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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	For all CyTOF analyses, all data were acquired using Helios™ at the Sidney Kimmel Comprehensive Cancer Center in Baltimore, Maryland. For preprocessing of CyTOF data, randomization, bead-based normalization, and bead removal of data were performed in CyTOF software (Standard Biotools™) v6.7 followed by gating of cell events (event length vs. 103Rh) and viability (Platinum 194-198) in FlowJo (BD Biosciences) v10.5. Individual samples were debarcoded by hierarchal gating (three positive and two negative CD45 axes) and exported as separate FCS files for analysis. RNA sequencing (RNA-seq) of pre- and post-treatment biopsy specimens was performed using the Personalis NeXT Dx™ Tumor Profiling platform (PMID: 26269718). This assay includes whole-exome and transcriptome sequencing with coverage across ~20,000 genes (median sequencing depth ~500×) and enhanced coverage of 247 cancer-related genes (median sequencing depth >1500×). RNA was extracted from formalin-fixed, paraffin-embedded (FFPE) or fresh-frozen tumor specimens and processed for library preparation using the manufacturer's proprietary protocols. Paired-end sequencing was performed on an Illumina platform. Raw gene expression files, including raw gene counts, FPKM, CPM, and TPM, were provided by Personalis and used for downstream analyses. TIRTL-seq analyses were performed as described previously [PMID: 39345544] as detailed in Methods. Final TCR libraries were sequenced on Illumina NovaSeq platform. Flow cytometry data were acquired on either a BD FACS Aria cell sorter or a BD Symphony flow cytometer using BD FACSDiva software. For 10x Genomics scRNAseq, libraries GEX, TCR, and surface feature (cell hashing) libraries were prepared according to the manufacturer's protocol. Libraries were sequenced on Illumina NovaSeq at read length 2x100bp for GEX libraries and 2x100-150bp for TCR and surface feature libraries.
Data analysis	For all CyTOF analyses, a computational pipeline based on diffcyt2 was employed using R. For unsupervised clustering, the FlowSOM3 algorithm was used to identify meta clusters that were then annotated and merged into final cell subtypes based on published literature.

Analysis of RNA sequencing data analysis was performed in R version 4.4.2. Gene expression data from RNA sequencing were analyzed to assess changes in antigen presentation machinery gene expression between pre- and post-treatment biopsy specimens. A curated list of antigen presentation genes was selected from the full transcriptome dataset (Personalis NeXT Dx™ platform). Raw transcript per million (TPM) values were extracted for these genes and reshaped for analysis. For each gene across each patient, log10 fold change of on-treatment relative to baseline expression was calculated. A heatmap was generated using pheatmap in R to visualize log10 fold change by patients.
TIRTL-seq TCR repertoire analyses: Raw fastq files from each well were processed with mixcr - v 4.6.03 package using the analyze tool. Optional switches were set to analyze the 'generic- amplicon' preset with floating-left-alignment-boundary, floating-right-alignment-boundary for C segment and split-by-sample. All other parameters were set to default. Tag-pattern switch was used to match and split the samples based on plate barcodes incorporated during the PCR I step. Processing steps included demultiplexing raw reads by plate barcode, aligning V, D and J segments, assembling identical reads into clonotypes and frequency-based error-correction. TCR pairs were called from TIRTL-seq data as described previously [PMID: 39345544] using T-SHELL and reimplemented MAD-HYPE algotrithms. To identify expanded/contracted clones between timepoints with TIRTL-seq we first average relative frequency (defined as sum of reads mapping to given CDR3β divided by sum of reads mapping to all CDR3βs) across all wells in a plate, including wells where the clone is not present. We filter out clones found in less than 5 wells in either time point to limit the number of comparisons. For each clonotype we calculated standard
error of the mean on both timepoints, and called clones significantly expanding/contracting if log2fold change between average frequencies on two timepoints is larger than 3 and 2.5 SEM intervals do not overlap. If clone has very low frequency on one of the timepoints and found in fewer than 3 wells we assumed that SEM is twice SEM of clones found in 3 wells at this time point, and pseudocount of 10^-6 was added to all clonal frequencies to get reasonable fold change estimates. TCR similarity networks were constructed by first measuring the the distance between TCR α/β clonotypes using the TCRdist algorithm
implementation from the conga python package [PMID PMID: 34426704]. Further analysis was performed using the R language for statistical computing, with merging and subsetting of data performed using the
data.table and dplyr packages. stringdist and igraph R packages were used to build TCR similarity networks, and gephi software was used for TCR similarity network layout and visualization.
Hierarchical decision tree analysis: Decision tree analysis was carried out with the rpart R package. The model was built with classification method "Class"; parameters were controlled by setting a minimum of 3 observations to attempt a split and a complexity parameter of 0.1.
For 10x Genomics scRNAseq, raw data were processed using CellRanger version 7.0.1 and aligned to
GRCh38-2020-A and vdj_GRCh38_alts_ensembl-7.0.0 Cell Ranger references. The resulting GEX matrices were analyzed using the Seurat R package version 5.0.17, and associated TCR clonotypes were added to each Seurat object via the AddMetaData function. Following standard quality control filtering, we discarded low quality cells (nFeatures<200 or >7500, MT% >5%) and non-T cells (CD3E<0), and normalized the data via the NormalizeData function using the default parameters. We then performed cell cycle regression to mitigate effects of cell cycle heterogeneity using the CellCycleScoring and ScaleData functions and found 2000 variable features via the FindVariableFeatures function. Cells were clustered with the resolution parameter set to 0.5. UMAP feature plots were visualized using Seurat.

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 raw RNA sequencing data, 10x genomics scRNAseq data, and raw TIRTL-seq will be publicly available in dbGaP repository (accession number phs003970)
The remaining data are available within the Article, Supplementary Information 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	Three(18.8%) of the patients were female. Both male and female participants could be included in the trial. The findings from this trial don't apply to only one gender.
Reporting on race, ethnicity, or other socially relevant groupings	Fifteen patients were White and 1 was Hispanic or Latin. Race and ethnicity data were based on self-report data. Baseline demographics are provided in the Table 1.
Population characteristics	All patient demographics are shown in detail in Table 1. in summary, we enrolled patients at least 12 years old with confirmed diagnosis of fibrolamellar hepatocellular carcinoma that that was metastatic or unresectable; median age was 23.5 (range 12-47).
Recruitment	Eligible patients were 12 years of age or older, had an Easter Cooperative Oncology Group performance status score of q o1 (on a 5-point scale in which higher numbers reflect greater disability), and had adequate hematological, renal, and hepatic function. Eligible patients had histologically and molecularly confirmed fibrolamellar hepatocellular carcinoma, locally advanced or metastatic; participants were also required to have measurable disease . Patients with a history of active autoimmune disease or uncontrolled intercurrent illness were excluded. The attached Clinical protocol provides full details on inclusion and exclusion criteria.

Ethics oversight	All procedures were conducted in accordance with the Declaration of Helsinki and the International Conference on Harmonization Guidelines for Good Clinical Practice. The protocol and all amendments were reviewed by the scientific review committee and institutional review board at the Johns Hopkins Sidney Kimmel Cancer Center. All patients provided written informed consent before enrollment.

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	This is an open-label, pilot study trial of neoantigen-targeted vaccine targeting the DNAJB1- PRKACA fusion kinase (FLC peptide vaccine) in combination with nivolumab and ipilimumab. The primary endpoint for safety is percent of adverse events by grade and for immunogenicity of the vaccine is the fold change in IFN-γ-producing DNAJB1-PRKACA chimeric transcript-specific CD8 and CD4 T cells at 10 weeks after vaccination compare to pre-vaccination baseline. The evaluable population of the endpoint of T cell response consists of all patients who receive at least one dose of vaccine and have baseline and post-treatment T cell measures at 10 weeks. Previous studies showed 2-4 fold increase of IFN-γ-producing T cells after vaccination. We anticipated that our regimen will result in at least 2.5-fold increase of IFN-γ-producing DNAJB1-PRKACA chimeric transcript-specific CD8 and CD4 T cells 10 weeks with treatment in each cohort. We assume, conservatively, that the coefficient of variation of IFN-γ-producing DNAJB1-PRKACA chimeric transcript-specific T cell density is 100%, and the correlation of the pre- and post-treatment measure is weak at 0.2. In each cohort, a sample size of 12 evaluable patients provided 81% power to detect a 2.5-fold increase based on a two-sided paired-sample t-test at significance level 0.05.
Data exclusions	No data were excluded from the analysis
Replication	N/A - The clinical trial was a non-randomized, single arm study. Correlative analyses were based on all samples available.
Randomization	N/A - The clinical trial was a non-randomized, single arm study.
Blinding	The clinical trial was a single arm study and response outcome were not blinded to the investigators during data collections or analyses

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	Antibodies used include: anti-human CD28 (BD Biosciences 555725, clone CD28.2, 1 ug/mL), anti-human CD49d (BD Biosciences 555501, clone 9F10, 1 ug/mL), anti-human CD40 (Miltenyi 130-094-133, clone HB14, 1 ug/mL), anti-human Fc block (Biolegend 422302, 1:50), TotalSeq-C anti-human hashtag antibody 5 (Biolegend 394609, 1:50), TotalSeq-C anti-human hashtag antibody 7 (Biolegend 394613, 1:50), TotalSeq-C anti-human hashtag antibody 8 (Biolegend 394615, 1:50), TotalSeq-C anti-human hashtag antibody 10 (Biolegend 394619, 1:50), anti-human CD3 (Brilliant Violet 421-conjugated, Biolegend 344834, clone SK7, 1:20), anti-human CD8 (Brilliant Violet 785-conjugated, Biolegend 344740, clone SK1, 1:20), anti-human CD4 (PerCP/Cy5.5-conjugated, Biolegend 344608, clone SK3, 1:20), anti-human CD154/CD40L (Brilliant Violet 605-conjugated, Biolegend 310826, clone 24-31, 1:20), anti-human CD137/4-1BB (APC-conjugated, Biolegend 309810, clone 4B4-1, 1:20), anti-human CD19 (APC/Fire750-conjugated,

Biolegend 302258, clone HIB19, 1:20), anti-human TCRαβ (BV780, Thermo Fisher Scientific 78-9986-42, clone IP26, 1:100), anti-pan-HLAII antibody (5 µg/1x106 cells; US Biological 367160, clone IVA12), anti-HLA-DP (5 µg/1x106 cells; Abcam ab20897, clone B7/21), anti-HLA-DQ (1 µg/1x106 cells; Novus Biologicals NBP2-45041, clone SPV-L3), anti-HLA-DR (5 µg/1x106 cells; Biolegend 307666, clone L243), anti-HLA-I (Clone EMR8-5), anti-HLA-II (Clone CR3/43), anti-CD3 (Clone LN10), anti-TIM3 (Clone BLR033F), anti-LAG3 (Clone 17B4), Cd-110 CD45 (Standard Biotools 3110001B, clone HI30, 1:200), Cd-111 CD45 (Standard Biotools 3111001B, clone HI30, 1:200), Cd-112 CD45 (Standard Biotools 3112001B, clone HI30, 1:200), Cd-113 CD45 (Standard Biotools 3113001B, clone HI30, 1:200), Cd-114 CD45 (Standard Biotools 3114001B, clone HI30, 1:200), Ir-115 CD45 (Biolegend 304002, clone HI30, 1:200), Cd-116 CD45 (Standard Biotools 3116001B, clone HI30, 1:200), Pr-141 CCR6 (Standard Biotools 3141003A, clone G034E3, 1:120), Nd-142 CD19 (Standard Biotools 3142001B, clone HIB19, 1:100), Nd-143 HLA-DR (Standard Biotools 3143013B, clone L243, 1:200), Nd-144 CCR5 (Standard Biotools 3144007A, clone NP-6G4, 1:120), Nd-145 CD4 (Standard Biotools 3144007A, clone RPA-T4, 1:200), Nd-146 CD8 (Standard Biotools 3146001B, clone RPA-T8, 1:200), Sm-147 CD7 (Standard Biotools 3147006B, clone CD7-6B7, 1:200), Nd-148 CD14 (Standard Biotools 3148010B, clone RMO52, 1:100), Sm-149 CD25 (Standard Biotools 3149010B, clone 2A3, 1:400), Nd-150 OX40 (Standard Biotools 3150027B, clone ACT35, 1:200), Eu-151 CD2 (Standard Biotools 3151003B, clone TS1/8, 1:400), Sm-152 TNFa (Standard Biotools 3152002B, clone Mab11, 1:133), Eu-153 TIM3 (Standard Biotools 3153008B, clone F38-2E2, 1:100), Sm-154 TIGIT (Standard Biotools 3154016B, clone MBSA43, 1:100), Gd-155 CD56 (Standard Biotools 3155008B, clone B159, 1:400), Gd-156 PDL1 (Standard Biotools 3156026B, clone 29E3.2A3, 1:100), Gd-158 IL2 (Standard Biotools 3158007B, clone MQ1-17H12, 1:133), Tb-159 CCR7 (Standard Biotools 3159003A, clone G043H7, 1:120), Gd-160 CD28 (Standard Biotools 3160003B, clone CD28.2, 1:200), Dy-161 CTLA4 (Standard Biotools 3161004B, clone 14D3, 1:264), Dy-162 Foxp3 (Standard Biotools 3162011A, clone PCH101, 1:133), Dy-163 CXCR3 (Standard Biotools 3163004B, clone G025H7, 1:120), Ho-165 CD45RO (Standard Biotools 3165011B, clone UCHL1, 1:100), Er-166 NKG2D (Standard Biotools 3166016B, clone ON72, 1:200), Er-167 CD27 (Standard Biotools 3167002B, clone O323, 1:200), Er-168 IFNg (Standard Biotools 3168005B, clone B27, 1:66), Tm-169 CD45RA (Standard Biotools 3169008B, clone HI100, 1:400), Er-170 CD3 (Standard Biotools 3170001B, clone UCHT1, 1:200), Yb-171 Gzmb (Standard Biotools 3171002B, clone GB11, 1:66), Yb-172 Ki67 (Standard Biotools 3172024B, clone B56, 1:66), Yb-173 41BB (Standard Biotools 3173015B, clone 4B4-1, 1:200), Yb-174 PD1 (Standard Biotools 3174020B, clone EH12.2H7, 1:100), Lu-175 Lag3 (Standard Biotools 3175033B, clone 11C3C65, 1:100), Yb-176 CD127 (Standard Biotools 3176004B, clone A019D5, 1:100), Pt-194-198 Cell-ID Cisplatin (Standard Biotools 201064, clone , 1:1000), Bi-209 CD16 (Standard Biotools 3209002B, clone , 1:100), Y-89 CD45 (Cell Signaling Technology®, clone D9M8I, 1:125). In-113 Collagen (Cell Signaling Technology®, clone E8F4L, 1:250), In-115 E-cadherin (Cell Signaling Technology®, clone 24E10, 1:125), Pr-141 αSMA (Standard BioTools™, clone 1A4, 1:500), Nd-142 Podoplanin (Biolegend®, clone D2-40, 1:125), Nd-143 Vimentin (Standard BioTools™, clone D21H3, 1:500), Nd-144 CD14 (Standard BioTools™, clone EPR3653, 1:125), Nd-145 CD45RO (Biolegend®, clone UCHL1, 1:250), Nd-146 CD16 (Standard BioTools™, clone EPR16784, 1:100), Sm-147 CD163 (Standard BioTools™, clone EDHu-1, 1:125), Nd-148 Pan-Keratin (Standard BioTools™, clone C11, 1:125), Sm-149 CD137 (Cell Signaling Technology®, clone D2Z4Y, 1:250), Nd-150 PD-L1 (Cell Signaling Technology®, clone E1L3N, 1:125), Eu-151 PD1 (Cell Signaling Technology®, clone D4W2J, 1:125), Sm-152 CD57 (Standard BioTools™, clone NK/804, 1:250), Eu-153 Tox/Tox2 (Cell Signaling Technology®, clone E6I3Q, 1:250), Sm-154 DC-LAMP (Novus Biologicals, clone 1010E1.01, 1:250), Gd-155 FoxP3 (Standard BioTools™, clone PCH101, 1:75), Gd-156 CD4 (Standard BioTools™, clone EPR6855, 1:125), Gd-158 pSTAT3 (Cell Signaling Technology®, clone D3A7, 1:62.5), Tb-159 CD68 (Standard BioTools™, clone KP1, 1:100), Gd-160 Syndecan 1 (CD138) (Cell Signaling Technology®, clone IHC138, 1:125), Dy-161 CD20 (Standard BioTools™, clone H1, 1:125), Dy-162 CD8α (Standard BioTools™, clone C8/144B, 1:250), Dy-163 CD21 (Biolegend®, clone Bu32, 1:250), Dy-164 ARG1 (Standard BioTools™, clone D4E3M, 1:75), Ho-165 CD33 (LS Bio, clone 44M12D3, 1:125), Er-166 CD45RA (Standard BioTools™, clone HI100, 1:250), Er-167 Granzyme B (Cell Signaling Technology®, clone D6E9W, 1:125), Er-168 Ki-67 (Standard BioTools™, clone B56, 1:250), Tm-169 PTPN22 (Atlas Antibodies, clone Polyclonal, 1:62.5), Yb-170 CD3ε (Standard BioTools™, clone Polyclonal, C-terminal, 1:125), Yb-171 Lag3 (GeneTex, clone 17B4, 1:125), Yb-172 CD15 (Biolegend®, clone SSEA-1, 1:100), Yb-173 DC-SIGN (Ionpath, clone DCN46, 1:62.5), Yb-174 HLA-DR (Standard BioTools™, clone LN3, 1:250), Lu-175 CD86 (Cell Signaling Technology®, clone E2G8P, 1:125), Yb-176 CD206 (Cell Signaling Technology®, clone E2L9N, 1:125), Pt-195 Plasma Membrane 2 (Standard BioTools™, clone 1A36, 1:250), Pt-196 Plasma Membrane 3 (Standard BioTools™, clone 1A37, 1:250), Pt-198 Plasma Membrane 4 (Standard BioTools™, clone 1A38, 1:250)

Validation

The following manuscripts detail antibody validation and protocols for IHC: Tsujikawa, Takahiro, et al. "Quantitative multiplex immunohistochemistry reveals myeloid-inflamed tumor-immune complexity associated with poor prognosis."Cell reports. 19.1 (2017): 203-217.

Banik, Grace, et al. "High-dimensional multiplexed immunohistochemical characterization of immune contexture in human cancers."Methods in enzymology635 (2020): 1-20.

Liudahl SM, Betts CB, Sivagnanam S, et al. "Leukocyte Heterogeneity in Pancreatic Ductal Adenocarcinoma: Phenotypic and Spatial Features Associated with Clinical Outcome." Cancer Discov. 2021;11(8):2014-2031. doi:10.1158/2159-8290.CD-20-0841

All antibodies used for flow cytometry were validated for use in flow cytometry and have relevant validation flow plots available on the manufacturer's website.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

E6.1 Jurkats containing the pASP90 reporter system (TCR-null, constitutive mCherry, NFAT-eGFP reporter) were obtained from The University of Pennsylvania.
Bear, A.S., Blanchard, T., Cesare, J. et al. Biochemical and functional characterization of mutant KRAS epitopes validates this oncoprotein for immunological targeting. Nat Commun 12, 4365 (2021). https://doi.org/10.1038/s41467-021-24562-2
2D3 Jurkats containing an NFAT-eGFP reporter were obtained from Fumihiro Fujiki ,Department of Cancer Immunology, Osaka University Graduate School of Medicine, Suita, Japan.
Morimoto S, Fujiki F, Kondo K, et al. Establishment of a novel platform cell line for efficient and precise evaluation of T cell receptor functional avidity. Oncotarget. 2018;9(75):34132-34141. doi:10.18632/oncotarget.26139

Authentication

E6.1 Jurkats were confirmed to be TCR null, express mCherry and when activated with PMA/ionomycin express eGFP via flow cytometry.
2D3 Jurkats were confirmed to be TCR null and when activated with PMA/ionomycin express GFP as measured via flow

	cytometry. No further authentication was performed.
Mycoplasma contamination	None identified, mycoplasma testing last performed on 03/2025
Commonly misidentified lines (See ICLAC register)	No commonly misindentified cell lines were used in this study.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	NCT04248569
Study protocol	Protocol is available as Supplementary information
Data collection	This was an open-label, single arm phase 1 clinical trial of a DNAJB1PRKACA vaccine in combination with nivolumab and ipilimumab, in children or adults with advanced FLC (NCT03299946), conducted at the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, Maryland. Patients were enrolled between April 2020 and September 2022.
Outcomes	The primary trial endpoints focused on Safety, defined as the frequency of toxicities by grade by documenting the drug-related adverse events using the NCI CTCAE Version 5.0, and The T cell response evaluated by the fold change in interferon-producing DNAJB1-PRKACA chimeric transcript-specific CD8 and CD4 T cells after vaccination at 10 weeks compare to pre-vaccination baseline. Secondary endpoints include objective response rate (ORR) assessed by RECIST 1.1), duration of response (DoR) among subjects who demonstrate an objective response to treatment; disease control rate (DCR); progression-free survival (PFS) and overall survival (OS)

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	For AIM assay and sorting for 10x Genomics scRNAseq: Patient PBMCs were expanded in vitro as described in the Methods. After antigen-specific expansion, expanded cells were restimulated with either 1 µM and 1 µg/mL each of anti-human CD28 (BD Biosciences 555725), CD49d (BD Biosciences 555501), and CD40 (Miltenyi 130-094-133) made up in complete RPMI (RPMI 1640, 10% FBS, 2mM L-glutamine, 100 U/mL/100 µg/mL penicillin/streptomycin). An unstimulated negative control condition (complete RPMI, CD28, CD49d, CD40) was included for each pool. Cells were incubated at 37 °C, 5% CO2, for 6 hours. After incubation, IFNγ-producing cells were labeled using an IFNγ Cytokine Secretion Assay Kit (Miltenyi 130-054-202) according to the manufacturer's instructions. Cells were then washed with FACS buffer (PBS, 2% FBS, 2 mM EDTA), blocked using anti-human Fc block (Biolegend 422302) for 10 minutes at 4 °C, and stained with a cocktail of surface antibodies for 30 minutes at 4 °C. This staining cocktail included 2.5 µL Ghost Violet 510 viability dye (Tonbo Biosciences 13-080-T100), 2 µL TotalSeq-C anti-human hashtag antibody 5, 7, 8, or 10 (one per stimulation condition; Biolegend 394609, 394613, 394615, 394619), and 5 µL each of anti-human CD3 (Brilliant Violet 421-conjugated, Biolegend 344834, clone SK7), anti-human CD8 (Brilliant Violet 785-conjugated, Biolegend 344740, clone SK1), anti-human CD4 (PerCP/Cy5.5-conjugated, Biolegend 344608, clone SK3), anti-human CD154/CD40L (Brilliant Violet 605-conjugated, Biolegend 310826, clone 24-31), anti-human

CD137/4-1BB (APC-conjugated, Biolegend 309810, clone 4B4-1), and anti-human CD19 (APC/Fire750-conjugated, Biolegend 302258, clone HIB19). After staining, cells were washed three times with FACS buffer.

For NFAT-GFP reporter TCR screening assays: TCR-expressing Jurkat cells were cocultured with autologous lymphoblastoid cell lines at a 1:1 Jurkat:LCL ratio or with healthy donor PBMCs at a 1:5 Jurkat:PBMC ratio. For HLA blocking assays, LCLs were blocked with anti-pan-HLAII antibody (5 µg/1x10^6 cells; US Biological 367160, clone IVA12), anti-HLA-DP (5 µg/1x10^6 cells; Abcam µg/1x10!^6 cells; Novus Biologicals NBP2-45041, clone ab20897, clone B7/21), anti-HLA-DQ (1 SPV-L3), or anti-HLA-DR (5 µg/1x10! cells; Biolegend 307666, clone L243) in cRPMI for 2 hours (37 °C, 5% CO") prior to coculture with Jurkats. Each well was pulsed with a stimulation cocktail containing either 10 µM FLC fusion peptide fused to HIV-tat to enhance uptake and presentation (RKREIFDRYGEEVKEFLAKAKEDF-RKKRRQRRR, Genscript) or one of six tiling 15mer peptides crossing the fusion junction (REIFDRYGEEVKEFL, IFDRYFGEEVKEFLAK, DRYGEEVKEFLAKAK, RYGEEVKEFLAKAKE, YGEEVKEFLAKAKED, or GEEVKEFLAKAKEDF; Genscript) made up in complete RPMI (RPMI 1640, 10% FBS, 2mM L-glutamine, 100 U/mL/100 µg/mL penicillin/streptomycin), an unstimulated negative control cocktail (complete RPMI), or PMA/Ionomycin positive control (1X Cell Stimulation Cocktail [eBioscience] in complete RPMI).Cells were incubated overnight (37 °C, 5% CO2), then washed with FACS buffer (PBS, 2% FBS, 2 mM EDTA) after incubation before flow cytometry analysis.

Instrument

For AIM assay and sorting for 10x Genomics scRNAseq: BD Biosciences FACS Aria cell sorter.

For NFAT-GFP reporter TCR screening assay: BD Symphony flow cytometer using FACSDiva software.

Software

All flow cytometry data were analyzed using FlowJo v 10.10.0 (BD Biosciences)

Cell population abundance

Viability of samples was determined using Ghost Dye Violet 510 (Tonbo Biosciences 13-0870-T100). For scRNAseq experiments, sorter counts and viability of live, CD3+, AIM+ cells were confirmed using a hemocytometer. For NFAT-GFP reporter TCR experiments, TCR-transgenic Jurkats and relevant antigen presenting cells were counted prior to coculture using a Vi-Cell BLU automatic cell counter. Abundance of each population is reported as frequency of parent gate.

Gating strategy

For AIM assay and sorting for 10x Genomics scRNAseq: An initial gate using FSC-A and SSC-A was used to select all cells. Live cells were identified with a Ghost Dye 510 (Tonbo Biosciences) gate. Singlets were identified using a SSC-A vs SSC-w gate. CD3 + T cells were identified by gating on a clear discrete BV421-CD3+ population. AIM+ cells were selected using an L-shaped gate covering both single- and double-positive cells on a plot of PE-IFNg vs BV605-CD40L.

For NFAT-GFP reporter TCR screening assays: An initial gate using FSC-A and SSC-A was used to select all cells. Singlets were identified using a FSC-A vs FSC-H gate. Transduced T cells were identified by gating s clear discrete population of mCherry+ cells. The gate on NFAT-GFP to define activated cells was defined using no peptide negative controls and PMA-Ionomycin positive control samples.
We have checked the box to indicate that gating strategy is shown but please note that it is in the primary figure (3G) rather than the supplementary.

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