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Reporting Summary

Nature Portfolio 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 Portfolio policies, see our Editorial Policies 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	Data for Sperm motility, average speed, and sperm concentration were obtained using CASA (SMAS3(ver.3.1.11.357), DITECT, Tokyo, Japan). Gross morphology, tissue and immunohistochemical data were obtained by using a digital charge-coupled device camera DP-71 (Olympus, Tokyo, Japan). The lengths of the fin and tooth of each fish were calculated using Adobe Photoshop CC (2019) using a picture taken with DP-71. The micro CT-scanned image was taken by Phoenix nanotom m (Baker Hughes, Houston, TX) at the JMC Corporation (Yokohama, Japan). The videos for mating behaviour and mate-choice test were recorded for 30 min using a digital video camera HDR-PJ800, (Sony, Tokyo, Japan). The movie of aggressive male-male competition was taken using a high-speed camera system (HAS-L2, DITECT).

Data analysis	For the screening of the Ar mutants, we used the Light scanner 96 software v2.0. The statistical analysis for the frequency of reproduction and the frequency of mating that fish exhibited courtship display was conducted using R version 3.6.2. For the other behavioral data, the statistical analysis was done using generalized liner mixed models (GLMMs) and linear mixed models (LMMs) in R version 4.1.0 with the package lme4 version 1.1.27.1. For the mate choice test, the statistics was analysed using the chi-squared ($\chi 2$) test of independence in R version 4.1.0. For RNA-seq, the adapter and quality trimming was done by using Trim Galore 0.6.4_dev with Cutadapt 1.18, and then the transcripts were quantified using salmon v1.3.0 by mapping the trimmed reads to the transcriptome sequences of Oryzias latipes (Hd-rR; ASM223467v1) with the Ensembl gene annotation (Release 105; https://www.ensembl.org/). The statistical analysis for the RNA-seq data was performed using the R package edgeR v3.34.1 and the gene ontology (GO) enrichment analysis was conducted using ShinyGO version 0.75 (http://bioinformatics.sdstate.edu/go/) using the Ensembl gene IDs of the differentially expressed genes as input. For qPCR primer design, we used Applied Biosystems Primer Express 2.0.0 (Thermo Fisher Scientific). The statistical analysis of LC/MS data and qPCR data was done with R version 3.6.2. For the statistical analysis of the fertilization rate, gonad/body weight, sperm motility test fin and tooth morphology, reporter gene assay, and the female fecundity test, we used Excel 2011 (Microsoft Corp., Redmond, WA) with an add-in software Statcel 3 (Yanai H. 2011. Statcel—the useful add-in software forms on Excel. 3. Tokyo (Japan): OMS.).

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 and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio 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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

RNA-seq data for the whole brain with a pituitary gland are available from DDBJ (Accession No. DRA013672). The transcriptome sequences of Oryzias latipes (Hd-rR; ASM223467v1)in Ensembl Release 100 (http://dec2021.archive.ensembl.org/) were used for the RNA-seq analysis.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	We do not include any human data in our manuscript.
Population characteristics	See above.
Recruitment	See above.
Ethics oversight	See above.

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

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	RNA-seq analysis, we used lab-raised fish with expected small variability and were interested in large differences in expression between wild type males and Ar mutant males, so we used three individuals for each group as the recommended minimum number of replicate in previous publications (e.g. Lamarre et al. 2018 Front Plant Sci). For experiments to quantify the gene expression levels, sample sizes were determined based on the expected variability and the number of fish available according to other studies with similar methodology (e.g.Ogino et al. 2014 Endocrinology). For behavioral experiments, we used as many fish as available.
Data exclusions	In the mating experiment of ara KO and arb KO males, the pairs that did not spawn eggs within the 30 min test were excluded from the data collection regarding the frequency of courtship display, mating latency, total number of wrapping rejection, and duration of wrapping with spawning, because such female may not have been ready for spawning. In other experiments, no data were excluded.
Replication	For the gene expression analysis with RNA-seq and quantitative PCR, each experiment was conducted using multiple sample fish as biological replicates. All images of the knockout and knock-in fishes were obtained from multiple fishes (n>3 in each group) except micro CT image. The micro CT-image was obtained from a single specimen in each group. To confirmed the data of tooth morphology taken by micro CT, we

	performed the bone staining of the teeth by using multiple fishshes (n = 9 for each group). Behavioral experiments were conducted with each fish as a replicate.
Randomization	In all behavioral experiments, each fish was randomly chosen from one of the families (i.e., family was randomized) and then allocated into the experimental group. In the behavioral experiments, each compartment was assigned randomly to the wild-type or the knockout fish.
Blinding	All behavioral experiments were conducted as blinded experiments.

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
[ ]	[x] Antibodies
[ ]	[x] Eukaryotic cell lines
[x]	[ ] Palaeontology and archaeology
[ ]	[x] Animals and other organisms
[x]	[ ] Clinical data
[x]	[ ] Dual use research of concern

Methods

n/a	Involved in the study
[x]	[ ] ChIP-seq
[x]	[ ] Flow cytometry
[x]	[ ] MRI-based neuroimaging

Antibodies

Antibodies used	We used the primary antibodies, anti-DDDDK-tag (FLAG) mouse mAb monoclonal antibody (M185-3S, Clone: FLA-1, Lot: 004, MBL, Nagoya, Japan), anti-GFP D5.1XP rabbit mAb monoclonal antibody having cross-reactivity to the mClover3 (#2956, Clone:D5.1, Lot: 2, Cell Signaling, Danvers, MA, USA. As the secondary antibodies, we used Alexa 555-conjugated goat anti-mouse IgG(H+L), F(ab')2 fragment (#4409, Lot: 18, Cell Signaling), and Alexa 488-conjugated anti-rabbit IgG(H+L), F(ab')2 fragment (#4412, Lot:4, Cell Signaling).
Validation	The GFP (D5.1) XP® Rabbit mAb detects GFP, YFP, and CFP-tagged proteins exogenously expressed in cells, whose antigen is perfectly conserved with the GFP tag used in this study (mClover3). The manufacturer confirmed that this antibody is available for immunohistochemistry with paraffin-embedded sections. The anti-DDDDK-tag (FLAG) mouse mAb reacts with the N-terminal, internal, and C-terminal DDDK-tgged (DYKDDDDK) proteins, whose availability for immunohistochemistry was validated by the manufacturer.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	ATCC
Authentication	The cell line (COS-7) was obtained from ATCC, and we checked the growth and morphology.
Mycoplasma contamination	The cell line was not tested by PCR but we checked it by hoechst staining.
Commonly misidentified lines (See ICLAC register)	*Name any commonly misidentified cell lines used in the study and provide a rationale for their use.*

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals	The following fishes (Cab strain of Japanese medaka Oryzias latipes) was used for this study: wild type (113 males; 244 females), ara KO (86 males; 12 females), arb KO (78 males; 12 females), ara/arb double KO (32 males; 12 females). All used fishes were at the adult stage (> 4 month old after hatching). They were maintained at aquarium under artificial reproductive conditions with 14 and 10 h of light and dark cycles at 26–28˚C.
Wild animals	No wild animals were used in this study.
Reporting on sex	Medaka has a male heterogametic (XX/XY) system, in which dmy/dmrt1bY on the Y chromosome determines their sexes. Therefore, In this study, we analysed the genetic sex by the amplification of dmy from the fin clips of all adult fishes. The males were used for the behavioral and morphological analyses. The females were used for the morphological analysis and fecundity test.

Field-collected samples	No field collection samples were used in this study.
Ethics oversight	Animal experiments were conducted under approval by the Institutional Animal Care and Use Committee of the National Institute for Basic Biology (15A005, 14A003, 13A023, 12A020, 11A028) and Kyushu University (A21-043-0, A19-137-0, A19-137-1, A29-088-0).

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