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Reporting Summary

Ctatictics

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 <u>Authors & Referees</u> and the <u>Editorial Policy Checklist</u>.

Ju	atio	LICS				
For	all st	atistical anal	yses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.			
n/a	Cor	nfirmed				
		The exact sa	ample size (n) for each experimental group/condition, given as a discrete number and unit of measurement			
		A statemen	t on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly			
		The statistic	cal test(s) used AND whether they are one- or two-sided n tests should be described solely by name; describe more complex techniques in the Methods section.			
\boxtimes		A description	on of all covariates tested			
		A description	on 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>				
\boxtimes		For Bayesia	n analysis, information on the choice of priors and Markov chain Monte Carlo settings			
\boxtimes	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes					
\boxtimes	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.			
So	ftw	are and	code			
Poli	cy in	formation ab	pout availability of computer code			
D	Data collection LSM700 (Carl Zeiss Microscopy), ELYRA PS.1 (Carl Zeiss Microscopy), Axio Zoom.V16 (Carl Zeiss Microscopy), JSM-7600F (JEOL) field emission scanning electron microscopy (FE-SEM), JEM1010 (JEOL) transmission electron microscopy (TEM), Talos L120C (FEI) transmission electron microscopy (TEM), MRI was conducted using a 9.4 T horizontal-bore Bruker Avance III HD imaging system (Bruker Biospin)					
D	TEN black (8.1) software 7EN blue (1.1.2.0) software Image! (Fiii) software IMARIS (8.0.2) software AngioTool (0.6 alpha) software					

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

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.

- Accession codes, unique identifiers, or web links for publicly available datasets

GraphPad Prism (5.01), SigmaPlot (7.0),

- A list of figures that have associated raw data
- A description of any restrictions on data availability

All data supporting the findings of this study are available from the corresponding author upon reasonable request.

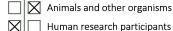
Field-specific	reporting
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	<u> </u>
Please select the o	ne below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.
Life sciences	Behavioural & social sciences Ecological, evolutionary & environmental sciences
For a reference copy of t	the document with all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>
Life scier	nces study design
All studies must dis	close on these points even when the disclosure is negative.
Sample size	Sample size was determined to be based on the magnitude and consistency of measurable differences between groups. For statistical significance, at least three independent experiments were subjected.
Data exclusions	No data were excluded.
Replication	Each experiment presented in the paper was repeated in multiple times and/or across multiple animals. Replicate experiments were successful. The precise number of repeats are given in the figure legend.
Randomization	We did not use randomization to assign animals to experimental groups. Mice analyzed were litter mates and sex-matched whenever possible.
Blinding	All investigators were blinded to group allocation during data collection and analysis.
Reportin	g for specific materials, systems and methods
	on from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, ted 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.

IVId	ter	Idis	α	exper	ime	IIIai	Sys	sten	15
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n/a	Involved in the study
П	Antibodies

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X		Eukarvotic cell	lin



Clinical data

Methods

/a | Involved in the study

ChIP-seq

Flow cytometry

MRI-based neuroimaging

Antibodies

Antibodies used

The following antibodies were used: rabbit IgG anti-Anks1a (1:100, Bethyl, A303-049A), rabbit IgG anti-Anks1a (1:100, Bethyl, A303-050A), mouse IgG1 anti-CNTRL (1:100, Santa Cruz, sc-365521), mouse IgG2b anti-FOP (1:250, Abnova, H00011116-M01), rabbit IgG anti-CEP164 (1:200, Atlas antibodies, HPA037606), mouse IgG2a anti-Centrin (1:100, Millipore, 04-1624), rabbit IgG anti-ODF2 (1:100, Atlas antibodies, HPA001874), mouse IgG1 anti-GT335 (1:200, Adipogen, AG-20B-0020), mouse IgG2b anti-Actubulin (1:200, Sigma Aldrich, T7451), mouse IgG1 anti-Y-tubulin (1:100, Abcam, ab11316), phalloidin-Atto647N anti-F-actin (1:50, Sigma Aldrich, 65906), mouse IgG1 anti-ZO-1-488 (1:100, Invitrogen, 339188), chick anti-GFP (1:250, Abcam, ab13970). rabbit anti-CEP19 (1:100, Proteintech, 26036-1-AP), mouse IgG2a anti-a-tubulin (1:200, Santa Cruz, SC5286), mouse IgG2a anti-Frizzled 3 (1:100, Sigma Aldrich, SH0007976M9), rabbit anti-Vangl1 (1:100, Atlas antibodies, HPA025235), rabbit anti-CEP350 (1:1000, Novus, NB100-59811).

Validation

Anks1a antibody was validated in Anks1a KO mice from our group. FOP, CEP19 antibodies were validated in each KO hTERT-RPE cells. (Reference: PubMed: 28625565)

ODF2 antibody was validated in ODF2 KO mice. (Reference: PubMed: 22265411)

When available, we purchased commercial antibodies that have been previously validated in multiple independent studies,

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

Laboratory mice were maintained on a congenic C57BL/6 background. Both male and female mice were used in this study. Mice were sacrificed at P0-P2 for ependymal cell cultures. MRI analysis was performed on old mice of 18-22 months of age. Anks1a+/lacZ gene trap mice have been previously described, (Kim, J. et al., 2010).

	nks1a-CreER mice were generated in this paper using the same approach as we previously do 26-stop-ETFP mice were purchased from The Jackson Laboratory, RPID: IMSR_JAX:006148 nks1af/+ mice were generated in this paper, enopus laevis (females for eggs collection and males for sperm collection) from the Jaebong'				
Wild animals	/ A				
Field-collected samples	MA.				
Ethics oversight	nd Use Committee (SWU-IACUC-2001-029). All mice were housed and handled at the animal iniversity. enopus embryo study was conducted in accordance with the regulations of the Institutional ACUC) of Hallym University (Hallym2019-81). All the research members attended both the ed	eriments were approved by and were in compliance with the Sookmyung Women's University Institutional Animal Care to Committee (SWU-IACUC-2001-029). All mice were housed and handled at the animal facility of Sookmyung Women's sity. Is embryo study was conducted in accordance with the regulations of the Institutional Animal Care and Use Committees of Hallym University (Hallym2019-81). All the research members attended both the educational and training courses for propriate care and use of experimental animals at our institutions in order to receive an animal use permit.			
ote that full information on the	roval of the study protocol must also be provided in the manuscript.				
Magnetic resonanc	maging				
xperimental design					
Design type	Indicate task or resting state; event-related or block design.				
Design specifications	Specify the number of blocks, trials or experimental units per session and/or subject, or block (if trials are blocked) and interval between trials.	Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.			
Behavioral performance me	State number and/or type of variables recorded (e.g. correct button press, response ti to establish that the subjects were performing the task as expected (e.g. mean, range, subjects).	•			
cquisition					
Imaging type(s)	Structural MRI				
Field strength	9.4T				
Sequence & imaging parame	Turbo spin echo sequence. Coronal: field of view(FOV) 20 x 20 mm, matrix 256 x 256, TR/TE = 4300/26 ms, slice thickness 0.25 mm				
Area of acquisition	Whole-brain scan was performed, followed by volume analysis of brain ventricle.				
Diffusion MRI Us	Not used ■ Not used				
reprocessing					
Preprocessing software	Provide detail on software version and revision number and on specific parameters (mesegmentation, smoothing kernel size, etc.).	nodel/functions, brain extraction,			
Normalization	If data were normalized/standardized, describe the approach(es): specify linear or not used for transformation OR indicate that data were not normalized and explain ration				
Normalization template	Describe the template used for normalization/transformation, specifying subject spacoriginal Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.				
Noise and artifact removal	Describe your procedure(s) for artifact and structured noise removal, specifying motion physiological signals (heart rate, respiration).	Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).			
Volume censoring	Define your software and/or method and criteria for volume censoring, and state the	Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.			
tatistical modeling & in	ence				
Model type and settings	Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essention and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).	al details of the model at the first			

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Effect(s) tested

Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.

Specify type of analysis: Whole brain

ROI-based

Both

Statistic type for inference (See <u>Eklund et al. 2016</u>)

Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.

Correction

Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).

Models & analysis

/a	Involved in the study
X	Functional and/or effective connectivity
\times	Graph analysis
X	Multivariate modeling or predictive analysis