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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 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	SRS images and fluorescence images were collected with Olympus FluoView software (FV3000). STORM images were taken with Applied Scientific Imaging platform
Data analysis	We used the newly developed deconvolution algorithm Adam optimization-based Pointillism Deconvolution (A-PoD) based on python for imaging analysis, we published it in Github. https://github.com/lingyanshi2020/A-PoD

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 the data supporting the findings of this study are available within the paper and its supplementary information files.

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 sample size calculation was performed. Our sample size was selected for adequately demonstrating the technical performance of our probes under the context of SRS metabolic imaging, with considering previous Raman imaging studies in a relevant design (Li et al. Aging Cells, 2022, Zhang et al. Nat Biomed Eng, 2019 and Shi et al. Nat Comm, 2018).
Data exclusions	No data were excluded.
Replication	Three replicate were usually performed for cell-culture experiments with independent measurement and analysis. The results were reproducible.
Randomization	For Drosophila experiments, control and high sugar diet treated larval were separated into the two groups, however, the covariates is not relevant to those experiments, because the read out we concern was direct visualization of spatial distribution from newly synthesized lipid.
Blinding	Blinding was not needed to this study. For experiments were to image the spatial distribution information from the newly synthesized brain lipid droplets, neither the animals nor the investigator would be influenced by blind experiments. The images and the algorithms cannot be biased in this study. Therefore, blinding experiments are not applicable in this case.

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
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Cell lines were purchased from ATCC including Hela (ATCCCCL-2), MCF7 (ATCCHTB-22), HEK (HEK 293 cell, CRL-1573)
Authentication	ATCC does not authenticate the cells.
Mycoplasma contamination	Cell lines were not tested for mycoplasma contamination.
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	The w1118parent female Drosophila were raised in vials containing the standard food (Bloomington cornmeal-yeast-sugar recipe) at 25°C in a controlled light (12/12-h light/dark cycle) and humidity (>70%) environment for several generations.
Wild animals	The study did not involve wild animals.

Field-collected samples	The study did not involve samples collected from the field.
Ethics oversight	There is no animal protocol needed for Drosophila model. All drosophila experiments followed UCSD Lab safety and ethics rules.

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