nature portfolio

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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	No software was used for data collection.
Data analysis	nSolver analysis software (v 4.0) was used to analyse gene-expression data according to NanoString-provided guidelines. Plots were generated in MATLAB (R2021b).

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 supporting the results in this study are available within the paper and its Supplementary Information. Source data for the main figures are provided with this paper. Sequencing data are available from the NCBI SRA with BioProject ID PRJNA923785. Cas9 plasmids are available on Addgene (ID# 196245 and 196246). All data, including the pre-processed nCounter data, are available from the corresponding authors on reasonable request.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	The study did not involve human research participants.
Population characteristics	—
Recruitment	—
Ethics oversight	—

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	Sample sizes were determined on the basis of preliminary experiments and/or experimental designs used in related studies.
Data exclusions	No data were excluded.
Replication	In vitro experiments used immune cells from 2, 3 or 4 healthy human donors. The capability of the A5K peptide to deliver RNPs was replicated across experiments. For all cell types investigated, there were no experiments in which the A5K peptide was not capable of delivering RNPs.
Randomization	After the first BLI measurement, which took place after NALM6 injection and before T-cell injection, mice were assigned to each T-cell condition so as to maintain a similar average mass and tumour burden across conditions.
Blinding	Blinding was not used. Experimental readouts were primarily instrument measurements such as NGS and flow cytometry.

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

Methods

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

Antibodies

Antibodies used	Antibodies are listed in Supplementary Table 3.
Validation	The antibodies were validated by the manufacturers.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	NALM6 cells were from the Justin Eyquem lab. A549 cells were provided by the Kole Roybal lab. Primary cells were derived from healthy human donors (female and male).
Authentication	The cell lines were not authenticated.
Mycoplasma contamination	Cell lines were not tested for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

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	Male NSG mice were between 8–12 weeks old at the start of the experiment.
Wild animals	The study did not involve wild animals.
Reporting on sex	The applicability of the findings is not sex-specific; no sex-based analysis was performed.
Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	The study was conducted in accordance with a protocol approved by the UCSF Institutional Animal Care & Use Committee.

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	Samples were prepared as described in Methods.
Instrument	Data were collected on an Attune NxT flow cytometer (Thermo Fisher Scientific) or an LSRFortessa X-50 flow cytometer (BD Biosciences; special order research product).
Software	Flow-cytometry data were collected using either Attune NxT software or FACSDiva software and analysed using FlowJo software.
Cell population abundance	Flow-activated cell sorting was not conducted.
Gating strategy	For T cells, lymphocytes were gated using FSC-A vs. SSC-A; single cells were gated using FSC-W vs. FSC-H followed by SSC-W vs. SSC-H; and live cells were gated as low for GhostDye Red 780. CD4+ and CD8+ cells were identified. Gating for phenotypes used CD62L vs. CD45RA. Gating for editing varied by the genes targeted for knockout and transgenes that were knocked in.

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