

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 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

- | | | |
|-------------------------------------|-------------------------------------|--|
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | The statistical test(s) used AND whether they are one- or two-sided <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i> |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | A description of all covariates tested |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | 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) |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> | For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted <i>Give P values as exact values whenever suitable.</i> |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> | 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 Raw data collection was done using imaging software Nikon Elements (version 5.11.01, Build 1367)

Data analysis
 1) Image analysis Python (version 3) based code to extract CTL positions from the original images (see code at https://github.com/BaroudLab/CTL_tracking_in_droplets)
 2) Data processing and data analysis codes (available at https://github.com/BaroudLab/CTL_analysis)
 3) custom made Macro is used in Imagej (version 1.53 f51) to analyze the spheroid size distribution.

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 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 raw data to reproduce the graphs in the figures are supplied. Any further data required can be supplied upon request.

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](https://www.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 | Throughout the study, averages over multiple data points were performed and the results were successfully reproduced. Sample sizes (n) are indicated at the relevant location in manuscript. We performed experimental replicates with total of 84 droplets for B16-OVA experiments and 54 droplets for B16-WT experiments. |
| Data exclusions | No data were excluded for analysis. |
| Replication | The data for the B16-Ova originate from 3 independent microfluidic chips. The data for the B16-WT originate from 2 independent microfluidic chips. |
| Randomization | Randomization is not applicable for single cell studies, it is used in clinical studies. |
| Blinding | Blinding is not applicable for single cell studies, it is used in clinical studies. |

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 |
|-------------------------------------|---|
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Antibodies |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> Eukaryotic cell lines |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Palaeontology and archaeology |
| <input type="checkbox"/> | <input checked="" type="checkbox"/> Animals and other organisms |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Human research participants |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Clinical data |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Dual use research of concern |

Methods

| n/a | Involved in the study |
|-------------------------------------|---|
| <input checked="" type="checkbox"/> | <input type="checkbox"/> ChIP-seq |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Flow cytometry |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> MRI-based neuroimaging |

Eukaryotic cell lines

Policy information about [cell lines](#)

| | |
|---|--|
| Cell line source(s) | B16 melanoma cells (B16-WT and B16-Ova) were kindly provided by Claude Leclerc (Institut Pasteur). |
| Authentication | B16 (B16.F0) melanoma cells previously authenticated by ATCC (see vendor website). The phenotype of the cell lines used in the study was checked after thawing of the cells. |
| Mycoplasma contamination | Cells were tested negative for mycoplasma contamination (using Venor™ GeM Mycoplasma Detection Kit from MINERVA bio labs) |
| Commonly misidentified lines (See ICLAC register) | No commonly misidentified cell lines were used. |

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

| | |
|--------------------|---|
| Laboratory animals | Male, Rag1 ^{-/-} OT-I TCR, 6-8-week-old. The mice were bred and managed by Institut Pasteur's animal facility with a central air conditioning equipment which maintains constant temperature of 22 ± 2°C. Air is renewed at least 20 times per hour in animal rooms. Fluorescent light is provided with a 14:10 h light:dark cycle. Humidity is monitored but not controlled and in the range of 25-65%. |
|--------------------|---|

Wild animals

The study did not involve any wild animals

Field-collected samples

The study did not involve any collected from the field

Ethics oversight

All animal studies were approved by the Institut Pasteur Safety Committee in accordance with French and European guidelines (CETEA 2017-0038)

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