

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
<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. 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> |
| ☒ | ☐ 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 S.Bench 6.0 was used to collect the fluorescence and scattering time series.

Data analysis Python 3.11 was used for denoising, time series analysis and data visualization. The code for the denoiser, along with example Jupyter notebooks, can be found at https://github.com/krulllab/Deep_Nanometry.

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](#)

The raw data of temporal signals before and after denoising and the table for the flow cytometric features, generated in this study have been deposited in the Zenodo database along with the analysis code and made publicly available with open access.

Links to the data used in the figures 59–62 of main manuscript are as follows: [<https://doi.org/10.5281/zenodo.14616191>, <https://doi.org/10.5281/zenodo.10521246>, <https://doi.org/10.5281/zenodo.10521358>, <https://doi.org/10.5281/zenodo.10521083>].

A link to the data used in the figures 63 of Supplementary Information is as follows: [<https://doi.org/10.5281/zenodo.14615945>].

The code for denoising and basic analysis of the measurement data was uploaded to GitHub [<https://zenodo.org/records/14610019>], and made publicly available with open access.

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	The sex and gender information is provided in Table 4.
Reporting on race, ethnicity, or other socially relevant groupings	No information about race, ethnicity, and other socially relevant groupings is provided.
Population characteristics	No information about population characteristics is provided.
Recruitment	Information about recruitment is not provided.
Ethics oversight	No ethics oversight is provided.

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	No statistical methods were used to pre-determine the sample size. Experiments were done in triplicate.
Data exclusions	Typical flow cytometric gating was performed to exclude obviously noise data points. In addition, we perform width-based typical flow cytometric gating to exclude doublets, multiplets, in Figure S1. Most data are within the gate. The gating method was specified in the method.
Replication	The results were successfully replicated.
Randomization	Using the measurement technique we developed, we randomly sample at least 10,000 data points per experiment at a constant sampling frequency.
Blinding	No blinding was employed.

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	To analyze EVs by DNM, EVs derived from CM or serum were incubated with anti-human CD9 antibodies-conjugated Tide FluorTM5WS (Cosmo Bio, cat #: SHI-EXO-M01-TF5, clone: 12A12, lot: 23D21CB) and anti-human CD147 antibodies-conjugated Alexa488 (abcam, cat #: ab187600, clone: MEM-M6/1, lot: 1034033-3) for 2 hours at room temperature in the dark. The antibody concentration used during labeling was 10 g/mL (CD9) and 40 g/mL (CD147).
Validation	It is quality-controlled by the manufacturer and the data sheet is available on its website. https://www.abcam.co.jp/products/primary-antibodies/alexa-fluor-488-cd147-antibody-mem-m61-prediluted-ab187600.html https://search.cosmobio.co.jp/cosmo_search_p/search_gate2/docs/CAC_/SHIEXOM01TF5.20220210.pdf?_gl=1*pkh8un*_gcl_au*NTgxMDMzOTA2LjE3MTQ3NDc0MDc

Eukaryotic cell lines

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

Cell line source(s)	Human ovarian cancer cell line ES-2 (ATCC catalogue numbers: CRL-1978) and human colorectal cancer cell line HCT116 (ATCC catalogue numbers: CCL-247)
Authentication	All cell lines used in this study were purchased from the American Type Culture Collection (ATCC), which performs cell line authentication using STR profiling.
Mycoplasma contamination	We routinely monitor cell cultures for mycoplasma contamination
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used in this study.

Plants

Seed stocks	<i>Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.</i>
Novel plant genotypes	<i>Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.</i>
Authentication	<i>Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.</i>

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	<i>Describe the sample preparation, detailing the biological source of the cells and any tissue processing steps used.</i>
Instrument	Proposed method in this paper
Software	S.Bench 6.0 was used to collect the fluorescence and scattering time series.
Cell population abundance	No sorting experiments is provided.
Gating strategy	If the scatter trigger criteria were not met, no detection was made. Gating lines were established based on noise from blank sample measurements, and typical flow cytometry gating was applied to exclude obvious noise data points. In addition, standard flow cytometry gating based on width was performed in Figure S1 to exclude doublets and multiplets. Most of the data fell within the gate. Details are provided in the manuscript.

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