaert L, Laurent C, Leseux L, *et al*. Involvement of the Syk-mTOR pathway in follicular lymphoma cell invasion and angiogenesis. *Leukemia*. 2012; 26(4): 795-805.
3. Wang W, Corrigan-Cummins M, Hudson J, Maric I, Simakova O, Neelapu SS, *et al*. MicroRNA profiling of follicular lymphoma identifies microRNAs related to cell proliferation and tumor response. *Haematologica*. 2012; 97(4): 586-94.

Figure S1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p-Tyr531-CD19
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FL-3
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ABC-DLBCL-7
ABC-DLBCL-11
ABC-DLBCL-27
GC lymphoma
non-GC lymphoma
10x
40x
B
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p-Thr202/Tyr204-ERK1/2
p = 0.1753
p-Tyr531-CD19
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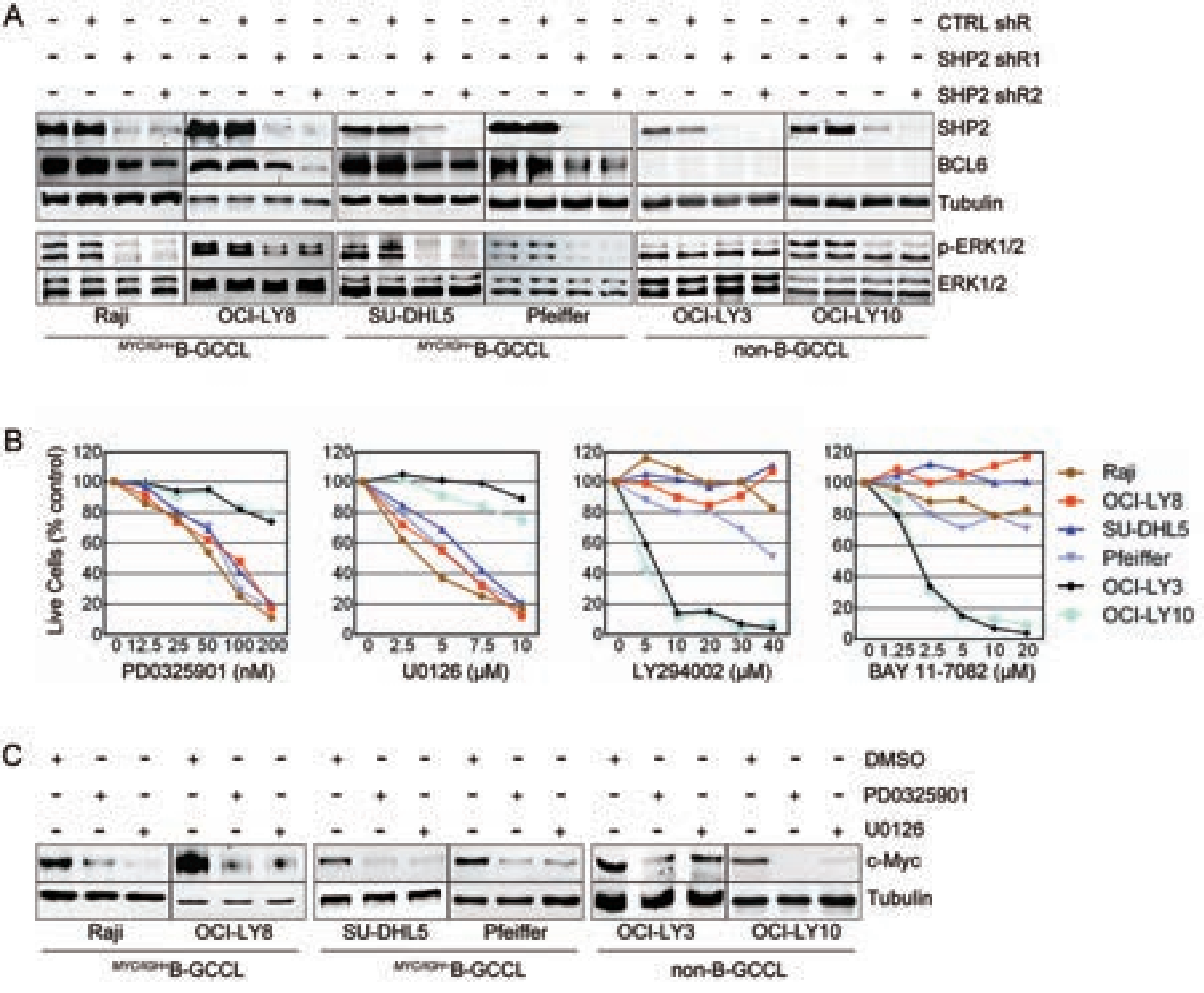
80%
60%
40%
20%
0%
GC lymphoma
non-GC lymphoma
Strong
Moderate
Negative

Figure S2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SHP2 shR2
SHP2
BCL6
Tubulin
p-ERK1/2
ERK1/2
Raji
OCI-LY8
SU-DHL5
Pfeiffer
OCI-LY3
OCI-LY10
MYC/IGH+ B-GCCL
MYC/IGH+ B-GCCL
non-B-GCCL
B
Live Cells (% control)
PD0325901 (nM)
U0126 (μM)
LY294002 (μM)
BAY 11-7082 (μM)
Raji
OCI-LY8
SU-DHL5
Pfeiffer
OCI-LY3
OCI-LY10
C
DMSO
PD0325901
U0126
c-Myc
Tubulin
Raji
OCI-LY8
SU-DHL5
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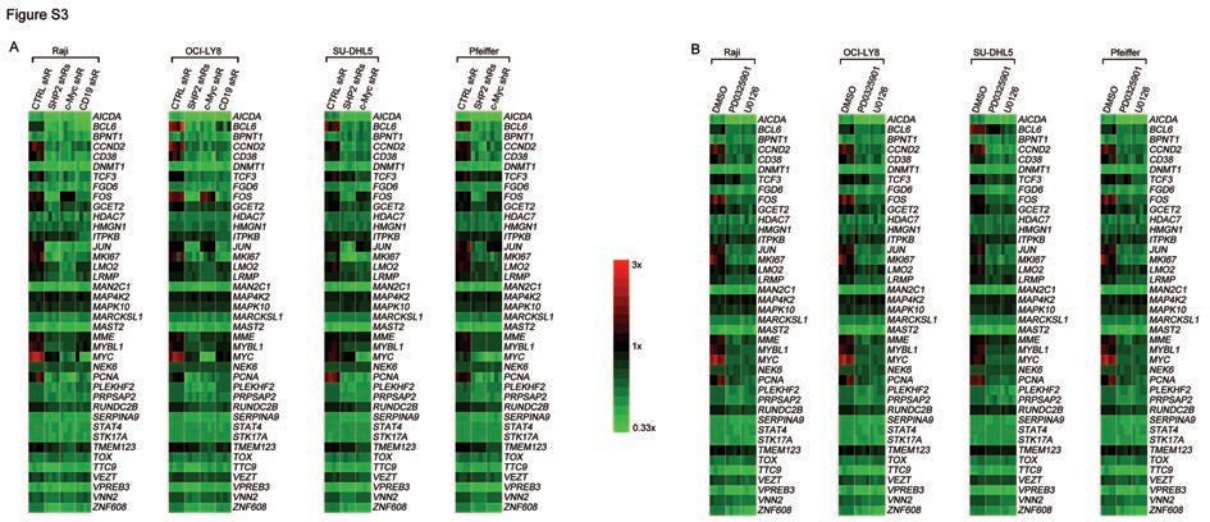
MYC/IGH+ B-GCCL
MYC/IGH+ B-GCCL
non-B-GCCL

Figure S3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OCI-LY8
SU-DHL5
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cMyc shR
CD19 shR
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U0126
AICDA
BCL6
BPNT1
CCND2
CD38
DNMT1
TCF3
FGD6
FOS
GCET2
HDAC7
HMGN1
ITPKB
JUN
MKI67
LMO2
LRMP
MAN2C1
MAP4K2
MAPK10
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MAST2
MME
MYBL1
MYC
NEK6
PCNA
PLEKHF2
PRPSAP2
RUNDC2B
SERPINA9
STAT4
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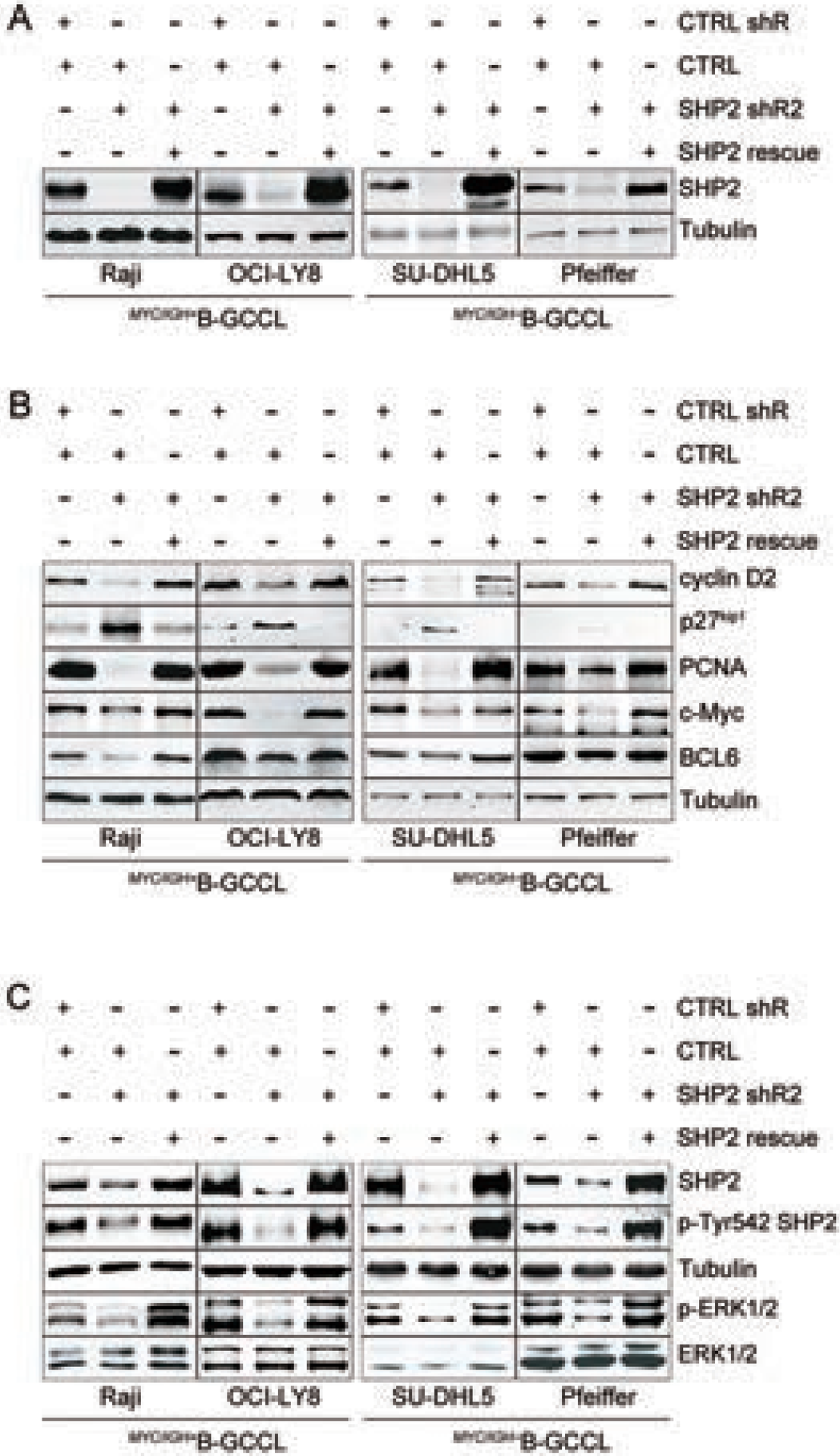
Figure S4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CTRL
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SHP2
Tubulin
Raji
OCI-LY8
SU-DHL5
Pfeiffer
MYC/BCL2 B-GCCL
MYC/BCL2 B-GCCL
B
CTRL shR
CTRL
SHP2 shR2
SHP2 rescue
cyclin D2
p27kip1
PCNA
c-Myc
BCL6
Tubulin
Raji
OCI-LY8
SU-DHL5
Pfeiffer
MYC/BCL2 B-GCCL
MYC/BCL2 B-GCCL
C
CTRL shR
CTRL
SHP2 shR2
SHP2 rescue
SHP2
p-Tyr542 SHP2
Tubulin
p-ERK1/2
ERK1/2
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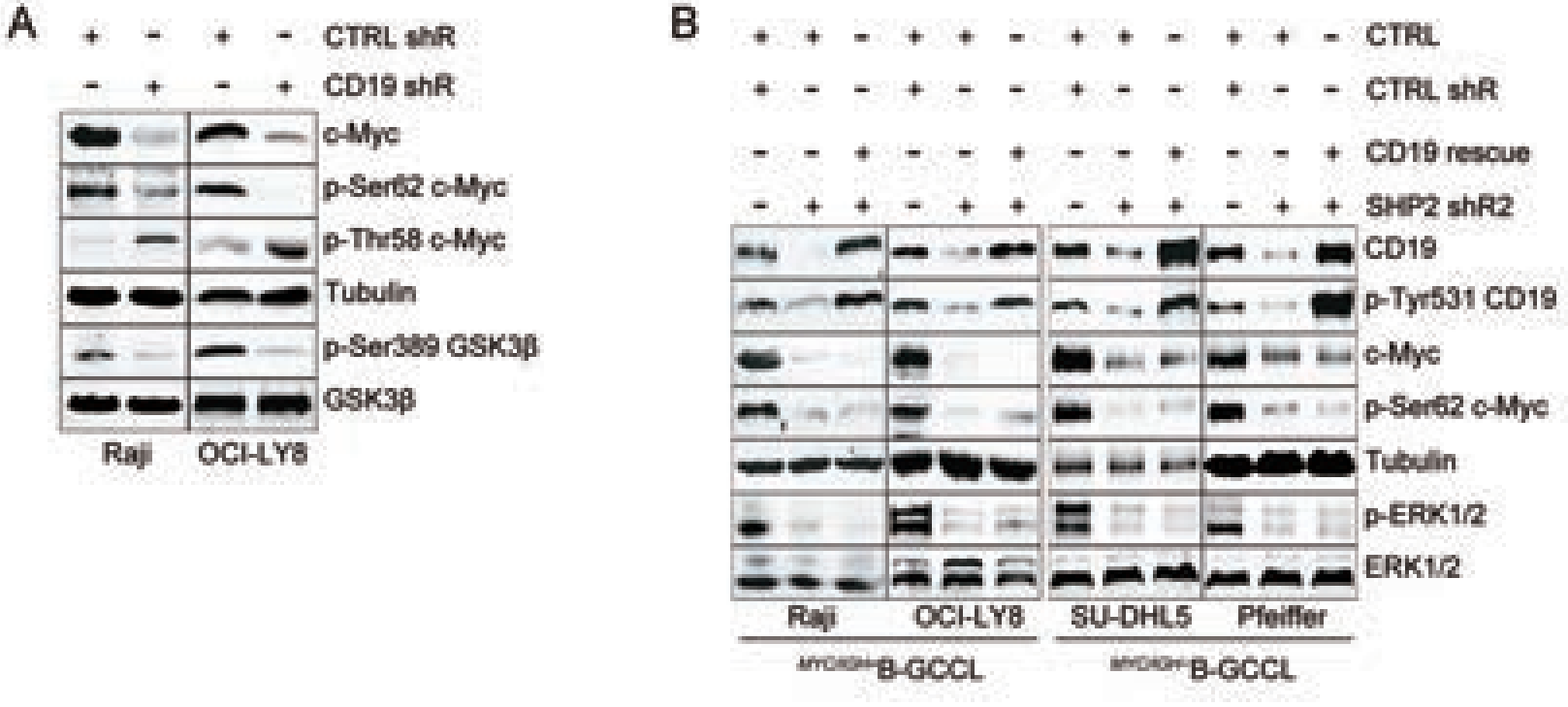
MYC/BCL2 B-GCCL
MYC/BCL2 B-GCCL

Figure S5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- + - + CD19 shR
c-Myc
p-Ser62 c-Myc
p-Thr58 c-Myc
Tubulin
p-Ser389 GSK3β
GSK3β
Raji
OCI-LY8
B
+ + - + + - + + - + + - CTRL
+ - - + - - + - - + - - CTRL shR
- - + - - + - - + - - + CD19 rescue
- + + - + + - + + - + + SHP2 shR2
CD19
p-Tyr531 CD19
c-Myc
p-Ser62 c-Myc
Tubulin
p-ERK1/2
ERK1/2
Raji
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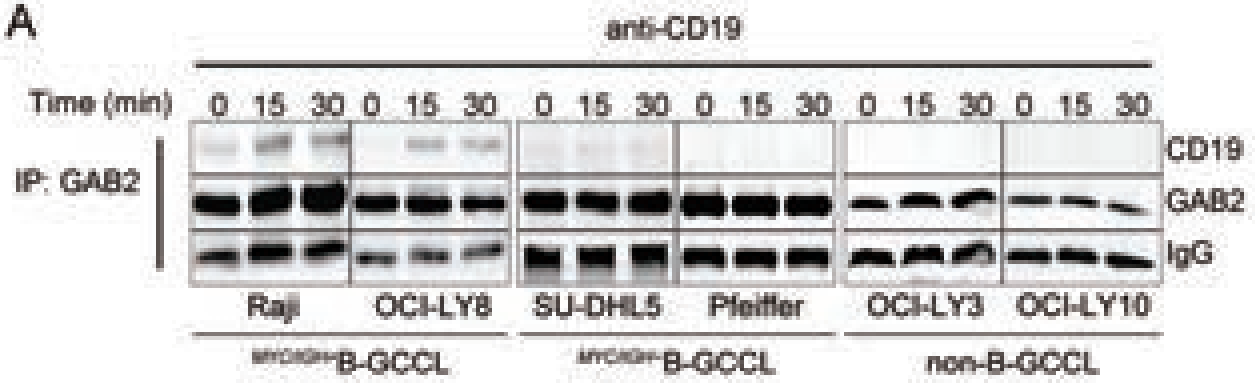
MYD88L265P B-GCCL
MYD88L265P B-GCCL

Figure S6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Time (min)
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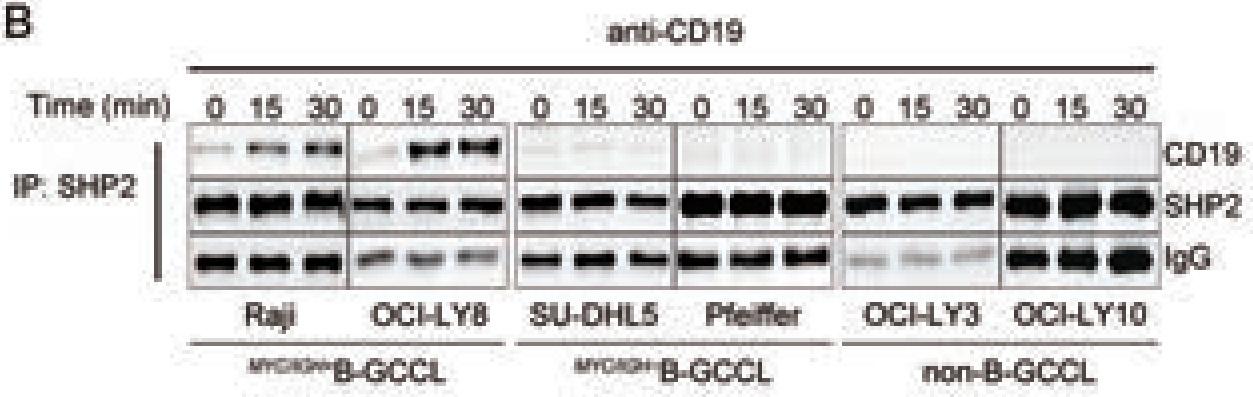
CD19
GAB2
IgG
Raji
OCI-LY8
SU-DHL5
Pfeiffer
OCI-LY3
OCI-LY10
MYC/BCL2-B-GCCL
MYC/BCL2-B-GCCL
non-B-GCCL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anti-CD19
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CD19
SHP2
IgG
Raji
OCI-LY8
SU-DHL5
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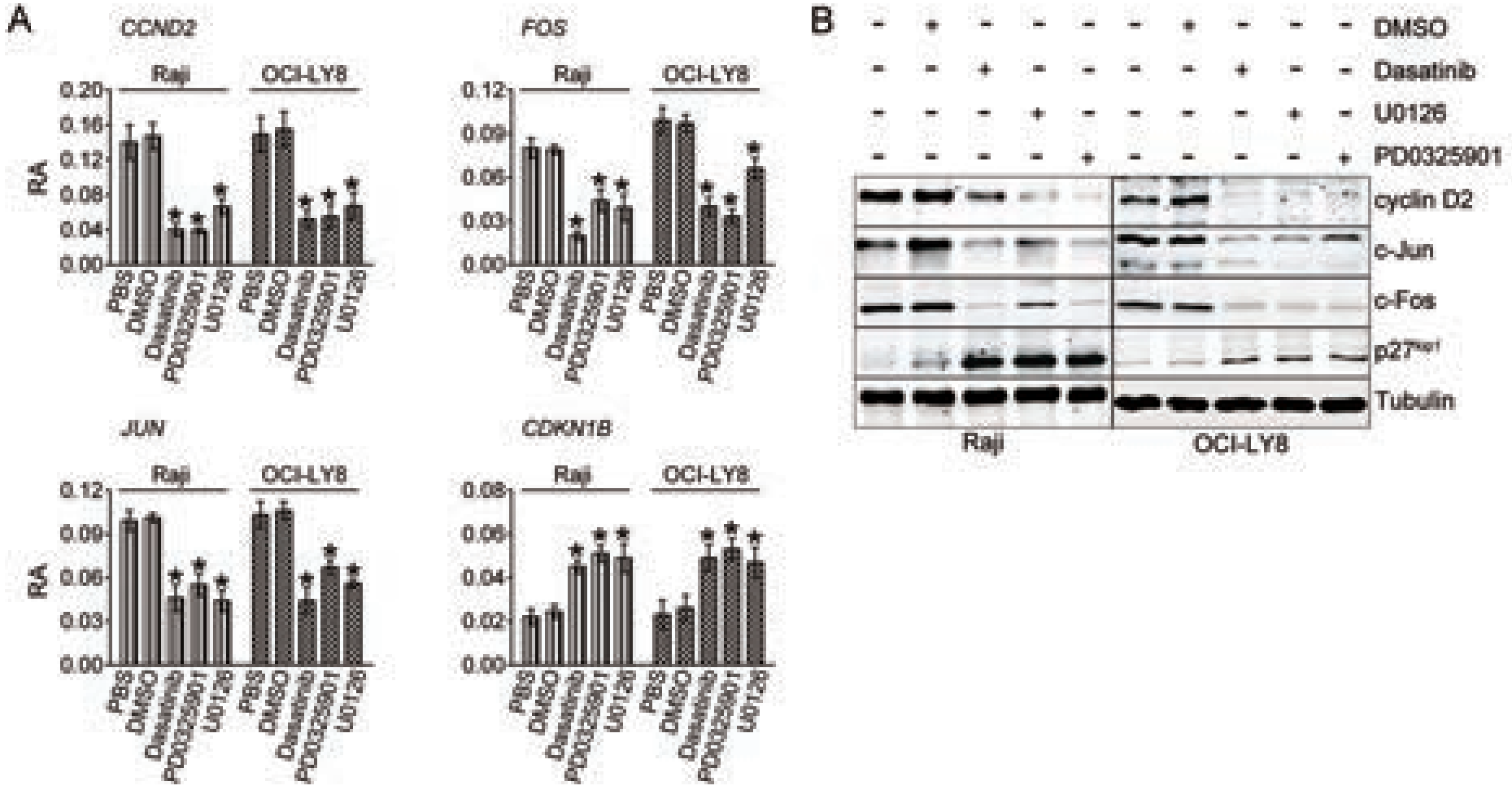
OCI-LY10
MYC/BCL2-B-GCCL
MYC/BCL2-B-GCCL
non-B-GCCL

Figure S7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DMSO
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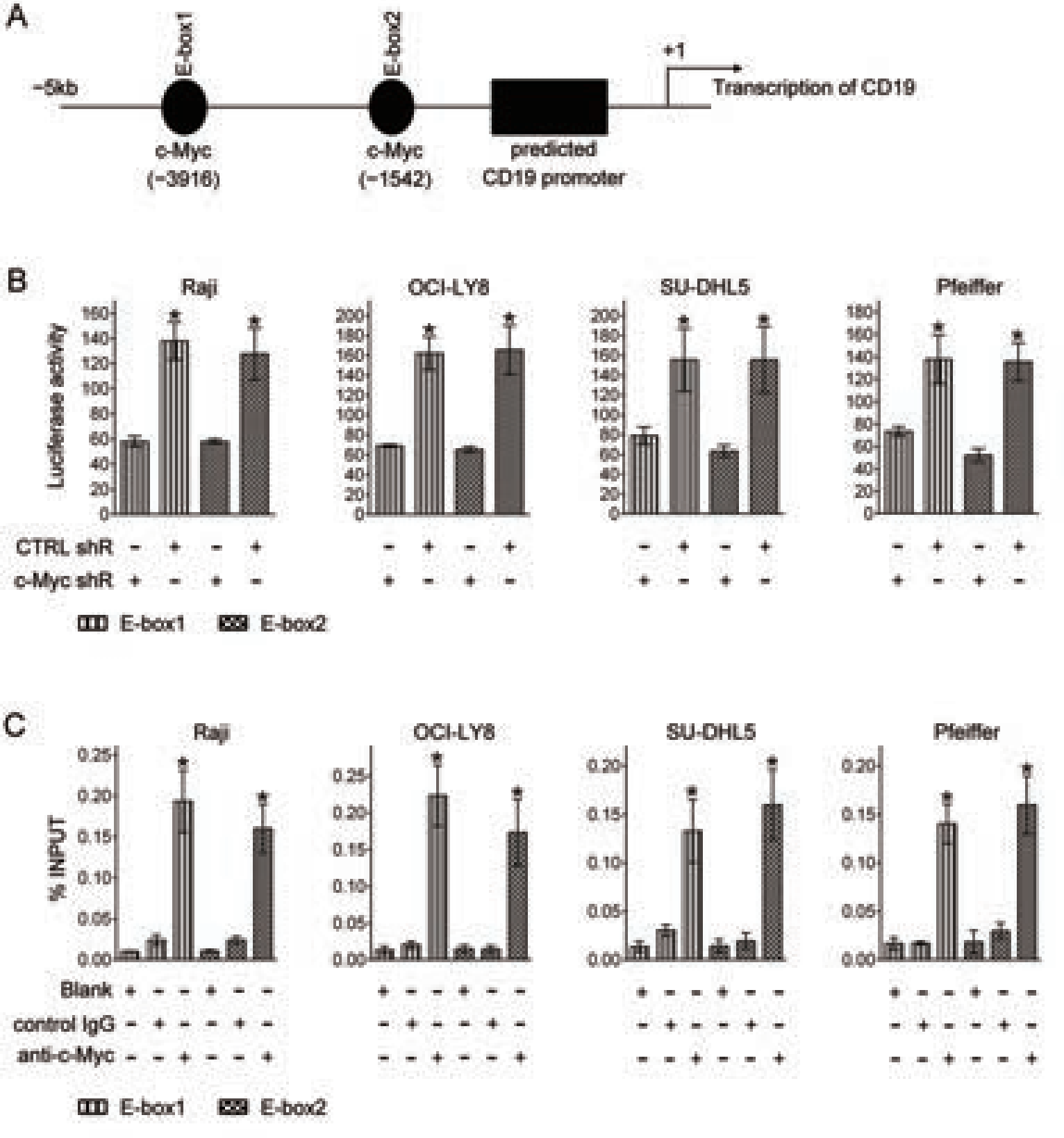
Raji
OCI-LY8

Figure S8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c-Myc
(-1542)
predicted
CD19 promoter
B
Raji
OCI-LY8
SU-DHL5
Pfeiffer
Luciferase activity
CTRL shR
c-Myc shR
E-box1
E-box2
C
Raji
OCI-LY8
SU-DHL5
Pfeiffer
% INPUT
Blank
control IgG
anti-c-Myc
E-box1
E-box2