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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.

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	Cell Ranger 1.0.0 – Barcode Identification, Alignment, Filter, Deduplication
Data analysis	macs2 2.1.1.20160309 – Peak Calling R version 3.5.1 – R environment for all custom code Motifmatchr 1.2.0 – Matching TF Motifs within peak regions Seurat_2.3.4 – SNN Graph Clustering Implementation GenomicFeatures 1.32.2 – Genomic Ranges Operations used for overlap analyses GenomicRanges 1.32.7 - Genomic Ranges Operations used for overlap analyses Matrix 1.2-14 – Sparse Matrix math implementations. BSgenome 1.48.0 – Toolkit used for getting Genomic DNA sequences for motif matching and footprinting. Rsamtools 1.32.3 – For manipulating BAM files within R. Irlba 2.3.2 – Running PCA/SVD on large matrices. Reticulate 1.10 – Used for running Python UMAP implementation within R. Rcpp 1.0.0 – Used for writing helpful C++ code to speed up operations. Rtsne 0.13 – Used for t-SNE embeddings. matrixStats 0.54.0 – Used for mathematical operations on large matrices. Cicero 1.0.13 – Used for calculating gene activity scores. chromVAR_1.2.0 – Calculating TF deviation scores which can be associated with TF activity. SingleCellExperiment 1.2.0 – R Data Class Environment used throughout analyses. Loupe Cell Broswer 1.0 – Used to visualize scATAC-seq data. Custom code for main analyses used in this work has been deposited on Github: https://github.com/GreenleafLab/10x-scATAC-2019.

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

All single-cell sequencing data are available through the Gene Expression Omnibus (GEO) under accession GSE129785. There are no restrictions on data availability or use.

Additionally, species mixing and PBMC datasets are available in pre- and post-processed formats here: https://support.10xgenomics.com/single-cell-atac/datasets.

WashU browser sessions of aggregated scATAC-seq data (by cluster, as shown in each Figure) are available here:
Figure 2 single-cell clusters-
http://epigenomegateway.wustl.edu/legacy/?genome=hg19&session=HcbHMSgBCc&statusId=28207718
Figure 4 single-cell clusters-
http://epigenomegateway.wustl.edu/legacy/?genome=hg19&session=tYJVrV7zzk&statusId=834543265
Figure 5 single-cell clusters-
http://epigenomegateway.wustl.edu/legacy/?genome=hg19&session=7UZG0iF90b&statusId=807471043

WES data from patients SU006 and SU008 were obtained from the Sequence Read Archive (SRA) under accession PRJNA533341.

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 chosen to provide sufficient confidence to validate the performance of single-cell ATAC-seq on cell lines and primary human samples. For cell lines, sample sizes were calculated in order to test technical reproducibility and the range of loading concentrations on the 10X Chromium platform. For primary healthy immune cells, sample sizes were calculated to sample each cell population from at least 3 individual volunteers with at least 200 cells per cell cluster. The rationale for obtaining 200 cells per cluster is presented in Supplementary Fig 1. For BCC cells, sample sizes were mainly determined by the availability of primary human tissues. However, we also ensured that multiple patients were included for pre- and post-therapy timepoints, and for responder and non-responder outcomes.
Data exclusions	No inclusion or exclusion criteria were used for human studies. No data were excluded from the manuscript.
Replication	All results presented in manuscript were reliably reproduced.
Randomization	No randomization of human participants was used.
Blinding	No blinding was used.

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 on blood cells included anti-CD45RA-PERCPCy5.5 (clone HI100, cat. no. 304107, lot no. B213966, Biolegend), anti-CD4-APC-Cy7 (clone OKT4, cat. no. 317417, lot no. B207751, Biolegend), anti-CD25-FITC (clone BC96, cat no. 302603, lot no. B168869, Biolegend), anti-CD11C-PECy7 (clone B-ly6, cat. no. 561356, lot no. 4125556, BD Biosciences), anti-HLA-DR-APCCy7 (clone G46-6, cat. no. 335796, BD Biosciences), anti-CD123-BV421 (clone 6H6, cat. no. 306018, lot no. B156518, Biolegend), anti-CD3-FITC (clone OKT3, cat. no. 11-0037-41, lot no. 2007722, Invitrogen), and anti-CD19-AlexaFluor 488 (clone HIB19, cat. no. 302219, lot no. B238185, Biolegend). Antibodies used on BCC cells included anti-CD45 V500 (clone HI30, cat. no. 560779, lot no. 7172744, BD Biosciences), anti-CD3 FITC (clone OKT3, cat. no. 11-0037-41, lot no. 2007722, Invitrogen), anti-CD8 Pacific Blue (clone 3B5, cat. no. MHCD0828, lot no. 1964935, Invitrogen), anti-PD-1 APC/Cy7 (clone EH12.2H7, cat. no. 329921, lot no. B245235, BioLegend), and anti-HLA-DR eVolve 605 (clone LN3, cat. no. 83-9956-41, lot no. 1949784, Affymetrix-Ebioscience). All antibodies were used at a 1:200 dilution, with the exception of anti-CD45 and anti-HLA-DR antibodies which were used at a 1:100 dilution.

Validation

All antibodies were validated by the manufacturer directly in human peripheral blood mononuclear cells, and antibody-specific staining was compared to isotype and no staining control samples.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

GM12878 cells were obtained from the Coriell Institute. A20 cells were obtained from ATCC (TIB-208).

Authentication

Both cell lines were immediately used for experiments after acquisition from the commercial source and were not further authenticated.

Mycoplasma contamination

All cell lines tested negative for mycoplasma contamination prior to use in experiments.

Commonly misidentified lines
(See ICLAC register)

None of the cell lines used in this study are listed in this database.

Human research participants

Policy information about studies involving human research participants

Population characteristics

Healthy human subjects were male and female, ages 30-50. BCC patients were male and female, ages 50-75. BCC patients were previously diagnosed with locally advanced or metastatic BCC and not previously treated with any immunotherapeutic agent.

Recruitment

No selective recruitment of healthy subjects was performed. BCC samples were obtained from patients at Stanford Hospital and Clinics with locally advanced or metastatic BCC. These patients were not eligible for surgical removal of their tumor and therefore were treated with PD-1 blockade. BCC is extremely rare in adults under the age of 40 and children under the age of 18. Therefore, the research topic is not directly relevant to children. This study did not attempt to exclude healthy volunteers or patients based on sex, race, or ethnicity.

Ethics oversight

This study was approved by the Stanford University Administrative Panels on Human Subjects in Medical Research, and written informed consent was obtained from all participants.

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