

Materials Design Analysis Reporting (MDAR)

Checklist for Authors

The [MDAR framework](#) establishes a minimum set of requirements in transparent reporting mainly applicable to studies in the life sciences.

eLife asks authors to **provide detailed information within their article** to facilitate the interpretation and replication of their work. Authors can also upload supporting materials to comply with relevant reporting guidelines for health-related research (see [EQUATOR Network](#)), life science research (see the [BioSharing Information Resource](#)), or animal research (see the [ARRIVE Guidelines](#) and the [STRANGE Framework](#); for details, see *eLife's* [Journal Policies](#)). Where applicable, authors should refer to any relevant reporting standards materials in this form.

For all that apply, please note **where in the article** the information is provided. Please note that we also collect information about data availability and ethics in the submission form.

Materials:

Newly created materials	Indicate where provided: section/figure legend	N/A
The manuscript includes a dedicated "materials and data availability statement" providing transparent disclosure about availability of newly created materials including details on how materials can be accessed and describing any restrictions on access.	Line 845	

Antibodies	Indicate where provided: section/figure legend	N/A
For commercial reagents, provide supplier name, catalogue number and RRID , if available.	Used antibodies are listed in Table 5 . Line 604	

DNA and RNA sequences	Indicate where provided: section/figure legend	N/A
Short novel DNA or RNA including primers, probes: Sequences should be included or deposited in a public repository.	Protospacer sequences are listed in Table 2 , line 598 Primer sequences used for genotyping are listed in Table 3 , line 600	

	<p>Primer sequences used for qRT-PCR are listed in Table 4, line 602</p> <p>Primers used for Sanger sequencing are provided in Materials and Methods section, line 826-831</p>	
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Cell materials	Indicate where provided: section/figure legend	N/A
Cell lines: Provide species information, strain. Provide accession number in repository OR supplier name, catalog number, clone number, OR RRID.		X
Primary cultures: Provide species, strain, sex of origin, genetic modification status.		X

Experimental animals	Indicate where provided: section/figure legend	N/A
Laboratory animals or Model organisms: Provide species, strain, sex, age, genetic modification status. Provide accession number in repository OR supplier name, catalog number, clone number, OR RRID.	Materials and Methods: Animals Line 607-629	
Animal observed in or captured from the field: Provide species, sex, and age where possible.		X

Plants and microbes	Indicate where provided: section/figure legend	N/A
Plants: provide species and strain, ecotype and cultivar where relevant, unique accession number if available, and source (including location for collected wild specimens).		X
Microbes: provide species and strain, unique accession number if available, and source.		X

Human research participants	Indicate where provided: section/figure legend) or state if these demographics were not collected	N/A
If collected and within the bounds of privacy constraints report on age, sex, gender and ethnicity for all study participants.	line 807	

Design:

Study protocol	Indicate where provided: section/figure legend	N/A
If the study protocol has been pre-registered, provide DOI. For clinical trials, provide the trial registration number OR cite DOI.		X

Laboratory protocol	Indicate where provided: section/figure legend	N/A
Provide DOI OR other citation details if detailed step-by-step protocols are available.	Line 651: qRT-PCR cited as 42 Line 630: HotShot DNA extraction protocol cited as 45 Line 641: Squash testis protocol cited as 46	

Experimental study design (statistics details) *		
For in vivo studies: State whether and how the following have been done	Indicate where provided: section/figure legend. If it could have been done, but was not, write "not done"	N/A
Sample size determination	Sample size used for each	

	experiment can be found in materials and methods section as well as in figure legends. Minimum biological replicate of 3 was used through the study.	
Randomisation		X
Blinding		X
Inclusion/exclusion criteria	No animals have been excluded from the study	

Sample definition and in-laboratory replication	Indicate where provided: section/figure legend	N/A
State number of times the experiment was replicated in the laboratory.	Statistics, line 842	
Define whether data describe technical or biological replicates.	Materials and methos section as well as figure legends include information on biological/technical replicates	

Ethics	Indicate where provided: section/submission form	N/A
Studies involving human participants: State details of authority granting ethics approval (IRB or equivalent committee(s), provide reference number for approval.	Study cohort and ethical approval, line 801-810	
Studies involving experimental animals: State details of authority granting ethics approval (IRB or equivalent committee(s), provide reference number for approval.	Methods, Animals Line: 607-629	
Studies involving specimen and field samples: State if relevant permits obtained, provide details of authority approving study; if none were required, explain why.		X

Dual Use Research of Concern (DURC)	Indicate where provided: section/submission form	N/A
If study is subject to dual use research of concern regulations, state the authority granting approval and reference number for the regulatory approval.		X

Analysis:

Attrition	Indicate where provided: section/figure legend	N/A
Describe whether exclusion criteria were pre-established. Report if sample or data points were omitted from analysis. If yes, report if this was due to attrition or intentional exclusion and provide justification.	No data was excluded from the study	X

Statistics	Indicate where provided: section/figure legend	N/A
Describe statistical tests used and justify choice of tests.	Statistics, line 842	

Data availability	Indicate where provided: section/submission form	N/A
For newly created and reused datasets, the manuscript includes a data availability statement that provides details for access (or notes restrictions on access).		X
When newly created datasets are publicly available, provide accession number in repository OR DOI and licensing details where available.		X
If reused data is publicly available provide accession number in repository OR DOI, OR URL, OR citation.		X

Code availability	Indicate where provided: section/figure legend	N/A
For any computer code/software/mathematical algorithms essential for replicating the main findings of the study, whether newly generated or re-used, the manuscript includes a data availability statement that provides details for access or notes restrictions.	Line 848, 851	
Where newly generated code is publicly available, provide accession number in repository, OR DOI OR URL and licensing details where available. State any restrictions on code availability or accessibility.	https://github.com/hansenjn/SpermQ https://github.com/hansenjn/ColorStackByTimeAndProject	
If reused code is publicly available provide accession number in repository OR DOI OR URL, OR citation.	Nuclear Morphology software cited in 51. PAML software cited in 53 and 54 SpermQ software cited in 22 https://github.com/hansenjn/SpermQ	

Reporting:

The MDAR framework recommends adoption of discipline-specific guidelines, established and endorsed through community initiatives.

Adherence to community standards	Indicate where provided: section/figure legend	N/A
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State if relevant guidelines (e.g., ICMJE, MIBBI, ARRIVE, STRANGE) have been followed, and whether a checklist (e.g., CONSORT, PRISMA, ARRIVE) is provided with the manuscript.	Provided at the end of this document	
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* We provide the following guidance regarding transparent reporting and statistics; we also refer authors to [Ten common statistical mistakes to watch out for when writing or reviewing a manuscript](#).

Sample-size estimation

- You should state whether an appropriate sample size was computed when the study was being designed
- You should state the statistical method of sample size computation and any required assumptions
- If no explicit power analysis was used, you should describe how you decided what sample (replicate) size (number) to use

No computational method was used for sample-size estimation. Most experiments were performed at least three times and using at least three biological replicates. The sample size for each experiment can be found in Material and Methods section as well as in figure legends or figure themselves (graphs).

Replicates

- You should report how often each experiment was performed
- You should include a definition of biological versus technical replication
- The data obtained should be provided and sufficient information should be provided to indicate the number of independent biological and/or technical replicates
- If you encountered any outliers, you should describe how these were handled
- Criteria for exclusion/inclusion of data should be clearly stated
- High-throughput sequence data should be uploaded before submission, with a private link for reviewers provided (these are available from both GEO and ArrayExpress)

Biological and/or technical replicates can be found within each figure legend or in the Materials and Methods section. No data have been excluded from the analysis.

Statistical reporting

- Statistical analysis methods should be described and justified
- Raw data should be presented in figures whenever informative to do so (typically when N per group is less than 10)
- For each experiment, you should identify the statistical tests used, exact values of N, definitions of center, methods of multiple test correction, and dispersion and precision measures (e.g., mean, median, SD, SEM, confidence intervals; and, for the major substantive results, a measure of effect size (e.g., Pearson's r, Cohen's d)
- Report exact p-values wherever possible alongside the summary statistics and 95% confidence intervals. These should be reported for all key questions and not only when the p-value is less than 0.05.

Graphs that represent quantified data show individual data point or value of N is shown in figure legend. For all analyses mean values +/- SD were calculated. Statistical significance was determined by One-way ANNOVA

analyses using Bonferroni correction.

Group allocation

- Indicate how samples were allocated into experimental groups (in the case of clinical studies, please specify allocation to treatment method); if randomization was used, please also state if restricted randomization was applied
- Indicate if masking was used during group allocation, data collection and/or data analysis

Experimental groups are represented by genotypes: wild-type, Cylc1-/y, Cylc2+/-, Cylc2-/-, Cylc1-/y Cylc2+/-, Cylc1-/y Cylc2-/-. Animals used were selected based on the sex (male) and age at the time of the analysis (8-15 weeks old).

Materials availability statement:

Custom made antibodies used in this study were developed against murine Cylicin 1 and Cylicin 2. A limited amount of antibody can be provided upon request.



The ARRIVE guidelines 2.0: author checklist

The ARRIVE Essential 10

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

Item	Recommendation	Section/line number, or reason for not reporting
Study design	1 For each experiment, provide brief details of study design including: <ol style="list-style-type: none"> The groups being compared, including control groups. If no control group has been used, the rationale should be stated. The experimental unit (e.g. a single animal, litter, or cage of animals). 	Results section Materials and methods, line 607-629
Sample size	2 <ol style="list-style-type: none"> Specify the exact number of experimental units allocated to each group, and the total number in each experiment. Also indicate the total number of animals used. Explain how the sample size was decided. Provide details of any <i>a priori</i> sample size calculation, if done. 	Number of animals used in each experiment is provided in Methods section and figures No prior sample calculation was done
Inclusion and exclusion criteria	3 <ol style="list-style-type: none"> Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established <i>a priori</i>. If no criteria were set, state this explicitly. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so. For each analysis, report the exact value of <i>n</i> in each experimental group. 	No animals were excluded Number of animals used in each experiment is provided in Methods section
Randomisation	4 <ol style="list-style-type: none"> State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence. Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly. 	not applicable - evident by genotype not applicable
Blinding	5 Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).	not applicable
Outcome measures	6 <ol style="list-style-type: none"> Clearly define all outcome measures assessed (e.g. cell death, molecular markers, or behavioural changes). For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size. 	Results section (line 91)
Statistical methods	7 <ol style="list-style-type: none"> Provide details of the statistical methods used for each analysis, including software used. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met. 	line 842
Experimental animals	8 <ol style="list-style-type: none"> Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures. 	Materials and methods section Animals 607-629 Materials and methods section Animals 607-629
Experimental procedures	9 For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including: <ol style="list-style-type: none"> What was done, how it was done and what was used. When and how often. Where (including detail of any acclimatisation periods). Why (provide rationale for procedures). 	Materials and methods Materials and Methods Materials and Methods
Results	10 For each experiment conducted, including independent replications, report: <ol style="list-style-type: none"> Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range). If applicable, the effect size with a confidence interval. 	Figures/Figure legends