nature portfolio

Corresponding author(s): Jeremy M. Baskin

Last updated by author(s): 10/31/2025

# 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<br>*Only common tests should be described solely by name; describe more complex techniques in the Methods section.* |
| ☒ | ☐ 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<br>*Give P values as exact values whenever suitable.* |
| ☒ | ☐ 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 | Imaging data were collected using Zeiss ZEN microscopy software (blue edition); Western blotting data were collected using Bio-Rad GelDoc EZ Gel Imaging System; Proteomics data were collected with the help of Cornell Proteomics Facility, who performed data collection using Orbitrap Eclipse (Thermo-Fisher Scientific) mass spectrometer equipped with a nanospray Flex Ion Source coupled with the UltiMate 3000 RSLCnano (Dionex); Lipidomics data were collected using Agilent MassHunter Workstation software (version B.07.00). |
| Data analysis | Imaging data were analyzed using ImageJ/FIJI; Western blotting data were analyzed using ImageJ/FIJI; Proteomics data were analyzed with the help of Cornell Proteomics Facility, who performed data analysis using Sequest HT search engine within the Proteome Discoverer 2.5 (PD 2.5, Thermo); Lipidomics data were analyzed using Agilent MassHunter Quantitative Analysis software (version B.07.00; with Find Compounds by Formula as described in the method section). |

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

All data supporting the findings of this study are available within the paper and its associated Source Data and Supplementary Information. Proteomics data are deposited to the PRIDE. Plasmids generated during the current study are listed along with their source information in the Supplementary Information. Custom Python scripts developed for proteomics and lipidomics analyses are publicly available at GitHub (https://github.com/teipanda/Membrane-Turbo), along with the corresponding example datasets.

## Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

| | |
|---|---|
| Reporting on sex and gender | 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 | Sample sizes were determined empirically to account for technical variability and assay reproducibility, which can be influenced by experimenter technique, experimental setup, and other conditions. For each assay, pilot experiments using well-established positive and negative controls were performed to estimate variability and guide sample size selection for subsequent experiments. |
| Data exclusions | In the analysis of proteomics data, proteins hits with only 1 identified unique peptide were excluded from the results to minimize false-positive hits. Corneocyte proteins, keratin and filaggrin, were considered to be detected due to contamination and removed from the search. |
| Replication | Replicate tests were generally conducted three times per assay, except for certain experiments with multiple samples supporting similar conclusions, where replicates were performed twice. The number of replicates is noted in the figure legends. |
| Randomization | Sample preparation and data collection orders were randomized across repeated independent experiments. In each experiment, replicates were distributed across different wells or gel lanes to detect and eliminate position-related bias. |
| Blinding | Proteomics data were collected and analyzed blindly by the Cornell Proteomics Facility. Other data were collected non-blindly but consistently across samples, and analyses were performed systematically using automated macros and scripts. |

# 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 |

## Methods

| n/a | Involved in the study |
|---|---|
| ☒ | ☐ ChIP-seq |
| ☒ | ☐ Flow cytometry |
| ☒ | ☐ MRI-based neuroimaging |

# Antibodies

Antibodies used

Western blotting:
phospho-p70 S6 kinase (Thr389; Cell Signaling Technology, #9205), 1:1000; β-actin (Cell Signaling Technology, #5125), 1:1000; anti-Lipin-1 polyclonal antibody (Proteintech, 27026-1-AP), 1:1000; anti-Lipin-2 monoclonal antibody (Santa Cruz Biotechnology, sc-514353), 1:100; anti-Nir2 polyclonal antibody (Proteintech, 26983-1-AP), 1:1000; anti-PDZD8 polyclonal antibody (Proteintech, 25512-1-AP), 1:1000; anti-SCP-x polyclonal antibody (Proteintech, 14397-1-AP), 1:1000; anti-SCP-2 polyclonal antibody (Proteintech, 23006-1-AP), 1:1000; anti-DGKD polyclonal antibody (Abcepta, AP8126b), 1:1000; anti-DGKH polyclonal antibody (Proteintech, 13873-1-AP), 1:1000; anti-β-Tubulin monoclonal antibody (Cell Signaling Technology, #86298).

Immunofluorescence:
Streptavidin–AlexaFluor 488 (Invitrogen, S11223), 1:50; anti-V5 antibody (Bio-Rad, MCA1360GA), 1:100; anti-Calnexin antibody as an ER marker (Thermo Fisher Scientific, PA534754), 1:100; anti-LAMP2 antibody as a lysosomal marker (Santa Cruz Biotechnology, sc-18822), 1:100; anti-GRASP65 antibody as a Golgi marker (Santa Cruz Biotechnology, sc-374423), 1:100; anti-Lipin-1 antibody (Cell Signaling Technology, #14906), 1:100; anti-mouse–Alexa Fluor 488 antibody conjugate (Invitrogen, A21202), 1:500; anti-mouse–Alexa Fluor 647 (Invitrogen, A31571), 1:500.

Validation

All antibodies were commercially validated by the manufacturer and further validated in our laboratory by Western blotting or immunofluorescence, confirming that the observed molecular weight or localization matched the manufacturer's specifications. The p-S6K antibody was verified by Western blotting under control conditions (serum-starved vs stimulated cells). Nir2, PDZD8, SCP-x, and SCP2 antibodies were validated by Western blotting in control versus siRNA knockdown cells. β-Actin and β-tubulin antibodies were routinely used as loading controls and consistently produced uniform, expected bands. V5 antibody was verified by immunofluorescence in control versus V5-overexpressing cells. Calnexin, LAMP2, and GRASP65 antibodies were routinely used as organelle markers and consistently showed expected localization patterns. No specific additional validation was performed for Lipin-1, Lipin-2, DGKD, or DGKH antibodies.

# Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

| | |
|---|---|
| Cell line source(s) | HEK 293T cells were obtained from ATCC; HEK 293TN cells were obtained from the Bretscher lab (Cornell). |
| Authentication | Cell lines were not authenticated. |
| Mycoplasma contamination | Tested negative. |
| Commonly misidentified lines (See ICLAC register) | None. |