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☐ Initial submission ☐ Revised version ☐ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

Describe how sample size was determined.

The experiments described in this exploratory study were done for the first time. No pre-specified effect size could be determined a priori.
For MS, in general, two replicates are acceptable if the overlap between them is good (e.g. r2 greater than or equal to 0.80). In this study we used a minimum of two replicates per proteome and up to 6 replicates. We had excellent reproducibility between replicates, with r2 greater than 0.90.

2. Data exclusions

Describe any data exclusions.

No data were excluded from the study.

3. Replication

Describe whether the experimental findings were reliably reproduced.

The isolation of specific proteomes was reproducible as is shown in the paper and described above. Nevertheless due to the several steps of the purification protocol, the purification of some samples failed and there was no mass spec analysis.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

As a general rule, mice (wt or transgenic) were all exposed to the same treatment. In the case of EE and HC experiments mice were randomly distributed in each of the cage types.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

Investigators were not blinded.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

Nature Biotechnology: doi:10.1038/nbt.4016

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	ImageJ64. Prism 6. Origin 2015G. MaxQuant 1.5.3.8. Perseus 1.5.5.3.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.	No unique materials are used.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	Mouse anti-biotin (IF and IB, 1:1000, Sigma, cat:033700). Rabbit anti-biotin (IF and IB, 1:1500, Cell Signaling, cat:5567). Rabbit anti-GFP (IF and IB 1:750, Invitrogen, cat:A11122). Chicken anti-GFP (IF, 1:500; IB: 1:1000; Aves,cat:1020). Gp anti-MAP2 (1:1000, SYSY,cat:188004). Antibodies used for the PLA experiments: rabbit anti-Lamin 1:1000 (abcam, cat:ab16048), rabbit anti-Debrin 1:500 (sigma, cat:d3816), rabbit anti-GluA1 1:500 (abcam, cat:ab31232). Mouse anti-far red (1:100, abcam, cat:ab52060). Secondary antibodies Goat anti-chicken Alexa 647 (IF, 1:750; IB, 1:7500; Invitrogen, a21449). Goat anti-rabbit Rhodamine RRX (IF, 1:800, Jackson laboratory, cat:711295152). Goat anti-rabbit FITC (IF, 1:800, Jackson laboratory, cat:111095003). Goat anti-mouse or anti-rabbit IR680 or IR800 (IB, 1:10.000, Licor,cat:296-32213 and 296-32211). Antibody specificity was evaluated using the proper negative controls.

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10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

Hek, HeLa and Cos7 were obtained from ATCC.

b. Describe the method of cell line authentication used.

None of the lines have been authenticated.

c. Report whether the cell lines were tested for mycoplasma contamination.

Lack of cell line contamination with mycoplasma was checked by PCR (eMyco detection kit, Intron Biotechnology).

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No commonly misidentified cell lines were used.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

All the mice used in this study were C57BL/6 from Jackson Laboratory. Animals used were 6 weeks old at the beginning of the experiments. Both males and Females were used.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

Study did not involved humans.

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