nature research

Corresponding author(s): Peter W. Reddien

☐ Initial submission ☐ Revised version ☒ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

Describe how sample size was determined.

Visual estimation of preliminary experiments indicated large effect sizes with greater than 2-fold differences on number of fibers and length-to-width ratios, therefore a minimum sample size of 6 was chosen to give a 99% power with a 1% false positive error rate. This size estimation applies for measurements taken in Figure 1c,d, Figure 2i, Figure 3b,c and Extended Data Figure 5g, Extended Data Figure 10b, and Extended Data Figure 11f. For RNA-sequencing of pooled wound sites, biological triplicates were used as in Wurtzel et al, 2015 and for whole animal RNA-seq, six animals were used instead to increase statistical power.

2. Data exclusions

Describe any data exclusions.

All animals were included in the analysis. For the sequencing data, one biological replicate was excluded from analysis and the explanation is in Methods.

3. Replication

Describe whether the experimental findings were reliably reproduced.

All experimental findings were reproduced at least in two independent experiments.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

Animals for all experiments were randomly selected from a large collection of clonal animals for all experiments.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

Investigators were not blinded during data collection and analysis.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	Prism 7 was used for all statistical analysis. Zeiss ZEN was used for image acquisition and ImageJ was used for contrast adjustment and cell counting. bowtie 1 was used for mapping reads. R packages DESeq and pheatmap were used to analyze differential expression between conditions and to generate scaled heatmaps. MUSCLE was used to align protein sequences. PhyML was used to calculate phylogenetic trees which were then visualized in FigTree. Further details and citations are in Methods.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.	No restrictions

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	The antibodies used were monoclonal (mouse 6G10 (DSHB; Ross et al, 2014)) and polyclonal rabbit V5277; these recognized unknown epitopes in planarian muscle. The stained structures in S. mediterranea were identified as planarian muscle by the very distinct, well-established appearance of muscle, similarity of the staining to f-actin (phalloidin) staining, and similarity to anti-planarian MHC (TMUS13) staining.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	n/a
b. Describe the method of cell line authentication used.	n/a
c. Report whether the cell lines were tested for mycoplasma contamination.	n/a
d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.	n/a

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.	A clonal asexual line (CIW4) of planarians was used.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.	n/a