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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	Mass photometry data acquisition was conducted using AcquireMP software (Refeyn, Ltd, v1.2.3). In vivo imaging of fluorescence signal was performed using a Xenogen IVIS Imaging System 200 and the Living Image software (PerkinElmer, v4.8.0).
Data analysis	For FRET assays, flow cytometry, transwell invasion assays, viability assays and in vivo experiments, data were analyzed and plotted using the software GraphPad Prism 9. For X-ray data processing and analysis: data were processed with XDS and structures were solved by a combination of Se-SAD phasing in Phenix and molecular replacement with PHASER. Refinement was performed with Phenix. Iterative cycles of model building with Coot and restrained refinement were performed. Figures related to X-ray analyses of crystal structures were prepared using PyMOL (The PyMOL Molecular Graphics System, Schrödinger, LLC., v2.5.5). The mass spectrometry spectra were deconvoluted using Protein Deconvolution Software (Thermo Fisher Scientific, Waltham, MA, USA, v4.0). Mass photometry data were processed and analyzed with Discover MP software (Refeyn Ltd, v1.2.3). In vivo imaging data were analyzed with Living Image software (PerkinElmer, v4.8.0). Flow cytometry data was analysed using FlowJo (Becton Dickinson and Co., USA, v10.9.0). Image preparation and analysis for PET/SPECT was done using the PMOD software (PMOD technologies, version 3.709, Zurich, Switzerland).

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 atomic models related to this study have been deposited to the Protein Data Bank (PDB, https://www.wwpdb.org/) under accession codes 8PI3 and 8RND. Raw and analysed data associated to Figure 1 and Extended Data Figure 2, as well as NNPI sequences and original gel scans are provided as Source Data files.

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	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

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	For vavP-Bcl2 mice, the estimated effect size for the difference in the number of B-cells in the spleen of animals is large when comparing wild-type and genetically modified CTSS KO mice, which meant that a sample size of 3 mice per group could be enough to observe a significant difference in case of a full CTSS KO. Even though we were aiming to obtain the same phenotype, the experimental set up was different thus the sample size could not be directly transposed. Since we treated the mice with an inhibitor for a limited time-frame , starting only at 18-30 weeks after birth, we expected a smaller effect size when assessing the difference in tumor development. Since we were working with pharmacological inhibitors that are never 100% effective and can introduce additional variability, we hypothesized based on our experience, that 5 mice per treatment group would be sufficient to detect a significant difference between treatment groups if the inhibitor was effective. For the imaging of CTSS enzymatic activity, we did not find in the literature examples of in vivo comparison of cathepsin activity in wild-type versus cathepsin KO models or upon CTSS inhibitor treatment, so we could not estimate the effect size a priori. Based on similar experiments reported in the literature to study the activity of other cathepsins in vivo using the same molecular probe (Verdoes et al., J Am Chem Soc., 2013), we decided to use 3 mice per treatment group. For the biodistribution experiments, the number of animals was chosen based on what is suggested by the literature and normally used for these standard experiments (see for example Delage et al., Cancers, 2021).
Data exclusions	No data were excluded from the analyses.
Replication	All molecular, biochemical and cellular assays presented in this study were replicated independently twice or more, and the results of all the replicate experiments are described in the article.
Randomization	vavP-Bcl2 mice were randomized in the treatment groups without any knowledge about tumor development. They were allocated randomly to the treatment groups, ensuring that mice of comparable age were assigned to each group. For the imaging of CTSS enzymatic activity in vivo, allocation to treatment groups was not random but based on tumor volume, to have mice with similar tumor volumes in each group. Analogously, for the biodistribution experiments, allocation to treatment groups was not random but based on tumor volume, to have mice

with similar tumor volumes in each group.
No special randomization criterion was needed for our molecular, biochemical and cellular experiments.

Blinding

For the in vivo experiments, during data collection, blinding of the experimenter was not relevant because data was collected in parallel for all animals using the same appropriate instruments and keeping the same acquisition parameters for all samples, therefore data collection could not be biased.

For all the other experiments, data collection was performed for all samples in parallel with automatized instruments (e.g. SpectraMax iD3 plate reader for fluorescence detection), therefore blinding of the experimenter was not required.

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

PE mouse anti-human CD74 (clone Pin.1, Biolegend, cat #357604); BV711 rat anti-mouse CD74 (Clone In-1, BD Biosciences, cat #740748, diluted 1:200); APC-Cy7 rat anti-mouse CD45 (clone 30F11, BD Bioscience, cat #557659); PE-CF594 rat-anti mouse B220 (clone RA3-6B2, BD Bioscience, cat #562313); BV786 hamster anti-mouse CD3 (clone 145-2C11, BD Bioscience, cat #564379); PerCP efluor710 rat anti-mouse CD4 (clone RM4-5, Thermo Fisher Scientific, cat #46-0042-82); PE-Cy7 rat anti-mouse CD8a (clone 53-6.7, BD Bioscience, cat #552877).
Anti-his-Tag primary antibody (Cell Signaling, cat #D3I10); IRDye 800CW goat anti-Rabbit secondary IgG antibody (LICOR, cat #926-32211); goat anti-human Cathepsin S (Bio-Techne, cat #AF1183); rabbit anti-human Cathepsin B XP Rabbit mAb (clone D1C7Y, Cell Signaling, cat #31718), anti-His-Tag XP rabbit mAb (clone D3I1O, Cell Signaling, cat #12698), mouse anti-human alpha-Tubulin (clone B-5-1-2, Sigma Aldrich, cat #T6074); IRDye 680RD goat anti-Mouse IgG (LI-COR, cat #926-68070); goat anti-rabbit antibody (H+L) (Merck Millipore, cat #AP307P), goat anti-mouse antibody (H+L) (Merck Millipore, cat #AP308P), rabbit anti-goat IgG (H+L) (ThermoFisher scientific, cat #31402).

Validation

All the antibodies used in this study were validated for use in the context of the species of interest by the manufacturer. Furthermore, all the primary and secondary antibodies used are standard products already reported multiple times in the literature for the same applications, and we used them only for applications for which they had been validated.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

Mouse Diffuse Large B cell Lymphoma cell line A20 were obtained from ATCC (at #ATCC-TIB208, BALB/c background) .
A20-HA-GFP cell line expressing hemagglutinin (HA) gene from influenza virus strain A/Puerto Rico/8/1934 H1N1 (UniProtKB-P03452) were originally provided by Novimmune SA.
HEK293E cells were obtained from Life Technologies (Invitrogen).
ExpiCHO cells were obtained as part of the ExpiCho Expression System from Thermo Fisher Scientific (cat #A29133).
Human Raji, BT-474, Karpas-422, HCC-1569, SU-DHL-4, RT-4 and PC-3 cell lines were obtained from ATCC, whereas human WSU-DLCL2 were obtained from the DSMZ repository.

Authentication

The cell lines used in this study were authenticated by STR DNA profiling.

Mycoplasma contamination

The cell lines used in this study were tested for mycoplasma contamination when they were obtained and all tested negative.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used in this 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	In vivo experiments involving subcutanous injection of Raji cells were performed on 8-10-week-old NSG mice (NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ). For the experiments involving treatment of a transgenic lymphoma mouse model, vavP-Bcl2 mice were used and treatment was started when the mice were 21-30 weeks old. For the T-cell assay: HA-specific TCR CD8+ T cells were isolated from the spleen of CL4 TCR transgenic mice (8-12 weeks old). Mice were kept in a 12 hours-light / 12 hours-dark cycle, at 18-23°C with 40-60% ambient humidity, as recommended and in accordance with the regulations of the Animal Welfare Act (SR 455) and Animal Welfare Ordinance (SR 455.1).
Wild animals	The study did not involve wild animals.
Reporting on sex	In vivo studies were performed on female mice.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	All animal experiments were performed in accordance with the Swiss Federal Veterinary Office guidelines and as authorized by the Cantonal Veterinary Office (animal licenses VD2932.1, VD2993, VD3631 and VD3701).

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	For the T-cell assay: HA-specific TCR CD8+ T cells were isolated from the spleen of CL4 TCR transgenic mice. The spleen was smashed, the cells were washed in PBS, resuspended in RBC lysis buffer (Miltenyi Biotec, cat #130-094-183) for 4 minutes at room temperature to lyse red blood cells, PBS was added to stop lysis and cells were filtered through a 40 µm filter (Falcon, #352350) to generate a single cell suspension. Single cell suspension was then processed through a negative mouse CD8 T cell (Miltenyi Biotec, cat #130-104-075) isolation kit following the manufacturer's instructions. A20-HA lymphoma cells WT, Ctss KO, or B2m KO, expressing HA (hemagglutinin) as antigen were pre-treated with the indicated compounds for 16 hours and then co-incubated for 72 h with CellTrace Violet-stained (Thermo Fisher, cat #C34557) CD8+ T cells isolated from CL4-HA transgenic animals, in presence or absence of treatment. At the end of the lymphoma cells/T cells co-culture, cells were collected, stained with DAPI viability dye (Thermo Fisher, cat #D1306) and analyzed for the proliferation of T cells by quantifying CellTrace Violet proliferation peaks, using a LSR Fortessa flow cytometer (BD Biosciences). The absolute number of A20 lymphoma cells in each sample was also assessed through the use of CountBright Absolute Counting Beads (Thermo Fisher, cat #C36950), by comparing the ratio of bead events to cell events for each sample. For flow cytometry analysis of spleens harvested from in vivo experiments: the spleens were smashed, the cells were washed in PBS, resuspended in RBC lysis buffer (Miltenyi Biotec, cat #130-094-183) for 4 minutes at room temperature to lyse red blood cells, PBS was added to stop lysis and cells were filtered through a 40 µm filter (Falcon, #352350) to generate a single cell suspension. After cell counting, 2x10e6 cells were incubated with 50 µl of Fc Block (BD Biosciences, cat #553142) 1:50 solution in FACS buffer [PBS (Gibco/Thermofisher scientific, cat #10010-015), 2% BSA (Sigma Aldrich, cat #A7906] for 15 min

	on ice. Samples were subsequently supplemented with 50 µl of the following antibody mixture: APC-Cy7 rat anti-mouse CD45 (clone 30F11, BD Bioscience, cat #557659), PE-CF594 rat-anti mouse B220 (clone RA3-6B2, BD Bioscience, cat #562313), BV786 hamster anti-mouse CD3 (clone 145-2C11, BD Bioscience, cat #564379), PerCP efluor710 rat anti-mouse CD4 (clone RM4-5, Thermo Fisher Scientific, cat #46-0042-82), PE-Cy7 rat anti-mouse CD8a (clone 53-6.7, BD Bioscience, cat #552877). Samples were then washed three times, resuspended in 200 µl of FACS buffer containing DAPI viability marker (Molecular probes, cat #D1306), and analyzed using LSR Fortessa flow cytometer (BD Biosciences).
Instrument	LSR Fortessa flow cytometer (BD Biosciences)
Software	Flow cytometric analyses were performed with FlowJo V10.9.0 software (Tree Star, Ashland, OR USA).
Cell population abundance	Cell sorting was not performed in this study.
Gating strategy	The complete gating strategy is exemplified in the Supplementary Information. In brief, single cells were gated using FSC-H/FSC-A and SSC-H/SSC-A gating; then, live cells were gated using DAPI-negative gating; among live cells, leukocytes were then gated using CD45+ gating; among leukocytes, CD3/B220 gating allowed to separate T cells (CD3+) from B cells (B220+); finally, among CD3+ T cells, CD4/CD8 gating was used to identify CD4 T cells and CD8 T cells (as CD4+ and CD8+ cells respectively).

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