# **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.

### 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	firmed
	×	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.
X		A description of all covariates tested
X		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, t, 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>
	×	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
X		For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
X		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 statistics for biologists contains articles on many of the points above.

# Software and code

Policy information about availability of computer code

Zen core 3 (Zeiss), GeoStar µGIS Software (Norris Scientific, Australia), EAGER (version 1.92.37), Clip&Merge, Burrows-Wheeler Aligner (BWA Data collection version 0.6.2), samtools (version 1.7), DeDup, pileupCaller (https://github.com/stschiff/sequenceTools), Edmond Data Repository of the Max Planck Society (https://edmond.mpdl.mpg.de/dataset.xhtml?persistentId=doi:10.17617/3.Y1KJMF), AADR v44.3 (https:// reichdata.hms.harvard.edu/pub/datasets/amh\_repo/curated\_releases/index\_v44.3.html) lolite Software (Igor Pro, WaveMetrics, Inc.), Software R (ver. 4.3.1, R-Core-Team 2023), smartpca software (EIGENSOFT v7.2.1), ADMIXTOOLS Data analysis (version 7.0.1), ANGSD pipeline, contamLD, mapDamage2.0, Ry value, Rx value, Sex DetERRmine, Yleaf (version 2.2), schmutzi (version 1.5.4), Haplogrep2 (version 2.4.0), MUSCLE, MEGAX, jModelTest v2.1.7, BEAST (version 1.10.4), BEAST's treeannotator program, Figtree (v1.4.4, https://github.com/rambaut/figtree/), BEAST's Tracer program, cmdscale() function of R, python tool (https://github.com/TCLamnidis/ pMMRCalculator), hapROH (version 0.4a1), SNPediaR (version 1.16.0), HIrisPlex-S, IntCal2020 and OxCal platform (version 4.4).

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.

### 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

All software packages used for analysis are publicly available and cited in the Methods section or in the Supplementary Notes. Source data are either provided in the Supplementary Data file or as a Source Data file. The new genomic data reported in this study is available at the European Nucleotide Archive (ENA) under accession number PRJEB66279.

# 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</u>, <u>ethnicity and racism</u>.

Reporting on sex and gender	N/A
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

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 science	s 🗌	Behavioural & social sciences	×	Ecological,	evolutionary	/ & environmental	sciences
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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	Describe how sample size was determined, detailing any statistical methods used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.
Data exclusions	Describe any data exclusions. If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.
Replication	Describe the measures taken to verify the reproducibility of the experimental findings. If all attempts at replication were successful, confirm this OR if there are any findings that were not replicated or cannot be reproduced, note this and describe why.
Randomization	Describe how samples/organisms/participants were allocated into experimental groups. If allocation was not random, describe how covariates were controlled OR if this is not relevant to your study, explain why.
Blinding	Describe whether the investigators were blinded to group allocation during data collection and/or analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.

# Behavioural & social sciences study design

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

Study description

Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sectional, quantitative experimental, mixed-methods case study).

Research sample	State the research sample (e.g. Harvard university undergraduates, villagers in rural India) and provide relevant demographic information (e.g. age, sex) and indicate whether the sample is representative. Provide a rationale for the study sample chosen. For studies involving existing datasets, please describe the dataset and source.
Sampling strategy	Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient. For qualitative data, please indicate whether data saturation was considered, and what criteria were used to decide that no further sampling was needed.
Data collection	Provide details about the data collection procedure, including the instruments or devices used to record the data (e.g. pen and paper, computer, eye tracker, video or audio equipment) whether anyone was present besides the participant(s) and the researcher, and whether the researcher was blind to experimental condition and/or the study hypothesis during data collection.
Timing	Indicate the start and stop dates of data collection. If there is a gap between collection periods, state the dates for each sample cohort.
Data exclusions	If no data were excluded from the analyses, state so OR if data were excluded, provide the exact number of exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.
Non-participation	State how many participants dropped out/declined participation and the reason(s) given OR provide response rate OR state that no participants dropped out/declined participation.
Randomization	If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if allocation was not random, describe how covariates were controlled.

# Ecological, evolutionary & environmental sciences study design

Study description	Palaeogenomics, dental palaeohistology, spatially-resolved geochemical analyses, and direct AMS radiocarbon dating of the skeletal remains of the Upper Palaeolithic human infant of Grotta delle Mura
Research sample	Archaeological bones and teeth of Le Mura 1, Upper Palaeolithic Homo sapiens of Grotta delle Mura. The pars petrosa was selected for palaeogenomic extraction for the better chance of extracting well-preserved DNA. The deciduous incisor and the permanent molar were selected to cover a broad lifespan of the infant and estimate its age at death.
Sampling strategy	Selected bones and teeth from the skeletal remains of Le Mura 1 in the best possible state of preservation
Data collection	The teeth were embedded and sectioned by O.A.H. The resulting thin sections of the teeth were analysed for histomorphometry by O.A.H. and measured with LA-ICPMS by F.L. Fragments of vertebrae were sampled for AMS radiocarbon dating. The left petrous bone was sampled for aDNA analysis. All is described in detail in the Methods section.
Timing and spatial scale	Sample preparation and data collection were performed from October 2019 to July 2022. Sampling occurred in a single occasion. Data were analyzed from July 2021 to May 2023.
Data exclusions	No data was excluded from the analyses.
Reproducibility	n/a
Randomization	n/a
Blinding	n/a

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

Did the study involve field work? Yes

# Field work, collection and transport

Field conditions	Describe the study conditions for field work, providing relevant parameters (e.g. temperature, rainfall).
Location	State the location of the sampling or experiment, providing relevant parameters (e.g. latitude and longitude, elevation, water depth).
Access & import/export	Describe the efforts you have made to access habitats and to collect and import/export your samples in a responsible manner and in compliance with local, national and international laws, noting any permits that were obtained (give the name of the issuing authority, the date of issue, and any identifying information).
Disturbance	Describe any disturbance caused by the study and how it was minimized.

× No

# 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
	<ul> <li>Palaeontology and archaeology</li> </ul>
×	Animals and other organisms
×	Clinical data
×	Dual use research of concern
×	Plants

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- n/a Involved in the study
- Flow cytometry
- ■ MRI-based neuroimaging

# Antibodies

Antibodies used	Describe all antibodies used in the study; as applicable, provide supplier name, catalog number, clone name, and lot number.
Validation	Describe the validation of each primary antibody for the species and application, noting any validation statements on the manufacturer's website, relevant citations, antibody profiles in online databases, or data provided in the manuscript.

# Eukaryotic cell lines

# Policy information about <u>cell lines and Sex and Gender in Research</u>

Cell line source(s)	State the source of each cell line used and the sex of all primary cell lines and cells derived from human participants or vertebrate models.
Authentication	Describe the authentication procedures for each cell line used OR declare that none of the cell lines used were authenticated.
Mycoplasma contamination	Confirm that all cell lines tested negative for mycoplasma contamination OR describe the results of the testing for mycoplasma contamination OR declare that the cell lines were not tested for mycoplasma contamination.
Commonly misidentified lines (See <u>ICLAC</u> register)	Name any commonly misidentified cell lines used in the study and provide a rationale for their use.

# Palaeontology and Archaeology

Specimen provenance	The specimen was excavated from and is housed at the University of Siena (Siena, Italy). All the relevant permissions for the described study were obtained from the Soprintendenza Archeologia, belle arti e paesaggio per la città metropolitana di Bari within the Ministry of Cultural Heritage and Activities and Tourism (MiBACT) (protocol number 4997).
Specimen deposition	The specimen is deposited at the University of Siena (Siena, Italy) with free access following a research application
Dating methods	The dating was performed on the vertebral samples, which were pretreated at MPI-EVA Leipzig. The pretreatment of R-EVA 3275 followed the protocol described in Fewlass et al. (2019) since it was a small amount of bone material. Whereas R-EVA 3276 followed the standard procedure described in Talamo et al. (2021). The collagen was combusted and converted to graphite using the AGE system before being measured at the AMS at the Klaus-Tschira-Labor für Physikalische Altersbestimmung (Curt-Engelhorn- Zentrums für Archaometrie), Mannheim, Germany. Radiocarbon dates were calibrated in OxCal v4.4.416 using the IntCal20 calibration curve.

**X** Tick this box to confirm that the raw and calibrated dates are available in the paper or in Supplementary Information.

Ethics oversight Ethical approval and guidance was not required, as we sampled archaeological collection specimens only.

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; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	For laboratory animals, report species, strain and age OR state that the study did not involve laboratory animals.	
Wild animals	Provide details on animals observed in or captured in the field; report species and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.	
Reporting on sex	Indicate if findings apply to only one sex; describe whether sex was considered in study design, methods used for assigning sex. Provide data disaggregated for sex where this information has been collected in the source data as appropriate; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex-based analyses where performed, justify reasons for lack of sex-based analysis.	
Field-collected samples	For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.	
Ethics oversight	Identify the organization(s) that approved or provided guidance on the study protocol, OR state that no ethical approval or guidance was required and explain why not.	

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 ICMJEguidelines for publication of clinical research and a completed <u>CONSORT checklist</u> must be included with all submissions.

Clinical trial registration	Provide the trial registration number from ClinicalTrials.gov or an equivalent agency.	
Study protocol	Note where the full trial protocol can be accessed OR if not available, explain why.	
Data collection	Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.	
Outcomes	Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.	

# Dual use research of concern

Policy information about dual 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:



### 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	Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.
Novel plant genotypes	Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.
Authentication	Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.

# 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 deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links May remain private before publication.	For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.	
Files in database submission	Provide a list of all files available in the database submission.	
Genome browser session (e.g. UCSC) Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents		

### Methodology

Replicates	Describe the experimental replicates, specifying number, type and replicate agreement.	
Sequencing depth	Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.	
Antibodies	Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.	
Peak calling parameters	Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.	
Data quality	Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.	
Software	Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.	

# 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	Describe the sample preparation, detailing the biological source of the cells and any tissue processing steps used.	
Instrument	Identify the instrument used for data collection, specifying make and model number.	
Software	Describe the software used to collect and analyze the flow cytometry data. For custom code that has been deposited into a community repository, provide accession details.	
Cell population abundance	Describe the abundance of the relevant cell populations within post-sort fractions, providing details on the purity of the samples and how it was determined.	
Gating strategy	Describe the gating strategy used for all relevant experiments, specifying the preliminary FSC/SSC gates of the starting cell population, indicating where boundaries between "positive" and "negative" staining cell populations are defined.	

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

# Magnetic resonance imaging

### Experimental design

1 0		
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, and specify the length of each trial or block (if trials are blocked) and interval between trials.	
Behavioral performance measure	State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).	
Acquisition		
Imaging type(s)	Specify: functional, structural, diffusion, perfusion.	
Field strength	Specify in Tesla	
Sequence & imaging parameters	Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.	
Area of acquisition	State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined	
Diffusion MRI Used	□ Not used	
Preprocessing		
Preprocessing software	Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).	
	If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.	
Normalization template	Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original 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 parameters, tissue signals and	

physiological signals (heart rate, respiration).

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Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.

# Statistical modeling & inference

Model type and settings	Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).		
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: 🗌 🛛	hole brain 🔲 ROI-based 🔲 Both		
Statistic type for inference	tic type for inference (Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.		
(See <u>Eklund et al. 2016</u> )			
Correction	Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).		
Models & analysis n/a Involved in the study Functional and/or effective Graph analysis Multivariate modeling or p			
Functional and/or effective conn	Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).		
Graph analysis	Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).		

Specify independent variables, features extraction and dimension reduction, model, training and evaluation

Multivariate modeling and predictive analysis