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Life Sciences Reporting Summary

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For final submission: please carefully check your responses for accuracy; you will not be able to make changes later.

ü Experimental design

1. Sample size

   Describe how sample size was determined.

   Sample size was not determined a priori - we combined all data fitting our inclusion criteria.

2. Data exclusions

   Describe any data exclusions.

   We included only experiments that fitted our inclusion criteria, which were pre-established and are described in the Methods section.

3. Replication

   Describe the measures taken to verify the reproducibility of the experimental findings.

   For treeClust machine-learning it was verified that that repetition with different random seeds generated nearly identical results. Also alternative methods to infer co-regulation, e.g. Pearson and Spearman correlation, were tested and yielded similar results. Where they disagreed it was validated that treeClust learning produced more accurate results. TreeClust learning was also applied to different datasets, e.g. RNA coexpression and TMT-based protein coexpression data. Microscopy-based experiments were repeated three times to ensure reproducibility.

4. Randomization

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

   This is not relevant to our study since we did not need to allocate experimental groups.

5. Blinding

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

   Only applicable to the mitochondria - peroxisome interaction part of the study. The analysis was made blind in different areas of the coverslip.

Note: all in vivo studies must report how sample size was determined and whether blinding and randomization were used.

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 *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ Test values indicating whether an effect is present *Provide confidence intervals or give results of significance tests (e.g. P values) as exact values whenever appropriate and with effect sizes 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 in all relevant figure captions (with explicit mention of central tendency and variation)

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

ü Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

Data were analysed in R 3.5.1, as described in the methods section. Mass spectrometry files were processed using MaxQuant 1.5.2.8 as described in the methods section. The interactive co-regulation map was created using the Python Flask 0.10.1 Web framework, as described in methods section. Microscopy images were taken and processed with Olympus Soft Imaging Viewer, MetaMorph 7, and VisiView as described in the methods section. GraphPad Prism 5 was used to analyse statistical significance relating to peroxisome - mitochondria interactions, as described in the methods section.

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.

ü 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 third party.

No restrictions.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

Mouse monoclonal antibody 9E10 against the Myc epitope (1:200, Santa Cruz Biotechnology, Inc., sc-40) was validated by the manufacturer for use in Western blots, immunoflourescence, immunoprecipitation and others. Rabbit monoclonal antibody against PEX11β (1:1000, Abcam, ab181066) was tested by the manufacturer for reactivity against human and mouse, as well as by us in and others in previous publications (e.g. Dahabieh et al, Cell Death Differ, 2017). Rabbit polyclonal antibody against GAPDH (1:2000, ProSci, Cat. No. 3783) was tested by the manufacturer for reactivity against human, mouse and rat and validated for Western blotting and immunoflourescence. Secondary anti-IgG antibodies against rabbit (Alexa 594, 1:1000, Molec. Probes/Life Technol. A21207) and mouse (Alexa 488, 1:400, Molec. Probes/ Life Technol. A21202) were obtained from ThermoFisher Scientific and were validated by the manufacturer for use in immunoflourescence. HRP-coupled donkey polyclonal antibody against rabbit IgG (1:5000) was obtained from Biorad (172-1013) was validated for Western blotting in a previous publication (Costello et al, J Cell Sci, 2017). Rabbit polyclonal antibody against PEX14, a gift from D. Crane, Griffith University, Australia, was successfully tested in the following publications: Nguyen T, Bjorkman J, Paton BC, Crane DI (2006) Failure of microtubule-mediated peroxisome division and trafficking in disorders with reduced peroxisome abundance. J Cell Sci 119(Pt 4):636–645, and Grant P, Ahlemeyer B, Karnati S, Berg T, Stelzig I, Nenicu A,Kuchelmeister K, Crane DI, Baumgart-Vogt E (2013) The biogenesis protein PEX14 is an optimal marker for the identification and localization of peroxisomes in different cell types, tissues, and species in morphological studies. Histochem Cell Biol 140 (4):423–442

10. Eukaryotic cell lines

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

COS7 cells were from ATCC, PEX5-deficient fibroblasts were kindly provided by H. Waterham, as described in the Methods section.

b. Describe the method of cell line authentication used.

PEX5 patients cell lines were authenticated through PEX5 expression analysis

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

All cell lines were routinely tested for mycoplasma using a PCR-based assay, confirming the absence of mycoplasma contamination

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.

These cell lines are not in the ICLAC list.

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

No animals were used for this study.

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.

No humans were used for this study.