

## 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](#).

### Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
- An indication of 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 of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistics 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.  $F$ ,  $t$ ,  $r$ ) with confidence intervals, effect sizes, degrees of freedom and  $P$  value noted  
*Give  $P$  values as exact values whenever suitable.*
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- 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  $d$ , Pearson's  $r$ ), indicating how they were calculated
- Clearly defined error bars  
*State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on [statistics for biologists](#) may be useful.

### Software and code

Policy information about [availability of computer code](#)

Data collection

NA

Data analysis

Link to the code and usage instructions are provided, as well as detailed description of the algorithm (Appendix S1).

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 upon request. 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 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 list of figures that have associated raw data
- A description of any restrictions on data availability

All data was deposited in GEO with the accession number GSE111068. The MetaCell package, UMI tables and annotation files are available on our group website: [http://compgenomics.weizmann.ac.il/tanay/?page\\_id=99](http://compgenomics.weizmann.ac.il/tanay/?page_id=99)

## Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf](https://www.nature.com/authors/policies/ReportingSummary-flat.pdf)

## Life sciences study design

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

Sample size	Sequencing depth and number of libraries were defined to allow support for the paper's main conclusions (there is no "sample size" in this paper).
Data exclusions	No data were excluded from the analysis.
Replication	NA
Randomization	NA
Blinding	NA

## Reporting for specific materials, systems and methods

### Materials & experimental systems

n/a	Involvement in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> Unique biological materials
<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
<input type="checkbox"/>	<input checked="" type="checkbox"/> Animals and other organisms
<input checked="" type="checkbox"/>	<input type="checkbox"/> Human research participants

### Methods

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

## Antibodies

Antibodies used	We employed antibodies against histone H3 and specific modifications of histone H3: anti-H3 antibody (Abcam, #ab1791) anti-H3K4me2 antibody (Abcam, #ab3236) anti-H3K4me3 antibody (Millipore, #07-473)
Validation	These antibodies have a wide species spectrum (paneukaryotic) and have been extensively used and validated in iChIP studies.

## Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals	Placozoan ( <i>Trichoplax adhaerens</i> ) specimens were cultured in the lab.
Wild animals	Sponge ( <i>Amphimedon queenslandica</i> ) specimens were collected from Heron Island Reef, Great Barrier Reef, Queensland, Australia. Ctenophore ( <i>Mnemiopsis leidyi</i> ) specimens originated from L. Friis-Møller, Kristineberg, Sweden.
Field-collected samples	Sponge and ctenophore specimens were maintained in filtered artificial sea water until further processing (dissociation for single-cell analysis or for chromatin extraction).

## 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.*

GSE111068

#### Files in database submission

Mnemiopsis\_genes.bed  
Mnemiopsis\_genome\_sequence.fasta  
Mnemiopsis\_original\_scaffolds\_edges.bed  
Trichoplax\_genes.bed  
Trichoplax\_genome\_sequence.fasta  
Trichoplax\_original\_scaffolds\_edges.bed

Mnemiopsis\_H3K4me2\_peaks.bed  
Mnemiopsis\_H3K4me3\_peaks.bed  
Trichoplax\_H3K4me2\_peaks.bed  
Trichoplax\_H3K4me3\_peaks.bed

Mnemiopsis\_input\_all\_RPM.bw  
Mnemiopsis\_me2\_all\_RPM.bw  
Mnemiopsis\_me3\_all\_RPM.bw  
Trichoplax\_input\_all\_RPM.bw  
Trichoplax\_me2\_all\_RPM.bw  
Trichoplax\_me3\_all\_RPM.bw

Mnemiopsis\_iChIP1\_input\_R1.fastq.gz  
Mnemiopsis\_iChIP1\_input\_R2.fastq.gz  
Mnemiopsis\_iChIP1\_K4me2\_R1.fastq.gz  
Mnemiopsis\_iChIP1\_K4me2\_R2.fastq.gz  
Mnemiopsis\_iChIP1\_K4me3\_R1.fastq.gz  
Mnemiopsis\_iChIP1\_K4me3\_R2.fastq.gz

Mnemiopsis\_iChIP2\_input\_R1.fastq.gz  
Mnemiopsis\_iChIP2\_input\_R2.fastq.gz  
Mnemiopsis\_iChIP2\_K4me2\_R1.fastq.gz  
Mnemiopsis\_iChIP2\_K4me2\_R2.fastq.gz  
Mnemiopsis\_iChIP2\_K4me3\_R1.fastq.gz  
Mnemiopsis\_iChIP2\_K4me3\_R2.fastq.gz

Trichoplax\_iChIP1\_input\_R1.fastq.gz  
Trichoplax\_iChIP1\_input\_R2.fastq.gz  
Trichoplax\_iChIP1\_K4me2\_R1.fastq.gz  
Trichoplax\_iChIP1\_K4me2\_R2.fastq.gz  
Trichoplax\_iChIP1\_K4me3\_R1.fastq.gz  
Trichoplax\_iChIP1\_K4me3\_R2.fastq.gz

Trichoplax\_iChIP2\_input\_R1.fastq.gz  
Trichoplax\_iChIP2\_input\_R2.fastq.gz  
Trichoplax\_iChIP2\_K4me2\_R1.fastq.gz  
Trichoplax\_iChIP2\_K4me2\_R2.fastq.gz  
Trichoplax\_iChIP2\_K4me3\_R1.fastq.gz  
Trichoplax\_iChIP2\_K4me3\_R2.fastq.gz

#### Genome browser session (e.g. [UCSC](#))

NA

### Methodology

#### Replicates

Two replicates of iChIP experiments. Reads were pooled for downstream analysis.

#### Sequencing depth

12 cycles of library PCR. 37nt Paired-End Reads. For M.leidy, the total number of reads was: 21M (H3K4me2), 12M (H3K4me3) and 10M (input). For T.adhaerens, the total number of reads was: 24M (H3K4me2), 14M (H3K4me3), and 11M (input).  
For T.adhaerens, % of single-mapping reads ranged 65-68%. For M.leidy, % of single-mapping reads ranged 40-42%.

#### Antibodies

anti-H3 antibody (Abcam, #ab1791)

Antibodies	anti-H3K4me2 antibody (Abcam, #ab3236) anti-H3K4me3 antibody (Millipore, #07-473)
Peak calling parameters	We transformed raw coverage values to quantile values. H3K4me3 and H3K4me2 peaks were defined as regions with coverage quantiles over 0.97 (in <i>M.leidyii</i> ) or 0.94 (in <i>T.adhaerens</i> ), merging peaks located at <200bp. To account for mappability/assembly problems, we defined “peaks” using input data and excluded those regions from our H3K4me3/me2 peaks.
Data quality	NA
Software	Reads mapped using bowtie v1.1.1 with parameters -m1 -v3. Duplicated reads removed using SAMtools v1.1.