nature portfolio

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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.

Please do not complete any field with "not applicable" or n/a. Refer to the help text for what text to use if an item is not relevant to your study. For final submission: please carefully check your responses for accuracy; you will not be able to make changes later.

Statistics

For	all st	atistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.
n/a	Cor	nfirmed
	\square	The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
	\square	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.
\checkmark		A description of all covariates tested
	\square	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. <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> .
\checkmark		For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
\checkmark		For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
\checkmark		Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i>), indicating how they were calculated
		Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.

Software and code

Policy information about availability of computer code Data collection All data was collected using "MinKNOW" software v4.2 (or higher), available from Oxford Nanopore Technologies All data was analysed with "The nanopore app" v7 (or higher), which runs on Matlab 2021A (or higher), available from corresponding authors upon request. The nanopore app used "Guppy" v5 for sequencing and basecalling, available from Oxford Nanopore Technologies. Data analysis

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

The data that support the plots within this paper for figure 4 were supplied as part of the submission. Other findings of this study are available from the corresponding author upon reasonable request. Source data for the figures and supplementary figures are provided as a Source Data file.

Research involving human participants, their data, or biological material

Policy information about studies with <u>human participants or human data</u>. See also policy information about <u>sex, gender (identity/presentation)</u>, <u>and sexual orientation</u> and <u>race, ethnicity and racism</u>.

Reporting on sex and gender	We do not report the sex or gender of any donors within our study. We did not consider sex or gender within the study design. The study did not aim to determine any clinically relevant information but was to esablish a techology therefore consideration of gender or sex at this stage was irrelevant.
Reporting on race, ethnicity, or other socially relevant groupings	We did not report the race, ethnicity, or other socially relevant groupings within our study, for reasons detailed above
Population characteristics	Age: 26-35 years; Gender: male/female,50/50;
Recruitment	Participants were recruited from Students and Staff of Imperial College London via word of mouth and posters. All participants identified as "healthy", using the study inclusion criteria. Participant inclusion criteria were: >18 years old; No known cardiovascular disease; no COVID-19 symptoms; no blood borne disease; not pregnant/ breast feeding; not prescribed diuretics/beta-blockers/ACE inhibitors/ Ca channel blockers/Dpostn
Ethics oversight	Human samples used in this research project were obtained from the Imperial College Healthcare Tissue Bank (ICHTB). ICHTB is supported by the National Institute for Health Research (NIHR) Biomedical Research Centre based at Imperial College Healthcare NHS Trust and Imperial College London. ICHTB is approved by Wales REC3 to release human material for research (22/WA/0214), and the samples for this project (R22016) were issued from subcollection reference number NHL_FN_021_028.

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 <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No sample size calculation was performed, there was no comparison between study groups. The use of human samples was to establish the technology, rather than observe clinical characteristics
Data exclusions	No data was excluded from the study
Replication	All experiements were performed with technical triplicates successfully
Randomization	There was no randomisation as the study was observational (there was no treatment performed on any samples)
Blinding	Blinding was not relevant to the human sample arm of the study as there were no groups allocated. We indicate in the manuscript where experiments have been blinded

Behavioural & social sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	
Research sample	
Sampling strategy	
Data collection	
Timing	
Data exclusions	
Non-participation	
Randomization	

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description		
Research sample		
Sampling strategy		
Data collection		
Timing and spatial scale		
Data exclusions		
Reproducibility		
Randomization		
Blinding		
Did the study involve field work?		

Field work, collection and transport

Field conditions	n/a
Location	n/a
Access & import/export	n/a
Disturbance	n/a

Reporting for specific materials, systems and methods

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	n/a Involved in the study
∇	Antibodies	ChIP-seq
\bigtriangledown	Eukaryotic cell lines	V Flow cytometry
\checkmark	Palaeontology and archaeology	MRI-based neuroimaging
\checkmark	Animals and other organisms	
\mathbf{V}	Clinical data	
\bigvee	Dual use research of concern	
\checkmark	Plants	

Antibodies

Antibodies used
Validation

Eukaryotic cell lines

Policy information about <u>cell lines and Sex and Gender in Research</u>			
Cell line source(s)			
Authentication			
Mycoplasma contamination			
Commonly misidentified lines (See ICLAC register)			
(000 100 10 100.000)			

Palaeontology and Archaeology

Specimen provenance			
Specimen deposition			
Dating methods			
Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.			
Ethics oversight			
Note that full information on the approval of the study protocol must also be provided in the manuscript			

Animals and other research organisms

Policy information about studies involving animals; <u>ARRIVE guidelines</u> recommended for reporting animal research, and <u>Sex and Gender in</u> <u>Research</u>

Laboratory animals	
Wild animals	
Reporting on sex	
Field-collected samples	
Ethics oversight	

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about <u>clinical studies</u>

All manuscripts should comply with the ICMJE	guidelines for publication of clinical	research and a completed CONSORT	checklist must be included with all submissions.

Clinical trial registration	
Study protocol	
Data collection	
Outcomes	

Dual use research of concern

Policy information about <u>dual use research of concern</u>

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:

Yes
Public health
National security
Crops and/or livestock
Ecosystems
Any other significant area

Experiments of concern

Does the work involve any of these experiments of concern:

No	Yes
	Demonstrate how to render a vaccine ineffective
	Confer resistance to therapeutically useful antibiotics or antiviral agents
	Enhance the virulence of a pathogen or render a nonpathogen virulent
	Increase transmissibility of a pathogen
	Alter the host range of a pathogen
	Enable evasion of diagnostic/detection modalities
	Enable the weaponization of a biological agent or toxin
	Any other potentially harmful combination of experiments and agents

Plants

Seed stocks	
Novel plant genotypes	
Authentication	

ChIP-seq

Data deposition

Confirm that both raw and final processed data have bee	n deposited in a public database such as GEO.

Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links May remain private before publication.	
Files in database submission	
Genome browser session (e.g. <u>UCSC</u>)	
Methodology	
Replicates	
Sequencing depth	
Antibodies	
Peak calling parameters	
Data quality	
Software	

nature portfolio | reporting summary

Flow Cytometry

Plots

Confirm that:

The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

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	
Instrument	
Software	
Cell population abundance	
Gating strategy	

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

Magnetic resonance imaging

Specify type of analysis: 🗌 Whole brain

Experimental design

Design type	
Design specifications	
Behavioral performance measures	
Imaging type(s)	
inidging type(3)	
Field strength	
Sequence & imaging parameters	
Area of acquisition	
Diffusion MRI Used	Not used
Preprocessing	
Preprocessing software	
Normalization	
Normalization template	
Noise and artifact removal	
Volume censoring	
Statistical modeling & inference	
Model type and settings	
Effect(s) tested	

Both

ROI-based

Statistic type for inference		
(See <u>Eklund et al. 2016</u>)		
Correction		
Models & analysis		
n/a Involved in the study		
Functional and/or effective	e connectivity	
Graph analysis		
Multivariate modeling or p	redictive analysis	
Functional and/or effective conn	ectivity	
Graph analysis		
Multivariate modeling and predic	ctive analysis	

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