

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 statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

|                                     |                                                                                                                                                                                                                                                                                                |
|-------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| n/a                                 | Confirmed                                                                                                                                                                                                                                                                                      |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> The exact sample size ( <i>n</i> ) for each experimental group/condition, given as a discrete number and unit of measurement                                                                                                                               |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly                                                                                                                                    |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided<br><i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>                                                               |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> A description of all covariates tested                                                                                                                                                                                                                                |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons                                                                                                                                                   |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> 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) |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> 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<br><i>Give P values as exact values whenever suitable.</i>                     |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings                                                                                                                                                                      |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes                                                                                                                                                |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> 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](#)

|                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
|-----------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Data collection | Software used to collect/preprocess data in this study (package/version/source):<br>bcl2fastq 2.20 Illumina<br>Guppy 6.0.1 Oxford Nanopore<br>Guppy 5.0.17 Oxford Nanopore<br>BD FACSDiva 6.1.3 BD Biosciences<br>FlowJo v10.7 BD Biosciences<br>Megalodon 2.5 Oxford Nanopore<br>Proteome Discoverer v2.5 Thermo Fisher Scientific<br>Mascot search engine v2.6 Matrix Science                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| Data analysis   | Open source software/packages used in for data analysis in this study (package/version/source):<br>Medaka 1.5.0 Oxford Nanopore<br>NextDenovo 2.5.0 <a href="https://github.com/Nextomics/NextDenovo">https://github.com/Nextomics/NextDenovo</a><br>Flye 2.9-b1768 <a href="https://github.com/mikolmogorov/Flye">https://github.com/mikolmogorov/Flye</a><br>NECAT 0.0.1 <a href="https://github.com/xiaochuanle/NECAT">https://github.com/xiaochuanle/NECAT</a><br>minimap2 2.18-r1015 <a href="https://github.com/lh3/minimap2">https://github.com/lh3/minimap2</a><br>Shasta 0.8.0 <a href="https://github.com/paoloshasta/shasta">https://github.com/paoloshasta/shasta</a><br>purge_dups 1.2.5 <a href="https://github.com/dfguan/purge_dups">https://github.com/dfguan/purge_dups</a><br>BRAKER2 <a href="https://github.com/Gaius-Augustus/BRAKER">https://github.com/Gaius-Augustus/BRAKER</a><br>TSEBRA <a href="https://github.com/Gaius-Augustus/TSEBRA">https://github.com/Gaius-Augustus/TSEBRA</a> |

StringTie <https://github.com/gpertea/stringtie>  
 LiftOff 1.6.1 <https://github.com/agshumate/LiftOff>  
 Broccoli 1.2 <https://github.com/rderelle/Broccoli>  
 Cactus 2.6.4 <https://github.com/ComparativeGenomicsToolkit/cactus>  
 EDTA 2.1.0 <https://github.com/oushujun/EDTA>  
 biobambam2 (<https://github.com/gt1/biobambam2>)  
 Juicer 1.6 <https://github.com/aidenlab/juicer>  
 Juicebox Assembly Tools 1.11.08 <https://github.com/aidenlab/Juicebox>  
 METALoci 0.3.0 <https://github.com/3DGenomes/METALoci>  
 pybbi 0.4.0 <https://github.com/nvictus/pybbi>  
 metacell 0.3.41 <https://github.com/tanaylab/metacells>  
 bwa 0.7.17 <https://github.com/lh3/bwa>  
 pairtools 0.2.2 <https://github.com/open2c/pairtools>  
 cooler 0.8.11 <https://github.com/open2c/cooler>  
 cooltools 0.5.1 <https://github.com/open2c/cooltools>  
 coolpuppy 1.1.0 <https://github.com/open2c/coolpuppy>  
 coolbox 0.3.8 <https://github.com/GangCaoLab/CoolBox>  
 SIP\_HiC 1.6.1 <https://github.com/PouletAxel/SIP>  
 TOBIAS 0.13.3 <https://github.com/loosolab/TOBIAS>  
 MACS2 2.2.6 <https://github.com/macs3-project/MACS>  
 Phast toolkit <http://compugen.cshl.edu/phast/>  
 HOMER 4.11.1 <http://homer.ucsd.edu/homer/motif/>  
 Trimmomatic 0.39 <http://www.usadellab.org/cms/?page=trimmomatic>  
 BUSCO 5.1.2 [https://busco.ezlab.org/busco\\_userguide.html](https://busco.ezlab.org/busco_userguide.html)  
 HISAT2 <https://daehwankimlab.github.io/hisat2/>  
 bedGraphToBigWig 2.9 UCSC  
 data.table 1.13.0 [cran.r-project.org](https://cran.r-project.org)  
 topGO 2.34.0 Bioconductor  
 ggplot2 3.4.4 Bioconductor  
 ggprism 1.0.4 Bioconductor  
 plyr 1.8.9 Bioconductor  
 rtracklayer 1.62.0 Bioconductor  
 GenomicRanges 1.54.1 Bioconductor  
 GenomicFeatures 1.54.3 Bioconductor  
 mixtools R 2.0.0 Bioconductor  
 mclust 6.1.1 Bioconductor  
 monaLisa 1.12.0 Bioconductor

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](#)

All sequencing data is deposited in GEO under accession number GSE260572. Processed data, annotation tables, and code for reproducing the analysis are available on GitHub <https://github.com/sebepedroslab/early-metazoa-3D-chromatin>. All generated datasets can be explored in interactive genome browsers: <https://sebelab.crg.eu/3d-genomes-arc-jb2>. The chromatin proteomics data is deposited to the PRIDE repository with the dataset identifier PXD056500. The de novo sequenced genome of *C. owczaraki* is deposited under BioProject PRJDB19057; *M. leidy* genome: BioProject PRJNA1174117 (genome accession number JBMABS000000000); *E. muelleri* genome: BioProject PRJNA1175447. We used publicly available RepeatMasker (version open-4-0-3) annotation of GRCh38 genome released by UCSC <https://hgdownload.soe.ucsc.edu/goldenPath/hg38/bigZips/>.

## Human research participants

Policy information about [studies involving human research participants and Sex and Gender in Research](#).

|                             |    |
|-----------------------------|----|
| Reporting on sex and gender | NA |
| Population characteristics  | NA |
| Recruitment                 | NA |
| Ethics oversight            | NA |

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 [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.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     | No statistical method was used to predetermine sample size. Sample size was determined based on preliminary experiments. Multiple nuclei (n = 2,000,000 per replicate) from multiple animals were used to prepare Micro-C libraries. For ATAC-seq at least 50,000 nuclei from minimum 2 individuals were used per replicate. ChIP-seq libraries were prepared from chromatin obtained from at least 4 specimens per replicate or at least 500,000 cells.          |
| Data exclusions | No data were excluded from the analysis.                                                                                                                                                                                                                                                                                                                                                                                                                          |
| Replication     | Each Micro-C dataset is represented by at least 2 replicates. The reproducibility of replicates is confirmed by the stratum adjusted correlation coefficient. ChIP-seq and ATAC-seq datasets are represented by at least two replicates. The MARS-seq experiment was conducted using four 384-well MARS-seq plates. The DAP-seq binding assay was performed three times in independent experiments. All replication attempts were successful for all experiments. |
| Randomization   | For each Micro-C, ChIP-seq, ATAC-seq, and RNA-seq experiment, we used more than six animals per biological replicate. Each animal was randomly selected from the laboratory culture. For unicellular organisms, at least two independent cell cultures were pooled for each biological replicate.                                                                                                                                                                 |
| Blinding        | Data collection and analysis was not performed blind. The experimental design of this study required direct observation, handling, and processing of morphologically distinct organisms, which prevented blinding.                                                                                                                                                                                                                                                |

## 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                                           |
|-------------------------------------|-----------------------------------------------------------------|
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Antibodies                  |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Eukaryotic cell lines                  |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Palaeontology and archaeology          |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Animals and other organisms |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Clinical data                          |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Dual use research of concern           |

### Methods

| n/a                                 | Involved in the study                              |
|-------------------------------------|----------------------------------------------------|
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> ChIP-seq       |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Flow cytometry |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> MRI-based neuroimaging    |

## Antibodies

|                 |                                                                                                                                                                            |
|-----------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Antibodies used | anti-H3K4me1 (Cell Signaling, 5326)<br>anti-H3K4me2 (Abcam, ab32356)<br>anti-H3K4me3 (Millipore, 07-473)<br>anti-SMC1 (ThermoFisher, A300-055A)<br>anti-H3 (Abcam, ab1791) |
| Validation      | All antibodies used in this study are commercially available products. Their validation statements are provided on the manufacturer's websites.                            |

## 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      | Sphaeroforma arctica; Salpingoeca rosetta; Capsaspora owczarzaki strain ATCC30864; Ephydatia muelleri, 7 days old specimens; Mnemiopsis leidyi, adult specimens; Hormiphora californensis, adult specimens; Trichoplax adhaerens H1 strain, adult specimens; Cladertaria collaboinventa, adult specimens; Nematostella vectensis NvElav1::mOrange transgenic line, 1.5-2 month old adult specimens.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| Wild animals            | No wild animals were used in this study.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |
| Reporting on sex        | The sex of the sampled animals was not recorded during collection. In unicellular holozoans, sex determination is either unreported (S. arctica, C. owczarzaki) or may represent a temporal differentiation state, where the same genotype can produce both male and female gametes (S. rosetta). Both ctenophores (M. leidyi and H. californensis) are simultaneous hermaphrodites. The sponge E. muelleri and placozoans (T. adhaerens and C. collaboinventa) do not complete their sexual cycle under laboratory conditions and reproduce asexually. N. vectensis specimens were collected before sexual maturity, making it impossible to distinguish between male and female individuals. However, we assume an equal representation of both sexes in the sample. The findings of this study reveal global genome organization principles at the whole-genome level that apply to both sexes where relevant. |
| Field-collected samples | No field collected samples were used in this study.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
| Ethics oversight        | This study does not require an ethical approval.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |

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

## 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<br><i>May remain private before publication.</i> | The raw and processed sequencing data are deposited in GEO under accession number GSE260572.      |
| Files in database submission                                       | NA                                                                                                |
| Genome browser session<br>(e.g. <a href="#">UCSC</a> )             | <a href="https://sebelab.crg.eu/3d-genomes-arc-jb2">https://sebelab.crg.eu/3d-genomes-arc-jb2</a> |

### Methodology

|                         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
|-------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Replicates              | For each species, we performed at least two replicates per antibody used in this study.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| Sequencing depth        | 15-20M reads/experiment.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |
| Antibodies              | anti-H3K4me1 (Cell Signaling, 5326)<br>anti-H3K4me2 (Abcam, ab32356)<br>anti-H3K4me3 (Millipore, 07-473)<br>anti-SMC1 (ThermoFisher, A300-055A)<br>anti-H3 (Abcam, ab1791)                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| Peak calling parameters | we used MACS2 2.2.6 to call peaks using the concatenated libraries for each species. Specifically, we used the callpeak utility to identify peaks from the filtered BAM files, with the following options: (i) an effective genome size equal to the ungapped genome length of each species (i.e. removing uncalled N bases), (ii) keeping duplicates from different libraries (--keep-dup all flag), (iii) retaining peaks with a false discovery rate $\leq 0.01$ , (iv) enabling multiple summit detection (--call-summits flag), and (v) disabling the modelling of peak extension for ChIP-seq libraries (--nomodel flag). |
| Data quality            | FDR 5% for peak selection.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| Software                | macs2 version 2.2.6                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |

# 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

To sort choanocytes of *E. muelleri*, 7 days post hatching sponges were fed with 0.5  $\mu\text{m}$  fluorescent carboxylate-modified FluoSpheres (Invitrogen, F8813). Next, animal tissue was dissociated into cells and crosslinked with formaldehyde (1%) for 10 min and DSG (3mM) for 40 min. Crosslinked cells were filtered through 40  $\mu\text{m}$  cell strainer and stained with 2  $\mu\text{g}/\text{ml}$  Hoechst 33342. For each replicate, we sorted 2,000,000 cells.

To sort the neuronal cell population from the *N. vectensis* transgenic line *NvElav1::mOrange*, tissue from one-day starved animals was crosslinked with 1% formaldehyde for 10 minutes under vacuum. The crosslinked tissue was then dissociated into single cells by incubating it with 10 mg/mL Protease XIV (Sigma-Aldrich, P5147) in 1/3 CMF and 1 mM  $\text{CaCl}_2$  for 5 minutes at 24°C. Following digestion, the tissue was pelleted at 800 g for 5 minutes, reconstituted in 1/3 CMF supplemented with 2 mM EDTA and 2  $\mu\text{g}/\text{mL}$  Hoechst 33342 (Thermo Scientific, 62249), and triturated for an additional 5–10 minutes. The crosslinked cells were passed through a 40  $\mu\text{m}$  cell strainer and stained with 2  $\mu\text{g}/\text{mL}$  Hoechst 33342. For each replicate, 2,000,000 cells were sorted.

Instrument

BD FACS Aria II

Software

BD FACSDiva 6.1.3 BD Biosciences

Cell population abundance

Choanocytes constituted 17-20% of total cells. Neuronal *NvElav+* cell population of *N. vectensis* constituted 1.5-2% of total cells.

Gating strategy

For *E. muelleri*, cells positive for nuclei staining (Hoechst 33342) staining together with fluorescent beads (FITC channel) were sorted. For *N. vectensis*, we sorted cells positive for nuclei staining (Hoechst 33342) together with the fluorescent mOrange protein (PE-Texas Red channel).

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