

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	For mass spectrometry-based proteomics: Xcalibur software package, version 2.1.0 (Thermo Fisher Scientific, Bremen, Germany), to control the mass spectrometer. Whole-cell recordings: IGOR Pro 6.06 (Wavemetrics) using NeuroMatic 2.03, for the detection of miniature excitatory postsynaptic currents. Protein detection in gel electrophoresis and in immunoblot membranes: Image Studio Lite (version 3.1 LI-COR Biosciences) Image Lab (version 3 bio Rad) Immunofluorescence image acquisition: Leica Standard Software TCS-AOBS, to operate the confocal microscope
Data analysis	For mass spectrometry-based proteomics: Progenesis QI software (Nonlinear Dynamics, Newcastle, UK), for peptide and protein quantification. Scaffold (version Scaffold_4.8.5, Proteome Software Inc., Portland, OR), for validation of peptides identified by LC-MS/MS. Mascot (Matrix Science, London, UK; version"2.5.1), for peptide identifications. NextProt tool (Expasy) or the Peptide Search tool from Uniprot, for peptide identification MSqROB (version 1), for the statistical comparison of differentially expressed proteins.

Protein quantification in gel electrophoresis and in immunoblot membranes:

Image Studio Lite (version 3.1 LI-COR Biosciences)

Image Lab (version 3 bio Rad)

Immunofluorescence image analysis:

Fiji/ImageJ (version 2.1.0)

Bioinformatic tools and databases:

pathfinder, for overrepresentation statistical analysis of signalling pathways and GO terms.

StringDB (version 11), database to retrieve protein-protein interaction information.

BioGrid (build 4.3.196), database to retrieve protein-protein interaction information.

Reactome DB, database of signaling pathways

WikiPathways DB, database of signaling pathways

KEGG DB, database of signaling pathways

GO DB, database of gene ontology terms.

Statistical analysis and graphs production:

GraphPad/Prism (version 8.0.1)

Inkscape (version 1.3.2)

Gimp (version 2.1)

R packages used:

scrattch.hicat R for generation of heatmaps

Seurat R package, for the analysis of single cell RNA expression data

Python packages used:

scipy.stats, for the permutation test.

umap-learn package (<https://pypi.org>), to generate u-maps

Random Forest Classifier' function within the scikit-learn, to perform random forest analysis

Custom made code

All custom made software has been deposited in github:

<https://github.com/Alex-Bayes/Synaptic-Proteome-Diversity>

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](#)

Data and code Availability

All the data generated by the bioinformatics analysis performed in this manuscript can be found in the supplementary tables.

Mass spectrometry proteomics data has been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifiers PXD052901 and PXD052913.

All custom-made code is available from GitHub:

<https://github.com/Alex-Bayes/Synaptic-Proteome-Diversity>

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

Population characteristics

administrative data, social media data, etc.)
Please provide details about how you controlled for confounding variables in your analyses.

Recruitment

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."

Ethics oversight

Identify the organization(s) that approved the study protocol.

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 was determined based on our previous experience in the different experimental procedures undertaken in this manuscript. Power analysis was also taken into consideration and used as a reference to pre-determine sample size, with the goal to minimize the number of animals used.

For power analysis we considered the minimum effect size (i.e. difference between the means of two or more experimental groups) that results in statistically significant differences. For the various methodologies used in this manuscript the effect size ranges between 15 and 25%. With each approach having its own effect size and standard deviation. These values, together with our previous research experience, were taken into consideration in order to pre-determine sample sizes. Significance level (alpha) was always set to 0.05 (as stated in figure legends) and power (1-beta) was set to 0.8, which is the standard procedure.

Pre-determined sample sizes were used as a reference, the exact number of animals used in each experiment is presented in the methods section. We do not include in the manuscript pre-determined samples sizes.

Data exclusions

No data points were excluded from the analysis

Replication

Mass spectrometry (MS) based findings were successfully replicated by immunoblot (Figure 2) and immunofluorescence (Figure 2 and Suppl. Figure 4, 5 and 6). In total, we validated 12 individual proteins identified as differentially expressed by MS, with all replication attempts proving successful.

Randomization

Organisms (mice) were randomized in biological replicates based on their sex. Each of the three experimental groups had similar numbers of male and female animals.

Blinding

Investigators were not blinded to group allocations as the results generated involve little human intervention, beyond handling, and do not rely on subjective interpretations. Proteomics data is mostly machine-generated and computationally analysed and the risk of bias due to human intervention is minimal.

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

Antibodies used in immunoblot:

PSD95 (1:1000, #3450; Cell Signaling, [RRID:AB_2292883]);
 Synaptophysin (1:1000, Ab8049; Abcam [SY38], [RRID:AB_2198854]);
 GluA2 (1:1000, MAB397; Millipore [RRID:AB_2113875];
 Shisa6 (1:250, NBP2-85726; Novus Biologicals [RRID:AB_3427376]);
 mGluR2 (1:1000, # 191 103; Synaptic Systems [RRID:AB_2232859];
 Prkar2a (1:500, ab32514; Abcam [RRID:AB_777289]);
 Ptprd (1:1000, NBP2-94767; Novus Biologicals [RRID:AB_3464681]).
 Vamp1 (1:1000, #13151; Cell Signalling [RRID:AB_2798132])
 anti-rabbit (1:7500, 926-68073, IRDye 680CW, [AB_10954442]),
 anti-mouse (1:7500, 926-32212, IRDye 800CW [RRID:AB_621847]
 anti-mouse (1:7500, 925-68072, IRDye 680RD, [RRID:AB_2814912])
 anti-goat (1:7500, 926-32214, IRDye 800CW, [RRID:AB_621846]).

Antibodies used in immunofluorescence:

PSD95 (1:100 dilution, Thermofisher MA1045; [RRID:AB_325399]),
 vGluT1 (1:750, Merck Millipore AB5905; [RRID:AB_2301751]),
 Homer2 (1:100, 160203, Synaptic Systems; [RRID:AB_10807099]),
 Calcineurin (1:100, 387002, Synaptic Systems; [RRID:AB_2661875]),
 Homer3 (1:100, 160303, Synaptic Systems; [RRID:AB_10804288]),
 Synaptoporin (1:100, 102003, Synaptic Systems; [RRID:AB_2619748]),
 Epb41l1 (1:100, 276103, Synaptic Systems; [RRID:AB_2620007]) and
 Mpp2 (1:50, HPA073483, Merck, No RRID codes).
 Anti-Rabbit Alexa-Fluor-488 (1:500, 160203, Invitrogen),
 anti-Mouse Alexa-Fluor-594 (1:500, 387002, Invitrogen)
 anti-Guinea pig Alexa-Fluor-647 (1:1000, AB5905, Invitrogen)

Validation

We provide identification codes from the database 'Research Resource Identification Portal' (RRID codes) for all primary antibodies used in this manuscript. This database allows for systematic tracing of the antibodies used. Furthermore it provides all validation information provided by the manufacturers. We did not directly validate any of the primary antibodies used in this study. This is a summary of the available validation information provided by the vendors, gathered from RRID:

PSD95 (#3450; Cell Signaling, [RRID:AB_2292883]); Can be used for WB as reported by vendor - Referenced in 74 PubMed Articles
 Synaptophysin (Ab8049; Abcam [SY38], [RRID:AB_2198854]); Can be used for WB as reported by vendor - Referenced in 47 PubMed Articles
 GluA2 (MAB397; Millipore [RRID:AB_2113875]; Can be used for WB as reported by vendor - Referenced in 91 PubMed Articles
 Shisa6 (NBP2-85726; Novus Biologicals [RRID:AB_3427376]); Can be used for WB as reported by vendor
 mGluR2 (# 191 103; Synaptic Systems [RRID:AB_2232859]; Can be used for WB as reported by vendor
 Prkar2a (ab32514; Abcam [RRID:AB_777289]); Can be used for WB as reported by vendor
 Ptprd (NBP2-94767; Novus Biologicals [RRID:AB_3464681]). Can be used for WB as reported by vendor

Antibodies used in immunofluorescence:

PSD95 (1:100 dilution, Thermofisher MA1045; [RRID:AB_325399]), Can be used for IHC as reported by vendor - Referenced in 66 PubMed Articles
 vGluT1 (1:750, Merck Millipore AB5905; [RRID:AB_2301751]), Can be used for IHC as reported by vendor - Referenced in 280 PubMed Articles
 Homer2 (1:100, 160203, Synaptic Systems; [RRID:AB_10807099]), Can be used for IHC as reported by vendor
 Calcineurin (1:100, 387002, Synaptic Systems; [RRID:AB_2661875]), Can be used for IHC as reported by vendor
 Homer3 (1:100, 160303, Synaptic Systems; [RRID:AB_10804288]), Can be used for IHC as reported by vendor
 Synaptoporin (1:100, 102003, Synaptic Systems; [RRID:AB_2619748]), Can be used for IHC as reported by vendor
 Epb41l1 (1:100, 276103, Synaptic Systems; [RRID:AB_2620007]) and Can be used for IHC as reported by vendor
 Mpp2 (1:50, HPA073483, Merck, [RRID:AB_3678682]). Can be used for IHC as reported by vendor

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	All animals used were wild-types, from the species <i>Mus musculus</i> ; strain/substrain C56BL/6J. Mice were obtained from Jackson Laboratories. (Jackson Laboratories strain code #000664; Research Resource Identifier, RRID:IMSR_JAX:000664). Ages 9-14 weeks. We used animals from both sexes in similar numbers.
Wild animals	The study did not involve wild animals.
Reporting on sex	We used animals from both sexes in similar numbers. We pooled tissue from both sexes in each sample in order to explore proteomics differences in synaptic composition which affected both sexes. We did not consider sex as a variable in the study design. Since we pooled tissue from both sexes in the same samples we do not have data disaggregated by sex.
Field-collected samples	Study did not involve samples collected from the field.
Ethics oversight	Animal handling All animal research was done with C56BL/6J mice (Jackson Laboratories, Research Resource Identifier, RRID:MGI:5656552) in accordance with national and European legislation (Decret 214/1997 and RD 53/2013). Research procedures were approved by the Ethics Committee on Animal Research from the: i) Institut de Recerca de l'Hospital de la Santa Creu i Sant Pau (IR-HSCP) and from the ii) Universitat de Barcelona for whole-cell recording experiments. These procedures were also approved by the Departament de Territori i Sostenibilitat from the Generalitat de Catalunya (approval reference numbers 9,655 and 164.16). Maintenance and experimental procedures were conducted at the animal facilities of the IR-HSCP or the Faculty of Medicine of the Universitat de Barcelona, for whole-cell recording experiments.

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

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>