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**Sample-size estimation**

* You should state whether an appropriate sample size was computed when the study was being designed
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* If no explicit power analysis was used, you should describe how you decided what sample (replicate) size (number) to use

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Sample sizes (where appropriate) are listed within the specific ‘Materials and Methods’ section or explained within the Results section, when necessary to explain the statistical method and related samples sizes. In addition, the figure legends contain further information relevant to each test.

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* You should report how often each experiment was performed
* You should include a definition of biological versus technical replication
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* High-throughput sequence data should be uploaded before submission, with a private link for reviewers provided (these are available from both GEO and ArrayExpress)

Please outline where this information can be found within the submission (e.g., sections or figure legends), or explain why this information doesn’t apply to your submission:

This information is presented within the specific Methods of the Manuscript. We select at least two individual specimens/embryos (biological replication) for both immunohistochemistry and in situ hybridisation assays. We then generate paraffin thin sections (14uM) and prepare multiple slides for a range of assays, whether histology, insitu hybridization and immunohistochemistry. Each set of immunohistochemistry and in situ hybridization assays therefore compare at least two individual embryos, and then these experiments are run at least another two times for consistency.

**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.

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All statistical reporting relevant to this manuscript are present within the methods, figure legends, explained when necessary within the results sections and we have included Source Data Files as supplemental files linked to Figure 6.

(For large datasets, or papers with a very large number of statistical tests, you may upload a single table file with tests, Ns, etc., with reference to sections in the manuscript.)

**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

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Group allocation is not relevant to the data produced for this manuscript. For experimental treatments (i.e., pharmacological perturbations) efforts were made to stage match each specimen for group treatments – therefore each experimental group contained equivalently stage-matched specimens.

**Additional data files (“source data”)**

* We encourage you to upload relevant additional data files, such as numerical data that are represented as a graph in a figure, or as a summary table
* Where provided, these should be in the most useful format, and they can be uploaded as “Source data” files linked to a main figure or table
* Include model definition files including the full list of parameters used
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Please indicate the figures or tables for which source data files have been provided:

Figures 8 and 9 represent in silico simulations the parameters of which are included as supplemental files S1, and S2. Source Data Files to support Figure 6 (morphometrics) are included in supplemental source data files 1-5.

**Code Availability**

Our analysis code, plots and morphometrics data can be found and re-ran at [**https://github.com/alexthiery/scanicula-aek**](https://github.com/alexthiery/scanicula-aek). We have also included a Docker container for reproducibility **docker://alexthiery/scanicula-aek:v1.0**.