

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	Data analysis for cell culture EVs was done using the Spectrum Mill MS Proteomics Workbench software package v 4.2 beta (Agilent Technologies). All extracted spectra were searched against a UniProt database containing human reference proteome sequences. Database matches were validated at the peptide and protein level in a two-step process with identification FDR estimated by target-decoy-based searches using reversed sequences. For pooled CSF and plasma EV proteomes data analysis, raw data from several samples of EVs isolated from pooled plasma or CSF (all from BioIVT) were combined and submitted for analysis in Proteome Discoverer 2.4 (ThermoFisher Scientific). Assignment of MS/MS spectra was performed using the Sequest HT algorithm by searching the data against a protein sequence database including all entries from the Human Uniprot (2019 release) database and other known contaminants such as human keratins and common lab contaminants. A MS2 spectra assignment false discovery rate (FDR) of 1% on protein level was achieved by applying the target-decoy database search and filtering was performed using Percolator. For individual CSF and plasma samples, data were analyzed by PEAKS Studio 12.5 software (Bioinformatic Solutions Inc.) in label-free quantification mode using a false discovery rate (FDR) of 1% at the peptide and protein levels. For the different CSF and, separately, for the plasma samples, “match between runs” mode was used.% on protein level was achieved by applying the target-decoy database search and filtering was performed using Percolator.
Data analysis	Python scripts for the computational marker pipeline are available: https://github.com/Wyss/extracellular-vesicle-markers .

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](#)

Mass spectrometry data is available in Supplementary Information Tables S1, S2, and S10. Raw data will be uploaded to ProteomeXchange upon publication.

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

Use the terms *sex* (biological attribute) and *gender* (shaped by social and cultural circumstances) carefully in order to avoid confusing both terms. Indicate if findings apply to only one sex or gender; describe whether sex and gender were considered in study design; whether sex and/or gender was determined based on self-reporting or assigned and methods used. Provide in the source data disaggregated sex and gender data, where this information has been collected, and if consent has been obtained for sharing of individual-level data; provide overall numbers in this Reporting Summary. Please state if this information has not been collected.
Report sex- and gender-based analyses where performed, justify reasons for lack of sex- and gender-based analysis.

Reporting on race, ethnicity, or other socially relevant groupings

Please specify the socially constructed or socially relevant categorization variable(s) used in your manuscript and explain why they were used. Please note that such variables should not be used as proxies for other socially constructed/relevant variables (for example, race or ethnicity should not be used as a proxy for socioeconomic status). Provide clear definitions of the relevant terms used, how they were provided (by the participants/respondents, the researchers, or third parties), and the method(s) used to classify people into the different categories (e.g. self-report, census or administrative data, social media data, etc.)
Please provide details about how you controlled for confounding variables in your analyses.

Population characteristics

Describe the covariate-relevant population characteristics of the human research participants (e.g. age, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."

Recruitment

Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.

Ethics oversight

All human CSF or plasma samples were from pools or de-identified individuals.

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

Sample size depended on whether biofluids from individuals or pooled were used and the amount of biofluid available (CSF from humans is challenging to obtain).

Data exclusions

No data was excluded.

Replication

All Simoa measurements were performed in duplicate and averaged.

Randomization

n/a

Blinding

Blinding was used when comparing PD and control patient samples. For other experiments, data collection and analysis were not performed blind to the conditions of the experiment.

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		Methods	
n/a	Involved in the study	n/a	Involved in the study
☐	☒ Antibodies	☒	☐ ChIP-seq
☐	☒ Eukaryotic cell lines	☒	☐ Flow cytometry
☒	☐ Palaeontology and archaeology	☒	☐ MRI-based neuroimaging
☒	☐ Animals and other organisms		
☒	☐ Clinical data		
☒	☐ Dual use research of concern		
☒	☐ Plants		

Antibodies

Antibodies used	The following primary antibodies were used for Western blot: M38 for CD81 (Thermo Fisher Scientific), H5C6 for CD63 (BD), MM2-57 for CD9 (Millipore), EPR18998 for L1CAM (Abcam), ab47441 for GJA1 (Abcam), F-10 for albumin (Santa Cruz), 989529 for ApoB-100 (R&D Systems), EPR8102 (Abcam) for Syntenin, PA5-71367 (ThermoFisher), E7K3G (Cell Signaling Technology), or AF5269 (R&D Systems) for NRXN3. Antibodies used for imaging were the following: CD9 (HI9a, BioLegend), CD63 (H5C6, BioLegend), CD81 (M38, ThermoFisher Scientific), NRXN3 (AF5269, R&D Systems), ATP1A3 (MA3-915, Invitrogen), APLP1 (ab290677, Abcam), Mouse IgG isotype control (eBMG2b, Invitrogen), Rabbit IgG isotype control (ab37415, Abcam) and Sheep IgG isotype control (ab37385, Abcam). The following antibodies were used for immuno-isolation: mouse monoclonal CD81 (clone 1.3.3.22, ThermoFisher Scientific), CD63 (clone H5C6, BD Biosciences), CD9 (clone CBL162, Millipore), GFP (clone 1GFP63, BioLegend or clone 9F9.F9, Rockland) and mCherry (clone EPR20579, Abcam). The following antibodies were used for immuno-isolation of NRXN3 and controls: sheep polyclonal NRXN3 (cat. AF5269, R&D Systems), GFP (4745-1051, Rockland), and Isotype Control (AB37385, Abcam). The following antibodies were used for Simoa: For NRXN3, AF5269 (R&D Systems) was used as capture and 47714 (ThermoFisher Scientific) was used as detector. For SLC12A5, MAB8369 (R&D Systems) was used as capture and 821701 (BioLegend) was used as detector. For SLC32A1, NB110-55238 (Novus Biologicals) was used as capture, and mab6847 was used as detector (Novus Biologicals). For ATP1A3, sc71640 (Santa Cruz) was used as capture, and sc374050 (Santa Cruz) was used as detector. For APLP1, ab291097 (Abcam) was used as capture, and ab290677 (Abcam) was used as detector.
Validation	NRXN3 antibodies were validated by indirect ELISA and flow cytometry as described in SI Figure S13.

Eukaryotic cell lines

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

Cell line source(s)	K562 and HEK293T cells were obtained from ATCC and iNGN iPS cells were obtained from the Church lab (where they were originally generated and derived from PGP1).
Authentication	PGP1 cell lines were generated in the lab.
Mycoplasma contamination	All cells were tested for mycoplasma contamination and tested negative.
Commonly misidentified lines (See ICLAC register)	Name any commonly misidentified cell lines used in the study and provide a rationale for their use.

Plants

Seed stocks	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.
Novel plant genotypes	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.
Authentication	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.