

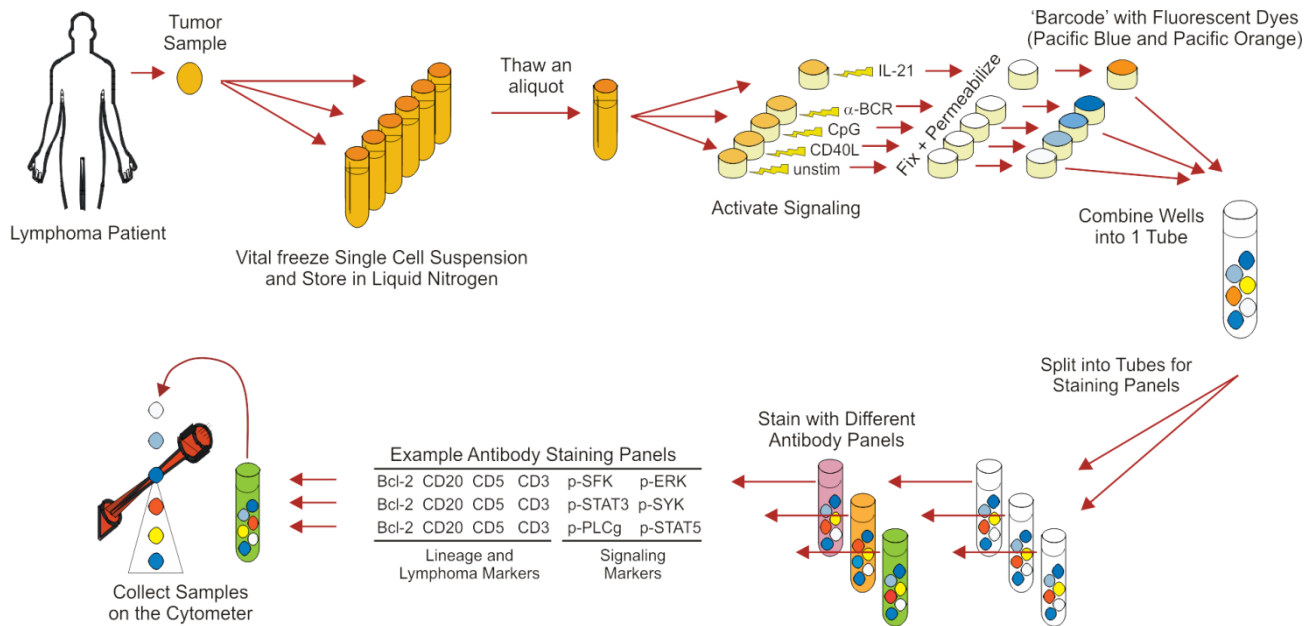
## Supplemental Methods

### Calculation of relative phosphorylation changes

Relative phosphorylation changes are calculated using arcsinh transformation of median fluorescence intensity (MFI) of the cell population of interest. Activation-induced phosphorylation levels were calculated as follows: Relative change = arcsinh (MFI of phospho-protein in activated lymphoma cells/scale argument) - arcsinh (MFI of phospho-protein in unstimulated lymphoma cells/scale argument). The scale argument numbers are channel-specific (here, ranged between 150 and 400). Basal phosphorylation levels (Figure 2) were calculated relative to healthy donor B cells as follows: Basal levels = arcsinh (MFI of phospho-protein in untreated lymphoma cells/scale argument) - arcsinh (MFI of phospho-protein in healthy donor B cells/scale argument).

### Sequencing of CD79B

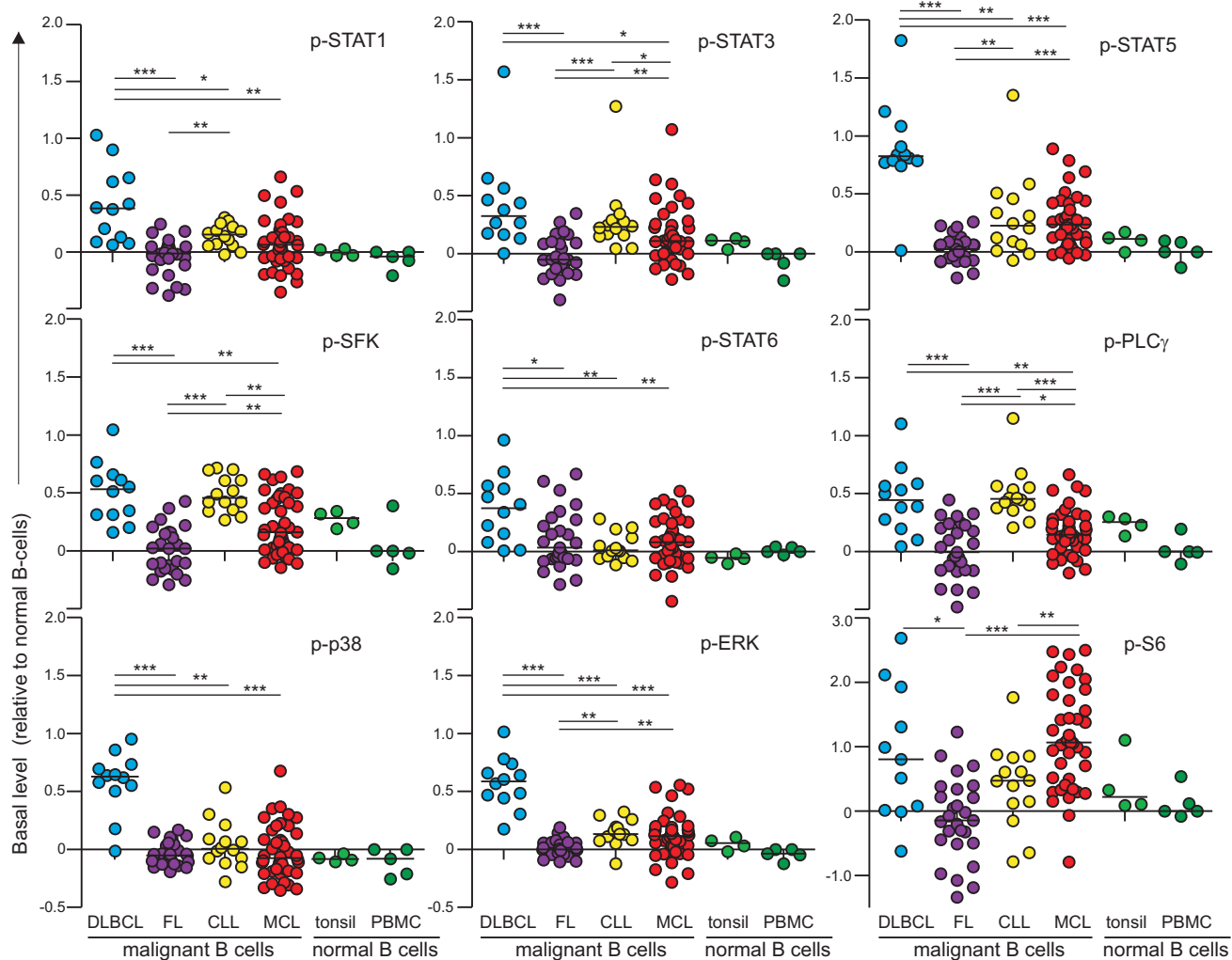
DNA was isolated from Granta 519 cells or from MCL samples depleted for CD3+ cells, using Dynabeads® CD3 (Thermo Fischer Scientific) for the following cases: MCL-R02, MCL-R03, MCL-R08, MCL-R09, MCL-R11. PCR was carried out using AmpliTaq Gold polymerase (Applied Biosystems, Weiterstadt, Germany) according to the supplier's instructions and using the following conditions: 94°C for 5 min followed by 34 cycles of denaturation 30s at 94°C, annealing 30s at 60°C and extension 45 s at 72°C. The primer pairs for CD79B, exon 5 and 6, (NM\_000626) were designed using Primer-BLAST (exon 5: 5'-tct tgc aga atg cac ctc ac-3', 5'-gca gcg tca cta tgt cct ca-3'; exon 6: 5'-tac gag gta agg aga ggg gc-3', 5'- aga caa atg gca gct ctg gt-3'). The PCR products were verified on an Agilent 2100 Bioanalyzer (Agilent Technologies, Waldbronn, Germany) and an aliquot of the products was directly sequenced from both ends on a 3130 Genetic Analyzer (Applied Biosystems) using BigDye Terminator v1.1 cycle sequencing kit (Applied Biosystems) and Sequencing Analysis v5.4 Software.



## Supplemental Figure 1

### Experimental overview of signaling-pathway profiling by phospho-specific flow cytometry in lymphoma.

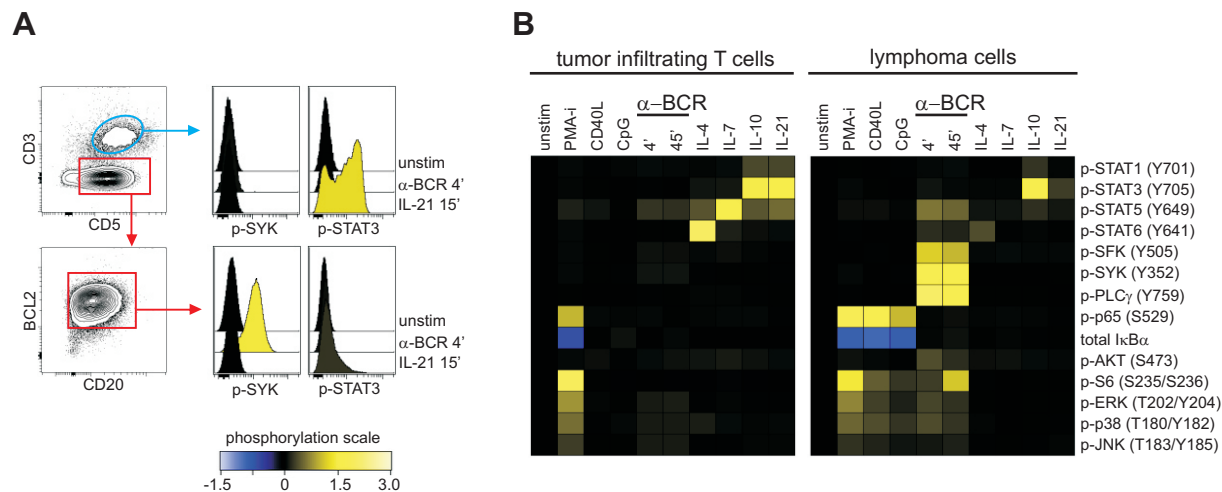
Briefly, the samples were thawed, redistributed at 5-10 million cells/mL and allowed to rest for 20 min at 37°C in RPMI 1640 (supplemented with 10% FCS), before redistribution at 200  $\mu$ L per well into v-bottomed 96 well plates and given another 20 min rest. Signaling was activated by  $\alpha$ -BCR for 4 min or 45 min, CD40L, IL-4, IL-7, IL-10 or IL-21 for 15 minutes, or CpG for 45 min. Paraformaldehyde (PFA) at a final concentration of 1.6 % was added to stop signaling and incubated for 5 minutes at RT, followed by permeabilization in > 90% freezer-cold methanol. At this point, the samples could be stored at -80 °C, before further processing. After rehydrating the cells by washing by centrifugation 2 times in PBS, the cells were fluorescent cell “barcoded”: The cells in each well (= each stimulation condition) were labeled with a unique combination of two different fluorescent dyes. Three different dye levels of Pacific Orange were used at final concentrations of 20.89 ng/mL (L3), 3.48 ng/mL (L2) or 0.348 ng/mL (L1), and 4 different concentrations of Pacific Blue were used at final concentrations of 31.2 ng/mL (L4), 7.8 ng/mL (L3), 1.56 ng/mL (L2) or 0.195 ng/mL (L1). Altogether, 3x4 different samples could be uniquely identified (= 12 different stimulation conditions per patient sample). The cells were labeled with the Pacific Blue and Pacific Orange esters for 30 minutes in the dark at RT. Cells were then washed twice in PBS containing 1% BSA by centrifugation, and all samples from one individual were combined into 1 tube before splitting into new tubes and proceeding with Ab staining, and collected on a LSR II flow cytometer. Data was analyzed using Cytobank Software ([www.Cytobank.org](http://www.Cytobank.org)).



## Supplemental Figure 2

### Elevated basal phosphorylation levels in subgroups of lymphoma patients.

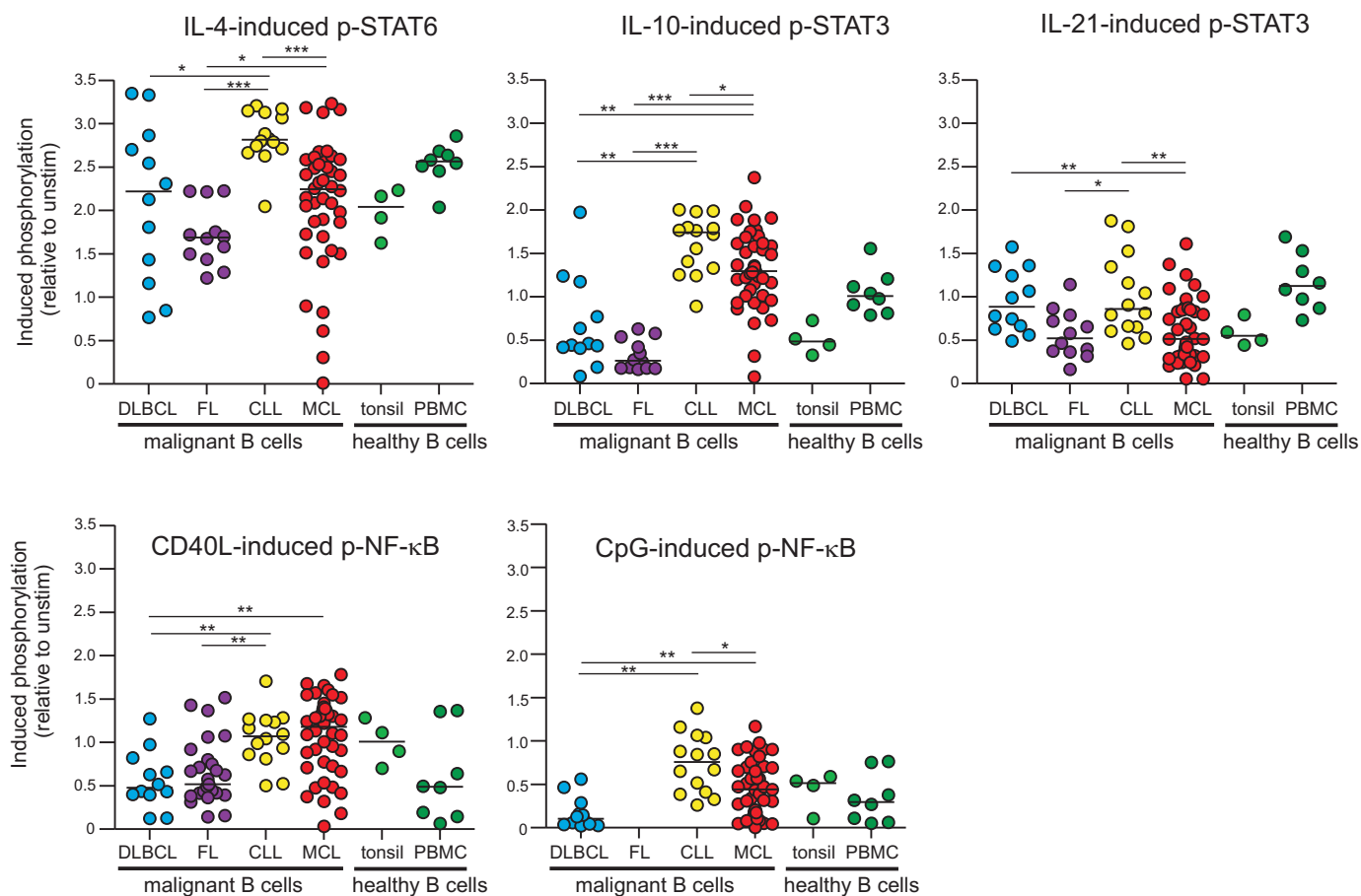
Single cell suspensions from lymphoma patients or healthy donors were thawed and rested for 90 min before fixation, permeabilization and staining with a combination of phospho-specific and lineage-marker specific antibodies, followed by fluorescent flow cytometric measurements (see Supplementary Figure1). Tumor cells were identified as CD20+BCL2+CD5-CD3- cells (FL, DLBCL) or CD20+BCL2+CD5+CD3- cells (MCL, CLL), healthy donor B cells as CD20+CD5-CD3- cells and T cells as CD20-CD3+CD5+. Shown is scatter plots of basal phosphorylation levels in lymphoma cells, relative to healthy donor control PBMC B cells. Each dot represents one patient sample. DLBCL: n=12, FL: n=27, CLL: n=14, MCL: n=42, Healthy donor controls: tonsillar B cells: n=4 and PBMC B cells: n=5. Statistical difference was calculated using Mann-Whitney non-parametric test, \*\*\*p < 0.0001, \*\*p < 0.005, \*p < 0.05.



### Supplemental Figure 3

Activation-induced signaling revealed cell-type specific signaling profiles.

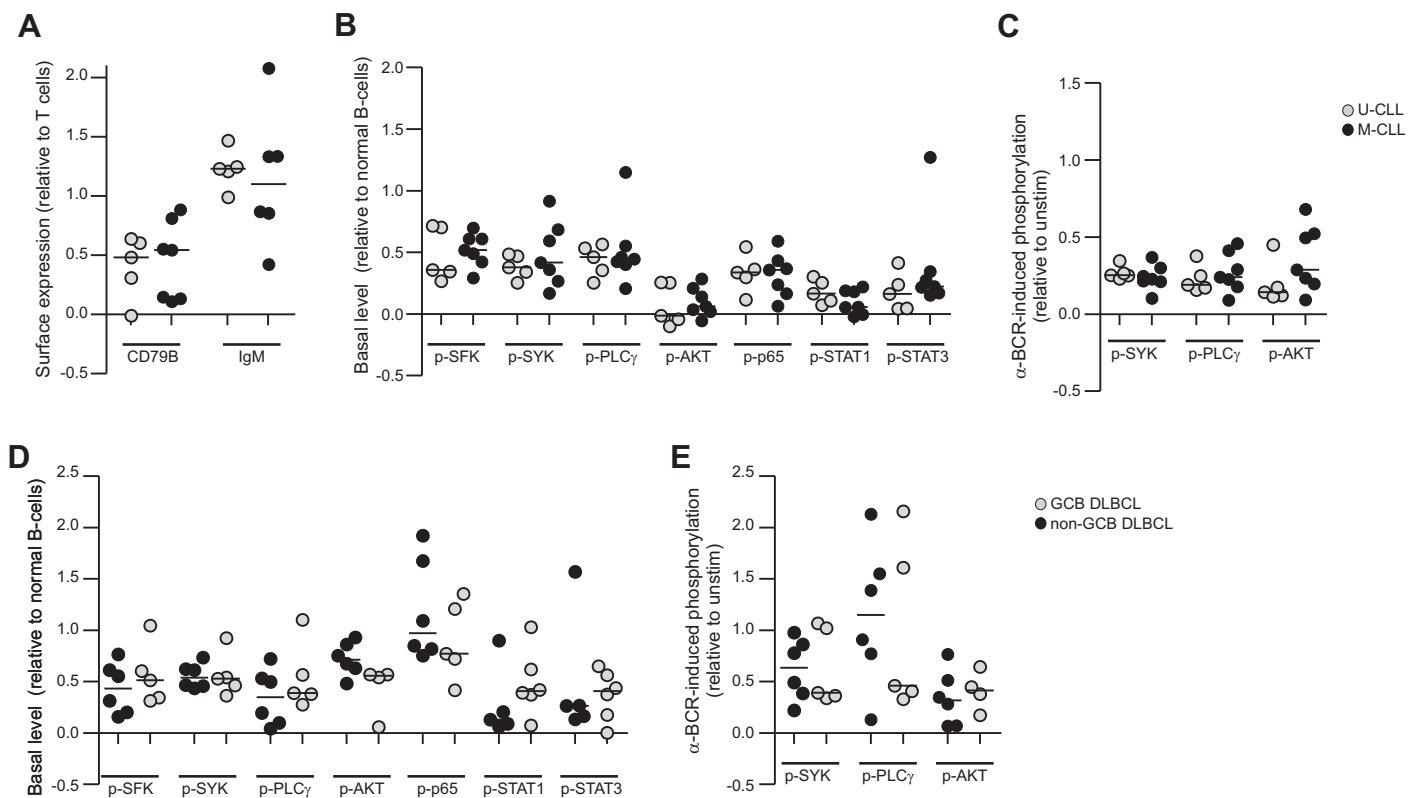
A MCL patient sample were thawed and rested for 20 minutes before activation with  $\alpha$ -BCR for 4 min or 45 min, CD40L, IL-4, IL-7, IL-10 or IL-21 for 15 minutes, CpG for 45 min, or left untreated (unstim), as described in Supplementary Fig. 1. Activation-induced signaling profiles are shown as (A) histogram overlays and (B) heatmaps of lymphoma cells (CD20+BCL2+CD5+CD3-) and intratumor T cells (CD20-CD5+CD3+). Phosphorylation levels are relative to unstimulated lymphoma cells or intratumor T cells, respectively.



## Supplemental Figure 4

### CLL cells shows potentiated signaling responses to IL-4, IL-10 and CD40L.

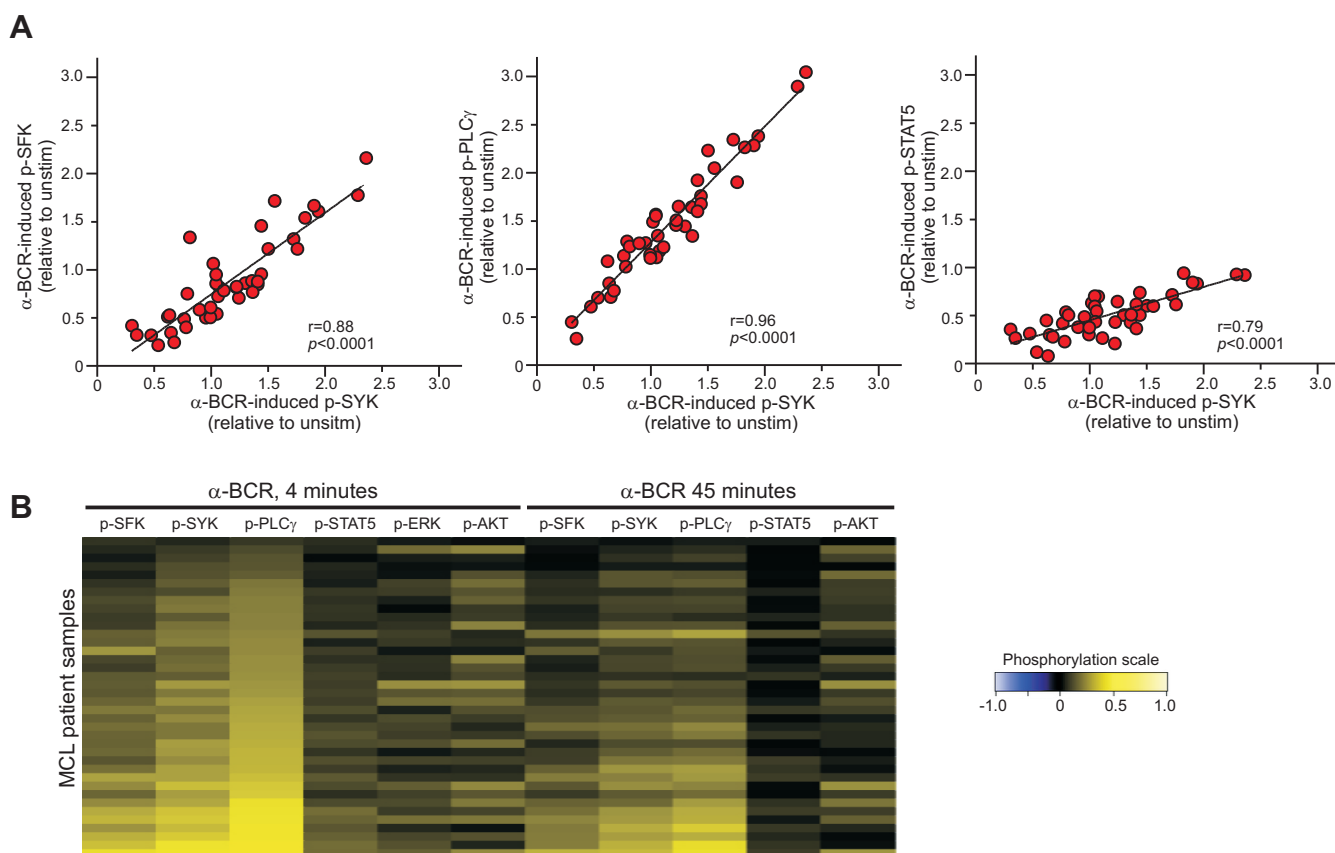
Patient samples and control samples were activated as previously described with CD40L, IL-4, IL-10 or IL-21 for 15 minutes, CpG for 45 min, or left untreated (unstim) as described in Supplementary Fig. 1. Shown are scatter plots of activation-induced phosphorylation levels in lymphoma cells, relative to unstimulated cells. Each dot represents a single patient sample. DLBCL: n=12, FL: n=27, CLL: n=14, MCL: n=42, healthy donor controls: tonsillar B cells: n=4 and PBMC B-cells: n=8. Statistical difference was calculated using Mann-Whitney non-parametric test, \*\*\* $p < 0.0001$ , \*\* $p < 0.005$ , \* $p < 0.05$ .



## Supplemental Figure 5

### Surface expression of CD79B and IgM, basal phosphorylation levels and anti-BCR-induced phosphorylation in subcategories of CLL and DLBCL

(A-C) CLL samples were grouped according to IGHV mutational status. U-CLL: unmutated CLL, <2% change in IGHV gene sequence compared with germline. M-CLL: Mutated CLL. (D-E) DLBCL were classified into GCB and non-GCB according to Choi and Hans algorithms. Statistical difference was calculated using Mann-Whitney non-parametric test, but no significant changes were found.

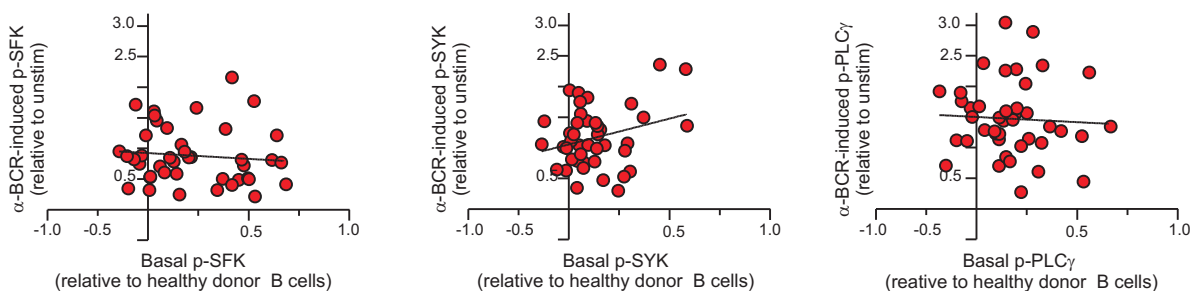


## Supplemental Figure 6

### BCR-proximal signaling events are highly correlated in MCL.

(A) Association between  $\alpha$ -BCR-induced p-SYK vs.  $\alpha$ -BCR-induced p-SFK, p-PLC $\gamma$  or p-STAT5 in MCL cells.

Each dot represents a MCL case. (B) Heatmap of  $\alpha$ -BCR-induced phosphorylation in MCL. Phosphorylation levels are relative to unstimulated cells. MC: n=42. Correlation was calculated by Spearman rank test.

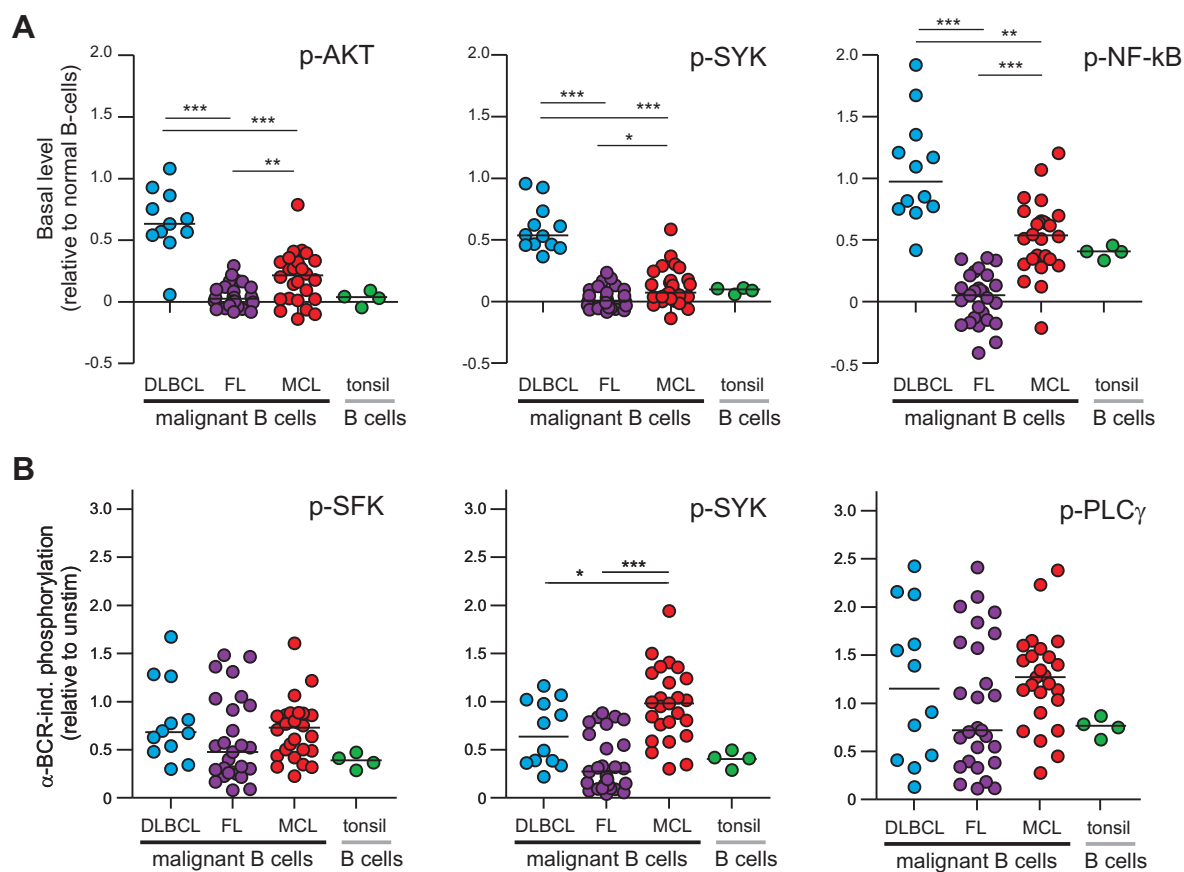


## Supplemental Figure 7

### No correlation between basal phosphorylation levels and $\alpha$ -BCR-induced phosphorylation in MCL.

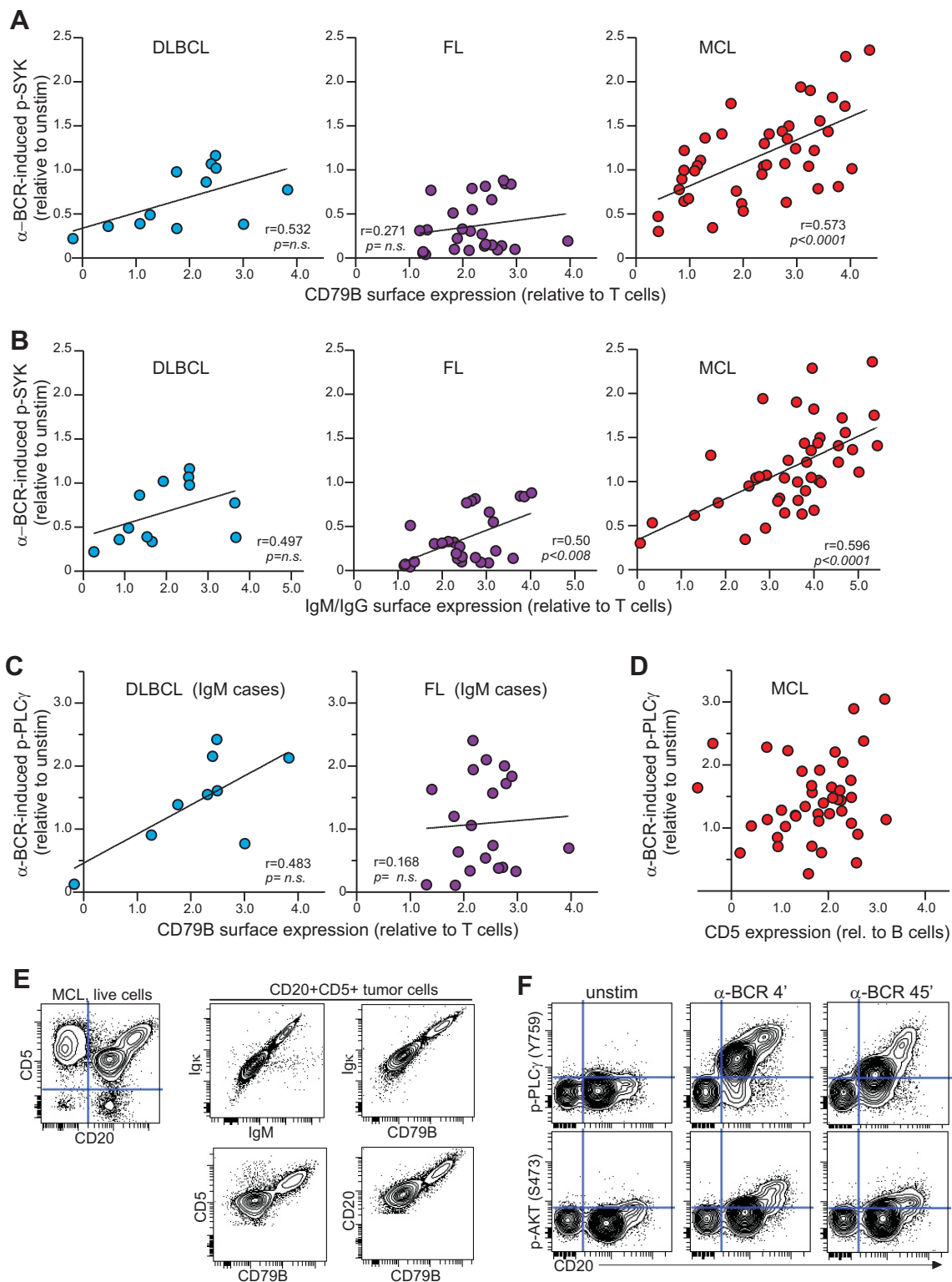
Shown is association between basal phosphorylation levels and  $\alpha$ -BCR-induced phosphorylation for SFK, SYK and PLC $\gamma$ . Each dot represents a MCL case. Basal phosphorylation levels are calculated relative to the levels in healthy donor B cells from a control sample, whereas  $\alpha$ -BCR-induced phosphorylation levels are relative to unstimulated cells. MCL: n=42. Correlation was calculated by Spearman rank test.





### Supplemental Figure 8

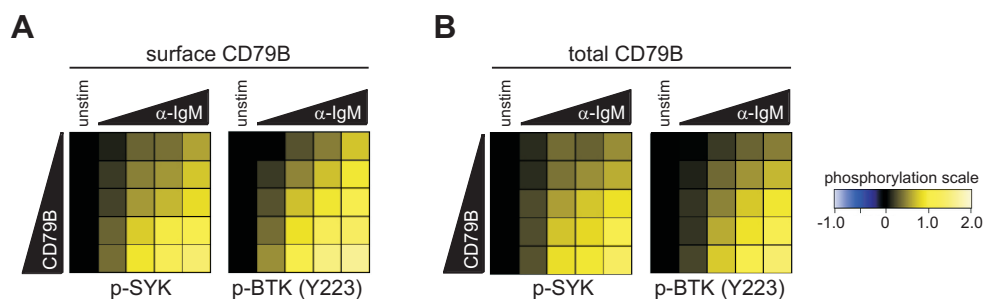
Same data as in Figure 1 and Figure 2, except only LN lymphoma cases are included, as well as healthy donor tonsils. (A) Shown is scatter plots of basal phosphorylation levels in lymphoma cells, relative to healthy donor control PBMC B cells. Each dot represents one patient sample. (B) Shown is scatter plots of  $\alpha$ -BCR-induced phosphorylation levels in lymphoma cells, relative to unstimulated cells. DLBCL: n=12, FL: n=27, and MCL: n=26. Healthy donor controls: tonsillar B cells: n=4. Statistical difference was calculated using Mann-Whitney non-parametric test, \*\*\* $p < 0.0001$ , \*\* $p < 0.005$ , \* $p < 0.05$ .



Supplemental Figure 9

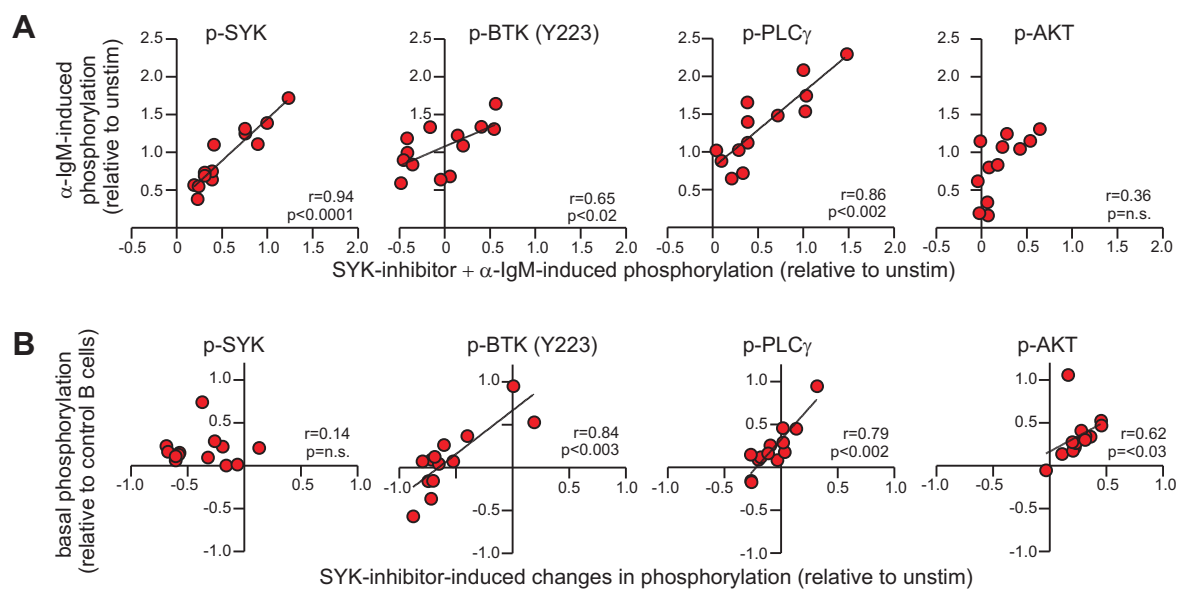
## Supplemental Figure 9

$\alpha$ -BCR-induced signaling strength in MCL cells is associated with increased surface expression of BCR subunits CD79B and IgM. Surface protein expression was not performed in the signaling assay, but was done as a separate immunophenotypic staining experiment. (A) Association between  $\alpha$ -BCR-induced p-SYK and CD79B surface expression. (B) Association between  $\alpha$ -BCR-induced p-SYK and tumor immunoglobulin heavy chain (IgM or IgG). Each dot represents a patient sample. Expression of CD79B or IgG/IgM was calculated relative to intratumor T cells within the same sample. (C) The presence of two distinct lymphoma subclones in a MCL patient (MCL-R001), based on expression of CD5 and CD20, demonstrates the association between CD79B and IgM surface expression, (D) potentiated  $\alpha$ -BCR-induced p-PLC $\gamma$  and p-AKT in the CD79B<sup>hi</sup>IgM<sup>hi</sup> tumor subclone. DLBCL: n=12, FL: n=27, MCL: n=42. Correlation was calculated by Spearman rank test.



### Supplemental Figure 10

Gating on specific expression levels of CD79B gives similar  $\alpha$ -IgM-induced phosphorylation of p-SYK and p-BTK, independent of surface staining or total protein detection. Granta 519 cells transduced with CD79 WT were stimulated with  $\alpha$ -IgM for 4 min, before fixation. (A) To be able to detect surface CD79B only, the cells were stained with  $\alpha$ -CD79B-Alexa 647, and then permeabilized before staining with phospho-specific antibodies. (B) To detect total level of CD79B, the cells were permeabilized after fixation, and then stained with  $\alpha$ -CD79B-Alexa 647 and phospho-specific antibodies. Shown is one representative experiment of 3 independent. Phosphorylation is relative to unstimulated cells, using archsinh transformed data.



## Supplemental Figure 11

### Efficacy of R406 to suppress $\alpha$ -BCR-induced signaling depends on signaling strength in MCL tumors.

Samples from 13 MCL patients were left untreated or treated with SYK-inhibitor Fostamatinib (R406, 2.5mM) for 60 minutes, prior to  $\alpha$ -BCR stimulation for 4 minutes. The cells were stained with lineage-specific markers and phospho-specific Abs as earlier described. (A) Scatter plots of  $\alpha$ -IgM-induced phosphorylation vs. SYK-inhibitor +  $\alpha$ -IgM-induced phosphorylation levels for SYK, PLC $\gamma$ , BTK (Y223) and AKT. Each dot represents a MCL patient sample. (B) Scatter plots of basal (untreated) phosphorylation levels in lymphoma cells relative to healthy donor B cells vs. phosphorylation levels in SYK-inhibitor treated lymphoma cells relative to untreated cells. Correlation was calculated by Spearman rank test.

**Supplemental Table 1.** Clinical characteristics of non-Hodgkins' lymphoma patients.

	Median Age, years (range)	Tissue specimen LN/ blood	Median Stage (range)	IGHV mutated/ not mutated (N/N)	Tumor IgH isotype IgM/ IgG	Sample acquired pre- treatment (N)	Total (N)
<b>DLBCL</b>	58 (40-82)	12/ 0	IVA (IIA-IVB)	n.d.	9/ 3	12	12
<b>FL<sup>a</sup></b>	44 (28-82)	27/ 0	IVA (IIA-IVB)	n.d.	20/ 7	27	27
<b>CLL</b>	64 (42-79)	0/ 14		7/ 5	14/ 0	13	14
<b>MCL</b>	61 (34-85)	24/ 18	IVA (IA-IVB)	n.d.	42/ 0	42	42

DLBCL: diffuse large B-cell lymphoma, FL: follicular lymphoma, CLL: chronic lymphocytic leukemia, MCL: mantle cell lymphoma.

<sup>a</sup>FL patient samples were from the testing set, reported in Irish, J.M., *et al.* B-cell signaling networks reveal a negative prognostic human lymphoma cell subset that emerges during tumor progression. *Proceedings of the National Academy of Sciences* **107**, 12747-12754 (2010).

**Supplemental Table 2.** Clinical characteristics of MCL.

Patient ID	Gender	Age at diagnosis	Stage	Ki67	MIPI	Sample	Overall Survival (years)	Censor for OS [1 = D]	1st Therapy	Rituximab (R)	Sum basal level
MCL-R002	male	59	IVB		high	LN	2.48	1	CVP		high
MCL-R003	male	55	IIA		low	LN	6.75	1	klorambucil		high
MCL-R008	female	76	IVA		high	LN	0.73	1	CHOP21		high
MCL-S029	male	85				blood	0.05	1	cvp		high
MCL-S039	male	57	IVB	90		blood	0.44	1	R-hCVAD	R	high
MCL-S055	male	65			intermediate	spleen	3.69	0	no chemo		high
MCL-S058	male	60	IVA	40	intermediate	LN	3.53	1	RCHOP/DHAP alt	R	high
MCL-S064	female	62		20	high	blood	0.15	1	RCHOP	R	high
MCL-S068		60	IIA	10	low	LN	1.85	0	RCHOP	R	high
MCL-S072	female	59		5	low	LN	3.44	0	R-hCVAD_mod	R	high
MCL-S076	male	61	IVA	10	low	LN	0.00	0			high
MCL-S032	male	68	IVA			LN	6.20	1	CHOP		intermediate
MCL-S035	male	48	IVA	10-20		LN	5.29	1	CHOP		intermediate
MCL-S037	male	43				LN	2.27	1	CHOP CVP		intermediate
MCL-S040	female	40	IV			blood	4.74	0	splenectomy / watchful waiting		intermediate
MCL-S041	male	57	IVB	30	high	blood	2.27	1	R-hCVAD	R	intermediate
MCL-S046	male	47	IVA	60	low	LN	3.86	1	mod RhCVAD	R	intermediate
MCL-S047	male	68	IVA	40	high	blood	5.07	0	mod RhCVAD	R	intermediate
MCL-S050	female	57	IVA	5	intermediate	blood	0.95	1	RCHOP	R	intermediate
MCL-S052	male	45	IVB	10	low	LN	1.05	0	mod RhCVAD	R	intermediate
MCL-S053	male	54	IVB		intermediate	blood	1.85	1	mod RhCVAD	R	intermediate
MCL-S057	male	67	IVB	10	high	blood	2.69	0	RCHOPx1, R-hCVAD mod	R	intermediate
MCL-S062	male	56	IVB		high	blood	1.56	0	RCHOP	R	intermediate
MCL-S065	female	69	IVA	10	intermediate	LN	0.53	0	RCHOP	R	intermediate
MCL-S067	female	56	IV	20	low	LN	2.42	0	RCHOP	R	intermediate
MCL-S069	female	67	IVA	5	high	blood	1.56	0	R-hCVAD_mod	R	intermediate
MCL-S070	female	70	IVA	30	intermediate	LN	3.76	0	R-hCVAD_mod	R	intermediate
MCL-S073	male	56	IVA	20	intermediate	LN	2.82	0	RCHOP/DHAP alt	R	intermediate
MCL-S074	female	34	IVA	90	low	LN	1.92	0	R-hCVAD_mod	R	intermediate
MCL-S077	male	62	IVB				0.93	0	RCHOP/ alt. with RDHAP	R	intermediate
MCL-S078	male	56	IVB				0.28	0	R-hCVAD	R	intermediate
MCL-R009	male	74	IIIA		intermediate	LN	7.43	1	klorambucil		low
MCL-R011	male	66	IVA		intermediate	LN	14.58	1	CHOP21		low
MCL-S028	female	66	IA	<5		LN	7.35	0	R-CVP	R	low
MCL-S030	male	52	IVA	30		LN	6.39	0	R-CHOP	R	low
MCL-S034	male	71	IVA			LN	4.01	1	CHOP		low
MCL-S043	female	63	IVA		intermediate	blood	4.05	0	mod RhCVAD	R	low
MCL-S048	male	62	IVA	10	intermediate	blood	3.92	0	mod RCHOP	R	low
MCL-S049	male	67	IVA	10	intermediate	LN	3.32	0	REPOCH	R	low
MCL-S051	female	69	IVA	20	intermediate	blood	4.30	0	mod RhCVAD	R	low
MCL-S056	male	65			high	blood	3.23	0	R-	R	low
MCL-S063		61	IVA			Blood	2.76	0	mod RhCVAD	R	low

Supplemental Table 3. Overview of antibodies used in the study

	Ab	Fluorophore	Catalog	Residue	Clone	Vendor	Stain (uL)/ 200 uL total volume
<b>Phospho-specific antibodies</b>	p-SFK	Ax488	557879	Y505	4/Lck-Y505	BD Biosciences	20
	p-ERK	Ax647	612593	T202/Y204	20a	BD Biosciences	20
	p-STAT6	Ax488	612600	Y641	18	BD Biosciences	20
	p-STAT1	Ax647	612597	Y701	4a	BD Biosciences	20
	p-S6	Ax488	4803	S235/S236	D57.2.2E+M40	Cell Signaling Technology	5
	p-AKT1	Ax647	2337	S473	193H12	Cell Signaling Technology	5
	p-ERK 1/2	Ax647	5376	T202/Y204	13148	Cell Signaling Technology	5
	p-STAT3	Ax488	557814	Y705	4/P-STAT3	BD Biosciences	20
	p-SYK	Ax647	557817	Y319/Y352	17a/P-ZAP70	BD Biosciences	20
	p-NFkB	Ax488	558421	S529	K10-895.12.50	BD Biosciences	20
	p-p38	Ax647	612595	T180/Y182	36/p38 (pT180/pY182)	BD Biosciences	20
	p-PLCg2	Ax488	558507	Y759	K86-689.37	BD Biosciences	10
	p-PLCg2	Ax647	558498	Y759	K86-689.37	BD Biosciences	10
	IKBa	Ax488	5743			Cell Signaling Technology	2
	p-Btk (Y223)	Ax647	564846	Y223	N35-86	BD Biosciences	2
	p-Btk (Y223)	PE	562753	Y223	N35-86	BD Biosciences	2
	p-BLNK	Ax647	558443	Y84	J117-1278	BD Biosciences	20
p-STAT5	Ax647	612599	Y694	47	BD Biosciences	20	
<b>Lineage markers, used with phospho-specific antibodies</b>	CD5	PE-Cy7	348790		L17F12	BD Biosciences	2
	BCL2	PE	556536		6C8	BD Biosciences	10
	CD20	PerCP-Cy5.5	558021		H1	BD Biosciences	10
	CD3	Q605	Q10054		UCHT1	Life Technologies, Invitrogen	0.25
<b>Antibodies used in Immunophenotyping</b>	CD3	PacBlue	558117		UCHT1	BD Biosciences	0.5
	CD4	PacBlue	558116		RPA-T4	BD Biosciences	0.5
	CD5	PE-Cy7	348790		L17F12	BD Biosciences	2
	CD20	PerCPCY5.5	340954		L27	BD Biosciences	5
	IgM	FITC	AHI1608		poly	Life Technologies, Invitrogen	0.25
	IgG	FITC	AHI1308		poly	Life Technologies, Invitrogen	0.25
	IgL	PE	AHI1907		poly	Life Technologies, Invitrogen	0.25
	IgK	APC	MH10515		HP6062	Life Technologies, Invitrogen	5
	CD79b	PE	557931		SN8	BD Biosciences	5
	CD10	PE-CY7	341102		HI10a	BD Biosciences	2
CD19	APC	340722		SI25C1	BD Biosciences	5	