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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.

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
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on 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 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)
☐	☒ 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

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

Skeletal computed tomography (CT) scans of notothenioids were processed in Siemens PETsyngo VG60A. No other software was used in the collection of the data

Data analysis

The following programs were used in the processing and analysis of the data:
BAMMtools (v2.1.0) -- lineage diversification rate analysis
BEAST (v2.4.8) -- substitution rate analysis
HYPHY (v2.3.9) -- positive selection through aBSREL program
PHAST (v1.4) -- accelerated sequence evolution through phyloP program
Python SciPy (v0.18.1) -- Fisher's exact test for GO-enrichment (fisher_exact)
Python statsmodels (v0.6.1) -- multiple hypothesis test correction (fdrcorrection0)
IQTree (v.1.6.3) -- gene trees
ASTRAL (v.5.6.2) --species tree from gene trees

Sequencing read processing and contig assembly programs:
Trimmomatic (v0.3)
FASTX-Toolkit (v0.0.13)
blastn (ncbi-blast-2.2.30+)
dc-megablast (v2.6.0+)
Biopython (v1.70) -- codonseq and pairwise2 modules
BEDtools (v2.23.0)
CAP3 (02/10/15)
Notung (v2.9)

Multiple sequence alignment and alignment pruning :
MACSE (v2.03)

Mafft (v7.313)
GUIDANCE (v2.02)

Read alignment:
NextGenMap (v0.5.5)
SAMtools (v1.9)
BCFtools (v1.9)

Processing and analysis of CT scans:
ImageJ (v1.51s)
Amira (v6.0.0)
Siemens PETsyngo VG60A

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. 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

The sequencing reads have been deposited on the NCBI SRA (Bioproject PRJNA531677). Assembled contigs and annotations for all species in the dataset have been uploaded to the Zenodo repository (doi: 10.5281/zenodo.2628936).

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

Life sciences study design

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

Sample size	We started with a goal of n>5 and >10 for control and experimental groups respectively for analysis of trip11 zebrafish mutants, with these numbers selected to capture variation in the phenotype while minimizing the usage of laboratory animals.
Data exclusions	No data were excluded from the analysis
Replication	Zebrafish trip11 mutant individuals were scanned from two independent genetic crosses.
Randomization	As the zebrafish work focused on defined genotypes, we did not randomize into experimental groups for zebrafish experiments. Further, no randomization was necessary in the genomic analysis, as this analysis is predicated on questions at specific places within the phylogeny
Blinding	Investigators were not blinded to the genotypic and genomic data analysis. As the research questions focused on specific lineages within the phylogeny, blinding would not be possible.

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
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	This study involves the use of zebrafish (Danio rerio). Both genders between the ages of 3-6 months were used. Mutant strains include col1a1a (dmh14) and col1a2 (dmh15). CRISPR mutants in trip11 were generated from wild-type strains.
Wild animals	This study did not involve wild animals
Field-collected samples	No tissue samples were directly collected from the field for the purposes of this study. Tissue samples were obtained from the Yale Peabody frozen tissue collection and from the laboratory collection of H.W. Detrich from previously published Antarctic expeditions. Adult notothenioid specimens were also loaned from the Yale Peabody Museum for CT scanning. Full sample and specimen identifiers are available in the Supplement.
Ethics oversight	Boston Children's Hospital Institutional Animal Care and Use Committee (IACUC).

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