

Reporting Summary

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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 (<i>n</i>) 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☐	☒ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ 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 <i>d</i> , Pearson's <i>r</i>), 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 Spectrometry: Orbitrap Ascend Tune v4.0.4091, Vanquish Neo: v2.1.1.51769 Flow Cytometry: iQue Forecyt Standard Edition 9.0 (R1), ProSort Software, BD FACSCorus 3.0, BD FACSDiva Software v9.3.1 Plate Reader: SPARKCONTROL v3.2 Live-Cell Imaging: Incucyte 2024A Surface Plasmon Resonance: Biacore Insight Control v5.0.18.22102 Cryo-Electron Microscopy: Legikon 3.6, MotionCor2 v1.6.4, CTFFIND4 v4.1.14 Liquid chromatography: ChromLab 6.1.29.0
Data analysis	Mass Spectrometry: Proteome Discoverer v3.0.1.27, Skyline v23.1.0.455 Flow Cytometry: iQue Forecyt Standard Edition 9.0 (R1), FlowJo v10.10.0 Live-Cell Imaging: Incucyte v2024A Data Analysis: Prism version 10.5.0, Microsoft Excel Version 16 Surface Plasmon Resonance: Biacore Insight Evaluation v5.0.18.22102 Cryo-Electron Microscopy: cryo-SPARC v4.6.2, Coot 0.9.8, UCSF Chimera 1.18, UCSF Chimera X-1.10, PDBePISA 1.48, TCR3d

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](#)

Mass spectrometry data files have been deposited in the MassIVE repository with the dataset identifier MSV000097193. Supplementary Data 1 contains a file map of MS files.

The cryo-EM maps and structure data have been deposited in the Electron Microscopy Database and PDB (EMD-4936187, EMD-49362, PDBID 9NFB, and PDBID 9NFC).

This manuscript contains a Source Data file.

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](https://www.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	In vivo studies: (1) CDX model. No statistical method was used to predetermine sample size. Based on our experience with testing TCEs in CDX models and based on comparable studies cited in the manuscript (reference 36: Giffin, M. J. et al. AMG 757, a half-life extended, DLL3-targeted bispecific T-cell engager, shows high potency and sensitivity in preclinical models of small-cell lung cancer. Clin Cancer Res 27, 1526–1537 (2021)), we assumed that 10 mice per cohort ensured that statistically significant results were obtained and that experiments did not need to be repeated unnecessarily. Thus, the NCI-H2122 CDX model used 10 mice per group in the initial study (shown in Figure 4d-l and Supplementary Figure 13a-e), resulting in significant differences ($p < 0.05$) in tumor growth and tumor weight. The subsequent study with 2 different human T-cell donors used 10 and 4 mice per group (shown in Supplementary Figure 13f-h), respectively, resulting in significant differences ($p < 0.05$) in tumor growth for both $n=10/\text{group}$ and $n=4/\text{group}$. However, only the study with $n=10/\text{group}$ resulted in a significant difference ($p < 0.05$) in tumor weight. (2) PK/PD study. No formal sample size calculation was performed. Based on our experience with antibody therapeutics, we assumed that blood drawn from 3 treated tumor-bearing mice and tested at 6 different time points (for blood PK) and tumors resected from 3 additional tumor-bearing mice and tested at 4 different time points (for tumor PK) would provide robust PK profiles with low standard deviation. This was achieved as shown in Supplementary Figure 12b. In vitro studies: For validity and reproducibility of the immunological studies, 4-6 different human T-cell donors were used (summarized in Supplementary Tables 1 and 2).
Data exclusions	In vivo studies: No mice were excluded from the analysis. In vitro studies: All biological and technical replicates were included with the exception of Figure 3a and Supplementary Figures 14f and 7b-c, where outliers were excluded. Outliers were defined as data points that were outside 1.5*IQR or were IncuCyte images (Supplementary Figure 14f) which contained an imaging error. These exclusion criteria were not pre-established.
Replication	In vivo studies: (1) CDX model. The initial study (shown in Figure 4d-l and Supplementary Figure 13a-e) was independently repeated and successfully reproduced using 2 different human T-cell donors (shown in Supplementary Figure 13f-h). (2) PK/PD study. Both blood and tumor PK profiles were based on mean $\pm$ standard deviation data of 3 biological replicates. In vitro studies: The number of biological and technical

replicates as well as the methods for determining significance are listed in the figure legends.

Randomization

For all in vivo studies involving tumor-bearing mice, subcutaneous xenograft size was measured prior to assigning mice to treatment groups, ensuring each group had a comparable average tumor size. In vitro studies: Not applicable.

Blinding

The Investigators were not blinded to allocation during experiments and outcome assessment.

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

Anti-human HLA-A3 (PE) (clone GAP-A3, 1:200, BD Pharmingen, #566605)
 Anti-human Vinculin (clone 42H89L44, 1:1000, Thermo Fisher, #700062)
 Anti-mouse Pan-Ras (clone Ab-3, 1:1000, MilliporeSigma, #OP40)
 Goat anti-rabbit IgG (HRP) (BioRad, 1:10,000, #1706515)
 Goat anti-mouse IgG (HRP) (BioRad, 1:10,000, #1706516)
 Anti-human CD3 (BB515) (clone SK7, 1:50, BD Pharmingen, #564560)
 Anti-human CD8 (RR780) (clone RPA-T8, 1:50, BD Pharmingen, #568684)
 Anti-human CD4 (PerCP-Cy5.5) (clone SK3/Leu3a, 1:50, BD Pharmingen, #566923)
 Anti-human CD25 (PE) (clone M-A251, 1:100, BioLegend, #985802)
 Anti-human CD69 (APC) (clone FN50, 1:100, BioLegend, #985206)
 Zombie Aqua Fixable Viability Kit (1:60, BioLegend, #423102)
 Anti-human TCR α/β (APC) (clone IP26, 1:10, BioLegend, #306718)
 Anti-human CD4 (APC) (clone RPA-T4, 1:200, BioLegend, #300518)
 Anti-human HLA-DR (PerCP-eFluor710) (clone L243, 1:20, Invitrogen, #46-9952-42)
 Anti-human CD25 (BV421) (clone M-A251, 1:60, BioLegend, #356114)
 Anti-human CD69 (BV711) (clone FN50, 1:40, BioLegend, #310944)
 Anti-human CD8a (BV650) (clone T8, 1:200 BioLegend, #301042)
 Anti-human/mouse/rat CD278 (ICOS) (PE) (clone C398.4A, 1:60, BioLegend, #313532)
 Anti-mouse CD16/32 (TruStain FcX PLUS) (clone S17011E, 1:100, BioLegend, #156604)
 Anti-human FOXP3 (PE) (clone 259D, 1:20, BioLegend, #320208)
 Fixable Viability Dye (eFluor 780) (Invitrogen, 1:2000, #65-0865-14)
 Goat anti-human IgG (HRP) (Jackson ImmunoResearch, #109-035-088)
 Anti-human B2m (PE) (clone 2M2, 1:500, BioLegend, #316305)
 Anti-human MHC Class I (clone W6/32, 100 μ g/5x10⁷ cells, BioXCell, #BE0079)
 AETX-R001, AETX-R023, AETX-R114, AETX-R302 (made in house, reported in this manuscript)

Validation

Clone GAP-A3 is a validated HLA-A3 binding antibody, reported and validated by AE Berger et al., Hybridoma, 1982, and validated by others. GAP-A3 was used to stain for HLA-A3 expression using flow cytometry by S Paul et al., Sci Transl Med., 2021 and others.
 Clone AB-3 is a validated RAS-binding antibody, reported and validated by P.J. Hamer et al., Hybridoma, 1990, and validated by others. AB-3 has been used as a western-blot antibody by E.O. dos Santos et al., Mol Cancer., 2016 and others.
 Clone SK7 was validated to bind CD3 by van Dongen et al., Blood, 1988 and others.
 Clone RPA-T8 has been shown to bind CD8 by S Jeong, J Virol, 200; Kmiecik, J Transl Med, 2009, and others.
 Clone SK3/Leu3a has been validated by R.L. Evans et al., PNAS, 1981; E.G. Engleman et al., J Exp. Med., 1981, and others to bind CD4.
 Clone M-A251 has been demonstrated to bind CD25 by M Abd Hamid et al., Cancer Immunol Res., 2019, and others.
 Immune cell staining utilizing clones SK7, RPA-T8, SK3, and M-A251 refers to guidance from Cossarizza et al., Eur J Immunol, 2024, which reports guidelines for immune cell phenotyping of human and murine immune cell subsets.
 Clone 2M2 has been used for B2M detection by E Jenkins et al., Nat Commun, 2023 (flow cytometry), Stopfer et al., Nat Commun, 2020 (ELISA), and others. Clone FN50 has been used for CD69 detection via flow cytometry by S Zhang et al., Nat Commun, 2021, C Yang et al., Nat Commun, 2021, and others. Clone IP26 has been used for CD3+ T cell staining by KR James et al., Nat Immunol, 2020, AS Bear et al., Nat Commun 2021, and many others. Clone RPA-T4 binds to the D1 domain of CD4 and has been used for flow cytometry staining by JC Charpentier., Nat Commun, 2020 et al and others. Clone 398.4A has been used for ICOS staining of T

cells by EA Kuczynski et al., EMBO Mol Med, 2022 and others. Clone S17011E is commonly used to block non-specific binding of immunoglobulin to FC receptors, and has been used by Y Wang et al., Nature, 2021, Y Jiang et al., Nat Commun, 2021, and many others. Clone 259D has been used for FOXP3 staining of T cells by E Weiss et al., J Immunol, 2011 and others. Clone W6/32 has been validated to bind human HLA-A/B/C by M.J. Shields and RK Ribaud, Tissue Antigens, 1998; P Pymm et al., J Immunol, 2024, and others. W6/32 has been used extensively for immunoprecipitations and surface staining of HLA molecules by flow cytometry (Choi Goulding et al., Cell Reports Methods, 2021, J.G. Abelin et al., Immunity, 2017). Clone L243 has been validated to bind a conformational epitope on HLA-DRa by M Maro et al., BMC Immunol., 2005.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	NCI-H2122 (CRL-5985), NCI-H2030 (CRL-5914), A375 (CRL-1619), and Raji (CCL-86) cells were purchased from the ATCC. HOP-62 cells were provided by the National Cancer Institute's Division of Cancer Treatment and Diagnosis (NCI-DCTD) Tumor Repository.
Authentication	Cell lines purchased for use in this study were authenticated by the vendor. All edited cell lines were authenticated through ATCC's STR Profiling Service.
Mycoplasma contamination	All cell lines were tested regularly for mycoplasma contamination (Lonza, MycoAlert Mycoplasma Detection Kit).
Commonly misidentified lines (See ICLAC register)	N/A

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	NOD SCID mice obtained from The Jackson Laboratory (JAX Mice Strain: NOD.Cg-Prkdcscid/J; stock number 001303). Mice were 8 weeks old at the time of study initiation.
Wild animals	n/a
Reporting on sex	Matching the XX genotype of the xenografted NCI-H2122 cancer cell line, only female mice were used.
Field-collected samples	n/a
Ethics oversight	All in vivo procedures were approved by the Institutional Animal Care and Use Committees of ATP R&D (IACUC #ATP-105) and Mispro (IACUC #2024-AET-01) and were performed according to the NIH Guide for the Care and Use of Laboratory Animals (https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf).

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

Plants

Seed stocks	n/a
Novel plant genotypes	n/a
Authentication	n/a

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	Detailed description of the sample preparation methods for flow cytometry of yeast cells, live T cells, live immune cell proportions in PBMCs, and HLA expression of tumor cells is described in detail in the manuscript methods section.
Instrument	BD FACSymphony A3, IntelliCyt iQue3, Melody FACS, Bio-Rad S3e
Software	Data collection: : iQue Forecyt Standard Edition 9.0 (R1), ProSort Software, BD FACSCorus 3.0, BD FACSDiva Software v9.3.1 Data analysis: iQue Forecyt Standard Edition 9.0 (R1), FlowJo v10.10.0 Live-Cell Imaging: Incucyte v2024A
Cell population abundance	Sorting was used for HLA isogenic cell line generation, where post sort fractions of HLA-A3 negative cell lines were isolated as single cells and clonally expanded.
Gating strategy	Exemplary gating strategies for all flow cytometry experiments is depicted in Supplementary Figure 19a-d.

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