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Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	N/A
Data analysis	R package corrplot; FlowJo; GraphPad Prism 9; NanoString's nSolverTM 4.0; Quality control of RNA-seq datasets was performed by FastQC. The reads were aligned to the National Center for Biotechnology Information (NCBI) genome browser build hg38 using TopHat v2.1.1 and reads were counted within features using HTSeq v2.0.1. Differential Expression analysis was done with DESeq2 v1.36.0 in R v4.2.0; Multiplex imaging cycle shift was corrected using a Fast Fourier transform (FFT) algorithm where the cross-correlation between two images is calculated to get translational offsets; Zeiss Zen software.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The raw RNA-seq data on organoids are available in the GEO database under accession code GSE209551. Remaining patient RNA sequencing data is available publicly through Hematologic Malignancies Research Consortium (HMRC, European Genome-phenome Archive EGAS00001002606), National Cancer Institute (NCI) Genomic Data Center (GSE99276) and with approval from the NCBI dbGaP Controlled Access portal, and Weill Cornell's Gene Expression Omnibus (GEO) GSE145043. The remaining data are available within the Article, Supplementary Information, or Source data file. Due to very large file sizes and volume of data, remaining raw data supporting the findings of this study are available from the corresponding author on reasonable request.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	For analysis of the patient cohort, the sample size was based on available data from the published cohort studies. Sample sizes of organoids were selected based on statistical power calculations and previous experience with these metrics (See Ref. 5, 52-55). The sample size of animal studies was based on published work (See Ref.5).
Data exclusions	No data from intact hydrogels or animal studies were excluded.
Replication	All studies were replicated 3 times and results were consistent across independent experimental runs, with the exception of the NSG mice in vivo tumor implantation studies that were performed once due to the associated costs and prior experience with published work(See Ref.5)
Randomization	After tumors developed, mice were randomized into five arms described in the manuscript. For organoids, randomization was not performed all the time but comparing those 96-well plates where samples from same group were placed next to each other versus far from each other produced no difference. Therefore, it was determined that randomization of organoids in 96-well plate was not necessary.
Blinding	Investigators were blinded during imaging analyses, mouse experiments, and drug response test. Blinding was performed without providing information of the type of organoids or treatment groups.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

CD4 (AF647; EPR6855; Abcam, catalog ab196147), CD20 (AF488; L26; Thermofisher, catalog 50-0202-82), CD40 (AF488; D8W3N; Cell Signaling Technology, catalog 40868S), CD40LG (AF647; D5J9Y; Cell Signaling Technology, catalog 15094), TRAF3 (AF488; 12H13L59; Thermofisher, catalog 700121). anti-IgM (clone SA-DA4; Thermo Fisher, catalog 14-9998-82), anti-CD20 (clone SP32; Thermo Fisher, catalog MA5-16334), and anti-CD40 (polyclonal; Thermo Fisher; catalog PA5-32325), anti-TRAF3 (clone 12H13L59; Thermo Fisher; catalog 700121). Secondary antibodies include donkey anti-rabbit IgG (Alexa Fluor Plus 488; Thermo Fisher, catalog A-21206), and donkey anti-mouse IgG (Alexa Fluor plus 647; Thermo Fisher, catalog A-31571), DAPI (Sigma, catalog D9542-10MG) and phalloidin (Alexa Fluor 555; Thermo Fisher; catalog A34055), anti-CD40L (Biotin; clone MK13A4; ThermoFisher, catalog BMS153BT), CD20 (Biotin; clone 2H7; ThermoFisher, catalog 13-0209-82), biotinylated anti-human CD40L (clone 24-31; BioLegend; catalog 310814). anti-BCL10 (AF488; clone EP606Y; abcam; ab200318), anti-CD20 (FITC, PE, PE-Cy7, APC; clone 2H7; Thermo Fisher; catalog 11-0209-42, 12-0209-42, 25-0209-42, 17-0209-42, respectively), anti-MALT1 (AF647; clone EP603Y; abcam; catalog ab217071), anti-phospho-BTK (PE; clone M4G3LN; Thermo Fisher; catalog 12-9015-42), BTK (PE; clone 53/BTK; BD; catalog 558527), phospho-NFkB p65 (PerCP-eFluor 710; clone B33B4WP; Thermo Fisher; catalog 46-9863-42), NFkB (BV421; clone K10-895.12.50; BD; catalog 56446), phospho-S6 (Ser235/236) (AF647; clone D57.2.E; Cell Signaling Technology; catalog 4851S), phosphor-SRC (Tyr418) (PE; clone SC1T2M3; Thermo Fisher; catalog 12-9034-42), Invitrogen Anti-CD29 (Integrin beta 1) Monoclonal (APC; clone TS2/16; eBioscience™, Thermo Fisher Catalog # 17-0299-42), Invitrogen Anti-CD51/CD61 (Integrin alpha v beta 3) Monoclonal (FITC; clone 23C6; eBioscience™, Thermo Fisher Catalog # 11-0519-42, anti-CD40L (BV421; clone: 4-31; Biolegend; catalog 310824), anti-CD19 (FITC; clone eBio1D3; Invitrogen, catalog 11-0193-85) and TLR9 (FITC, PE, APC; eB72-1665; Thermo Fisher; catalog 12-9099-82, 17-9099-82, respectively). Additional stains included CellTraceTM CFSE (Thermo Fisher; catalog C34554) to measure cell proliferation as well as LIVE/DEADTM fixable stains (Green Dead Cell, Far Red Dead Cell, Aqua Dead Cell; Thermo Fisher; catalog L23101, L10120, L34957) or propidium iodide (Thermo

Fisher; catalog P1304MP) to measure cell viability. Canine antibodies included mouse anti-dog CD20 (clone 6C12, Invivogen, catalog dcd20-mab11) with AlexaFluor488-labeled goat anti-mouse IgG secondary (clone Poly4053, Biolegend or Life Technologies) and anti-MHC II (FITC, Clone YKIX334.2, eBioscience, catalog MCA1044GA).

Validation

All antibodies and viability stains were validated by their respective manufacturers and utilized at the manufacturer's recommended dilution, in the event of no clear recommendation a dilution of 1:200 was used. Antibodies were also validated for use using positive and negative control samples using dilutions and conditions based on the manufacturer's recommendation or our previous experience. The absence of a primary antibody was used for validating staining for all antibodies tested for imaging. For flow, all antibodies were titrated using cells positive for the antibody marker.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Cell lines OCI-Ly3, OCI-LY7, OCI-Ly10, and HBL1 were obtained from Dr. Ari Melnick (Weill Cornell Medicine, New York, NY). OCI-Ly3, OCI-LY7, and OCI-Ly10 were originally provided by the University Health Network, Toronto, Canada, and transferring scientist Dr. Mark Minden. CD40L-expressing fibroblasts were obtained from Dr. Hideki Ueno.
Authentication	Validation of lines was performed using flow cytometry for expression of specific markers (example, CD40L)
Mycoplasma contamination	Cell lines cultured are routinely tested for mycoplasma, and all lines were verified to be negative for mycoplasma prior to experiments.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	Patient-derived xenografts (PDX) were first established in female and male NSG B2M mice (NOD.Cg-B2mtm1Unc Prkdcscid Il2rgtm1Wjl/SzJ). These PDXs were then serially propagated into NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG) mice (Jackson Laboratory). All mice were housed with following housing condition. Dark/light cycle: 12hr /12hr (8am ~ 8pm light on, 8pm ~ 8am light off) temperate: 22 degrees Celsius (± 2), humidity: 50% (± 10%).
Wild animals	This study did not involve wild animals.
Field-collected samples	No field collected samples were used in the study.
Ethics oversight	The research conducted in this report complies with relevant ethical regulations from Weill Cornell Medicine's IACUC-approved protocol 2014-0024 and Cornell's IACUC protocol number 2017-0035.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about studies involving human research participants

Population characteristics	HMRC Cohort: This cohort, published by Reddy et al.22, contains tumor biopsies (N=1001), paired-normal tissue (N=400), and clinical information from adult and pediatric patients with de novo DLBCL treated with a standard, rituximab-containing regimen. Tissue and clinical data were obtained from institutions participating in the HMRC, as detailed in Reddy et al.22. RNA-sequencing was performed in 775 patients for whom adequate material was available; 313 and 331 separate patients were respectively classified as ABC and GCB DLBCL. Cases were anonymized, shipped to Duke University, and processed in accordance with a protocol approved by the Institutional Review Board at Duke University. Clinical data including initial response to therapy, overall survival, gender, age, stage, performance status and number of extranodal sites were collected on nearly all cases, as detailed in Reddy et al.22 Cornell cohort: This cohort was obtained from Dr. Giorgio Inghirami and raw data of RNA-seq profiles was re-analyzed from Weill Cornell's Gene Expression Omnibus (GEO) GSE14504315 to identify and define ABC vs GCB DLBCLs. Deidentified patients' samples were obtained with informed consent under Weill Cornell Medicine's IRB-approved protocol 1410015560A002). Molecular and clinical details of these tumors are described in Kotlov et al.15
Recruitment	No human patient was recruited to this study. Established deidentified patients' samples were obtained from Dr. Giorgio Inghirami, with molecular and clinical details of these tumors described in his research Kotlov et al.15
Ethics oversight	The research conducted in this report complies with relevant ethical regulations from Weill Cornell Medicine's IRB-approved protocol 1410015560A002.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	For flow cytometry analysis, organoids were digested in TrypLE (Gibco) for Matrigel or collagenase (Worthington Biomedical) for PEG-MAL. After digestion, cells were spun down at 400x to attain a single cell suspensions. Cells were subsequently blocked for half an hour in PBS with 1% BSA (Sigma). Cells were subsequently washed (400x) and resuspended in FACS buffer. After an hour of incubation on ice, cells were washed 3x prior to being analyzed on an Accuri C6 flow cytometer (Becton Dickinson). Flow cytometry analysis was performed using FlowJo (Treestar). All antibodies or their fluorophore variations were used in these studies at a 1:250 dilution. A list of antibodies, clones, and vendors is available in the manuscript
Instrument	Accuri C6 flow cytometer or Beckman Coulter CytoFlex S
Software	All flowcytometry data was analyzed using FlowJo (Treestar).
Cell population abundance	Cells were not sorted. All data was performed on samples with at least 10,000 cells.
Gating strategy	Gating was established using negative controls to identify the positive population. For samples with multiple stains, single stain controls were used to establish compensation.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.