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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.

Ctatistics					
Statistics For all statistical analysis	es, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.				
n/a   Confirmed	es, commit that the following items are present in the figure legend, table legend, main text, or internous section.				
	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement				
	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	A description of all covariates tested				
A description	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons				
A full descript  AND variation	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 Give <i>P</i> values as exact values whenever suitable.					
For Bayesian	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings				
For hierarchic	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 <i>d</i> , Pearson's <i>r</i> ), indicating how they were calculated					
I	Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.				
Software and o	code				
Policy information about <u>availability of computer code</u>					
Data collection	No software was used for data collection.				
Data analysis	Data analysis was performed with Matlab.				
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 abo					
- Accession codes, un - A list of figures that	include a <u>data availability statement</u> . This statement should provide the following information, where applicable: ique identifiers, or web links for publicly available datasets have associated raw data restrictions on data availability				
All data is included (anon	ymized) in the supplementary information.				
Field-speci	fic 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	Life sciences Behavioural & social sciences Ecological, evolutionary & environmental sciences				

For a reference copy of the document with all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>

## Life sciences study design

Gating strategy

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All studies must disc	close on the	se points even when the disclosure is negative.		
	All samples were taken at n=4 technical replicates, with cell samples also having an additional n=2 biological replicates (i.e., 8 replicates total) The upper bound on replicates was determined by plate size, as an adding more replicates would require more than one 96-well plate.			
Data exclusions	Data exclusions are detailed in the Methods section.			
Replication	Reproducibility is tested through the collection of data by 244 separate participating teams.			
Randomization	Randomization is not relevant: all data collection teams were asked to measure the same constructs.			
Blinding	The expected performance of constructs was not disclosed to data collection teams in advance.			
We require information system or method listed  Materials & exp  n/a Involved in the Antibodies  Eukaryotic of Palaeontolo Animals and	perimental	n/a Involved in the study  ChIP-seq  Flow cytometry  MRI-based neuroimaging		
Flow Cytome	etry			
The axis scales  All plots are co	s are clearly ontour plots	narker and fluorochrome used (e.g. CD4-FITC).  visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).  with outliers or pseudocolor plots.  sheer of cells or percentage (with statistics) is provided.		
Methodology				
Sample preparation	on	See supplementary information with protocol		
Instrument		Different teams used a variety of different instruments.		
Software	Data was collected by each team with its own instrument's associated software. Data was analyzed with TASBE Flow Analytics 7.3			
Cell population ab	oundance	nce Not applicable: no sorting was performed.		

Gating was computed automatically as a gaussian mixture model fit to FSC-A and SSC-A for the negative control.

| Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.