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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
<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 Mass spectrometry data was acquired using an Exploris 480 (Thermo) using Xcalibur (Tune v2.11.0.3006) (Thermo)

Data analysis Raw data was processed with DIA-NN 1.8.1 and MaxQuant versions 2.0.1.0 and 2.4.2.0. Other analyses were done with Perseus v1.6.10.50

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 mass spectrometry proteomics data generated in this study have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifiers PXD062547 and PXD062477. Intracellular protein levels were obtained from the supplementary material of Frejno et al.. Total mRNA levels were retrieved from the Cancer Cell Line Encyclopedia at the Expression Atlas repository (<https://www.ebi.ac.uk/gxa/experiments/E-MTAB-2770/Results>). 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	n/a
Reporting on race, ethnicity, or other socially relevant groupings	n/a
Population characteristics	n/a
Recruitment	n/a
Ethics oversight	n/a

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	No statistical methods were used to predetermine sample size. The cohort of 15 distinct human cancer cell lines was selected to cover five diverse tissue origins (lung, breast, colon, melanoma, ovarian), ensuring sufficient biological variability to define a robust, universal sEV core proteome. This sample size strategy aligns with standard practices for large-scale exploratory proteomics. Additionally, human serum and urine were selected for analysis as they represent the most common and clinically relevant biofluids, demonstrating the workflow's applicability to complex biological matrices.
Data exclusions	No data was excluded throughout the manuscript except, as indicated in the manuscript, one density gradient fraction (F08 in Figure 6C) due to technical issue during LC-MS/MS
Replication	The density gradient profiles were measured in 15 independent cancer cell lines. Three of these cell lines were replicated as independent experiments. The density gradient upon Proteinase K degradation of crude sEVs was performed once (Figure 6C). The density gradient with spiked E. coli extract was performed once (Figure 6G). The density gradient from human serum was performed twice as independent biological replicates (Figure 8D). The density gradient from human urine was performed once (Figure 8E).
Randomization	Randomization was not applicable in the context of this study as each cell line could be analyzed independently. In experiments where a quantitative comparison was done (Figure 5C and 6C), density gradient fractions from control and treated conditions were alternated during LC-MS/MS to avoid technical biases.
Blinding	Blinding was not possible during sample preparation as knowledge of the experimental groups was necessary for the execution of the density gradient fractionation and enzymatic treatments. However, mass spectrometry data acquisition was performed using automated methods, and quantitative data analysis was conducted using computational algorithms (DIA-NN, Perseus).

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	Involvement 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	Involvement in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

Primary antibodies:

Abcam: anti-GM130 (1/1000; Cat# ab52649)
 Abcam: anti-Calreticulin (1/1000; Cat# ab2907)
 Abcam: anti-Syntenin (1/1000; Cat# ab133267)
 Abcam: anti-CD63 (1/1000; Cat# ab193349)
 Millipore: anti-CD9 (1/1000; Cat#CBL162)

Secondary antibodies used:

Jackson ImmunoResearch: anti-Mouse-HRP (1/10000; Cat#115-035-062)
 Jackson ImmunoResearch: anti-Rabbit-HRP (1/10000; Cat#111-035-045)

Validation

All primary antibodies used in this study are commercially available and were validated by the respective manufacturers for use in Western Blotting (see manufacturer websites for the catalog numbers listed above).

Eukaryotic cell lines

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

Cell line source(s)

Fifteen human cancer cell lines were used in this study. All cell lines were obtained as generous gifts from research groups at the Spanish National Cancer Research Centre (CNIO, Madrid, Spain). Female lines: HeLa (Cervix), JIMT1 (Breast), MCF7 (Breast), MDA-MB-231 (Breast), HT29 (Colon), SK-MEL-5 (Melanoma), UACC-62 (Melanoma), A2780 (Ovarian), SKOV-3 (Ovarian). Male lines: Colo205 (Colon), HCT116 (Colon), A549 (Lung), NCI-H460 (Lung), NCI-H522 (Lung), SK-MEL-28 (Melanoma).

Authentication

Cells were not authenticated.

Mycoplasma contamination

Cultures were routinely tested for mycoplasma contamination and consistently tested negative

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

None of the cell lines used in this study are listed in the ICLAC database of commonly misidentified cell lines.

Plants

Seed stocks

n/a

Novel plant genotypes

n/a

Authentication

n/a