# nature portfolio

corresponding author(s):	MARIA LLORENS-MARTIN
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## **Reporting Summary**

- 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 the materials are available from the corresponding author upon reasonable request

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 <u>Editorial Policies</u> and the <u>Editorial Policy Checklist</u>.

Statistics			
For all statistical an	alyses, 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 stateme	ent on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly		
The statist	tical test(s) used AND whether they are one- or two-sided non tests should be described solely by name; describe more complex techniques in the Methods section.		
A descript	ion of all covariates tested		
A descript	ion of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons		
A full desc	cription of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) tion (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)		
For null hy Give P value	ypothesis 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 es as exact values whenever suitable.		
For Bayes	ian analysis, information on the choice of priors and Markov chain Monte Carlo settings		
For hierar	chical 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		
1	Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.		
Software an	d code		
Policy information	about <u>availability of computer code</u>		
Data collection	Fiji (ImageJ 64 bits 1.53t) was used to analyze confocal images		
Data analysis	Data were analyzed using GraphPad Prism 9 software (GraphPad.v.9.4.1 (681) 2022; GraphPad Software, LLC).		
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			
All manuscripts m	about <u>availability of data</u> ust include a <u>data availability statement</u> . This statement should provide the following information, where applicable: s, unique identifiers, or web links for publicly available datasets		

<u>and sexual orientat</u>		with <u>human participants or human data</u> . See also policy information about <u>sex, gender (identity/presentation),</u> ethnicity and racism.	
Reporting on sex and gender		No human subjects participate in this study	
Reporting on race, ethnicity, or other socially relevant groupings		N/A	
Population characteristics N/A		N/A	
Recruitment N/A		N/A	
Ethics oversight		N/A	
Note that full informa	ation on the appr	oval of the study protocol must also be provided in the manuscript.	
E. 1.1			
Field-spe	ecitic re	porting	
Please select the o	ne below that is	s the best fit for your research. If you are not sure, read the appropriate sections before making your selection.	
X Life sciences	В	Behavioural & social sciences	
or a reference copy of t	the document with	all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>	
_ife scier	nces stu	udy design	
All studies must dis	sclose on these	points even when the disclosure is negative.	
Sample size	Sample size wa	s determined according to our knowledge from previous studies.	
	Atypical data were identified using using GraphPad Prism 9 software (GraphPad.v.9.4.1 (681), 2022; GraphPad Software, LLC) and extreme outlier values were eliminated when necessary.		
Data exclusions		vere eliminated when necessary.	
Data exclusions  Replication	outlier values w	biological replicates is detailed in the Material and Methods subsections	
	outlier values w  The number of		
Replication	The number of  Mice were rance	biological replicates is detailed in the Material and Methods subsections	
Replication Randomization Blinding  Reportin We require informati	outlier values w The number of Mice were rance No specific blin  g for sp on from authors	biological replicates is detailed in the Material and Methods subsections  domly allocated to the distinct experimental groups	
Replication Randomization Blinding Reportin We require informati	outlier values w The number of Mice were rance No specific blin  g for specific blin on from authors ted is relevant to	biological replicates is detailed in the Material and Methods subsections  domly allocated to the distinct experimental groups  ding strategy was used  Decific materials, systems and methods  about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.	
Replication Randomization Blinding  Reportin We require information rystem or method list  Materials & ex n/a Involved in the	outlier values w The number of Mice were rance No specific blin  g for sk on from authors ted is relevant to perimental sine study	biological replicates is detailed in the Material and Methods subsections  domly allocated to the distinct experimental groups  ding strategy was used  Decific materials, systems and methods  about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.  Systems  Methods  n/a Involved in the study	
Replication Randomization Blinding  Reportin We require informati ystem or method list	outlier values w The number of Mice were rance No specific blin  g for specific blin on from authors ted is relevant to perimental specific study	biological replicates is detailed in the Material and Methods subsections  domly allocated to the distinct experimental groups  ding strategy was used  Decific materials, systems and methods  about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.	

### **Antibodies**

Plants

Antibodies used

The following primary antibodies have been used in immunofluorescence:
Mouse anti-Calbindin, Sigma #C9848, RRID: AB\_476894, 1/400
Rabbit anti-Calretinin, Swant #CR-7697, RRID: AB\_2619710, 1/500

Chicken anti-Doublecortin, Synaptic Systems #326006, RRID: AB 2737040, 1/500 Goat anti-Doublecortin, Santa Cruz #8066 (discontinued), RRID: AB\_2088494, 1/1,000 Guinea Pig anti-Doublecortin, Millipore #ab2253, RRID: AB 1586992, 1/250 Guinea Pig anti-Doublecortin, Synaptic Systems #326004, RRID: AB\_2620068, 1/500 Mouse anti-Doublecortin, Santa Cruz #sc271390, RRID: AB 10610966, 1/50 Rabbit anti-Doublecortin, Abcam #ab18723, RRID: AB\_732011, 1/500 Rabbit anti-Doublecortin, Atlas #HPA036121, RRID: AB\_2674950, 1/100 Rabbit anti-Doublecortin, Cell Signalling #4604, RRID: AB 561007, 1/250 Rabbit anti-Doublecortin, Synaptic Systems #326003, RRID:AB 2620067, 1/500 Rabbit anti-Doublecortin, Abcam #ab207175, RRID: AB\_2894710, 1/1,000 Chicken anti-Iba1, Synaptic Systems #234006 (discontinued), RRID: AB 2619949, 1/1,000 Rabbit anti-Ki67, Abcam #ab15580, RRID: AB\_443209, 1/500 Mouse anti- Polysialylated neural cell adhesion molecule, Millipore #mab5324, RRID: AB\_95211, 1/1,000 Guinea pig anti-S100 calcium-binding protein β, Synaptic Systems #287004, RRID: AB 2620025, 1/500 Goat anti-SRY (sex determining region Y)-box 2 (Sox2), R and D Systems #AF2018, RRID: AB\_355110, 1/700 Rabbit anti-Vimentin, Abcam #ab193555, RRID: AB 2814713, 1/1,000 Rabbit anti-Vinculin, Abcam #ab129002, RRID:AB\_11144129, 1/7,500

The following secondary antibodies were used to detect the binding of primary antibodies: Donkey Alexa-488 Anti-Rabbit, Thermo Fisher #A-21206, RRID: AB\_2535792, 1/1,000 Donkey Alexa-555 Anti-Mouse, Thermo Fisher #A-31570, RRID: AB\_2536180, 1/1,000 Goat Alexa-555 Anti-Guinea Pig , Thermo Fisher #A-21435, RRID: AB\_2535856, 1/1,000 Donkey Alexa-555 Anti-Goat, Thermo Fisher #A-21432, RRID: AB\_2535853, 1/1,000 Donkey Alexa-647 Anti-Mouse, Thermo Fisher #A-31571, RRID: AB\_162542, 1/1,000 Donkey Alexa-647 Anti-Guinea Pig, Jackson ImmunoResearch #706-605-148, RRID: AB\_2340476, 1/1,000 Goat Alexa-647 Anti-Chicken, Thermo Fisher #A-21449, RRID: AB\_2535866, 1/1,000 Goat HRP Anti-Rabbit, Dako #P0448, RRID: AB\_2617138, 1/1,000

#### Validation

- Mouse anti-Calbindin, Sigma #C9848, RRID: AB\_476894, 1/400: Validated by manufacturer to detect Calbindin of mouse, human, guinea pig, canine, sheep, rat, pig, rabbit, feline, bovine, goat origin by IHC, WB and ELISA.
- Rabbit anti-Calretinin, Swant #CR-7697, RRID: AB\_2619710, 1/500: validated by manufacturer to detect Calretinin of human, monkey, rat, mouse, guinea pig, chicken, and fish origin by WB and IHC.
- Chicken anti-Doublecortin, Synaptic Systems #326006, RRID: AB\_2737040, 1/500: validated by manufacturer to detect Doublecortin of mice origin by ICC and IHC.
- Goat anti-Doublecortin, Santa Cruz #8066 (discontinued), RRID: AB\_2088494, 1/1,000: Validated by manufacturer to detect Doublecortin of mouse, rat, human, and avian origin by WB, IP, IF, IHC, and ELISA.
- Guinea Pig anti-Doublecortin, Millipore #ab2253, RRID: AB\_1586992, 1/250: Validated by manufacturer to detect Doublecortin of mouse and rat origin by WB, IHC and ICC.
- Guinea Pig anti-Doublecortin, Synaptic Systems #326004, RRID: AB\_2620068, 1/500: validated by manufacturer to detect Doublecortin of mice and rat origin by WB, IP, ICC and IHC.
- Mouse anti-Doublecortin, Santa Cruz #sc271390, RRID: AB\_10610966, 1/50: Validated by manufacturer to detect Doublecortin of mouse, rat, and human origin by WB, IP, IF, IHC, and ELISA.
- Rabbit anti-Doublecortin, Abcam #ab18723, RRID: AB\_732011, 1/500: Validated by manufacturer to detect Doublecortin of mouse, rat, and quail origin by WB, IHC and ICC.
- Rabbit anti-Doublecortin, Atlas #HPA036121, RRID: AB\_2674950, 1/100: Validated by manufacturer to detect Doublecoritn of human origin by IHC
- Rabbit anti-Doublecortin, Cell Signalling #4604, RRID: AB\_561007, 1/250: Validated by manufacturer to detect Doublecortin of mouse and rat origin by WB, IP, IF, IHC and ICC.
- Rabbit anti-Doublecortin, Synaptic Systems #326003, RRID: AB\_2620067, 1/500: validated by manufacturer to detect Doublecortin of mice, rat and human origin by WB, IP, ICC and IHC.
- Rabbit anti-Doublecortin, Abcam #ab207175, RRID: AB\_2894710, 1/1,000: validated by manufacturer to detect Doublecortin of mice, rat and human origin by WB, IF, ICC and IHC.
- Chicken anti-Iba1, Synaptic Systems #234006 (discontinued), RRID: AB\_2619949, 1/1,000: validated by manufacturer to detect Iba1 of mice, rat and human origin by WB, IF, ICC and IHC.
- Rabbit anti-Ki67, Abcam #ab15580, RRID: AB\_443209, 1/500: validated by manufacturer to detect Ki-67 of mice and human origin by ICC and IHC.
- Mouse anti- Polysialylated neural cell adhesion molecule (PSA-NCAM), Millipore #mab5324, RRID: AB\_95211, 1/1,000: validated by manufacturer to detect PSA-NCAM of mice, rat and human origin by ICC, IHC, RIA and WB.
- Guinea pig anti-S100 calcium-binding protein β (S100β), Synaptic Systems #287004, RRID: AB\_2620025, 1/500: validated by

	manufacturer to	detect S100β o	of mice and rat	origin	by ICC and	IHC
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- Goat anti-SRY (sex determining region Y)-box 2 (Sox2), R and D Systems #AF2018, RRID: AB\_355110, 1/700: validated by manufacturer to detect Sox2 of mice, rat and human origin by WB, ChIP and ICC.
- Rabbit anti-Vimentin, Abcam #ab193555, RRID: AB\_2814713, 1/1,000: validated by manufacturer to detect Vimentin of mice, rat, African green monkey and human origin by WB, Flow cytometry, IHC, IF and ICC.
- Rabbit anti-Vinculin, Abcam #ab129002, RRID:AB\_11144129, 1/7,500: validated by manufacturer to detect Vinculin of mice, rat and human origin by WB, Flow cytometry, IP, IF and ICC.

Secondary antibodies were validated by manufacturer and have been extensively validated in the literature.

Eul	kary	/otic	cel	Llines
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Eukaryotic cell lin	es			
Policy information about <u>ce</u>	ell lines	and Sex and Gender in Research		
Cell line source(s)		N/A		
Authentication		N/A		
Mycoplasma contaminati	ion	N/A		
Commonly misidentified lines (See <u>ICLAC</u> register)		N/A		
Palaeontology an	d Arc	chaeology		
Specimen provenance	N/A			
Specimen deposition	N/A			
Dating methods	N/A			
Tick this box to confir	m that	the raw and calibrated dates are available in the paper or in Supplementary Information.		
Ethics oversight	N/A			
Note that full information on t	he appro	oval of the study protocol must also be provided in the manuscript.		
Animals and othe	r res	earch organisms		
		nvolving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in		
Laboratory animals	Seven-	to nine-week-old C57BL/6J-OlaHsd mice		
Wild animals	N/A			
Reporting on sex	All mice included in the study were female.			
Field-collected samples	N/A			
Ethics oversight	Ethics oversight Animal experiments were approved by the CBMSO (AEEC-CBMSO-23/172) and National (PROEX 185.4/20) Ethics Committees.			
Note that full information on the approval of the study protocol must also be provided in the manuscript.				
Clinical data				
Policy information about <u>cl</u> All manuscripts should comply		tudies  E ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.		
Clinical trial registration	N/A			
Study protocol	N/A			
Data collection	N/A			

Outcomes	N/A				
Dual use research	of concern				
Policy information about de	ual use research of concern				
Hazards					
Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:					
No Yes	No Yes				
Public health	Public health				
National security					
Crops and/or lives	ock .				
Ecosystems					
Any other significa	nt area				
Experiments of concer	n				
Does the work involve an	y of these experiments of concern:				
No Yes					
- -	to render a vaccine ineffective				
-1-	to therapeutically useful antibiotics or antiviral agents				
	nce of a pathogen or render a nonpathogen virulent				
	ibility of a pathogen				
Alter the host rang					
-1-	diagnostic/detection modalities				
	nization of a biological agent or toxin				
Any other potentia	ally harmful combination of experiments and agents				
Plants					
Seed stocks	N/A				
Novel plant genotypes	N/A				
Authentication	N/A				
ChIP-seq					
Data deposition					
Confirm that both raw and final processed data have been deposited in a public database such as GEO.					
Confirm that you have	e deposited or provided access to graph files (e.g. BED files) for the called peaks.				
Data access links	N/A				
May remain private before publi					
Files in database submiss	ion N/A				
Genome browser session (e.g. <u>UCSC</u> )	N/A				
Methodology					

N/A Replicates N/A Sequencing depth

Peak calling parameters  N/A  Data quality  N/A  Software  N/A  Flow Cytometry  Plots  Confirm that:  The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
Software  N/A  Flow Cytometry  Plots  Confirm that:  The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
Software  N/A  Flow Cytometry  Plots  Confirm that:  The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
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The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
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The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
All plots are contour plots with outliers or pseudocolor plots.
A numerical value for number of cells or percentage (with statistics) is provided.
Methodology
Sample preparation N/A
Instrument N/A
Software N/A
Cell population abundance N/A
Gating strategy N/A
Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.
Magnetic resonance imaging
Experimental design
Design type N/A
Design specifications N/A
Behavioral performance measures N/A
Acquisition
Imaging type(s)  N/A
Field strength N/A
Sequence & imaging parameters N/A
Area of acquisition N/A
Diffusion MRI Used Not used
Preprocessing
Preprocessing software N/A
Normalization N/A
Normalization template N/A
Noise and artifact removal  N/A  N/A

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Volume censoring	N/A		
Statistical modeling & infere	nce		
Model type and settings	N/A		
Effect(s) tested	N/A		
Specify type of analysis: W	hole brain ROI-based Both		
Statistic type for inference	N/A		
(See Eklund et al. 2016)			
Correction	N/A		
Models & analysis			
n/a   Involved in the study			
Functional and/or effective connectivity			
Multivariate modeling or p	redictive analysis		