nature portfolio

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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: No software was used for data collection

Data analysis	ellRanger v6.l.1 (l0x Genomics) CellBender v.0.2.0 Seurat v4.0.l Slide-Seq V2 tools GISTIC v2.0 ichoCNA v0.2.0 control-FREEC vll.6 SCENIC 0.11.2 RCTDvl.2.0 R v4.0.2 Python v3.8.3 scanpy 1.7.2 scvi v0.8.1 Seurat 4.0.1 STACAS 1.1.0 velocyto 0.17.17 Code is publicly available via: https://github.com/IzarLab/fresh_vs_frozen_comparison and https://github.com/azizilab/starfysh

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

Processed data is available on GEO accession number: GSE192402. Reviewer Toke kbcpociyhhuzdmp. Raw data will be available on dbGAP: accession number phs003097.v1.p1. Code is publicly available via: https://github.com/IzarLab/fresh_vs_frozen_comparison and https://github.com/azizilab/starfysh

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	Available/collected data were collected and were reported in supplemental table
Population characteristics	Available/collected data were collected and were reported in supplemental table
Recruitment	n/a
Ethics oversight	Columbia University, UCLA, Dana-Farber Cancer Institute approved the study protocol.

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	No samples size calculation was performed. The presented analyses were performed to demonstrate technical feasibility
Data exclusions	No data were excluded.
Replication	Technical replicates were performed for snRNA-seq were feasible.

Randomization	No randomization was performed as no intervention was performed.
Blinding	No blinding was performed as no intervention was performed.

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

Methods

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

Antibodies

Antibodies used	Pacific-Blue-aCD45 (Biolegend, #304022) The cutaneous melanoma sample was stained with the following (all Biolegened): Human TruStain FcX (#422302) Pacific-Blue-aCD45 (#304022) PE-Dazzle594-aCD3 (#300450) PE-CY7-aCD66b (#305116) APC-aCD15 (#301908)
Validation	All antibodies were previously validated by the manufacturer(s).

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	To enrich viable immune cells (primary uveal melanoma sample) or sort viable immune and non-immune cells (cutaneous melanoma) the samples were sorted using a FACS Aria II (BD Biosciences) gating on viable CD45+ (immune) or viable CD45- (non-immune) cells (Extended Data Fig. 1a). First, cells were stained for viability (Zombie NIR, 1:500 in PBS; Biolegend, San Diego, Ca; #423106) for 10 min at room temperature in the dark. Thereafter, cells were washed once with sorting buffer, collected by centrifugation and surface antigens were stained for 15 minutes on ice in the dark. The primary uveal melanoma sample was stained with Pacific-Blue-aCD45 (Biolegend, #304022). The cutaneous melanoma sample was stained with the following (all Biolegened): Human TruStain FcX (#422302), Pacific-Blue-aCD45 (#304022), PE-Dazzle594-aCD3 (#300450), PE-CY7-aCD66b (#305116), APC-aCD15 (#301908). After staining, the samples were washed twice with ice-cold sorting buffer and 1.5x103 cells per population of interest were sorted and immediately processed for scRNA-sequencing.
Instrument	Influx (BD Biosciences)
Software	BD FACS software 1.2.0.142
Cell population abundance	CD45+ and CD45- negative cells were sorted for single-cell RNA-sequencing.
Gating strategy	We gated on viable CD45+ and CD45- cells for cell sorting.

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