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Reporting Summary

Nature Portfolio 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 Portfolio policies, see our Editorial Policies 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	Flow cytometry analysis was performed using a flow cytometer (Attune NxT, Thermo Fisher Scientific, Attune NxT Software version 3.2.1); ELISA color intensity was measured using a Cytation 5 Cell Imaging Multi-Mode Reader (Agilent Technologies, Software Gen5 2.0); Cell-cell conjugations were observed using a laser scanning confocal microscope (A1R, Nikon, Software NIS-Elements C-ER); In vivo tumor burden was measured by an IVIS Spectrum (PerkinElmer, Living Image® Software 4.3.1).
Data analysis	FlowJo_V10 software was used for the analysis of all flow cytometry data; All the statistical analyses were performed using GraphPad Prism version 8.4.2 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 and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio 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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

The data are available within the Article, Supplementary Information or Source Data file. Source data are provided with this paper.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	Eleven patient samples were used in this study, two were female and nine were male. Healthy volunteers were male and female. Sex was not used as a variable in study analyses, and study findings are not specific to one sex.
Reporting on race, ethnicity, or other socially relevant groupings	The patients or healthy volunteers used for the study were all Asian. Race was not used as a variable in the study analyses, and the findings were not specific to one racial, ethnicity, or other socially relevant groupings.
Population characteristics	Peripheral blood or bone marrow samples were collected from patients diagnosed with B-cell malignancies, following their informed consent. All the participants signed the Informed Consent Form.
Recruitment	Patients samples were provided by the Department of Hematology, Huazhong University of Science and Technology Union Shenzhen Hospital. Anonymized healthy donor blood was provided by the Department of Hematology, Huazhong University of Science and Technology Union Shenzhen Hospital.
Ethics oversight	All experiments using human derived cells were approved by the Ethics Committee of Huazhong University of Science and Technology Union Shenzhen Hospital.

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

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	The sample size for the in vivo study in mice was determined based on our extensive experience in preclinical models of B-cell hematological malignancies and a review of the relevant literature. To ensure data reproducibility, 4-5 mice were enough to distinguish the difference between normal tumor-bearing mice and healthy mice. For the comparison of anti-tumor efficacy between different drug candidates, a group of 5 mice was selected. For the gradient dose experiments of the same drug candidate, we chose to use a group of four mice. Sample size and number of independent experiments are stated in the figure legends.
Data exclusions	No data were excluded from analysis.
Replication	All experiments were repeated at least twice independently, and similar results were obtained.
Randomization	For the in vivo experiments, mice were randomly assigned before CAR-T treatment to ensure consistent mean tumor burden across groups. For in vitro experiments, randomization was performed.
Blinding	For the in vivo study, drug delivery and bioluminescent substrate injection were performed by one operator who was unaware of the group assignments. Tumor burden and other data analyses were based on objectively measurable data (e.g. fluorescence intensity). All in vitro data were instrument-collected data without bias, so blinding was not used.

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 and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Antibodies

Antibodies used

Western blot antibodies:
anti-human CD19 antibody (Boster, BM4935, 1:2000),
anti-human CD22 antibody (Boster, BM4178, 1:2000),
anti-human CD3ζ antibody (Santa Cruz Biotechnology, sc-1239, 1:200),
anti-mouse IgG-HRP (TransGen Biotech, HS201-01, 1:10000).

Flow cytometry antibodies:
APC anti-human BAFFR (BioLegend, 11C1, 316916, 1:50),
PE anti-human BCMA (BioLegend, 19F2, 357504, 1:200),
PE anti-human TACI (BioLegend, 1A1, 311906, 1:200),
APC anti-human CD3 (BioLegend, ,HIT3a, 300312, 1:50),
Pacific Blue anti-human CD3 (BioLegend, 300329, 1:200),
FITC anti-human CD45 (BioLegend, HI30, 304038, 1:200),
PE anti-human CD22 (BioLegend, HIB22, 302506, 1:200),
APC anti-human CD19 (BioLegend, HIB19, 302212, 1:100),
Alexa Fluor 647 goat anti-mouse IgG(H+L) (Invitrogen, A21235, 1:500),
Alexa Fluor 647 F(ab')2 fragment of goat anti-mouse IgG (Invitrogen, A21237, 1:500),
FITC goat pAb to Myc tag (abcam, ab1263, 1:500).

Immunofluorescence antibodies:
anti-human PKC-θ biotin-conjugated antibody (Signalway Antibody, C33151, 1:200).

Validation

All the antibodies used in this study were commercially available and purchased through the reagent manufacturers. Antibody validation was performed by the supplier and the relevant information is provided on the website and in the product information data sheet. A Certificate of Analysis (CoA) is provided to ensure the quality of each batch of antibodies.
Species, application validations and citations for primary antibodies can be found from the manufacturer's websites.
https://www.boster.com.cn/index/products/productsDetail?goods_sn=BM4935#yywx
https://www.boster.com.cn/index/products/productsDetail?goods_sn=BM4178
https://www.scbio.cn/zh/p/cd3-zeta-antibody-6b10-2
https://www.transgen.com/antibody_second/403.html
https://www.biolegend.com/en-us/products/apc-anti-human-cd268-baff-r-baffr-antibody-6705
https://www.biolegend.com/en-us/products/pe-anti-human-cd269-bcma-antibody-8446
https://www.biolegend.com/en-us/products/pe-anti-human-cd267-taci-antibody-3492
https://www.biolegend.com/en-us/products/apc-anti-human-cd3-antibody-749
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https://www.biolegend.com/en-us/products/fitc-anti-human-cd45-antibody-707
https://www.biolegend.com/en-us/products/pe-anti-human-cd22-antibody-723
https://www.biolegend.com/en-us/products/apc-anti-human-cd19-antibody-715
https://www.thermofisher.cn/cn/zh/antibody/product/Goat-anti-Mouse-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21235
https://www.thermofisher.cn/cn/zh/antibody/product/Goat-anti-Mouse-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-21237
https://www.abcam.cn/products/primary-antibodies/fitc-myc-tag-antibody-ab1263.html
https://www.sabbiotech.cn/g-192146-PKC-theta-Conjugated-Antibody-C33151.html

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	All human malignant hematologic cell lines were directedly obtained from the American Type Culture Collection (ATCC), including Nalm6, IM9, Raji, Jeko-1, MEC-1, RPMI8226, MM.1S, K562. HEK293T cells were purchased from Thermo Fisher Scientific. The FreeStyle 293 suspension cell line was obtained from Sino Biological Inc.
Authentication	Cell line authentication procedures were provided by their original providers, with Certificate of Analysis (CoA) provided. Properties pertinent cell lines to the study (CD19- or/and CD22-knockout Nalm6 cell lines, CD19-knockout Raji cell line) were further confirmed by flow cytometry and western blotting.
Mycoplasma contamination	All cell lines were tested routinely for mycoplasma and were negative.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in the study.

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	Six- to eight-week-old female NOD-Prkdc scidIl2rg em1/Smoc (NSG) mice were purchased from Shanghai Model Organisms Center, Inc.. Six- to eight-week-old female BALB/c mice were purchased from Zhejiang Vital River Laboratory Animal Technology Co., Ltd.. Other parameters included: ambient temperature 21 °C ± 1 °C, and humidity 40-70%, dark/light cycle 12/12 (6:00-18:00 light).
Wild animals	No wild animals were used in the study.
Reporting on sex	The construction of the disease model was independent of mouse sex. Female mice were used for the convenience of experimental operation.
Field-collected samples	No field collected samples were used in the study.
Ethics oversight	All in vivo experimental procedures were approved by the Peking University Shenzhen Graduate School Animal Care and Use Committee and performed according to the national and international guidelines for the ethical treatment of animals.

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

Plants

Seed stocks	*Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*
Novel plant genotypes	*Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*
Authentication	*Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*

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	Sample preparation was performed accordingly to the guidelines for Cell Surface Flow Cytometry Staining Protocol: https://www.thermofisher.cn/cn/zh/home/references/protocols/cell-and-tissue-analysis/protocols/staining-cell-surface-targets-flow-cytometry.html

Instrument	Attune NxT flow cytometer (Thermo Fisher Scientific)
Software	FlowJo_V10
Cell population abundance	Purity of cell population checked by flow cytometry
Gating strategy	For all experiments, FSC-H/SSC-H gating of the starting cell population was used to distinguish viable cells from cell debris. Single cell populations were detected using SSC-H/SSC-A or FSC-A/FSC-H gating.To exclude dead cells from the analysis, all samples were stained with 7-AAD staining (BD BioSciences, 51-68981E) prior to quantitative analysis. Positive and negative gates were determined based on isotype or WT control.

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