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Reporting Summary

Nature Research 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 Research policies, see Authors & Referees 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 *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 *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	Used software are described in the methods. Serial EM is an open source software regularly updated
Data analysis	Used software are described in the methods. Fiji, IMOD (regurarly updated), SoftWoRx (regularly updated) ICY 2.0.0.0, NovaCTF, Gctf 1.06, 3DCT, UCSF Chimera 1.13 are open source. Graph Pad Prism is a commercially available software available at https://www.graphpad.com/scientific-software/prism/. MATLAB is a commercial software available at https://www.mathworks.com/downloads/. Movavi Video Editor 15 is a commercially available software at https://www.movavi.com/mac-video-editor/. The input data and the scripts used for modeling are available at Zenodo (https://zenodo.org/) under the DOI: 10.5281/zenodo.3820319.

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/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

The three EM maps associated with this manuscript have been deposited in the Electron Microscopy Data Bank (EMD-10198, EMD-10660, EMD-10661). The integrative models of ScNPC are available at Zenodo (https://zenodo.org/) under the DOI: 10.5281/zenodo.3820319.

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 size were chosen from to the practical limitation of the methodology used it was sufficient to interpret the data in a robust way according to standard statistical tests like ANOVA or Mann-Whitney and to previous publications
Data exclusions	No data was excluded from the analyses
Replication	A minimum of three biological replicates were performed for the light microscopy and western blot experiments. Electron microscopy data comes from EM grids prepared in different days from different cell cultures (biological replicates). All attempts of replication were successful
Randomization	All light microscopy and electron microscopy data comes from randomly selected cells
Blinding	Blinding is not relevant for this study because cells are randomly distributed

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
☒	☐ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

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

Antibodies

Antibodies used	Monoclonal antibody against Dpm1 (1:10,000; clone 5C5A7) was purchased from Invitrogen (Catalog # A-6429) EGFP (1:500; clone B-2) was purchased from Santa Cruz Biotechnology
Validation	The antibodies used in this study were used and validated in previous studies of S. cerevisiae (DOI:10.1038/s41556-019-0459-2). The antibodies are against GFP (clone B-2, Santa Cruz, https://www.scbt.com/p/gfp-antibody-b-2) and against Dpm1 (clone 5C5A7, Invitrogen, https://www.thermofisher.com/antibody/product/DPM1-Antibody-clone-5C5A7-Monoclonal/A-6429).

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Mating_type / Strain_Name / Genotype / Created 1) alpha,WT MATα, his3-Δ200, leu2-3,2-112, lys2-801, trp1-1(am), ura3-52 Fig. 1, 2; Extended Data Fig.1-4 2)a WT MATa his3-Δ200, leu2-3,2-112, lys2-801, trp1-1(am), ura3-52 Fig. 4c, Extended Data Fig.7b 3)alpha Split-Venus-Nup159-Atg8 nup120Δ MATα his3-Δ200, leu2-3,2-112, lys2-801, trp1-1(am), ura3-52 Nup159-VC::HIS3MX6, natNT2::ADH::VN-Atg8, Nup170::mars::kanMX4, nup120Δ::hphNT1 Lee et al, 2020 Fig. 4b, Extended Data Fig. 8b 4) alpha Split-Venus-Nup159-Atg8 MATα his3-Δ200, leu2-3,2-112, lys2-801, trp1-1(am), ura3-52 Nup159-VC::HIS3MX6, natNT2::ADH::VN-Atg8, Nup170::mars::kanMX4 Lee et al, 2020 Revisions 5) a nup116Δ MAT a nup116::HIS3 ade2 his3 leu2 trp1 ura3 kind gift by Hurt lab Fig. 2, 3; Extended Data Fig. 5, 6 6) a atg15Δ Nup192p-yeGFP Nup159p-mars MATa atg15::HIS3MX6 NUP192GFP::kanMX6 NUP159mars::natNT2 or atg15Δ Nup192p-TagGFP Nup159p-mars, his3-Δ200, leu2-3,2-112, lys2-801, trp1-1(am), ura3-52 Lee et al, 2020 Fig. 4e, f

7) alpha nup116Δ Nup188p-yeGFP nup116::natNT2 NUP188GFP::klTRP1 This study Fig. 3f
8) alpha Nup188p-yeGFP NUP188GFP::klTRP1 This study Fig. 3f
9) a nup116Δ Nup133p-yeGFP nup116::natNT2 NUP133GFP::klTRP1 This study Extended Data Fig. 7a
10) alpha Nup133p-yeGFP NUP133GFP::klTRP1 This study Extended Data Fig. 7a
11) alpha Nup120p-yeGFP NUP120GFP::klTRP1 This study Fig. 4d
12) alpha atg8Δ atg8::KAN kind gift by Lusk lab Fig. 4c, Extended Data Fig. 7b
13) alpha atg8Δ Nup120p-yeGFP atg8::kanMX6 NUP120GFP::klTRP1 This study Fig. 4d
14) alpha atg8Δ Nup192p-mars Nsg1p-yeGFP atg8::kanMX6 NUP192Mars::natNT2 NSG1GFP::HIS3MX6 This study Extended Data Fig. 7d
15) alpha Nup192p-mars Nsg1p-yeGFP NUP192Mars::natNT2 NSG1GFP::HIS3MX6 This study Extended Data Fig. 7d
16) alpha vps4Δ vps4::HYG kind gift by Lusk lab Extended Data Fig.7b
17) alpha vps4Δ Nup192p-yeGFP vps4::natNT2 NUP192GFP::klTRP1 This study Extended Data Fig. 7c
18) alpha atg8Δ Nup192p-yeGFP atg8::kanMX6 NUP192GFP::klTRP1 This study Extended Data Fig. 7c
19) alpha Nup192p-yeGFP NUP192GFP::klTRP1 This study Extended Data Fig. 7c
20) alpha Nup84p-yeGFP Nup159p-mars NUP84GFP::klTRP1 NUP159mars::hphNT1 This study Fig. 3c, Extended Data Fig. 6e
21) a nup116Δ Nup84p-yeGFP Nup159p-mars nup116::natNT2 NUP84GFP::klTRP1 NUP159mars::hphNT1 This study Fig. 3c, Extended Data Fig. 6e

Authentication

Authentication of cell lines not created in this study was done on the basis of the expected phenotype of the line (PMID: 32029894)

Mycoplasma contamination

No test was needed since only S. cerevisiae strains were used

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were used