

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 (<i>n</i>) 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☐	☒ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☐ 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 <i>d</i> , Pearson's <i>r</i>), 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	Python version 3.8 Python Software Foundation https://www.python.org XC-SDK 2018 Sony https://www.image-sensing-solutions.eu/XCG-CG160.html flexImaging 7.5 R&D Prototype Bruker Daltonics timsControl 6.0.0 alpha Bruker Daltonics
Data analysis	Python version 3.8 Python Software Foundation https://www.python.org ImageJ Rasband 62 https://imagej.net/ij/ SCiLS Lab MVS, Version 2024b Pro Bruker Daltonics https://www.bruker.com/en/products-and-solutions/mass-spectrometry/ms-software/scils-lab.html OlyVIA 4.1 Evident https://www.olympus-lifescience.com/de/discovery/image-sharing-made-easy-meet-olyvia/ FISCAS Schwenzfeier et al. 48 https://github.com/BioMedMS/fiscas SimpleITK 2.3.1 NumFOCUS 63,64 https://simpleitk.org DeepCell Mesmer 0.12.9 Van Valen Lab 49 https://www.deepcell.org/ CellProfiler 4.2.1 Cimini Lab 50 https://cellprofiler.org/ LipostarMSI 2.0.1 Molecular Horizon 65 https://www.molhorizon.it/software/lipostar/ opencv-Python-headless 4.6.0.66 OpenCV Team https://pypi.org/project/opencv-python-headless/ imageio 2.22.4 Almar Klein https://pypi.org/project/imageio/ jupyterlab 4.2.3 Project Jupyter https://jupyter.org/ matplotlib 3.6.3 Matplotlib 66 https://matplotlib.org/ numba 0.56.4 Numba https://numba.pydata.org/

numpy 1.23.4 Numpy 67 <https://numpy.org/>
 pandas 1.5.1 Pandas 68 <https://pandas.pydata.org/>
 scikit-image 0.19.3 Scikit-Image 69 <https://scikit-image.org/>
 scipy 1.9.3 SciPy 70 <https://scipy.org/>
 seaborn 0.13.2 Seaborn 71 <https://seaborn.pydata.org/>
 tqdm 4.64.1 Tqdm 72 <https://pypi.org/project/tqdm/>
 umap-learn 0.5.6 UMAP 73 <https://pypi.org/project/umap-learn/>

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](#)

MALDI MSI data, fluorescence and brightfield microscopy images as well as processed data have been deposited at OMERO and are publicly available including an interactive data viewer at https://doi.org/10.57860/min_prj_000012. Any additional information required to open or reanalyze the data reported in this paper is available from the lead contact upon request. Source data are provided with this paper.

Research involving human participants, their data, or biological material

Policy information about studies with [human participants or human data](#). See also policy information about [sex, gender \(identity/presentation\), and sexual orientation](#) and [race, ethnicity and racism](#).

Reporting on sex and gender

Reporting on race, ethnicity, or other socially relevant groupings

Population characteristics

Recruitment

Ethics oversight

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](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

Data exclusions

Replication

Randomization

Blinding

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
☒	☐ Plants

Methods

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

Antibodies

Antibodies used

Anti-Calbindin antibody Abcam Cat# ab229915; RRID:AB_3086776
Goat Anti-Rabbit IgG H&L (Alexa Fluor® 594) Abcam Cat# ab150080; RRID:AB_2650602
Alexa Fluor® 594 Anti-Ly6g antibody [EPR22909-135] Abcam Cat# ab307167
DcTRAIL-R1 (TNFRH1) Antibody, anti-mouse (APC-Vio® 770) Miltenyi Biotec Cat# 130-110-873; RRID:AB_2651535
CD45 Antibody, anti-mouse, APC, REAfinity Miltenyi Biotec Cat# 130-110-798; RRID:AB_2658220

Validation

All antibodies were marked as tested (Abcam) or "Satisfaction guaranteed" (Miltenyi) upon purchase

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)

Human: THP-1 cells DSMZ ACC-16

Authentication

The cell line was purchased directly from the DSMZ and not authenticated after arrival.

Mycoplasma contamination

The cell line was not tested for mycoplasma contamination.

Commonly misidentified lines
(See [ICLAC](#) register)

no commonly misidentified lines were used.

Animals and other research organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research, and [Sex and Gender in Research](#)

Laboratory animals

Female BALB/c mice (Charles River Laboratories)

Wild animals

No wild animals were used.

Reporting on sex

The breast cancer model only applies to female mice. The brain study only concerns structures in the brain that are independent of the sex.

Field-collected samples

No field collected samples were used.

Ethics oversight

North Rhine-Westphalia State Agency for Nature, Environment and Consumer Protection, LANUV, Protocol No. 81-02.04.2018.A010

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Plants

Seed stocks

No plants were used.

Novel plant genotypes

No plants were used.

Authentication

No plants were used.