Supporting Information

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SI Materials and Methods

Primary Human Lymphoma Specimens and Healthy B Cells. Tumor specimens were obtained with informed consent in accordance with the Declaration of Helsinki and this study was approved by Stanford University's Administrative Panels on Human Subjects in Medical Research. Samples were transferred directly from the operating room to the laboratory and used for the preparation of viable, sterile single-cell suspensions. In a laminar flow hood, lymph node tissue was diced and forced through a metal sieve into RPMI tissue culture medium. Disaggregated lymphoma biopsy cells were then pelleted and resuspended in media composed of 90% FBS (HyClone) and 10% DMSO (Sigma), frozen slowly in the vapor phase of liquid nitrogen in multiple cryotubes, and stored in liquid nitrogen. PBMCs from healthy individuals were isolated using density gradient separation Ficoll-Paque Plus (Amersham Biosciences) and subsequently treated as described for FL specimens.

Stimuli and Inhibitors. The signaling profile assay began by thawing live cells from a lymphoma specimen, removing approximately 4 million cells for viability and immunophenotyping, aliquotting the remaining cells evenly into wells, and then querying the cell signaling network by stimulating cells with a panel of signaling inputs whose effects are measured at several phospho-protein readouts (Fig. 1).

Briefly, an individual CryoTube was resuspended in RPMI plus 10% FBS at 10×10^6 cells per mL, rested at 37 °C for 30 min in a 5% CO_2 tissue culture incubator, resuspended and transferred to v-bottom plates, and then rested once more for 45 min before stimulation. To stop signaling, cells were fixed by addition of paraformaldehyde (Electron Microscopy Services) at a final concentration of 1.6%. The total time from resuspension to fixation was 2 h. Cells were fixed for 5 min at room temperature, pelleted, permeabilized by resuspension in −80 °C methanol for 10 min, and stored at −80 °C for less than 3 d before being stained with phospho-specific and lineage antibodies (as detailed later) and collected on using a LSR II three-laser cytometer (Becton Dickinson).

Signaling inputs included PMA + iono (Sigma-Aldrich) at 1 μg/mL each; IL-2, IL-7, IL-15 (eBioscience) at 20 ng/mL; soluble CD40L trimer (Amgen-Immunex) at 100 ng/mL, BCR cross-linking by goat polyclonal $F(ab')_2$ against Igμ and Igγ (Invitrogen Biosource) at 10 μg/mL each, H_2O_2 (MP Biomedicals) at 3.3 mM, and α-BCR + H_2O_2. When used, 2.5 μM R406 (1) (Rigel Pharmaceuticals) was added to resting cells 30 min before stimulation by α-BCR + H_2O_2 (1). The DMSO vehicle for R406 was ultimately diluted to 0.5% and did not significantly affect BCR-mediated phosphorylation of any of the measured readouts when combined alone with α-BCR + H_2O_2 stimulation. Concentrations of all stimuli other than H_2O_2 were chosen to be in excess of the saturating concentration at the measured time point for healthy primary B and T cells.

As in previous studies of healthy and FL B cells, BCR cross-linking (α-BCR) was used alone and in combination with 3.3 mM H_2O_2 (α-BCR + H_2O_2). H_2O_2 is a reversible inhibitor of protein tyrosine phosphatases and potential second messenger in B cells (2). H_2O_2 is a milder oxidant than pervanadate that functions in a mechanistically similar, but reversible, manner. Past studies of FL also indicated that measuring activation kinetics was informative for BCR signaling (3, 4). Time points of 4, 15, and 45 min were therefore used for α-BCR, H_2O_2, and α-BCR + H_2O_2 in preliminary studies and time points of 4 and 45 min were used in the final signaling profile.

Antibodies. Antibodies (Becton Dickinson) were used to detect phosphorylated BTK/ITK(Y551) clone 24a/BTK, p38(T180/Y182) clone 36/p38, ERK(T202/Y204) clone 20A, SFK/LCK (Y505) clone 4/LCK-Y505, SYK/ZAP70(Y352/Y319) clone 17a/P-ZAP70, NFκB p65(S529) clone K10-895.12.50, STAT1(Y701) clone 4a, STAT3(Y705) clone 4/P-STAT3, STAT5(Y694) clone 47, STAT6(Y641) clone 18, CBL(Y700) clone 47/CBL, PLCγ (Y759) clone K86-689.37, BLNK(Y84) clone J117-1278. Antibodies from Cell Signaling Technology were used to detect phosphorylated AKT(S473) clone 193H12 and S6(S235/S236) clone D57.2.2E.

Antibodies from Becton Dickinson were used to detect expression of CD20 cytoplasmic tail clone ^{1}H, BCL2 clone 6C8, CD5 clone L17F12, CD3 clone UCHT1, CD20 (extracellular) clone L27, CD79β, CD38, CD40, CD81, CD19, CD10, HLA-DR, CD47, IL-10R, IL-4R, IL-21R, CD95, CD14, CD56, and CD22. Antibodies from Invitrogen BioSource were used to detect expression of Igμ, Igγ, and Igλ; all were goat polyclonal $F(ab')_2$, whereas Igκ was mouse IgG3 clone HP6062.

Fluorescent Cell Bar Coding. After stimulation, cells from each condition were given a fluorescent "bar code" indicating stimulation state and then combined for simultaneous antibody staining (5). Fluorescent cell bar coding (FCB) helps mitigate potential volume and loading errors when staining a large number of samples and allows for significant conservation of patient specimen cells. Stimulation conditions were encoded in a 3 × 3 × 3 bar-coding grid (27 stimulation states possible) or a 3 × 3 bar-coding grid (nine stimulation states) using three levels each of three succinimidyl ester dyes from Invitrogen (Pacific Orange, Pacific Blue, and Alexa 750) or two dyes (Pacific Orange and Pacific Blue), respectively. The dyes were used at the following final concentrations to obtain three bar-coding levels each of Pacific Blue (labeling concentrations of 0.78, 7.0, and 50 ng/mL), Pacific Orange (8.7, 87, and 520 ng/mL), and, optionally, Ax750 (8.3, 75, and 300 ng/mL). The labeling reaction was carried out at room temperature for 30 min in 200 μL PBS solution.

The bar-coding technique was critical to the feasibility of the project. Without FCB, the antibody costs for the initial signaling profile would have been approximately 20-fold higher, and 33 d in uninterrupted sample acquisition would have been required to collect more than 12,000 stained samples. Alternatively, a smaller signaling profile might have missed key findings by limiting the stimuli, signaling inputs, time points, or the number of phosphoproteins measured. As FCB introduces additional steps in the profiling protocol, we ensured that protocol variability and compensation were not adversely affected. First, the bar-coding protocol was optimized for effective separation between populations with wide variance in forward and side scatter. We left the "no dye" levels on each channel unused to exclude cells that were not labeled by bar-coding dye, and we used concentrations of dye that ensured our highest population of dyed cells had a median fluorescence of approximately 30,000 (at recommended voltages) to avoid error, signal spreading, and nonlinearity issues with very high fluorescent signals. Cells stained only with bar-coding dyes were collected to examine any false signal spillover into the channels used to measure phosphoprotein levels (Alexa 488 and Alexa 647). After compensation, no significant relationship between barcoding dyes (Pacific

Orange, Pacific Blue, and Alexa 750) and the channels used to measure signaling (Alexa 488 and Alexa 647) was observed and the signaling median was the same for all bar-coded populations.

Signaling Profile Design. Initially, many signaling inputs were studied in FL samples at 12 phospho-protein readouts (Fig. 1 and Table S4) in the first half of FL samples in the training set (samples with IDs between LP-J001 through LP-J023). Following this initial view, the set of input stimuli was eventually narrowed down to nine conditions and the set of readouts expanded to 15 phospho-proteins (Table S5) based on creating a panel focused on BCR signaling and a few key lymphoma B cell and tumor-infiltrating T cell stimuli. At this point, CD3 was measured using QDot605 and CD5 PE-Cy7 was added to the staining panel, as well as measurement of p-BLNK, p-S6, and p-PLCγ. This updated and focused signaling profile of nine inputs multiplied by 15 readouts was then uniformly applied to the remaining training set and all testing set samples. Correspondingly, the bar-coding approach was a $3 \times 3 \times 3$ grid for the early samples, allowing 27 possible stimulation conditions, and a 3×3 grid for the later samples in which only nine stimulation conditions were needed (Tables S4 and S5).

Scaling and Calculation of Fold Change. Signaling was quantified as the fold change in per-cell phospho-protein median fluorescence intensity (MFI) of stimulated samples compared with unstimulated samples using the scale of data display, as in previous studies (3, 6). The use of a BD LSR II here resulted in high-resolution, unbinned fluorescence intensity measurements that were negative for some channels, depending on background subtraction and compensation. To display and compare intensity values including negative numbers and correct for large variance with some fluorophores, we used the inverse hyperbolic sine (arcsinh) with a cofactor instead of the traditional $\log_{10}$ scale. The arcsinh median of intensity value × with cofactor c was calculated as $\text{arcsinh}_c(x) = \ln(x/c + \sqrt{((x/c)^2 + 1)})$. The cofactor c is a fluorophore-specific correction for signal variance that also provides a smooth display around the origin. With flow cytometers that record unbinned digital measurements (vs. older cytometers that record binned representations of analog measurements), it is critical to display data on an appropriate scale, to use a scale in which no more than 10% of events are binned together in the first or last bin, and to use the same scale for comparison of intensity as the scale for data display, especially for intensity measurements near the origin and for fluorophores with a broad autofluorescence peak.

Heat Map Plot Visualization and Mountain Plots. To compare subsets of lymphoma cells based on immunophenotype and to visualize signaling across such populations, we developed a mountain plot representation of the third dimension of information that essentially showed color as a surrogate for the activation level of the phosphorylated protein being measured (e.g., Fig. 2*B* in the main text). The median fluorescence intensity of a signaling measurement, such as p-ERK, was graphed across CD20 and BCL2. Heat map plots were created in Cytobank and made into mountain plots using ImageJ.

Scoring Rules for Quantification of the LNP Cell Subset. Criteria for inclusion were as follows: the thawed tumor specimen must consist of more than 70% viable cells as determined by flow cytometry membrane integrity stain. Furthermore, at least 25% of the tumor cells must respond to some stimulation condition.

Scoring procedure was as follows:

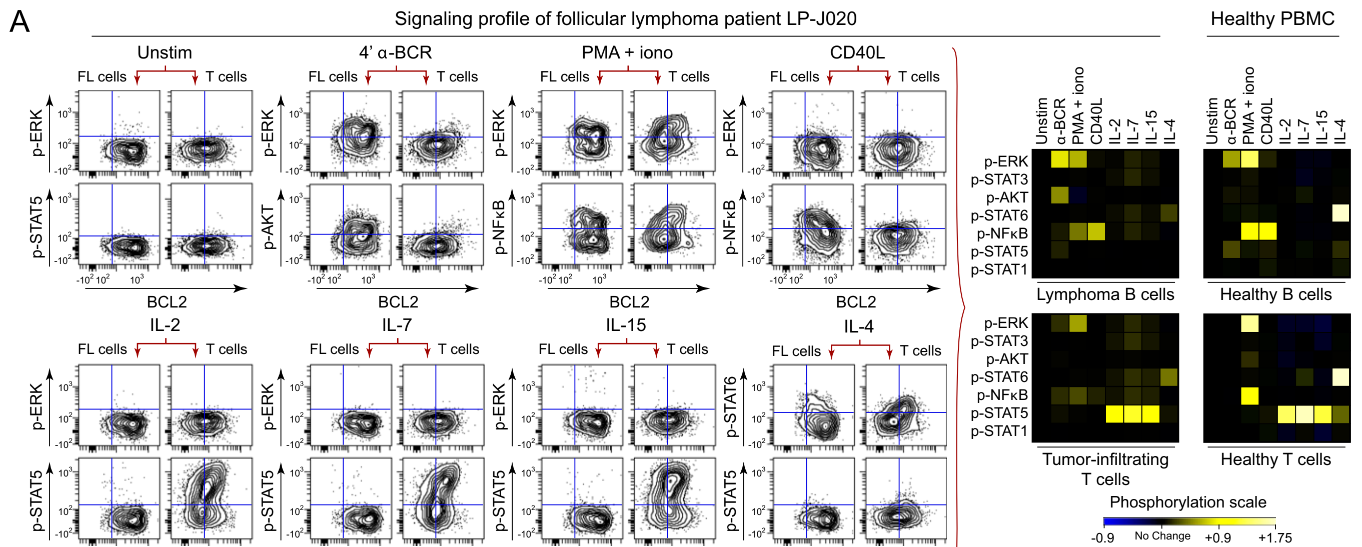
1. Set the compensation and scales for the run using healthy peripheral blood mononuclear cells.
2. Adjust the scales for the phospho-proteins so that the median signal intensity in the unstimulated condition (i.e., the basal level) has a black/purple color. For the α-BCR 4 min condition, draw a "non-LNP cells" gate around all tumor cells that have significantly higher induced signal compared with basal level (bright orange/red color on the mountain plot). Draw an "LNP cells" gate around the remainder of the cells. The percentage of events in these gates relative to their sum becomes the percentage of LNP cells and non-LNP cells. Favor continuous shapes when drawing these gates.
3. Classification.

a. Calculate percentage of LNP cells as: (% LNP cells) / (% LNP cells + % non-LNP cells)

i. Samples <40% LNP cells = profile 1

ii. Samples ≥40% LNP cells = profile 2

1. Braselmann S, et al. (2006) R406, an orally available spleen tyrosine kinase inhibitor blocks fc receptor signaling and reduces immune complex-mediated inflammation. *J Pharmacol Exp Ther* 319:998–1008.
2. Reth M (2002) Hydrogen peroxide as second messenger in lymphocyte activation. *Nat Immunol* 3:1129–1134.
3. Irish JM, Czerwinski DK, Nolan GP, Levy R (2006a) Altered B-cell receptor signaling kinetics distinguish human follicular lymphoma B cells from tumor-infiltrating nonmalignant B cells. *Blood* 108:3135–3142.
4. Irish JM, Czerwinski DK, Nolan GP, Levy R (2006b) Kinetics of B cell receptor signaling in human B cell subsets mapped by phosphospecific flow cytometry. *J Immunol* 177: 1581–1589.
5. Krutzik PO, Nolan GP (2006) Fluorescent cell barcoding in flow cytometry allows high-throughput drug screening and signaling profiling. *Nat Methods* 3:361–368.
6. Irish JM, et al. (2004) Single cell profiling of potentiated phospho-protein networks in cancer cells. *Cell* 118:217–228.



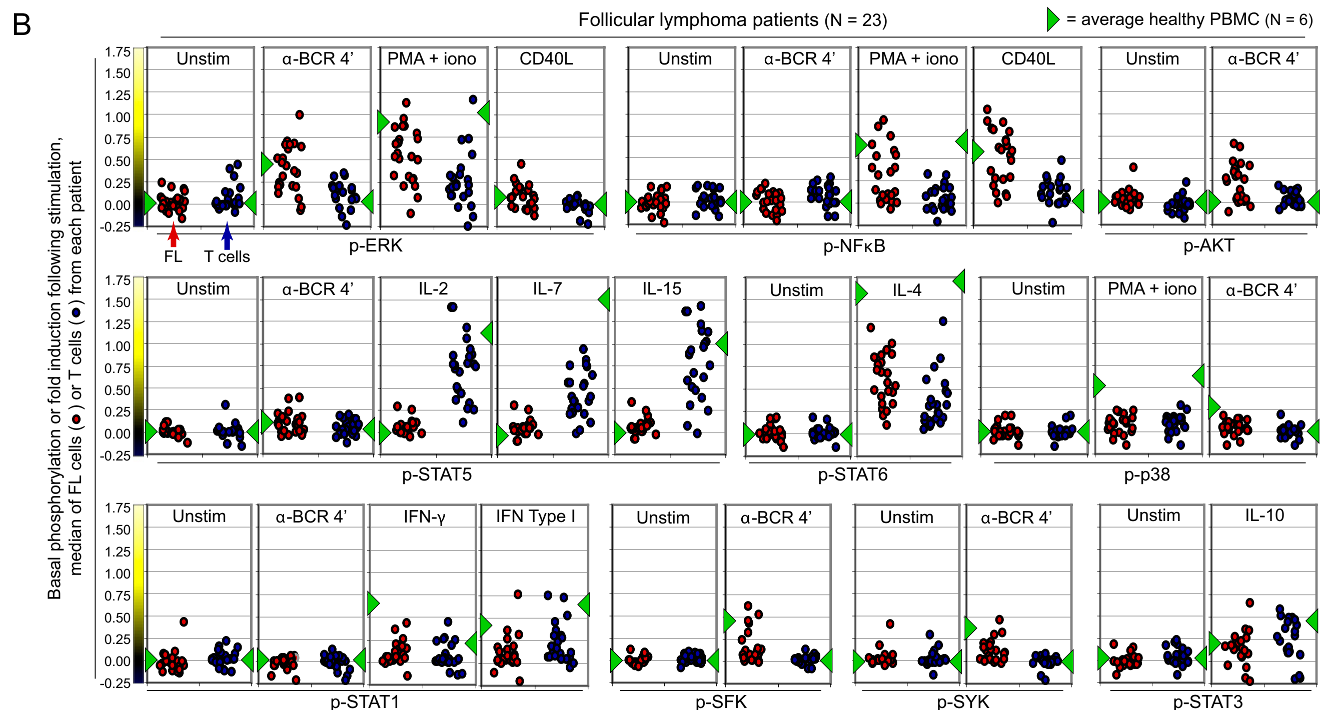
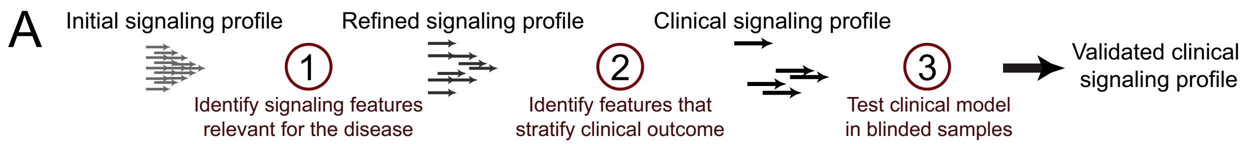
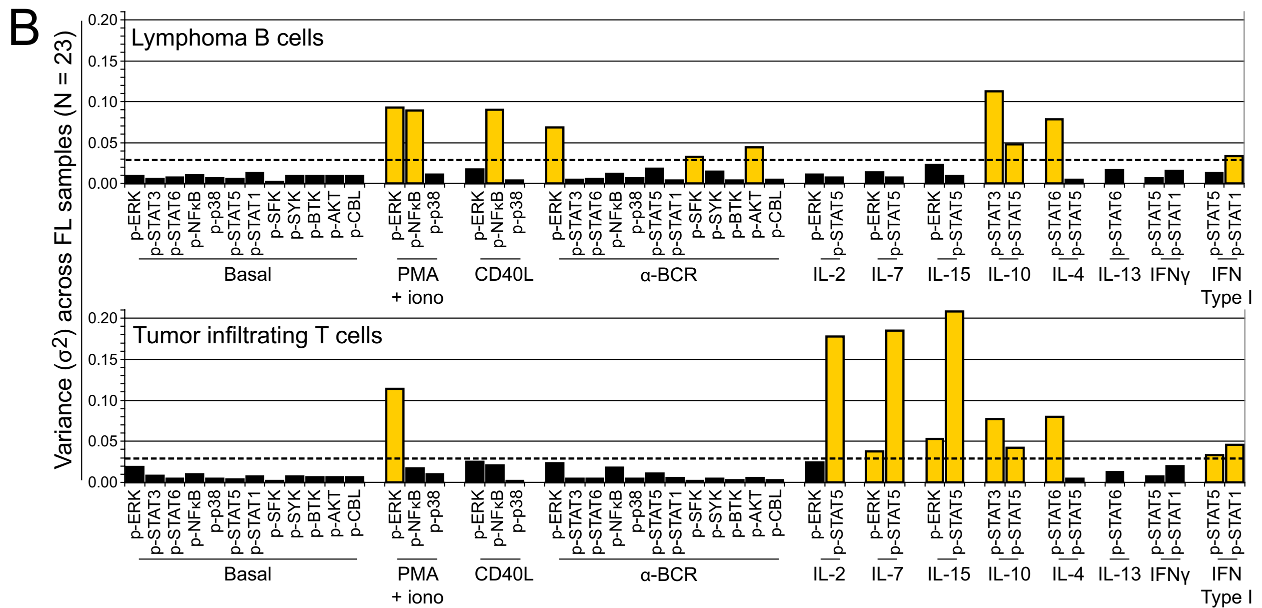


Fig. S1. (*A*) An outline of the data analysis plan is shown. A prognostic clinical signaling profile was finalized and tested in a set of patients in whom investigators were blinded to clinical outcome while scoring. (*B*) Variance of signaling features with significant signaling activity was compared across FL specimens to rule out features that did not vary significantly among patients (shown in black). (*C*) Contrasting signaling profiles of cell subsets within lymphoma tumors. (*A*) Contour plots show signaling and expression of BCL2 in lymphoma B cells and tumor-infiltrating T cells from an FL patient sample (LP-J020) that was stimulated as indicated. A heat map of fold induction of signaling was calculated for this patient and for a sample of healthy peripheral blood mononuclear cells (PBMCs). This a one representative example of the data graphed in *B*. (*B*) Scatter plots graph the basal phosphorylation (unstim) or fold induction over basal for lymphoma B cells (red dots) and tumor-infiltrating T cells (blue dots) from 23 FL patient samples stimulated as indicated. The average basal or fold signaling response of healthy B and T PBMCs is also indicated (green arrow, $n = 6$). For basal values, each patient's unstimulated MFI was compared with the median basal MFI of the cohort. For fold induction, each patient's stimulated MFI was compared with their own basal MFI. The scale transformation and positive range (1.75 fold) are the same here as in the contour plots (*y* axis) and heat map plots (*z* axis) and throughout the text.





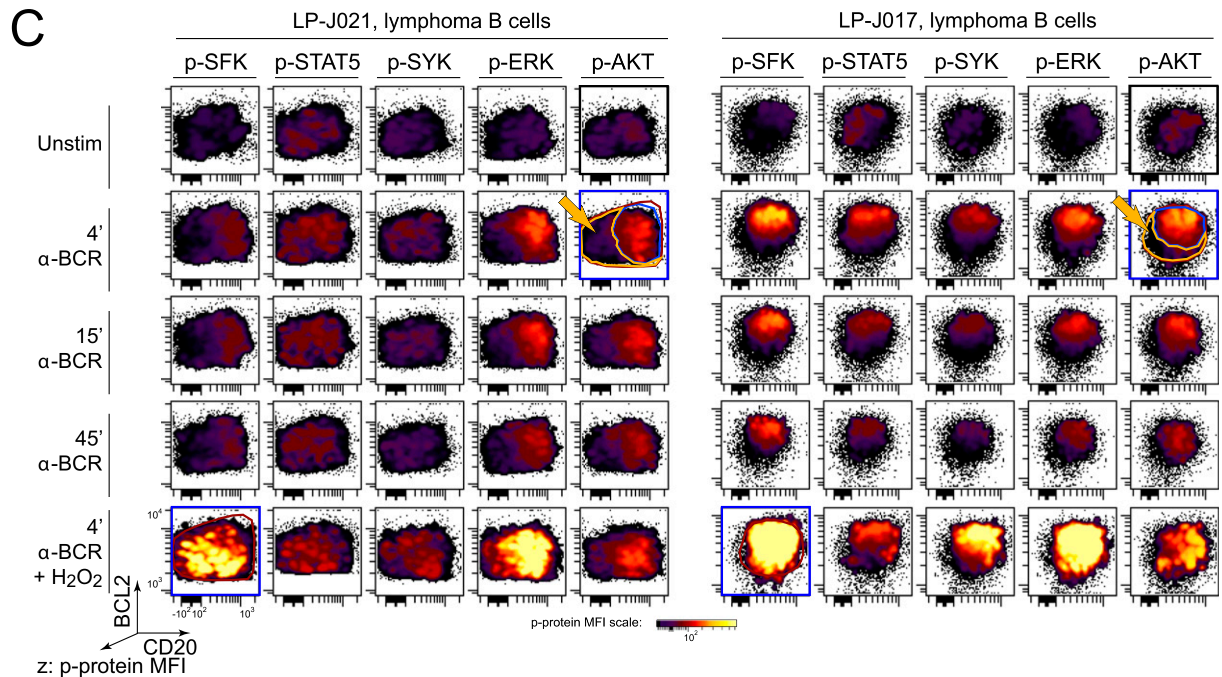


Fig. S2. (*A*) An outline of the data analysis plan is shown. A prognostic clinical signaling profile was finalized and tested in a set of patients in whom investigators were blinded to clinical outcome while scoring. (*B*) Variance of signaling features with significant signaling activity was compared across FL specimens to rule out features that did not vary significantly among patients (shown in black). (*C*) Signaling kinetics and additional phospho-protein phosphorylation of five proteins was examined at the standard time point of 4 min and two additional time points (15 min and 45 min) following α-BCR in two representative samples. For all samples studied (n = 74; Table S3), signaling at all phospho-proteins was examined at 4 min and 45 min following α-BCR. Here, representative phospho-proteins are shown; for quantification, all phospho-proteins were considered (*SI Materials and Methods*). LNP cells were distinguished by the absence of phosphorylation of all examined phospho-proteins at 4 min after α-BCR. As a control, stimulation by α-BCR + H_2O_2 for 4 min triggered intense signaling in both the LNP cell subset and other lymphoma B cells.

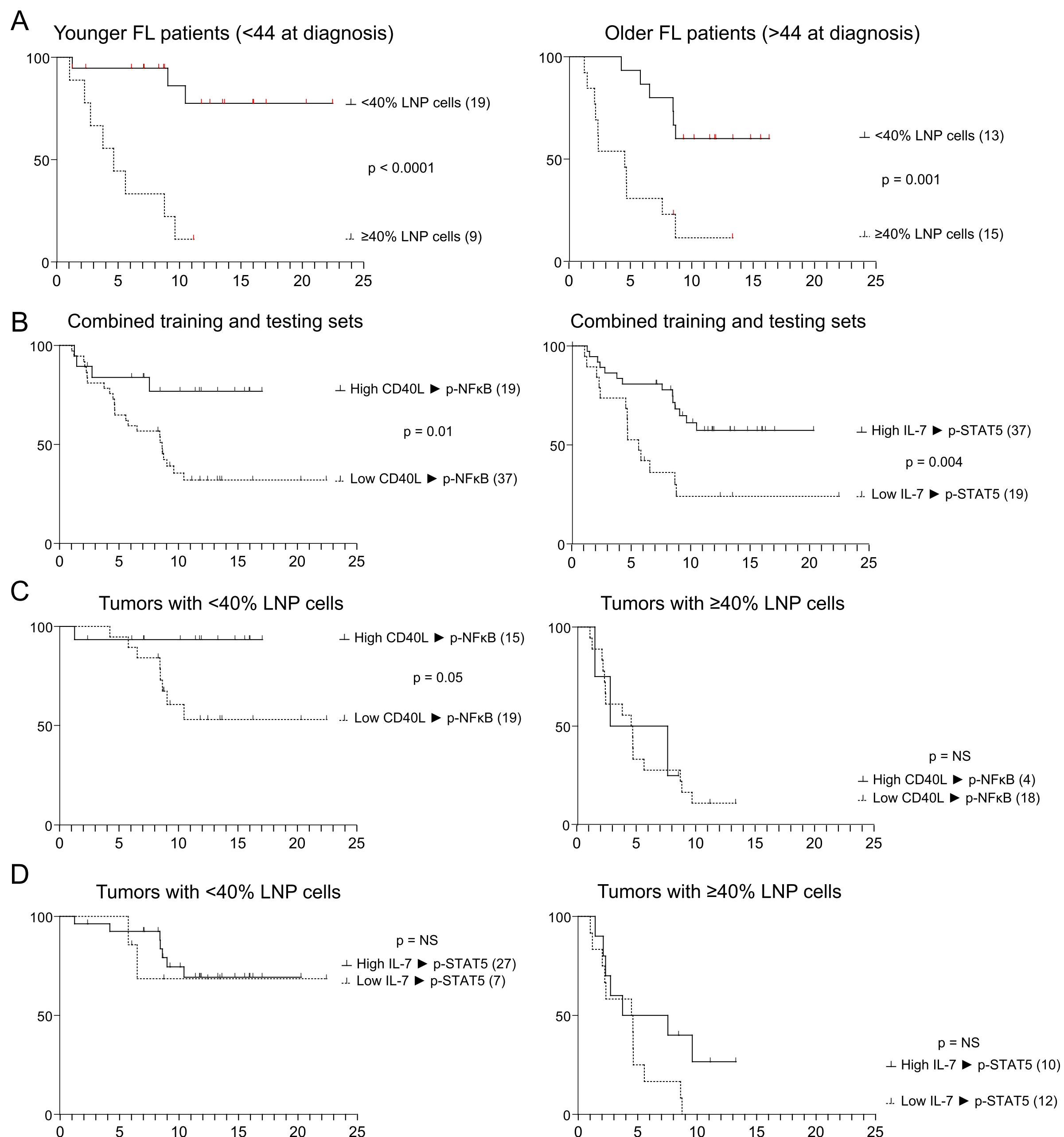


Fig. S3. LNP cells stratify survival risk independent of patient age at diagnosis and CD40L-mediated NF-κB signaling only stratifies survival of patients lacking an LNP cell subset. (*A*) The most significant component of clinical prognostic FLIPI (1) is age. Older patients with FL have inferior overall survival compared with younger patients. It might have been the case that patient age was related to the abundance of the LNP cell subset. However, age was not significantly different between patients whose tumors contained at least 40% LNP cells and fewer than 40% LNP cells (Table S1). As an additional test of the independence of the LNP cell subset from patient age, the training and testing datasets were combined into one cohort and then divided according to the median age at diagnosis of 44 y to produce two groups: younger and older FL patients. Overall survival was then compared for patients whose tumors contained at least 40% LNP cells and fewer than 40% LNP cells within the younger and older FL patient groups. LNP cells stratified the survival of both younger and older FL patients and were especially good at stratifying the outcome of younger patients ($P < 0.0001$). (*B*) Stratification of survival in the combined training and testing cohorts by CD40L and IL-7 signaling. (*C*) The independence of CD40L signaling from the LNP cell subset was examined by combining the training and testing sets, dividing patients into groups based on LNP cells (<40% and ≥40%), and then stratifying these groups by CD40L mediated phosphorylation of NF-κB p65. CD40L signaling was partially independent from the LNP cell model. "CD40L ▸ p-NFκB" stratified overall survival of patients lacking an LNP cell subset ($P < 0.05$), but CD40 signaling did not stratify survival of the patient group whose tumors contained at least 40% LNP cells. (*D*) The independence of IL-7 signaling from the LNP cell subset was examined as with CD40 signaling. IL-7 was closely related to the LNP cell model and did not stratify survival independent of the LNP cell model.

1. Solal-Céligny P, et al. (2004) Follicular lymphoma international prognostic index. *Blood* 104:1258–1265.

Fig. S4. Clinical outcomes for training and testing set patients (*A*). Overall survival for the combined group of training and test set patients (n = 56) in shown. (*B*) Initial CVP chemotherapy response and overall survival, shown as days from biopsy to last follow up (+) or death (D), are shown for the combined patient sets. Patients in the training and testing sets were treated with uniform initial CVP therapy. CR, complete response; CRu, unconfirmed CR; PR, partial response; NR, no response; PD, progressive disease.

Fig. S5. Median BCR signaling stratifies overall survival. The LNP cell subset displayed a characteristic lack of apparent response to α-BCR, so the median response to α-BCR of the entire lymphoma B cell population was examined as a potential classifier in the testing set. (*A*) FL patients in the testing set were clustered according to median phosphorylation of 15 phospho-proteins in lymphoma cells. Two main patient clusters were identified and designated cluster 1 and cluster 2. (*B*) Overall survival of patients in cluster 2 was worse than that of patients in cluster 1, although this stratification by median signaling was inferior to the stratification provided by the LNP cell subset. The patient groups defined by median signaling corresponded fairly well to groups identified by the LNP cell subset model. However, for five of the 28 patients, median BCR signaling failed to capture bimodal signaling responses, which are represented well in the LNP subset model. For example, LP-J114 was misclassified because outstanding signaling was observed in the non-LNP cells and the LNP cells constituted 40.7% of the tumor. Thus, the bulk tumor signaling obscured the LNP cell subset when median signaling was measured. More commonly, median signaling failed to distinguish between samples that lacked LNP cells and displayed a low to moderate signaling response in all of the lymphoma B cells (LP-J133) and samples with a bimodal pattern in which the apparent median signaling was low as a result the presence of a significant LNP cell subset.

Fig. S6. BCR expression and signaling networks over time and BCR-specific inhibition of ERK phosphorylation by R406. (*A*) MFI of Ig light chain and CD79 is shown for the same paired samples in Fig. 4*A* in the main text, in which measurement of LNP cells and Ig heavy chain was shown. (*B*) Signaling network models graph the response of measured BCR signaling network proteins in lymphoma B cells from a pretherapy sample (*Left*) and a sample taken after therapy and progression (*Right*). Dark pie shading indicates percentage of BCR-insensitive lymphoma B cells for that protein following α-BCR stimulation for 4′, as determined by phospho-specific flow cytometry. (*C*) Phosphorylation of ERK was measured in Ramos B lymphoma cells following 15 min of stimulation by either α-IgM or PMA in cells either pretreated by 2.5 μM R406 or vehicle control for 30 min (*SI Materials and Methods*). SYK inhibition by R406 specifically blocked BCR mediated ERK phosphorylation and not PMA mediated ERK phosphorylation. This was expected based on the known biochemistry of this signaling cascade, as PMA stimulates PKC, leading to SYK-independent ERK phosphorylation.

Table S1. Clinical features associated with LNP cells

Clinical feature*	Overall (*N* = 56)	≥40% LNP (*n* = 22)	<40% LNP (*n* = 34)	*P* value
Median FLIPI score (range)*	2 (1–4)	2 (1–3)	2 (1–4)	0.41
Age at diagnosis, y				0.68
≥60	8 (14%)	4 (18%)	4 (12%)	
Median (range)	45(27–75)	47(30–75)	44(27–63)	
Ann Arbor stage (%)				0.91
I/II	2 (4%)	1 (5%)	1 (3%)	
III/IV	52 (93%)	20 (91%)	32 (94%)	
Nodal count (%)*				0.08
>4	27 (69%)	8 (50%)	19 (83%)	
Median (range)	5 (2–10)	5 (2–9)	5 (2–10)	
LDH > ULN (%)*	5 (11%)	3 (17%)	2 (8%)	0.63
Hemoglobin <120 g/L	3 (8%)	1 (8%)	2 (9%)	0.97
B symptoms absent (%)	48 (86%)	20 (91%)	28 (82%)	0.49
Duration of watch and wait, mo	5.7 (0.2–135.7)	5.0 (0.2–25.9)	6.5 (0.4–135.7)	0.29
Response to first therapy, no. (%)				0.003†
CR/CRu	26 (46%)	6 (27%)	20 (59%)	
PR	21 (38%)	8 (36%)	13 (38%)	
NR/–	8 (14%)	8 (36%)	0 (0%)	
Median progression-free survival, mo (range)	17.2 (0.7–269.2)	13.2 (0.7–109.7)	25.4 (4.1–269.2)	0.03†
Time to transformation, mo	18.8 (10.2–133.4)	12.4 (10.2–112.4)	66.0 (18.8–133.4)	0.11
Survival at median, no. (%)				<0.0001†
Alive at median of 9.6 y	19 (34%)	2 (9%)	17 (50%)	
Deceased before 9.6 y	26 (46%)	19 (86%)	7 (21%)	
Censored before 9.6 y	11 (20%)	1 (5%)	10 (29%)	
Histological grade (%)				0.53
Grade 1	33 (59%)	11 (50%)	22 (65%)	
Grade 2	19 (34%)	8 (36%)	11 (32%)	
Grade 3	2 (4%)	1 (5%)	1 (3%)	
Median tumor infiltrating CD3+ cells, % (range)	22% (3–45%)	23% (8–44%)	22% (3–45%)	0.95
Median CD19+ cells, % (range)	75% (6–95%)	76% (6–95%)	73% (11–95%)	0.68
BCR heavy chain isotype				0.20
IgG	16 (29%)	9 (41%)	7 (21%)	
IgM	40 (71%)	13 (59%)	27 (79%)	

ULN, upper limit of normal.

*For some statistics, the value was not available for all patients.

†Significant difference.

Table S2. Clinical features of patients

Patient												Tumor							
							Initial therapy							Transformation			BCR signaling profile		
ID	Age, y	Stage	Nodal count	LDH	HGB	FLIPI	Regimen	Response	Progression-free survival	Overall survival	Mortality status	Histo.	Pre/post therapy		Time, y	Isotype		(% LNP)	ID
Training cohort																			
LP-J002	32	4a	8	Normal	Normal	2	CVP	PR	7.9	17.1	A+	LMFM	Pre	—	—	IgM	Sens.	1.2	9850a
LP-J003	36	4b	5	Normal	Abnormal	3	CVP	CRu	16.5	7.1	A+	LMFSC	Pre	—	—	IgM	Sens.	1.9	26062
LP-J004	61	3a	5	Normal	Normal	3	CVP	CRu	18.6	7	A+	LMFSC	Pre	T	1.6	IgG	Sens.	0.6	27863
LP-J005	46	4a	7	Normal	Normal	2	CVP	CR	12.1	6.5	D	LMFSC	Pre	—	—	IgM	Sens.	14.8	20455
LP-J006	52	2a	—	—	—	N.D.	CVP	PR	6	14.8	A+	LMFSC	Pre	T	11.1	IgG	Sens.	6.4	21161
LP-J008	56	4a	—	—	—	N.D.	CVP	PR	44.6	8.4	D	LMFSC	Pre	T	6.3	IgM	Sens.	6.8	9182
LP-J009	51	4a	6	Normal	—	2	CVP	PR	27	5.8	D	LMFM	Pre	T	4.7	IgM	Sens.	6.3	26820
LP-J010	31	4a	6	Normal	Normal	2	CVP	PR	161.1	9.8	A+	LMFM	Pre	—	—	IgM	Sens.	1.0	24874
LP-J013	36	3a	5	Normal	Normal	2	CVP	CR	147.3	9	A+	LMFSC	Pre	—	—	IgM	Sens.	9.7	25153
LP-J017	27	4a	8	Normal	Normal	2	CVP	CR	27	2.4	A+	LMFSC	Pre	—	—	IgM	Sens.	12.2	24999
LP-J018	36	3b	4	Normal	—	1	CVP	CR	10	6.1	A+	LMFSC	Pre	—	—	IgG	Sens.	0.6	27978
LP-J020	57	4a	5	Normal	—	2	CVP	CRu	13.9	4.2	D	LMFM	Pre	—	—	IgM	Sens.	3.3	27880
LP-J031	53	4a	8	Abnormal	Normal	3	CVP	CRu	16.2	15.1	A+	LMFSC	Pre	—	—	IgM	Sens.	28.7	20889
LP-J033	40	4a	—	—	—	—	CVP	CRu	9	16	A+	LMFM	Pre	—	—	IgM	Sens.	23.9	20953
LP-J035	44	4a	6	—	Normal	—	CVP	PR	20.1	10.5	D	LMFSC	Pre	—	—	IgG	Sens.	27.9	24863
LP-J037	49	4a	2	Normal	Normal	1	CVP	PR	186.4	15.6	A+	LMFSC	Pre	—	—	IgG	Sens.	25.2	20376
LP-J001	41	4a	—	Normal	—	2	CVP	CR	109.8	9.6	D	LMFSC	Pre	T	9.4	IgG	BCR ins.	100	9130
LP-J007	53	3a	4	Normal	Normal	1	CVP	NR	6.1	2	D	LMFSC	Pre	—	—	IgM	BCR ins.	59.6	22651
LP-J011	42	4a	3	Normal	—	1	CVP	—	11.8	1	D	LMFSC	Pre	T	1	IgM	BCR ins.	52.2	26805
LP-J012	56	3a	4	Normal	—	1	CVP	—	5.8	1.2	D	LMFM	Pre	T	0.9	IgG	BCR ins.	100	26956
LP-J014	47	4a	9	Normal	Normal	2	CVP	CR	12.9	8.6	D	LMFSC	Pre	—	—	IgM	BCR ins.	68.9	24933
LP-J022	49	4a	—	—	—	N.D.	CVP	PR	13.5	4.7	D	LMFM	Pre	—	—	IgG	BCR ins.	70.8	25424
LP-J023	54	—	—	—	—	N.D.	CVP	NR	0.7	4.7	D	LMFM	Pre	—	—	IgM	BCR ins.	58.5	28599
LP-J027	40	4a	8	Normal	Normal	2	CVP	CRu	14	5.6	D	LMFSC	Pre	T	5.4	IgM	BCR ins.	47.1	10318
LP-J028	67	3a	3	Normal	Normal	2	CVP	CRu	3.9	2.4	D	LMFM	Pre	—	—	IgM	BCR ins.	59.6	20609
LP-J029	30	4a	9	Normal	Normal	2	CVP	PR	17.2	3.8	D	LMFSC	Pre	—	—	IgM	BCR ins.	66.7	25429
LP-J030	45	4a	—	—	—	—	CVP	PR	45.6	8.8	D	LMFSC	Pre	—	—	IgG	BCR ins.	67.1	20297
LP-J032	62	4a	4	Normal	Normal	2	CVP	CR	46.2	8.5	A+	LMFSC	Pre	—	—	IgM	BCR ins.	100	25250
Testing cohort																			
LP-J105	63	3a	—	Normal	Normal	2	CVP	CR	4.1	11.4	A+	LMFSC	Pre	—	—	IgM	Sens.	7.1	25414
LP-J106	43	3b	—	Normal	—	2	CVP	CR	37	17.1	A+	LMFM	Pre	—	—	IgM	Sens.	2.1	10523
LP-J108	36	4a	—	—	—	—	CVP	PR	7.1	9	D	LMFSC	Pre	—	—	IgM	Sens.	30.3	24936
LP-J111	62	3a	5	—	Normal	—	CVP	CR	17.1	11.9	A+	LMFSC	Pre	—	—	IgG	Sens.	21.9	21097
LP-J112	40	4a	3	Normal	Normal	1	CVP	PR	190.1	16	A+	LMFM	Pre	—	—	IgM	Sens.	20.3	19808
LP-J116	61	4b	7	Normal	Normal	2	CVP	CRu	62.7	9.3	A+	LMFSC	Pre	—	—	IgM	Sens.	4.8	25350
LP-J117	35	3a	10	Normal	Normal	2	CVP	CR	42.5	8.3	A+	LMFSC	Pre	—	—	IgM	Sens.	4.2	26954
LP-J120	32	4a	7	Normal	Normal	2	CVP	PR	6	1.3	D	LMFM	Pre	—	—	IgM	Sens.	9.8	28768
LP-J122	44	4a	5	Normal	Normal	2	CVP	CR	104.9	8.8	A+	LMFSC	Pre	—	—	IgM	Sens.	21.9	25260
LP-J123	37	3a	3	Normal	Normal	1	CVP	PR	162.8	13.7	A+	LMFM	Pre	—	—	IgM	Sens.	16.8	20153
LP-J124	46	3b	5	Normal	Normal	2	CVP	N.D.	23.9	11.8	A+	LMFLC	Pre	—	—	IgM	Sens.	29.7	24066
LP-J126	57	4a	—	—	—	—	CVP	CR	5.1	8.7	D	LMFSC	Pre	—	—	IgM	Sens.	24.1	23728

Table S2. Cont.

Patient												Tumor							
							Initial therapy							Transformation			BCR signaling profile		
ID	Age, y	Stage	Nodal count	LDH	HGB	FLIPI	Regimen	Response	Progression-free survival	Overall survival	Mortality status	Histo.	Pre/post therapy		Time, y	Isotype		(% LNP)	ID
LP-J127	46	4a	5	Abnormal	Abnormal	4	CVP	CRu	46.5	13.3	A+	LMFSC	Pre	—	—	IgM	Sens.	11.1	20477
LP-J131a	28	4a	—	Normal	Normal	2	CVP	PR	10	8.7	A+	LMFM	Pre	—	—	IgM	Sens.	24.1	25351
LP-J133a	33	—	10	—	Normal		CVP	CR	269.2	22.5	A+	LMFM	Pre	—	—	IgM	Sens.	23	9135
LP-J134	36	4a	—	Normal	—	2	CVP	CR	60.8	11.8	A+	LMFSC	Pre	—	—	IgM	Sens.	0	25254
LP-J140	42	4a		Normal	Normal	2	CVP	PR	50.4	7.1	A+	LMFM	Pre	—	—	IgM	Sens.	29	26050
LP-J141	51	4a	—	—	—	—	CVP	CRu	55.1	10.2	A+	LMFSC	Pre	—	—	IgG	Sens.	28.6	26041
LP-J101	75	4a	5	Normal	Normal	3	CVP	PR	17.2	1.5	D	LMFLC	Pre	—	—	IgM	BCR ins.	100	20381
LP-J102	38	4a	—	—	—	—	CVP	PR	12.3	2.3	D	LMFSC	Pre	T	1.1	IgG	BCR ins.	45.7	26033
LP-J103	38	4as	3	Abnormal	Abnormal	2	CVP	PR	23.2	4.6	D	LMFSC	Pre	—	—	IgM	BCR ins.	58.4	5527
LP-J109	46	4a	7	Normal	Normal	2	CVP	NR	9.9	2.3	D	LMFM	Pre	—	—	IgG	BCR ins.	48.4	23493
LP-J110	47	3a	-	Normal	Normal	1	CVP	PR	64.7	13.3	A+	LMFM	Pre	—	—	IgM	BCR ins.	60.7	24496
LP-J114	42	4as	8	Abnormal	Normal	3	CVP	NR	8	2.7	D	LMFM	Pre	—	—	IgM	BCR ins.	40.7	27905
LP-J119	56	4a	8	Abnormal	—	3	CVP	PR	21.4	2.1	D	LMFM	Pre	—	—	IgM	BCR ins.	54.1	26957
LP-J121	53	4a	7	Normal	—	2	CVP	NR	8.9	4.5	D	LMFSC	Pre	T	0.9	IgG	BCR ins.	58.1	25428
LP-J125	74	2a	2	Normal	Normal	1	CVP	—	7.9	7.6	D	LMFM	Pre	—	—	IgG	BCR ins.	60.2	24029
LP-J128a	40	4b	4	normal	Normal	1	CVP	CR	31.5	11.1	A+	LMFM	Pre	—	—	IgG	BCR ins.	60.7	25109
Pretraining and posttherapy samples (a = pretherapy, b = posttherapy)																			
LP-J039b	44	—	—	—	—	—	—	PR	6	4.1	A+	LMFM	Post	—	—	IgG	Sens.	35.8	29904
LP-J038b	39	3a	7	Normal	Normal	—	—	CRu	19.5	1.5	D	LMFSC	Post	—	—	IgM	BCR ins.	68.3	20608
LP-J040b	29	—	—	—	—	—	—	N.D.	77.9	3.3	D	LMDMXN	Post	T	0	IgM	BCR ins.	59.8	10504
LP-J041b	36	—	—	—	—	—	—	N.D.	6.7	2.3	D	LMDMXN	Post	T	−4	n.d.	BCR ins.	100	7307
LP-J016	36	3a	10	Normal	—	2	—	CR	158.4	13.4	A+	LMFM	Pre	—	—	IgM	Sens.	0.4	23934
LP-J019	45	4a	10	Normal	Normal	2	—	CR	144.2	13.2	A+	LMFSC	Pre	—	—	IgM	Sens.	6.2	24060
LP-J025	45	4a	7	Normal	—	2	—	PR	34.5	12.3	A+	LMFSC	Pre	—	—	IgG	Sens.	22.5	21468
LP-J026	40	4a	—	Normal	Normal	2	—	CR	130.1	10.9	A+	LMFM	Pre	—	—	IgM	Sens.	32.1	24490
LP-J036	48	4a	5	Normal	—	2	—	PR	10.4	10	A+	LMFM	Pre	—	—	IgG	Sens.	27.6	24840
LP-J039a	44	—	—	—	—	—	—	PR	6	15.6	A+	LMFM	Pre	—	—	IgG	Sens.	17.4	21595
LP-J015	48	3a	6	Normal	Normal	2	—	NR	99.4	9.4	D	LMFM	Pre	—	—	IgG	BCR ins.	45.2	22967
LP-J021	41	4a	8	Abnormal	Normal	3	—	PR	8.1	3.9	D	LMFSC	Pre	—	—	IgG	BCR ins.	46.7	24844
LP-J038a	39	3a	7	Normal	Normal		—	CRu	19.5	6.6	D	LMFSC	Pre	—	—	IgM	BCR ins.	47.5	6663
LP-J040a	29	—	—	—	—	—	—	N.D.	77.9	7.8	D	LMFM	Post	T	4.5	IgM	BCR ins.	59.9	2423
LP-J041a	36	—	—	—	—	—	—	N.D.	6.7	7	D	LMFSC	Post	T	0.7	N.D.	BCR ins.	59.6	2980
LP-J131b	28	4a	—	Normal	Normal	—	—	PR	10	7	A+	LMFM	Pre	—	—	IgM	Sens.	28.4	26061
LP-J128b	40	4b	4	Normal	Normal	1	—	CR	31.5	8.5	A+	LMFSC	Post	—	—	IgG	BCR ins.	100	27900
LP-J129b	45	4a	—	Normal	—	2	—	PR	23.8	7.6	A+	LMFSC	Post	—	—	IgM	BCR ins.	54.4	27882

N.D., not determined.

Table S3. Biological features in groups defined by LNP cells

Biological feature, lymphoma B cells, testing set	Overall (N = 28)	<40% LNP cells (n = 10)	≥40% LNP cells (n = 18)	P value
Ig heavy chain (IGH)				0.08
Median	1.3	1.1	1.3	
Range	0.1–2.3	0.3–1.5	0.1–2.3	
Ig light chain (IGL)				0.14
Median	1.2	0.9	1.4	
Range	0–2.9	0.3–2.4	0–2.9	
CD79b				0.14
Median	1.3	1.0	1.4	
Range	0.1–2.9	0.4–2.1	0.1–2.9	
p-PLCγ, resting level				0.94
Median	0.0	0.0	0.0	
Range	−0.2 to 0.3	−0.2 to 0.2	−0.2 to 0.3	
p-PLCγ, 4′ BCR stimulation				0.005[†]
Median	0.8	0.5	1.0	
Range	0.2–2.2	0.2–1.8	0.3–2.2	
CD19				0.40
Median	0.8	1.1	0.8	
Range	0–2.1	0.4–1.6	0–2.1	
CD22				0.22
Median	0.1	0.2	0.1	
Range	0–0.6	0.1–0.4	0–0.6	
CD10				0.40
Median	0.6	0.8	0.6	
Range	0–2	0.4–1.5	0–1.7	
CD38				0.28
Median	0.2	0.2	0.2	
Range	0–0.4	0.1–0.4	0.1–0.3	
CD20				0.71
Median	1.7	1.9	1.7	
Range	0–2.6	0.8–2.2	0–2.3	
HLA-DR				0.90
Median	1.2	1.3	1.2	
Range	0–2.7	0.4–2	0–2.7	
CD81				0.10
Median	1.7	1.9	1.6	
Range	0.3–2.8	0.9–2.8	0.3–2.7	
CD47				0.94
Median	0.4	0.5	0.4	
Range	0.2–1	0.2–0.8	0.3–1	
CD124 IL-4R				0.20
Median	0.2	0.2	0.2	
Range	0–0.5	0.1–0.4	0–0.5	
IL-10R				0.55
Median	0.1	0.1	0.1	
Range	0–0.2	0–0.2	0.1–0.2	
IL-21R				0.38
Median	0.2	0.2	0.2	
Range	0.1–0.4	0.2–0.3	0.1–0.4	
CD95 Fas				0.83
Median	0.2	0.3	0.2	
Range	0.1–1.1	0.1–0.7	0.1–1.1	
CD40				0.28
Median	0.1	0.2	0.1	
Range	0–0.6	0–0.3	0–0.6	
CD5				0.24
Median	0.0	0.0	0.0	
Range	0–0.8	0–0.1	0–0.8	
CD3				0.08
Median	0.1	0.1	0.0	
Range	0–0.1	0–0.1	0–0.1	
CD14				0.72
Median	0.0	0.0	0.0	
Range	0–0.1	0–0	0–0.1	

Table S3. Cont.

Biological feature, lymphoma B cells, testing set	Overall (N = 28)	<40% LNP cells (n = 10)	≥40% LNP cells (n = 18)	P value
CD56				0.46
Median	0.0	0.1	0.0	
Range	0–0.1	0–0.1	0–0.1	

†Significant difference.

Table S4. Signaling profile v1, applied to LP-J001–LP-J023: 27 stimuli × 12 phospho-proteins

Ch.	Parameter	Signaling profile
1	Pacific Blue	Barcoding channel 1
2	Pacific Orange	Barcoding channel 2
3	Alexa488	Phospho channel 1
4	PE	BCL2
5	PerCP-Cy5.5	CD20
6	Alexa647	Phospho channel 2
7	Ax750	Barcoding channel 3
8	Alexa700	CD3
9	Forward scatter area	Cell size/doublets
10	Forward scatter width	Cell size/doublets
11	Side scatter area	Cell granularity/doublets
12	Side scatter width	Cell granularity/doublets

Table S5. Focused signaling profile v2, applied to LP-J024 through LP-J140: nine stimuli × 15 phospho-proteins

Ch.	Parameter	Signaling profile
1	Pacific Blue	Barcoding channel 1
2	Pacific Orange	Barcoding channel 2
3	QDot605	CD3
4	Alexa488	Phospho channel 1
5	PE	BCL2
6	PerCP-Cy5.5	CD20
7	PE-Cy7	CD5
8	Alexa647	Phospho channel 2
9	Forward scatter area	Cell size/doublets
10	Forward scatter width	Cell size/doublets
11	Side scatter area	Cell granularity/doublets
12	Side scatter width	Cell granularity/doublets