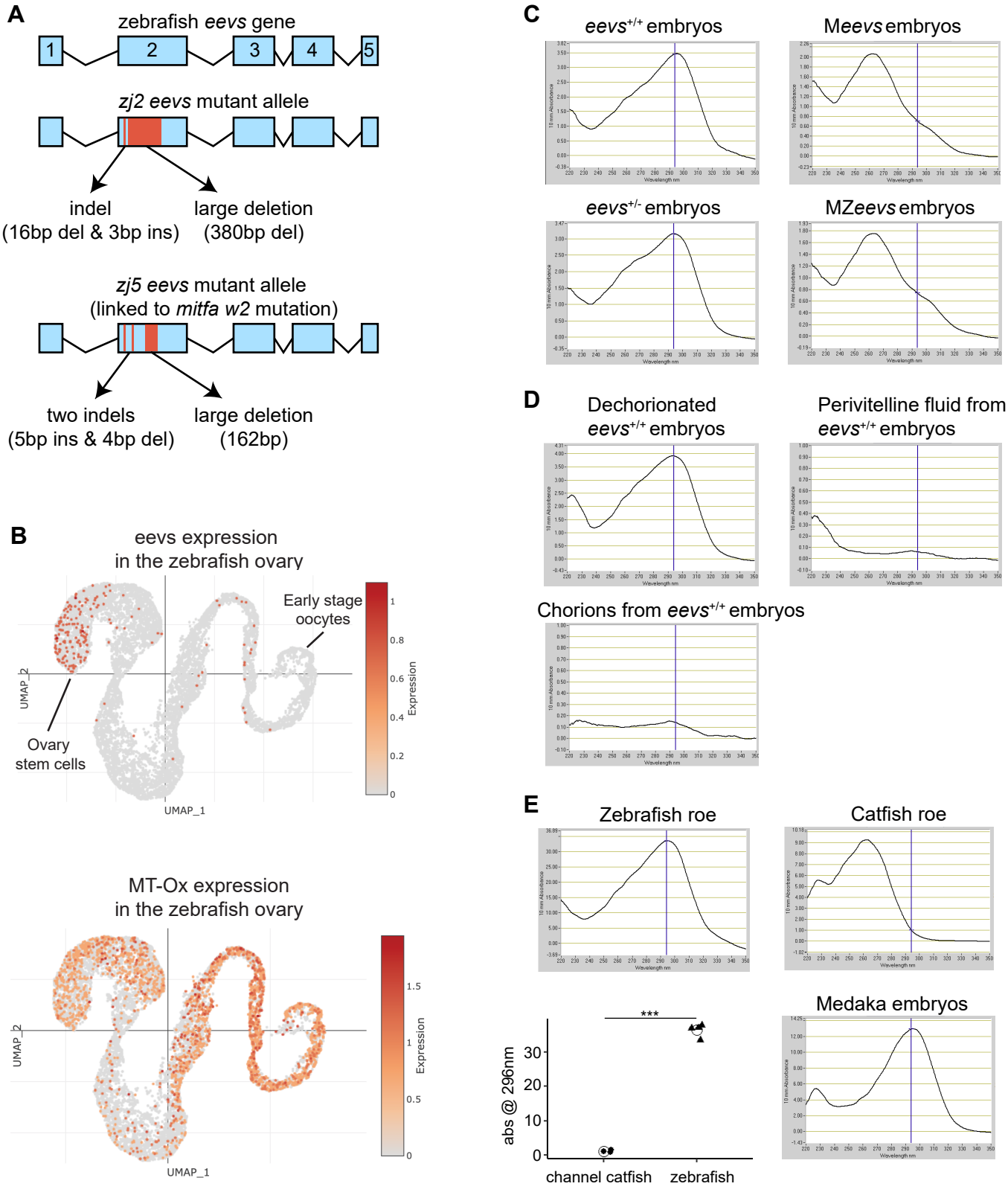

A
zebrafish eevs gene
1
2
3
4
5
zj2 eevs mutant allele
indel
(16bp del & 3bp ins)
large deletion
(380bp del)
zj5 eevs mutant allele
(linked to mitfa w2 mutation)
two indels
(5bp ins & 4bp del)
large deletion
(162bp)
B
eevs expression
in the zebrafish ovary
Early stage
oocytes
Ovary
stem cells
UMAP_2
UMAP_1
Expression
MT-Ox expression
in the zebrafish ovary
C
eevs+/+ embryos
Meevs embryos
eevs+/- embryos
MZeevs embryos
D
Dechorionated
eevs+/+ embryos
Perivitelline fluid from
eevs+/+ embryos
Chorions from eevs+/+ embryos
E
Zebrafish roe
Catfish roe
***
abs @ 296nm
channel catfish
zebrafish
Medaka embryos

Figure S1 *eevs* mutant alleles, *eevs* expression, and gadusol detection in various samples, Related to Figures 1 and 4.

A, (top) Diagram of the gene structure of the zebrafish wild type *eevs* allele, with exons depicted as numbered boxes and introns depicted as connecting lines. (middle) Diagram of the zj2 mutant *eevs* allele with compound mutations that delete 393 bp (red) from exon 2, generated using CRISPR-Cas9. (bottom) Diagram of the zj5 mutant *eevs* allele with compound mutations that delete 161 bp (red) from exon 2, generated using CRISPR-Cas9.

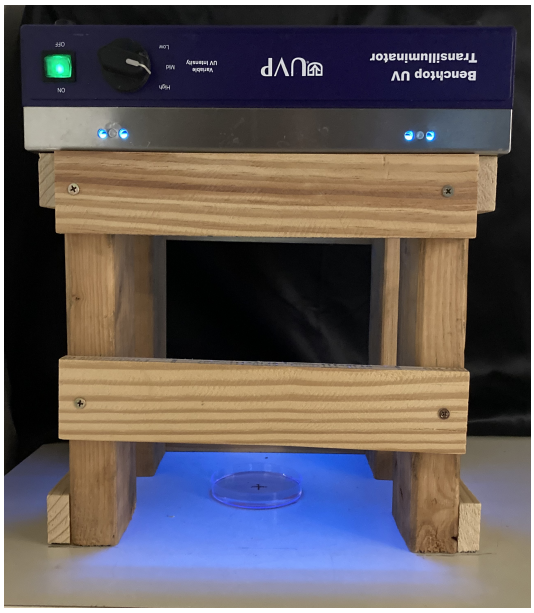
B, Expression of *eevs* (top) and MT-Ox (bottom) in a single-cell atlas of the juvenile zebrafish ovary (Liu et al., *eLife* 2022). *eevs* is only expressed during early stages of oogenesis.

C, Nanodrop UV-spectrograms of polar compounds from methanol-extracted embryos with the indicated genotypes: *eevs*$^{+/+}$, M*eevs*, *eevs*$^{+/-}$, and MZ*eevs*. The absorption peak for gadusol at neutral pH is 296 nm, indicated with a blue line. Absorption at 296 nm is absent in M*eevs* and MZ*eevs* embryos.

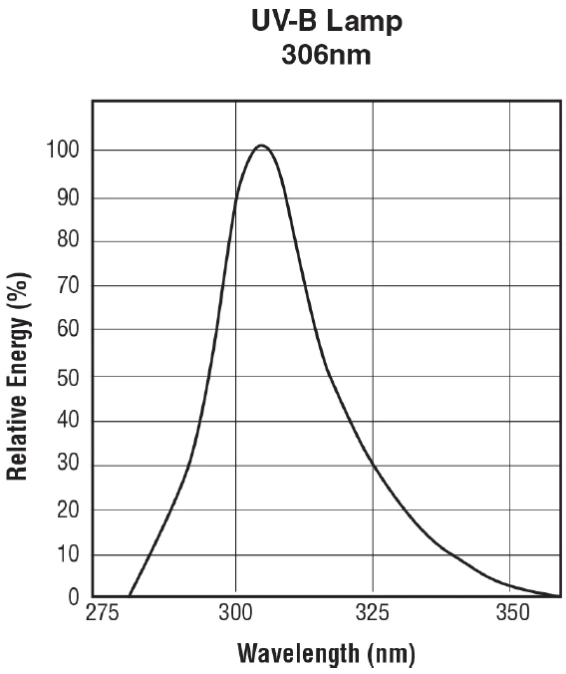
D, Gadusol is absent from the chorion and perivitelline fluid**.** Nanodrop UV-spectrograms of polar compounds from the following samples: dechorionated wild-type embryos, isolated chorions**,** and isolated perivitelline fluid. The absorption peak for gadusol at neutral pH is 296 nm, indicated with a blue line. Absorption at 296 nm is absent in chorion and perivitelline fluid samples.

E, Nanodrop UV-spectrograms of polar compounds from the following samples: Channel catfish (*Ictalurus punctatus*) ovaries (roe), zebrafish (*Danio rerio*) roe, and fertilized medaka (*Oryzias latipes*) eggs. The absorption peak for gadusol at neutral pH is 296 nm, indicated with a blue line. The channel catfish genome does not contain intact copies of *eevs* and MT-Ox, and absorption at 296 nm is absent in catfish ovaries.

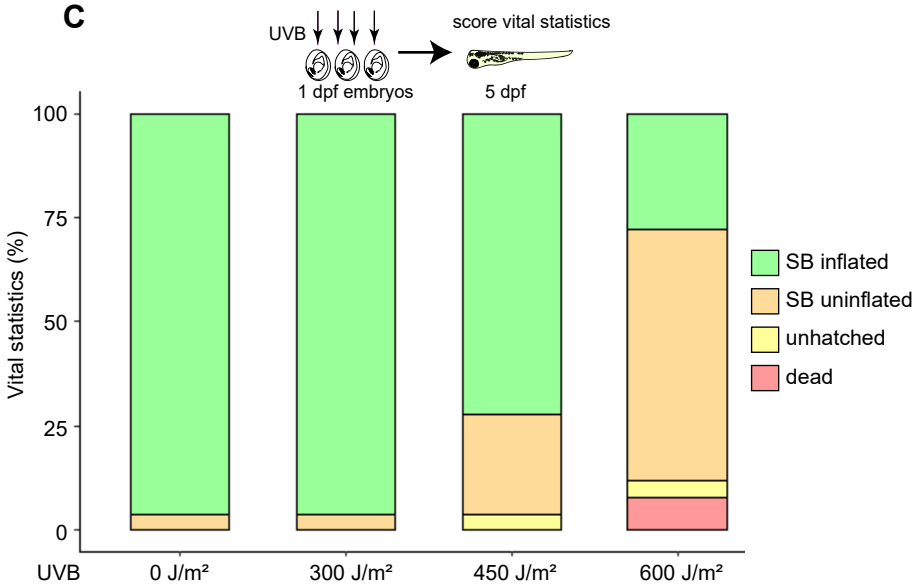
A


Benchtop UV
Transilluminator
UVP

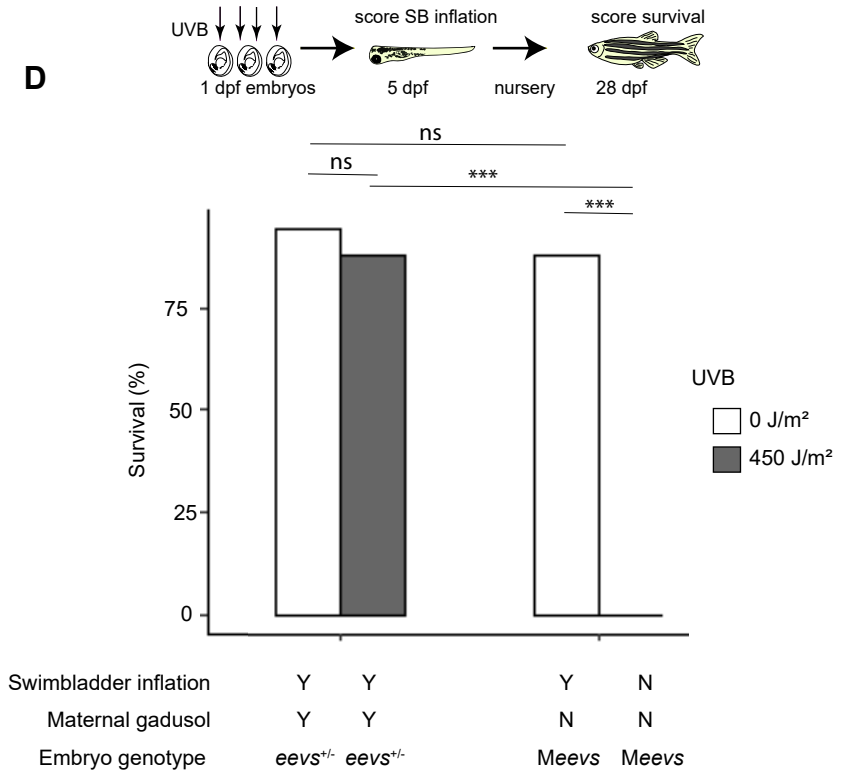
B


UV-B Lamp
306nm
Relative Energy (%)
100
90
80
70
60
50
40
30
20
10
0
275
300
325
350
Wavelength (nm)

C


UVB
1 dpf embryos
score vital statistics
5 dpf
Vital statistics (%)
100
75
50
25
0
UVB
0 J/m²
300 J/m²
450 J/m²
600 J/m²
SB inflated
SB uninflated
unhatched
dead

D


UVB
1 dpf embryos
score SB inflation
5 dpf
nursery
score survival
28 dpf
ns
ns
***
***
Survival (%)
75
50
25
0
UVB
0 J/m²
450 J/m²
Swimbladder inflation
Y
Y
Y
N
Maternal gadusol
Y
Y
N
N
Embryo genotype
eevs+/-
eevs+/-
Meevs
Meevs

E

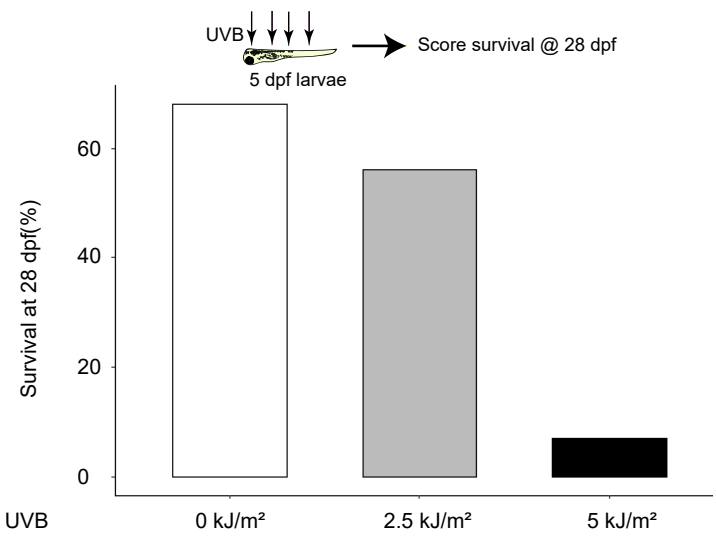

UVB
5 dpf larvae
Score survival @ 28 dpf
Survival at 28 dpf(%)
60
40
20
0
UVB
0 kJ/m²
2.5 kJ/m²
5 kJ/m²

Figure S2 UVR assay on embryonic and larval zebrafish, Related to Figure 1.

A, A UVP transilluminator rests inverted on a 30 cm wooden stand to lower the fluence rate to 2.5 W/m², an ecologically relevant rate. Embryos and larvae are placed in 30 ml of clear E3 buffer and lids removed during UVR exposure.

B, UVB spectrum of 8-Watt broadband 306 nm UVB bulbs (Ushio 30000318). Note absence of UVC light.

C, UVR dosage curve performed on 24 hpf embryos. Vital statistics were scored at 5 dpf. We scored swim bladder (SB) inflation, chorion hatching, and obvious mortality. We generated TU/AB hybrid strain embryos to mimic the background genetics of the M*eevs* and *eevs*$^{+/-}$ embryos used elsewhere in this paper. The parental cross was a wild-type TU strain female to a *mitfa*$^{-/-}$ AB strain male. From left to right, n = 25, 26, 25, 25. Based on these data, the 450 J/m$_2$ dose was selected for all UVB exposures to 24 hpf embryos as it resulted in modest defects in swim bladder inflation but did not result in immediate mortality.

D, Swimbladder inflation is a valid metric for embryonic/larval health and development. 5 dpf larvae that do not hatch or inflate their swim bladder will not survive. (Fisher's exact ***p<.0001). Mock-exposed embryos inflated their swim bladders and survived in the nursery at similar rates regardless of genetic condition. From left to right, n = 50 for all groups.

E, Dosage curve for UVR exposures on 5 dpf larvae. Wild-type TU 5 dpf larvae were exposed to various doses of UVB. The larvae were then placed in the nursery and survival was scored at 4 weeks post UVR exposure. 2.5 kJ/m$_2$ UVB was selected as an appropriate dose as it resulted in a modest decrease in survival in wild-type fish. n = 100 for all groups.

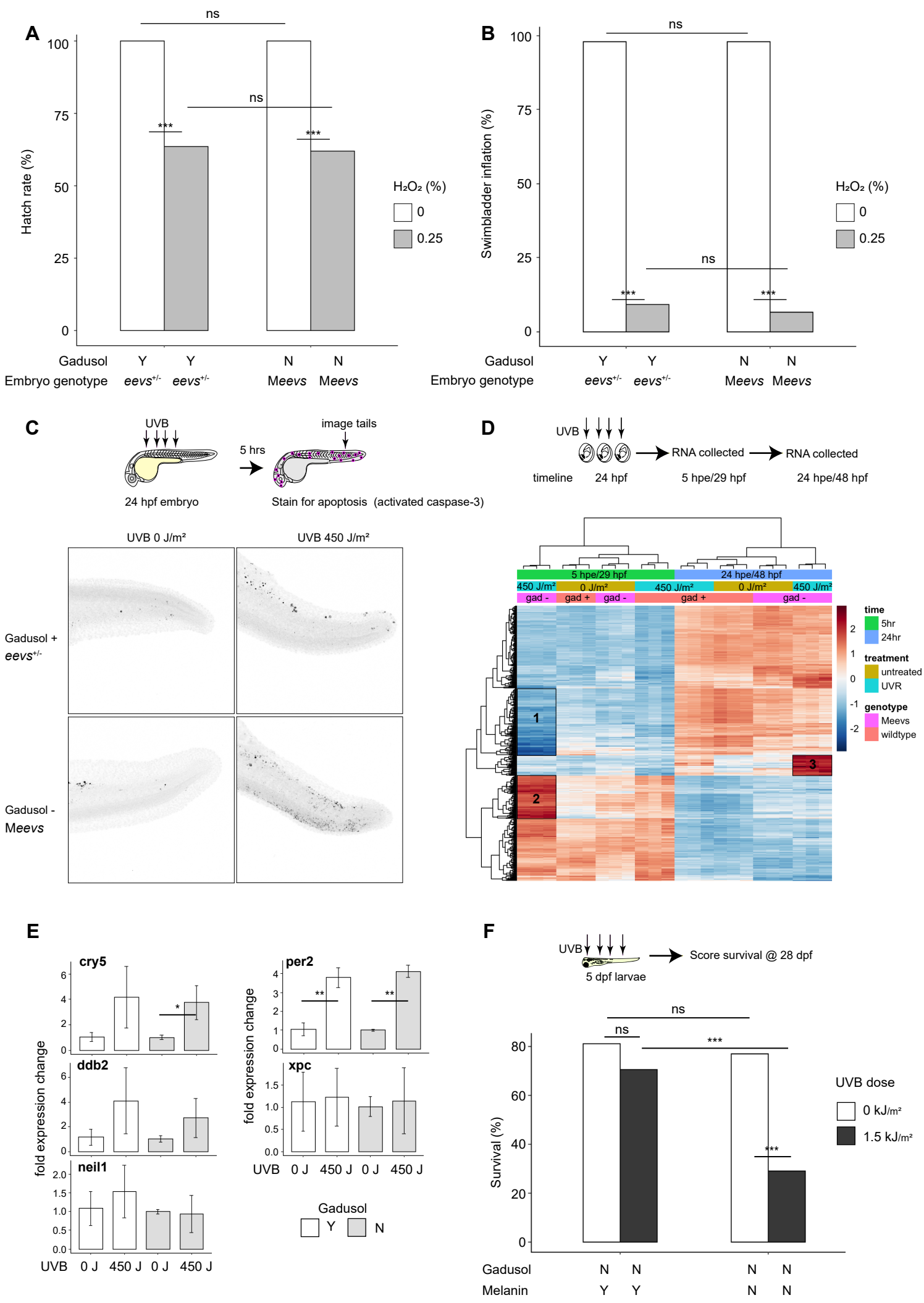

A
ns
ns
***
***
100
75
50
25
0
Hatch rate (%)
H₂O₂ (%)
0
0.25
Gadusol Y Y N N
Embryo genotype eevs+/- eevs+/- Meevs Meevs
B
ns
ns
***
***
100
75
50
25
0
Swimbladder inflation (%)
H₂O₂ (%)
0
0.25
Gadusol Y Y N N
Embryo genotype eevs+/- eevs+/- Meevs Meevs
C
UVB
5 hrs
image tails
24 hpf embryo
Stain for apoptosis (activated caspase-3)
UVB 0 J/m²
UVB 450 J/m²
Gadusol + eevs+/-
Gadusol - Meevs
D
UVB
RNA collected
RNA collected
timeline
24 hpf
5 hpe/29 hpf
24 hpe/48 hpf
5 hpe/29 hpf
24 hpe/48 hpf
450 J/m² 0 J/m² 450 J/m² 0 J/m² 450 J/m²
gad - gad + gad - gad + gad -
1
2
3
2
1
0
-1
-2
time
5hr
24hr
treatment
untreated
UVR
genotype
Meevs
wildtype
E
cry5
ddb2
neil1
per2
xpc
fold expression change
UVB 0 J 450 J 0 J 450 J
Gadusol
Y
N
F
UVB
Score survival @ 28 dpf
5 dpf larvae
ns
ns
***
***
80
60
40
20
0
Survival (%)
UVB dose
0 kJ/m²
1.5 kJ/m²
Gadusol N N N N
Melanin Y Y N N

Figure S3 Gadusol is a true sunscreen, Related to Figure 2 and Table S2.

A, B, 24hpf *eevs*$^{+/-}$ or M*eevs* embryos were incubated in 0% or 0.25% hydrogen peroxide for 1 hour, then washed in E3 embryo medium. **A,** Hatch rate displayed between groups showing no difference between embryos with or without gadusol. **B,** Swim bladder inflation rate is similar between embryos with or without gadusol. Hatch rate and swim bladder inflation was scored at 5 dpf. All embryos result from TU strain eevs$^{-/-}$ males or females outcrossed with AB strain males or females. From left to right, n = 45, 44, 45, 45. Two clutches of embryos were used for each group. Statistics: Fisher's exact ***p<0.0001.

C, Representative images of zebrafish embryo tails stained for a marker of apoptosis. 24 hpf *eevs*$^{+/-}$ or M*eevs* embryos within chorions were mock-exposed or exposed to 450 J/m$_2$ of UVB. 5 hours later, embryos were dechorionated, fixed and stained using an activated caspase-3 antibody. Fluorescence intensities were quantified as described in the Methods and displayed in **Figure 2C**.

D, Clusters of differentially-expressed genes emerge in UV-treated embryos that lack gadusol**.** Displayed is a heat map of the 500 genes with most variable expression levels across conditions. Each row is a gene, each column is a single RNAseq experiment. Each condition (M*eevs* or wild type, mock-exposed or exposed to UVB, 5 hours or 24 hour after exposure) is represented by three replicate RNAseq experiments. Three clusters of genes are highlighted that emerged from unbiased hierarchical clustering. Cluster 1 genes were strongly downregulated in embryos that lack gadusol 5 hours after exposure to UVB. Genes in cluster 1 are associated with GO terms that indicate downregulation of transcription. Cluster 2 genes were strongly upregulated in embryos that lack gadusol 5 hours after exposure to UVB. Genes in cluster 2 are associated with stress response and DNA damage response GO terms. Select genes from cluster 2 are shown in **Figure 2D**. Cluster 3 genes were strongly upregulated in embryos that lack gadusol 24 hours after exposure to UVB. Cluster 3 contains two genes associated with the wound healing GO term, suggesting that the UV-treated gadusol-lacking embryos are still regulating a response to wound healing 24 hours after UV exposure. See **Table S1** for a complete list of GO terms associated with each cluster.

E, M*eevs* and wild type embryos respond similarly to visible light. Gene expression of 5 genes quantified via qPCR. Embryos were exposed to visible light (cool white LED with no UVR) for 5hrs. Then RNA was extracted, cDNA synthesized and qPCR performed. Statistics: student t-test **p<.01, *p<.05.

F, A modest role for melanin as a sunscreen in larval fish. Larvae with or without maternal gadusol, and with or without melanin, were exposed to 1.5 kJ/m$_2$ UVB (a lower dose than in other experiments) and placed into the fish facility nursery. Survival was scored at 28 dpf. All larvae are siblings. n = 48 for all groups. Two clutches of embryos were used for each group. Statistics: fisher's exact *** p<.0001.

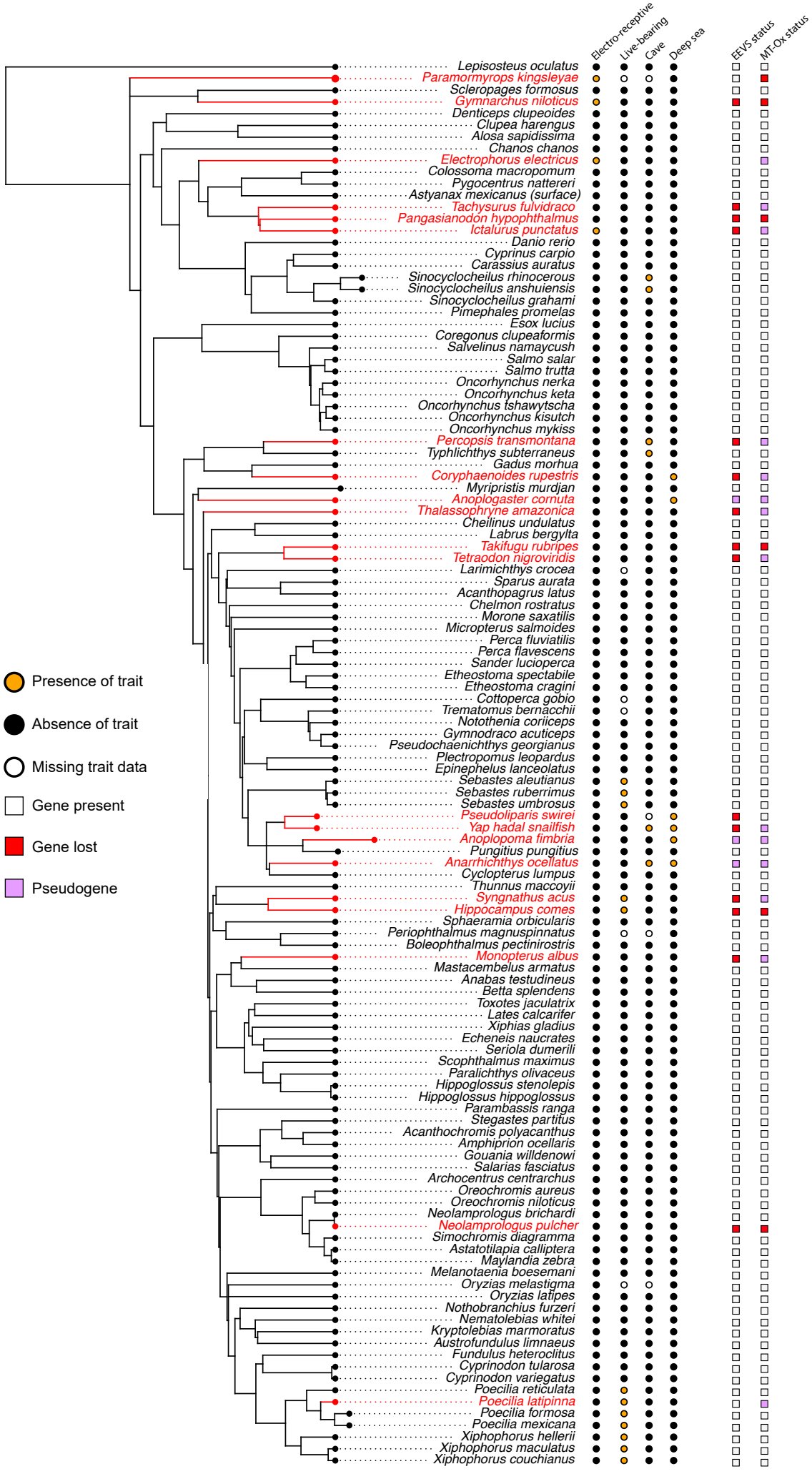

Electro-receptive
Live-bearing
Cave
Deep sea
EEVS status
MT-Ox status
Lepisosteus oculatus
Paramormyrops kingsleyae
Scleropages formosus
Gymnarchus niloticus
Denticeps clupeoides
Clupea harengus
Alosa sapidissima
Chanos chanos
Electrophorus electricus
Colossoma macropomum
Pygocentrus nattereri
Astyanax mexicanus (surface)
Tachysurus fulvidraco
Pangasianodon hypophthalmus
Ictalurus punctatus
Danio rerio
Cyprinus carpio
Carassius auratus
Sinocyclocheilus rhinocerous
Sinocyclocheilus anshuiensis
Sinocyclocheilus grahami
Pimephales promelas
Esox lucius
Coregonus clupeaformis
Salvelinus namaycush
Salmo salar
Salmo trutta
Oncorhynchus nerka
Oncorhynchus keta
Oncorhynchus tshawytscha
Oncorhynchus kisutch
Oncorhynchus mykiss
Percopsis transmontana
Typhlichthys subterraneus
Gadus morhua
Coryphaenoides rupestris
Myripristis murdjan
Anoplogaster cornuta
Thalassophryne amazonica
Cheilinus undulatus
Labrus bergylta
Takifugu rubripes
Tetraodon nigroviridis
Larimichthys crocea
Sparus aurata
Acanthopagrus latus
Chelmon rostratus
Morone saxatilis
Micropterus salmoides
Perca fluviatilis
Perca flavescens
Sander lucioperca
Etheostoma spectabile
Etheostoma cragini
Cottoperca gobio
Trematomus bernacchii
Notothenia coriiceps
Gymnodraco acuticeps
Pseudochaenichthys georgianus
Plectropomus leopardus
Epinephelus lanceolatus
Sebastes aleutianus
Sebastes ruberrimus
Sebastes umbrosus
Pseudoliparis swirei
Yap hadal snailfish
Anoplopoma fimbria
Pungitius pungitius
Anarrhichthys ocellatus
Cyclopterus lumpus
Thunnus maccoyii
Syngnathus acus
Hippocampus comes
Sphaeramia orbicularis
Periophthalmus magnuspinnatus
Boleophthalmus pectinirostris
Monopterus albus
Mastacembelus armatus
Anabas testudineus
Betta splendens
Toxotes jaculatrix
Lates calcarifer
Xiphias gladius
Echeneis naucrates
Seriola dumerili
Scophthalmus maximus
Paralichthys olivaceus
Hippoglossus stenolepis
Hippoglossus hippoglossus
Parambassis ranga
Stegastes partitus
Acanthochromis polyacanthus
Amphiprion ocellaris
Gouania willdenowi
Salarias fasciatus
Archocentrus centrarchus
Oreochromis aureus
Oreochromis niloticus
Neolamprologus brichardi
Neolamprologus pulcher
Simochromis diagramma
Astatotilapia calliptera
Maylandia zebra
Melanotaenia boesemani
Oryzias melastigma
Oryzias latipes
Nothobranchius furzeri
Nematolebias whitei
Kryptolebias marmoratus
Austrofundulus limnaeus
Fundulus heteroclitus
Cyprinodon tularosa
Cyprinodon variegatus
Poecilia reticulata
Poecilia latipinna
Poecilia formosa
Poecilia mexicana
Xiphophorus hellerii
Xiphophorus maculatus
Xiphophorus couchianus
Presence of trait
Absence of trait
Missing trait data
Gene present
Gene lost
Pseudogene

Figure S4 Gadusol production has been lost in several species no longer exposed to UVR, Related to Figure 4 and Table S3.

For each of 136 teleost species, we assessed various life history traits that identify habitats that may not require embryonic protection from UVR, including electroreception, live-bearing, cave dwelling, and deep-sea dwelling, indicated with colors to the left of the phylogeny. For each species, we identified the presence of intact open reading frames for *eevs* and/or MT-Ox. Species that have lost the genes required for gadusol production are indicated in red. We found 16 independent losses across this phylogeny. We found that fish with these traits are more likely than by chance to lose gadusol (p=0.012).