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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 (*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	Microsoft Excel, Microsoft Access
Data analysis	inForm v2.4.2 (Akoya Biosciences), HALO v2.3 (Akoya Biosciences), TIBCO Spotfire v7.8 (TIBCO), immunoSEQ Analyzer 3.0 (Adaptive Biotechnologies) and R 3.5.1 (The R project). All code underlying the analysis is available upon request from the corresponding author.

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. 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

TCR sequencing data that support the findings of this study have been deposited at the publicly available immuneACCESS platform under this URL https://clients.adaptivebiotech.com/pub/pruessmann-2019-nc and this DOI 10.21417/WP2019NC.
Imaging source data is available upon request from the corresponding author.
Numerical source data for Fig. 3c, 7a-d and 8 have been provided as Source Data files Kupper_SourceData_Fig.3c, ta-d and 8, respectively. All other data supporting the findings of this study are available from the corresponding author on reasonable request.
Referenced accessions: https://clients.adaptivebiotech.com/pub/weber-2018-cir, https://github.com/riazn/bms038_analysis
No restrictions on data availability.

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	At least 150 FFPE samples were required to achieve ≥80% statistical power with a type I error probability of 5% for a range of potential effect sizes (HR: 0.5 – 0.8; Suppl. Fig. 1). An interim analysis found that the success of TCRB sequencing depended on the age of the FFPE sample, and many of our samples dated back almost two decades (median 9 years). We therefore conservatively estimated that 30-50% might fail TCR sequencing quality controls and targeted a total collection of 300 primary melanomas, ultimately collecting 337.
Data exclusions	All of these exlusion criteria were pre-established. For the database searches we applied the following criteria: 1. Patients with single primary melanomas stage I – III at time of initial diagnosis. 2. Complete excision biopsies. 3. Similar number of patients per tumor thickness group: 1-2mm (T2), 2-4mm (T3), >4mm (T4). 4. Patients were selected for 50% deceased from melanoma : 50% alive per thickness category. Reasons for excluding any identified patient after the database search included: block could not be accessed, no tumor tissue left on block during visual inspection prior to cutting 5x10µm scrolls, no tumor tissue left on block after cutting. We now provide a flow scheme about the case selection in the supplement material of the manuscript and have clarified the methods section. For analytical robustness, TCR sequencing data was analyzed for T cell fraction (TCFr) if samples had a minimum of 500 total nucleated cells (n = 199) and were also analyzed for their repertoire clonality if they also had a minimum of 100 T cells (n = 158). These sample adequacy s reflect the DNA quality of the input material to be able to generate sufficient output such that metrics are robust across samples. T-cell fraction was calculated as the proportion of T cells in a sample relative to its total number of nucleated cells. Simpson clonality was calculated according to equation Simpson clonality= √(∑p_i^2) where p_i is the proportional abundance of clone i in a sample's repertoire and ranges in value from 0 to 1; a value closer to 0 indicates a highly polyclonal repertoire and a value closer to 1 indicates a highly oligoclonal repertoire. Only 17 samples failed internal QC methods, which are proprietary to Adaptive and are based on internal spike-in of a synthetic repertoire. Patients without follow up data for progression-free survival were excluded.
Replication	T cell fraction was an independent prognostic factor for progression-free survival in training and test group. TCFr HR ratios were within each other's 95% confidence interval (train group: HR 0.84 per 0.1 increase, CI 0.73 - 0.97, p = 0.0227, LRT p = 0.01; test group: HR 0.77 per 0.1 increase, CI 0.65 - 0.92, p = 0.00339, LRT p = 6.00E-4). TCFr was also predictive when applied to external validation cohorts of metastatic melanoma patients (https://clients.adaptivebiotech.com/pub/weber-2018-cir, https://github.com/riazn/bms038_analysis). Immunofluorescence staining of a subset of our FFPE samples (n=20) visualised abundant numbers of CD3+ T cells in samples with high TCFr but only few infiltrating T cells for tumors with low TCFr. Multiplex Immunohistochemistry (n=57) revealed correlation between TCFr and CD3 + T cell count.
Randomization	To reduce bias in selecting a TCFr that optimally identifies patients at risk for recurrence, we implemented a number of data partitioning methods (Fig. 4a). First, the cases were randomly partitioned into two groups, training (n = 100) and testing (n = 99), in a manner that preserved the proportions of tumor thickness and progressors in the aggregated collection of cases. We then bootstrapped the training group by randomly subsampling 80% of the data 100x. For each subsample, a grid search was performed across seven TCFr values (0.05, 0.1, 0.15, 0.2, 0.25, 0.3, 0.35) and the precision-recall F-score, true-positive rate (TPR: proportion of patients below the cut-off who experienced disease recurrence), and true-negative rate (TNR: proportion of patients above the cut-off who remain disease-free) were calculated for predicting disease progression at five years post-resection. The precision-recall F-score was chosen over the area under the receiver-operator curve due to its robustness against imbalanced data labels. The TPR (and not the TNR) was prioritized alongside the F-score as part of our heuristic for cutoff selection to ensure at-risk patients are identified. We then calculated the first and second order derivatives of the F-score and TPR versus the TCFr cut-off and selected the first (i.e., lowest) cutoff where the second order derivatives approximated zero. The chosen cutoff was then applied to the test group and considered valid if the Cox proportional-hazard model yielded a p-value ≤0.05, indicating a significant difference in progression-free survival intervals between patients above versus below the cutoff.
Blinding	All samples with usable TCRB HTS results were regraded for tumor infiltrating lymphocytes (TILs) by an experienced dermatopathologist (MCM) who was "blinded" to the identity of the samples. One diagnostic H+E stained slide was used and two different grading systems applied: (a) TIL briskness assessment originally proposed by Clark et al. 9 which differentiates between absent, non-brisk and brisk TIL infiltration (n = 199); (b) a recently proposed grading by the Melanoma Institute Australia which distinguishes four grades (0 to 3) based on density (mild, moderate or marked) and distribution (focal, multifocal or diffuse) of lymphocytes in the dermal tumor component (n = 153).

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
☒	☐ Animals and other organisms
☐	☒ Human research participants
☐	☒ Clinical data

Methods

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

Antibodies

Antibodies used

Antibodies used for IF staining: Primary rabbit anti-CD3 antibody (1:150, A0452, polyclonal, Dako, lot # 00052829) and mouse (mIgG1) anti-Sox10 antibody (1:25, ACI3099, clone BC34, BioCare Medical, lot # 070318). Secondary Alexa Fluor 594 conjugated goat anti-rabbit IgG antibody (1:1000, A11012, polyclonal, Invitrogen, lot # 1420898) and Alexa Fluor 488 conjugated goat anti-mIgG1 antibody (1:1000, A21121, polyclonal, Invitrogen, lot # 1820808).

Antibodies used for mIHC (further details Supplement Table 4):
Listed as follows: Antibody, Source, Clone, Manufacturer, Lot number, Dilution
FoxP3, Mouse, 236A1E7, Abcam, GR3268573-1, 1:1000
CD103, Rabbit, EPR4166(2), Abcam, GR3185003-11, 1:800
CD8, Rabbit, C8/144B, Dako, 20066516, 1:1000
CD4, Mouse, 4B12, Biocare Medical, 07231, 1:400
CD3, Rabbit, MRQ-39, Cell Marque, 0000054576, 1:2000
CD39, Rabbit, EPR20627, Abcam, GR3234300-1, 1:2000

Validation

IF primary antibodies:

CD3: https://www.citeab.com/antibodies/3382891-a0452-cd3

SOX-10: Quality Control: Refer to CLSI Quality Standards for Design and Implementation ofImmunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2)CLSI Wayne, PA USA (www.clsi.org). 2011. References: References: [1] Mohamed A, et al. SOX10 Expression in malignant melanoma, carcinoma, andnormal tissues. Appl Immunohistochem Mol Morphol. 2013 Dec; 21(6):506-10. [2] Pusch C, et al. The SOX10/Sox10 gene from human and mouse: sequence,expression, and transactivation by the encoded HMG domain transcription factor. HumGenet. 1998 Aug; 103(2):115-23. [3] Mollaaghababa R, Pavan WJ. The importance of havingyour SOX on: role ofSOX10 in the development of neural crest-derived melanocytes and glia. Oncogene.2003 May 19; 22(20):3024-34. [4] Bondurand N, et al. Expression of the SOX10 gene during human development.FEBS Lett. 1998 Aug 7; 432(3):168-72. [5] Bannykh SI, et al. Oligodendroglial-specific transcriptional factor SOX10 isubiquitously expressed in human gliomas. J Neurooncol. 2006 Jan; 76(2):115-27. [6] Britsch S, et al. The transcription factor Sox10 is a key regulator of peripheral glialdevelopment. Genes Dev. 2001 Jan 1; 15(1):66-78. [7] Feng Z, et al. Incidence and survival of desmoplastic melanoma in the United States,1992–2007. J Cutan Pathol. 2011 Aug; 38(8):616-24. [8] Center for Disease Control Manual. Guide: Safety Management, NO. CDC-22,Atlanta, GA. April 30, 1976 "Decontamination of Laboratory Sink Drains to RemoveAzide Salts." [9] Clinical and Laboratory Standards Institute (CLSI). Protection of LaboratoryWorkers from Occupationally Acquired Infections; Approved Guideline-Fourth EditionCLSI document M29-A4 Wayne, PA 2014.

mIHC primary antibodies:

FoxP3: https://www.citeab.com/antibodies/731387-ab20034-anti-foxp3-antibody-236a-e7?des=CBEE772CA3D27E22
CD103: https://www.citeab.com/antibodies/767425-ab129202-anti-cd103-antibody-epr4166-2?des=C45154040C2AE8A9
CD8: https://www.citeab.com/antibodies/2414791-m7103-cd8-concentrate?des=C57AEC823C0F1585

CD39:
Validated by Abcam for the following tested applications as listed on their website:
Application Abreviews Notes
WB, 1/1000. Detects a band of approximately 78 kDa (predicted molecular weight: 57 kDa).
IHC-P, 1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

CD3:
Denning SM, et al. Activation of human thymocytes via CD3 and CD2 molecules. In: McMichael AJ, et al. eds. Leucocyte Typing III, White Cell Differentiation Antigens. Oxford-New York-Tokyo. Oxford University Press. 1987; 144-7.
Beverley PC, et al. Distinctive functional characteristics of human "T" lymphocytes defined by E rosetting or a monoclonal anti-T cell antibody. Eur J Immunol. 1981; 11:329-34.
Clevers H, et al. The transmembrane orientation of the epsilon chain of the TcR/CD3 complex. Eur J Immunol. 1988; 18:705-10.
Campana D, et al. The cytoplasmic expression of CD3 antigens in normal and malignant cells of the T lymphoid lineage. J Immunol. 1987; 138:648-55.
Hedvat CV, et al. Application of tissue microarray technology to the study of non-Hodgkin's and Hodgkin's lymphoma. Hum

Pathol. 2002; 33:968-74.
Karube K, et al. Non-B, non-T neoplasms with lymphoblast morphology: further clarification and classification. Am J Surg Pathol. 2003; 27:1366-74.
Dogan A, et al. Micronodular T-cell/histiocyte-rich large B-cell lymphoma of the spleen: histology, immunophenotype, and differential diagnosis. Am J Surg Pathol. 2003; 27:903-11.
Axdorph U, et al. T-cell-rich B-cell lymphoma - diagnostic and therapeutic aspects. APMIS. 2002; 110:379-90.

CD4:
1. Leong A S-Y, Cooper K and Leong F J W-M eds. Manual of diagnostic antibodies for immunohistology, second edition 2003. Greenwich Medical Media Ltd: p. 65-6.
2. Izban KF, Hsi ED, Alkan S. Immunohistochemical analysis of mycosis fungoides on paraffin-embedded tissue sections. Mod Pathol. 1998 Oct; 11(10):978-82.
3. Macon WR, Salhany KE. T-cell subset analysis of peripheral T-cell lymphomas by paraffin section immunohistology and correlation of CD4/CD8 results with flow cytometry. Am J Clin Pathol. 1998 May; 109(5):610-7.
4. Uchiyama N, et al. CD2-, CD4+, CD56+ agranular natural killer cell lymphoma of the skin. Am J Dermatopathol. 1998 Oct;20(5):513-7.
5. Gordon SN, et al. Disruption of intestinal CD4+ T cell homeostasis is a key marker of systemic CD4+ T cell activation in HIV-infected individuals. J Immunol. 2010 Nov 1;185(9):5169-79.
6. Rathore AS, et al. CD3+, CD4+ & CD8+ tumour infiltrating lymphocytes (TILs) are predictors of favourable survival outcome in infiltrating ductal carcinoma of breast. Indian J Med Res. 2014 Sep;140(3):361-9.
7. Center for Disease Control Manual. Guide: Safety Management, NO. CDC-22, Atlanta, GA. April 30, 1976 “Decontamination of Laboratory Sink Drains to Remove Azide Salts.”
8. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.

Human research participants

Policy information about studies involving human research participants

Population characteristics	Supplement Table 1 lists all the patient characteristics for training and test group as well as the overall cohort. Overall patients had a median age of 66yrs (IQR 55 - 74 yrs, range 28 - 92 yrs), 68.2% were female. All of them were diagnosed with primary malignant melanoma, tumors were excised without any prior (neoadjuvant) therapy. If patients presented with positive regional lymph nodes at initial diagnosis, these were also completely resected. After surgery all patients were classified as disease-free. Because we only investigated progression-free survival (= recurrence of disease either locally or metastatically), we do not have any information about treatment regimens after surgery. At intial diagnosis 25.1% T1, 37.7% T2, and 37.2% T3 melanomas; 10.6% were Stage I, 48.7% Stage II and 40.7% Stage III (as classified according to 8th AJCC). 45% were ulcerated and 95.5% had >0 mitoses/mm2. Disease recurred in 46.2% of the cases, 37.7% died due to melanoma. For further details on co-variates please see Supplement Table 1.
Recruitment	Utilizing institutional databases, we identified archival primary melanomas with a tumor thickness > 1mm. All melanomas had been wide exitional biopsies. Samples were formalin-fixed and paraffin-embedded (FFPE) for routine histopathological examination and had been stored for 2-19 years. Patients were monitored for disease recurrence. Samples were derived from the Melanoma Institute Australia, Sydney, Australia, n = 271; Brigham and Women’s Hospital, Boston, USA, n = 18; and Zealand University Hospital, Roskilde, Denmark n = 48. All patients gave written informed consent prior to inclusion in the study.
Ethics oversight	Institutional review boards at all three collection centers: namely IRB of Brigham and Women's Hospital, Boston, Massachusetts, USA; IRB of Royal Prince Alfred Hospital, Camperdown, Australia and Ethics comittee of Zealand University Hospital, Roskilde, Denmark.

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

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	Not a clinical trial
Study protocol	Not a clinical trial
Data collection	Patients were monitored for disease recurrence and data was collected at all of their follow up visits. Patient metadata (age, sex, tumor thickness, ulceration status, mitotic rate, anatomic site, tumor type, nodal disease, sentinel lymph node (if applicable), PFS, OS) was derived from the Melanoma Institute Australia, Sydney, Australia, n = 271; Brigham and Women's Hospital, Boston, USA, n = 18; and Zealand University Hospital, Roskilde, Denmark n = 48.
Outcomes	Progression-free survival (PFS) is defined as the time from surgical resection of the primary melanoma

Outcomes

and any possible lymph node metastases, during which all patients of this study were classified as disease free, to disease progression or death from any cause. Overall survival (OS) is the same, except death from any cause rather than disease progression is used.