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Reporting Summary

Nature Research 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 Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	BD FACSDiva 8.0.2
Data analysis	bcl2fastq (version 2.18.0.12) STAR RNA-seq aligner (version 2.5.3a) LOHHLA (release 20180126) fastq-multx (part of ea-utils version 1.1.2-806) BWA-MEM (version 0.7.10) Picard tools (version 2.18.4) MiTCR (version 1.0.3) TCRprimer (version 5) Graphad Prism (version 7.03) Flowjo (version 10.0.7) HALO (version 2.2.1870.17) R (version 3.4.1)

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/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

DNA and RNA sequencing data have been deposited in the European Genome-phenome Archive (EGA) under accession code EGAS00001003119 and are subject to a controlled Data Access Agreement. These data are available from the corresponding authors to any party able to comply with the associated Data Access Agreement.

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	The number of patients and TCRs analyzed in this proof-of-concept work were reasonably limited by the current costs of TCR gene library synthesis. For each patient, we modeled the likelihood that the observed data are representative of the entire intratumoral TCR repertoire, and we present the respective likelihood intervals in the manuscript.
Data exclusions	No data were excluded from the analyses.
Replication	Every TCR selected for functional analysis was tested for reactivity against autologous tumor material at least twice. TCRs are presented as tumor-reactive only if they demonstrated reactivity to tumor in both replicate experiments.
Randomization	For each tumor sample analyzed, approximately 20 TCRs were randomly selected from identified TCRs (but included recurrent TCRs) and used for functional analysis.
Blinding	No experimental groups were employed in this work, and blinding was therefore not possible nor relevant.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☐	☒ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☐	☒ Human research participants

Methods

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

Unique biological materials

Policy information about availability of materials

Obtaining unique materials	Sequences and DNA preparations of unique patient-derived TCRs isolated in this work are available from the corresponding author upon request. Because of restrictions imposed by the informed consent form used for this study, the primary tumor specimens used in this study are not available for distribution.

Antibodies

Antibodies used	anti-CD3 (Alexa-Fluor 700; clone UCHT1; Life Technologies; catalog nr. CD0329; dilution 1:30) anti-CD8a (FITC; clone SK1; BD Biosciences; catalog nr. 345772; dilution 1:30) anti-CD8beta (Brilliant Blue 700; clone 2ST8.5H7; BD Biosciences; catalog nr. 745761; dilution 1:100) anti-PD-1 (PE; clone eBIOJ105; eBioscience; catalog nr. 12-2799-42; ; dilution 1:100) anti-LAG-3 (APC; R&D Systems; catalog nr. FAB2319A; dilution 1:75) anti-TIM-3 (PE-Cy7; clone F38-2E2; Biolegend; catalog nr. 345008; dilution 1:100) anti-CD137 (Brilliant Violet 421; clone 4B4-1; Biolegend; catalog nr. 309819; dilution 1:200) anti-CD103 (Brilliant Violet 711; clone Ber-ACT8; BD Biosciences; catalog nr. 563162; dilution 1:50) anti-CD45RO (PE-CF594; clone UCHL1; BD Biosciences; catalog nr. 562327; dilution 1:200) anti-mouse TCRβ constant domain (PE; clone H57-597; BD Biosciences; catalog nr. 553172; dilution 1:150) anti-mouse TCRβ constant domain (APC; clone H57-597; BD Biosciences; catalog nr. 553174; dilution 1:150) anti-IFNγ (PE or APC; clone B27; BD Biosciences; catalog nr. 554701 or 554702; dilution 1:150) anti-CD69 (APC; clone FN50; BD Biosciences; catalog nr. 555533; dilution 1:30) anti-CD3 (unconjugated; clone SK7; Spring/ITK; catalog nr. 344802; dilution 1:100) anti-CD8a (unconjugated; clone C8/144B; Dako; dilution 1:200) anti-PD–L1 (unconjugated; clone 22C3; Dako) anti-MHC-I (unconjugated; clones HC10; Nordic-Mubio; dilution 1:20,000) anti-MHC-I (unconjugated; clones HCA2; Nordic-Mubio; dilution 1:5,000)
Validation	Anti-CD3, anti-CD8a, anti-CD8b, anti-PD-1, anti-LAG-3, anti-Tim-3, anti-CD137, anti-CD103, anti-CD45RO, anti-IFNg, and anti-CD69 antibodies for flow cytometry were validated and titrated by staining PHA-stimulated and unstimulated human PBMCs. Anti-mouse TCRbeta constant domain was validated and titrated on TCR-transduced versus non-transduced human T cells.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Melanoma cell lines Mel063 and NKIRTIL064 were generated from patients treated at the Netherlands Cancer Institute. FLY-RD18 and Jurkat cell lines were commercially obtained from Sigma and ATCC, respectively.
Authentication	All cell lines were validated functionally: FLY-RD18 cells by their capacity to produce retrovirus, Jurkats by CD3 expression and their capacity to express CD69 upon activation, and patient cell lines by their capacity to serve as antigen-presenting cells for cognate T cell clones or TCRs.
Mycoplasma contamination	Cell lines were tested negative for Mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Human research participants

Policy information about studies involving human research participants

Population characteristics	Patient: NKIRTIL083; sex: male; age: 49; primary tumor location: skin of right shoulder; histology type: superficial spreading melanoma; MSI/MSS status: N/A; stage: Clark level 4; treatment: none; TMB: N/A; source: Netherlands Cancer Institute. Patient: OVC21; sex: female; age: 76; primary tumor location: ovary; histology type: high grade serous carcinoma; MSI/MSS status: N/A; stage: T1N0M0; treatment: none; TMB: 252; source: Netherlands Cancer Institute. Patient: OVC48; sex: female; age: 74; primary tumor location: ovary; histology type: high grade serous carcinoma; MSI/MSS status: N/A; stage: p3C; treatment: none; TMB: 75; source: BC Cancer Agency. Patient: CRC11; sex: female; age: 38; primary tumor location: colon (sigmoid); histology type: moderately differentiated adenocarcinoma; MSI/MSS status: MSS; stage: T3N0M0; treatment: none; TMB: 254; source: Netherlands Cancer Institute. Patient: CRC15; sex: male; age: 44; primary tumor location: colon (caecum); histology type: moderately differentiated adenocarcinoma; MSI/MSS status: MSS; stage: T4N2M1; treatment: none; TMB: 97; source: Netherlands Cancer Institute.
Recruitment	Treatment-naïve patients were selected to avoid potentially confounding effects of prior (chemo)therapy. Patients were not further selected for any particular tumor feature.

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	Resected tumor specimens were mechanically disrupted and digested overnight in RPMI 1640 medium supplemented with 1 mg/ml collagenase type IV, penicillin-streptomycin, and 0.01 mg/ml pulmozyme, and cryopreserved in liquid nitrogen. Before single cell sorting of CD8+ TIL, samples were thawed in RPMI 1640medium supplemented with 10% human serum and benzonase. Samples were stained with fluorescent antibodies in FACS buffer (PBS supplemented with 1% bovine serum albumin and 0.05% sodium azide), washed with FACS buffer, and resuspended in PBS for cell sorting. TCR-transduced T cell cultures as well as samples from coculture experiments were stained similarly, but were resuspended in FACS buffer before measurement.
Instrument	Single cell sorting was performed on a MoFlo Astrios sorter (Beckman Coulter), all other analyses were performed on Fortessa or LSRII flow cytometers (BD Biosciences).
Software	Summit 6.2 was used during single cell sorting on the MoFlo Astrios sorter. BD FACSDiva 8.0.2 and Flowjo 10.0.7 were used for the acquisition and analysis of the other flow cytometry experiments.
Cell population abundance	Sorted single intratumoral CD8+ T cells were directly used in TCR amplification by PCR, and assessment of purity after sorting was therefore not possible.
Gating strategy	For single cell sorting of intratumoral CD8+ T cells, stained total tumor populations were first gated based on FSC-A/SSC-A ("lymphocyte gate"), followed by gating on single cells based on SSC-A/SSC-H, gating on live cells based on IRDye, and finally on CD3+CD8+ cells. For measuring coculture experiments with TCR-transduced T cells and tumor material, cell populations were gated similarly as above, but with the final gate on CD8+mouseTCRbeta+ (instead of CD3+CD8+).

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