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<br>*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<br>*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 | Data obtained using flexImaging software (v7.2, Bruker Daltonics GmbH) and CyTOF software (7.0.5189, Standard Biotools) |
| Data analysis | Imaging data was merged in Python (version 3.9) and further analyses and visualisation with R (version 4.3.3) in R-studio (version 2023-1-12) using the following R packages: ComplexHeatmap (version 2.14.0), umap (version 0.2.10.0).<br><br>code avaible on https://github.com/deMirandaLab/MALDI-MSI-IMC |

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

Raw IMC and MSI data as well as combined datasets and source data are available via figshare: https://figshare.com/s/c58ddc70fa8dc0602842

## Human research participants

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

| | |
|---|---|
| Reporting on sex and gender | Data not collected |
| Population characteristics | NA |
| Recruitment | NA |
| Ethics oversight | Medical Ethical Committee of the Leiden University Medical Centre |

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 | Three samples, as the study is a methodological proof of principle. no differences were observed between samples and thus not more samples were included |
| Data exclusions | no data excluded |
| Replication | 2 areas imaged per sample, no areas or samples were discarded |
| Randomization | NA, no groups involved |
| Blinding | samples were anonimised and analysed as such |

# 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

CD4 EPR6855 Abcam
CD8a D8A8Y Cell Signaling Technology
ICOS D1K2T Cell Signaling Technology
CD204 J5HTR3 Thermofisher Scientific
CD163 D6U1J Cell Signaling Technology
HLA-DR TAL 1B5 Abcam
CD11b D6X1N Cell Signaling Technology

Granzyme B D6E9W Cell Signaling Technology
CD138 MI15 Biolegend
CD14 D7A2T Cell Signaling Technology
CD7 EPR4242 Abcam
CD11c EP1347Y Abcam
CD45 D9M8I Cell Signaling Technology
CD3 EP449E Abcam
FOXP3 D608R Cell Signaling Technology
CD27 EPR8569 Abcam
Vimentin D21H3 Cell Signaling Technology
Keratin C11 Cell signaling Technology
Keratin AE1/AE3 Biolegend
CD68 D4B9C Cell Signaling Technology
CD31 89C2 Cell Signaling Technology
CD57 HNK-1 / Leu-7 Abcam
Ki-67 8D5 Cell Signaling Technology
IgG1 EPR4417 Abcam
CD45RO UCHL1 Cell Signaling Technology
D2-40 D2-40 Biolegend
CD38 EPR4106 Abcam
Histone H3 D1H2 Cell Signaling Technology

Validation

All antibodies were selected based on thorough validation by the selling companies for human specificity and validated use for FFPE IHC. Antibodies were further tested in house by IHC and by multiplex imaging mass cytometry as shown in Ijsselsteijn et al 2019 (10.3389/fimmu.2019.02534) to confirm colocalisation with literature defined co expressed proteins.