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1 **The multi-level regulation of clownfish metamorphosis by thyroid hormones**

2

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27

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35 **Abstract:**

36 Most marine organisms have a biphasic life cycle during which a pelagic larva is transformed
37 into a radically different juvenile. In vertebrates the role of thyroid hormones (TH) in triggering this
38 transition is well known, but how the morphological and physiological changes are integrated in a
39 coherent way with the ecological transition remains poorly explored. To gain insight into this
40 question, we performed an integrative analysis of metamorphosis of a marine teleost, the clownfish
41 *Amphiprion ocellaris*. We reveal how TH coordinate a change in color vision as well as a major
42 metabolic shift in energy production, hence highlighting its central integrative role in regulating this
43 transformation. By manipulating the activity of LXR, a major regulator of metabolism, we also reveal
44 a tight link between metabolic changes and metamorphosis progression. Strikingly, we observed that
45 these regulations are at play in the wild revealing how hormones coordinate energy needs with
46 available resources during life cycle.

47

48

49 **Introduction**

50 In vertebrates, metamorphosis is a common post-embryonic transition regulated by thyroid
51 hormones (TH) during which a larva is transformed into a juvenile. The role of TH as the main trigger
52 and coordinator of the distinct biological processes that occurred during metamorphosis has been
53 extensively studied, mostly in anurans as well as in flatfish (Power et al., 2008; Tata, 2006). Both
54 biological models exhibit a spectacular metamorphosis during which major morphological,
55 physiological and ecological transitions occurred (Laudet, 2011). TH not only trigger but also
56 coordinate at the tissue and cellular level the transformation of the larva into a juvenile. For example,
57 in tadpole, cell-specific actions of TH are instrumental for the transformation of several organs
58 (intestine, limbs, tail) illustrating the pleiotropic action of this hormone (Grimaldi et al., 2013). This
59 action is mediated by the binding of TH to thyroid hormone receptors (TR) that act as transcription
60 factor and regulate the expression of target genes (Sachs and Buchholz, 2017). However, these
61 models (flatfish and amphibian) are more exceptions than the rule as in most vertebrate species
62 morphological changes are more subtle (Buchholz, 2015; Holzer and Laudet, 2013; Laudet, 2011).
63 Indeed, all chordates, including amniotes, are passing through such a post-embryonic transformative
64 period even if, in many species, it is not morphologically spectacular (Holzer and Laudet, 2013). In
65 addition, several reports suggest that the successful completion of metamorphosis is decisive for the
66 quality and therefore the ecological success of the juvenile emanating from it (Besson et al., 2020).
67 Therefore, understanding how TH control this transformation is critically important to better
68 understand the pleiotropic action of the hormone and how juvenile can cope with their new
69 environment (Lowe et al., 2021).

70 A particular challenge, common to all vertebrate species, is to make sure that the
71 transformation is well aligned with available environmental conditions and with the metabolic status
72 of the organism (Hulbert and Else, 2000; Sheridan and Kao, 1998). THs, with their well-known
73 metabolic effects, are critical for this action (Sayre and Lechleiter, 2012; Sinha et al., 2018). This has
74 been shown in several species; particularly in sticklebacks in which they control metabolic rate in
75 freshwater populations that are living in an energy-poor environment (Kitano et al., 2010). Similarly,
76 studies in several teleost fish have shown that TH impact the activity of enzymes involved in lipid
77 metabolism therefore influencing energy availability (Deal and Volkoff, 2020). However, these
78 metabolic actions of TH are mostly studied in juveniles or adults and how this occurred during
79 metamorphosis remain largely unknown (Deal and Volkoff, 2020). Shedding light on how TH control
80 metabolism during metamorphosis would be particularly desirable as it is known that metabolic
81 pathways are heavily modified during larval transformation (Darias et al., 2008). Therefore, it
82 remains to be determined: (i) how TH regulate metabolism during metamorphosis and (ii) how this

83 action is integrated with the transformation of the larvae into a juvenile, also controlled by the
84 hormone. To fulfil this gap, we carried out for the first time an integrative study of metamorphosis to
85 better understand the central role of TH in the coordination of larval transformation with metabolic
86 status. Understanding these processes is critical to better understand the ecological function of TH
87 but also the constraints and bias affecting metamorphosis, a key process ensuring natural population
88 replenishment. This is particularly important in the context of the global changes affecting natural
89 populations worldwide (Lowe et al., 2021).

90 We used the false clownfish *A. ocellaris* as a model system to explore the hormonal basis of
91 this metabolic integration. These fishes live in coral reefs, in symbiosis with sea anemones in which
92 they form colonies with a reproductively active pair and a variable number of juveniles (Buston,
93 2003). Every two to three weeks, the couple lays ca. 500 eggs on a substrate near their host. After
94 hatching the larvae disperse in the ocean for 10–15 days. At the end of this pelagic phase, the larvae
95 transform via metamorphosis into small juveniles, called young recruits, which must locate a reef and
96 actively look for a host sea anemone using their sensory abilities combining visual, chemical and
97 acoustic cues (Barth et al., 2015). During metamorphosis, the larvae lose their larval characteristics
98 (light pigmentation, elongated body shape) and transform into miniature adults, with an ovoid body
99 shape and a conspicuous pigmentation pattern displaying white bars on a bright orange background
100 (Roux et al., 2019). These pigmentation changes have been shown to be regulated by TH indicating
101 that as for other teleost fishes this metamorphosis is controlled by TH (Holzer et al., 2017; Salis et al.,
102 2021).

103 In this paper, we conducted an extensive study of vertebrate metamorphosis by combining
104 developmental transcriptome assembly, TH levels measurements, behavioral observations, lipid
105 content analysis, *in situ* observations, and functional experiments. By focusing on visual perception
106 and metabolic changes, we uncover how TH control gene regulatory programs, as well as behavioral
107 and physiological outputs, hence highlighting the central integrator role of TH in regulating the whole
108 larva to juvenile transformation. Our results demonstrated that TH link metabolic regulation,
109 morphological transformation and behavioral changes ensuring the full ecological transformation of
110 the pelagic larvae into a benthic reef associated juvenile.

111

112 Results

113 *Three distinct post-embryonic phases.*

114 The entire larval development period of *A. ocellaris* have been divided into seven distinct
115 developmental stages (named S1 to S7 for stage 1 to 7 in Fig. 1A, Roux et al. 2019). To obtain a global
116 perspective on gene expression levels during post-embryonic development, we performed a
117 transcriptomic analysis of these seven stages. RNAs from three entire larvae per stage were
118 extracted and sequenced on an Illumina platform giving a total of 21 samples (see Material and
119 Methods).

120 Principal component analysis (PCA) and global hierarchical clustering were first applied on the
121 1000 genes with the highest amplitude of expression (variance) without considering the
122 developmental stages (Fig. 1A and Supp. Fig. 1A). Both methods allowed distinguishing three distinct
123 groups: (i) early developmental stages which included stages 1, 2 and 3, (ii) late developmental
124 stages including stages 5, 6 and 7, and (iii) the pivotal stage 4. Of note, one stage 4 individual is
125 tending toward the late stages cluster, indicating that there may be a gap between transcriptional
126 regulation and the observed morphological changes (Fig. 1A and Supp. Fig. 1A).

127 The same three groups were also observed on the heat map clustering the 100 genes with the
128 highest variance (Fig. 1B). One group showed genes that are up regulated (in red) during early larval
129 development, corresponding to stages 1-3. Another group is formed by individuals in which genes
130 are up regulated during late larval development (stages 5-7). Finally, some genes appeared to be up-
131 regulated early on, at stage 4. Interestingly, when we considered the number of differentially
132 regulated genes between each consecutive stage, we observed that stage 4 coincides with a major
133 transition of the regulatory program with 178 significantly differentially expressed between stage 3
134 and 4 (Fig. 1E, Supp. Fig. 2).

135 The two periods we observed are distinct in terms of morphological transformation. On a PCA
136 performed with morphological measurements (body depth (BD)/standard length (SL); head length
137 (HL)/SL and snout vent length (SVL)/SL), we observed that the three first stages are aligned along the
138 main axis of variation (PC1), whereas after stage 4 there is an inflexion and the metamorphic stages
139 are, then, varying mostly along PC2 (Fig. 1C). These changes can be explained by a change in body
140 shape, metamorphic stages becoming more ovoid (increase of body depth). During the last stages,
141 we also observed major changes in body pigmentation. We conclude that stages S5 to S7 correspond
142 to the metamorphosis *per se*, whereas stage 4 appears to be a pivotal stage assuring the transition
143 between the larval period and the metamorphosis.

144 Interestingly, TH levels measurements showed a high level of T4 and T3 at stage 1 and 2 that
145 likely correspond to residues of maternal loading as observed in other species (Fig. 1D) (see for

146 example (Chang et al., 2012; Einarsdóttir et al., 2006a) on Atlantic halibut and Chang et al., 2012; on
147 zebrafish). Then, we observed a surge of T4 levels at stage 4 which precedes white bars appearance
148 occurring at stage 5 (Roux et al. 2019; Salis et al., 2020) followed by a decline in later stages. T3, the
149 most active form of TH, showed on the contrary more stable levels than T4, probably linked to the
150 fact that T3 is mostly produced and metabolized by deiodinases with different kinetics in different
151 peripheral tissues (Darras and Van Herck, 2012).

152 Overall, these results reveal that *A. ocellaris* post-embryonic development is characterized by
153 three distinct phases: (i) larval development, (ii) the pivotal stage 4 that marks the onset of
154 metamorphosis with a peak of TH and (iii) metamorphosis that corresponds to the actual
155 transformation.

156

157 *TH pathway is activated during metamorphosis*

158 To investigate the role played by TH in this process, we analyzed the expression levels of the TH
159 signaling genes. We selected genes from the hypothalamo pituitary thyroid (HPT) axis involved in the
160 central control of TH synthesis (*trh*: thyrotropin releasing hormone, and *tsh β* : thyrotropin stimulating
161 hormone subunit β); genes involved in TH synthesis in the thyroid follicles (*tg*: thyroglobulin, *tpo*:
162 thyroperoxidase, *nis* sodium/iodine symporter, *duox*: dual oxidase); genes involved in TH metabolism
163 (*dio1*, *dio2*, *dio3a* and *dio3b*: deiodinase 1, 2, 3a, 3b respectively); as well as the genes encoding
164 thyroid hormone receptors (*TR α a*, *TR α b* and *TR β* ; Fig.2).

165 The expression level of *trh* is relatively stable and *tsh β* expression levels rise from the onset of
166 metamorphosis (stage 4) and stabilize until stage 7. In the thyroid gland, *duox* have been shown to be
167 critical for TH production and is associated with high TH levels in clownfish (Salis et al., 2021).
168 Interestingly, we observed that its expression levels strongly rise during metamorphosis, starting at
169 stage 5. The expression level of *nis* (encoding for iodine transporter in thyroid follicles) increased, at
170 stage 6. In contrast, the expression of the gene encoding for the precursor of TH, *tg*, increased early
171 on (stage 3) and reached a peak at stage 4 as did the expression of *tpo*. These results clearly
172 corroborate the high production of T4 observed in stage 4 confirming that this corresponds to the
173 onset of metamorphosis.

174 Deiodinases are known to be key enzymes controlling the amount of T3, the most active thyroid
175 hormone, available in peripheral tissues and their expression in specific tissues are known to be
176 excellent indicators of the activity of the pathway (Bianco and Kim, 2006). We observed a spectacular
177 peak of expression of *dio3b*, one of the duplicates of the *dio3* gene which is coherent with the known
178 regulation of this gene by high TH levels (Russo et al., 2021). We also observed a strong two step
179 increase of *dio1* (known to either activate or inactivate TH depending on the context) at stage 4 and
180 stage 7, respectively. In contrast, both *dio3a* and *dio2* (which activates TH by converting T4 into T3)

181 expression remains constant. This lack of change in global expression levels for *dio3a* and *dio2* may
182 hide significant differences at tissues levels that are not visible in the context of entire larvae as it is
183 also the case for the global T3 levels.

184 Finally, we analyzed the expression levels of the three TR that are known to mediate the
185 action of TH in target cells: *TRβ* displayed a spectacular increase of expression starting at stage 4,
186 clearly in accordance with its role as a TH regulated gene in many species (McMenamin and Parichy,
187 2013). Its expression level remains high until stage 7. *TRαa* and *TRαb* expression peaked at the stage
188 4 and stage 6. The statistical significance of the variations of expression levels described above are
189 summarized in Supp. Fig. 1B. A global PCA analysis of TH signaling gene expression level again
190 separated the three first stages from S5-S7, indicating a clear activation of this pathway at stage 4
191 (Supp. Fig. 1C). Taken together, TH signaling gene expression levels are in accordance with the peak
192 of T4 observed at stage 4 confirming that clownfish metamorphosis starts at this stage.

193 The above results suggest that several of these TH signaling genes may be regulated by TH as
194 observed in other species (Tata 2006; Power et al. 2008). Additionally, after investigating the effects
195 of exogenous TH by treating stage 3 larvae with three different concentrations of T3 we observed a
196 positive or negative regulation of TH signaling genes. This is very similar to what has been observed
197 in other species (Campinho, 2019; McMenamin and Parichy, 2013). In summary, our analysis clearly
198 suggests that TH-signaling is activated during clownfish metamorphosis and that TH are the main
199 driver of the complex transformation of a pelagic larva to a reef associated juvenile.

200

201 *TH control a molecular and behavioral shift in vision*

202 During their transformation, clownfish larvae must switch from an oceanic (pelagic)
203 environment to a colorful reef environment. It is well known that in many fish species this ecological
204 transition is accompanied by a change in color vision (Cortesi et al., 2016). Since TH appeared critical
205 for larval to juvenile transition in clownfish, we investigated the regulation of genes encoding for
206 visual opsin during metamorphosis.

207 Eight visual opsin genes (*opnsw1-α*, *opnsw1-β*, *opnsw2B*, *rh1*, *Rh2A-1*, *rh2A-2*, *rh2B*, *opnlw*)
208 have been identified in *A. ocellaris* (Mitchell et al., 2021). In our transcriptomic data we observed a
209 reciprocal shift in their expression (Fig. 3A): short wavelength opsins (*opnsw1-α*, *opnsw1-β*,
210 *opnsw2B*), and mid-wavelength opsins (*rh2A*, *rh2B*) are highly expressed at the beginning of larval
211 development and are down-regulated after stage 4. Of note we observed a larval expression of both
212 duplicates of *opnsw1*, including *opnsw1-α* for which no clear expression was yet detected (Mitchell
213 et al., 2021). In contrast, the long wavelength opsin (*opnlw*) is poorly expressed in larval stages and

214 its expression strongly increase from stage 4 onwards and remains high throughout metamorphosis
215 (Fig. 3A).

216 This reciprocal shift (decrease in shortwave length and increase in long wavelength opsins,
217 respectively) suggested that there could be a shift in visual perception from blue/green to yellow/red
218 during metamorphosis. We therefore tested the visual preferences of *A. ocellaris* before and after
219 metamorphosis using a dual choice chamber (illustrated in Fig. 3B). Stage 2 larva spent significantly
220 more time in the blue compartment (50% vs. 23% in the orange compartment p -value = 0.004)
221 whereas stage 5 larva prefer the orange one (41% vs. 17% in the blue compartment, p -value = 0.009).
222 This indicated that opsin gene expression correlates with visual preference. This shift is also visible by
223 *in situ* hybridization: The *opnsw2B* gene is strongly expressed in photoreceptors at stage 2 and
224 slightly visible at stage 4 and 6 on the perimeter of the retina except on the ventral side whereas the
225 *opnlw* gene is not expressed at stage 2 and is detected in the photoreceptors at stage 6 (Fig. 3C).

226 We then tested if TH is controlling this molecular and behavioral shift. We observed that T3
227 strongly up regulated the expression levels of *opnlw* and down regulated *opnsw2B* from 12 to 72
228 hours after treatment (Fig. 3D, Supp. Fig. 4A). It also up regulated the expression of *Rh2A* after 48
229 hours of exposure (Supp. Fig. 4A). These data therefore suggest that the shift in opsin gene
230 expression is controlled by TH.

231 We next investigated if TH could also induce a shift in visual preferences. We treated stage 3
232 larvae (*i.e.*, before metamorphosis) for 72 hours with either T3 or DMSO as a vehicle and measured
233 the color preference of the larvae in a dual chamber, as described above. Interestingly, we observed
234 that control larvae spent significantly more time in the blue compartment than T3 treated larvae
235 (53% vs 1% respectively, p -value=5,2x10⁻⁷, Fig. 3E), which can be correlated with the higher
236 expression of *opnsw2B* observed in control larvae (Fig. 3D). Surprisingly, T3 treated larvae did not
237 spend much time in the orange compartment compared to DMSO treated larvae (11% vs 16%
238 respectively). Instead, they remained most of the time in the central compartment (88% vs 31% for
239 the DMSO controls, p -value=1,3x10⁻⁵). Observation of the larvae after 72 hours of T3 treatment
240 during the experimental trial revealed however that they remained close to the bottom of the
241 chamber, barely swimming, whereas control larvae were actively exploring the blue compartment.
242 The same behavior was observed when 30 days post hatching (dph) juveniles were similarly tested
243 (Supp. Fig. 4B). This indicated that, as expected, T3 accelerates the appearance of a juvenile-type
244 behavior which is in accordance with the benthic lifestyle of juvenile's clownfish, hiding in the
245 tentacles of their sea anemone host.

246 Taken together these results clearly demonstrate that TH control a shift in opsin gene
247 expression that coincides with a change in color preference occurring during metamorphosis. Even if
248 the shift of opsin gene expression cannot solely explain the visual preference, our results clearly

249 indicate that TH is coordinating a molecular, behavioral, and ecological transition essential for larval
250 survival in the wild.

251

252 *A TH regulated metabolic transition during metamorphosis*

253 Because TH is known to regulate metabolism in mammals, we investigated metabolic gene
254 expression during clownfish metamorphosis (Mullur et al., 2014). Figure 4 shows the expression
255 profile of the main genes involved in these pathways and highlights the rate limiting steps for
256 glycolysis (phosphofructokinase, *pfkma* and *pfkmb*), citric acid cycle (citrate synthase, *cs*; isocitrate
257 dehydrogenase, *idh3a*; oxoglutarate dehydrogenase, *ogdhl*, *dlst2*) and fatty acid β -oxidation
258 (carnitine palmitoyl transferase *cpt1aa*, *cpt1b*, *cpt2*). The expression profile of all the genes
259 implicated in these pathways are shown in Supplementary figures 5, 6 and 7.

260 These profiles revealed a clear overall pattern: glycolysis genes are highly expressed in larval
261 stages (S1 to S3) while their expression decreases during metamorphosis. This is particularly true for
262 the expression of the rate limiting enzymes (*pfkma* and *pfkmb*) which surges at S3 and strongly
263 decreases up to stage 7. In sharp contrast, the expression of fatty acid β -oxidation genes showed an
264 inverse profile with a low expression in larval stage and a sharp increase starting at stage 3 or 4. This
265 is especially the case for the *acads* gene that encodes acyl-coA dehydrogenase, an enzyme involved
266 in the second step of β -oxydation. This trend is again encountered on the rate limiting step (*cpt1aa*,
267 *cpt1b*, *cpt2*). The citric acid cycle genes are, in overall, expressed at lower level during larval stages
268 and their expression tends to increase early on during metamorphosis. This trend is more subtle and
269 early than the ones observed with glycolysis and fatty acid β -oxidation but again is observed on the
270 three rate limiting steps (*cs*, *idh3a*, *ogdhl* and *dlst2*). The lactic acid fermentation genes (lactate
271 dehydrogenase, *ldha*, *ldhba*, *ldhbb*, *ldhd*) are showing a more complex regulation.

272 Taken together, these results suggest that larval fish mainly rely on glycolysis and lactic acid
273 fermentation (considered as anaerobic energy production) whereas during metamorphosis, the
274 young juveniles rely more on fatty acid, degraded by the β -oxidation, and use the citric acid cycle for
275 aerobic energy production. Of note, this trend is the inverse to what is observed in some other fish
276 species such as the sea bass (Darias et al., 2008; Mazurais et al., 2011). This difference is likely to be
277 explained by a difference in life history trait, sea bass being a pelagic fish whereas clownfish is reef
278 associated.

279 We then studied if TH were instrumental in controlling this transition in energy production by
280 investigating their effects on the expression levels of *pfkma* and *pfkmb* (rate limiting step) and *pkmb*

281 for glycolysis, *acads* for fatty acid β -oxidation, *idh3a* for the citric acid cycle as well as *ldhba* and
282 *ldhbb* for lactic acid fermentation (Fig. 4B). In accordance with expression profile described above, TH
283 down regulated the expression of glycolysis gene and up regulated those involved in the citric acid
284 cycle and β -oxidation. For lactic acid fermentation we observed a dual effect with TH up regulating
285 *ldhbb* and down regulating *ldhba*, consistent with the respective increase and decrease of those two
286 genes during metamorphosis (Fig. 4A-B). This clearly indicates that TH control a switch between
287 glucose-based anaerobic toward a fatty acid, aerobic-energy production.

288

289 *TH favors a complexification of lipids during metamorphosis*

290 The previous data suggest a transition in energy source during metamorphosis with fatty acid
291 β -oxidation being the main energy pathway during metamorphosis. In line with this observation, we
292 noticed that the genes implicated in lipid biosynthesis and fat storage are not induced and
293 sometimes even repressed during metamorphosis.

294 Lipid biosynthesis requires the transport of citrate from the mitochondria to the cytosol by
295 the tricarboxylate carrier protein SLC25A1 and then the formation of acetyl-coA by the ATP citrate
296 lyase (*acly*) (Fig. 5A). This acetyl-coA is then used by acetyl-coA carboxylase (*acaca* and *b*) that form
297 malonyl-coA, the main starting substrate for fatty acid biosynthesis by fatty acid synthase (*fasn*). In
298 our transcriptomic data, we observed that *slc25a1* expression decreased regularly from stage 1 until
299 stage 6 with a final slight increase in stage 7 (Fig. 5A). The following genes in the pathway were either
300 slightly increasing (*aclya*, *acacb*) or constant (*aclyb*, *acaca* and *fasn*). All this suggest that *de novo*
301 fatty acid biosynthesis is minimal during metamorphosis. In accordance with this notion, TH
302 treatment decreased *fasn* expression, suggesting that TH does not favor *de novo* fatty acid
303 biosynthesis in this context (Fig. 5B). These data also suggest that the main source of fuel for fatty
304 acid β -oxidation are fatty acid coming from the diet, a situation that has already been observed in
305 many marine fish species as fatty acids are abundant in marine algae and zooplankton (Tocher, 2010).

306 In sharp contrast to these *de novo* fatty acid biosynthesis steps, we observed that the genes
307 implicated in the desaturation and elongation of fatty acids are regulated during metamorphosis.
308 This is important as long-chain fatty acid (polyunsaturated and highly unsaturated fatty acid,
309 respectively PUFA and HUFA) participate in many biological processes and are precursors of key
310 signaling molecules. PUFAs and HUFAS are generated by the action of front-end desaturases (FADS,
311 SCD) and elongase (ELOVL). In contrast to mammals, in clownfish there is only one FADS that is
312 encoded by the *fads2* gene, as in most marine fishes. This gene is up regulated during

313 metamorphosis (Fig. 5A). The other desaturases, stearoyl-coA desaturase (*scda* and *scdb*) are also
314 activated with similar dynamics. The many elongase genes, including *elovl5* acting on C₁₈ and C₂₀
315 PUFAs, *elovl4* also acting on PUFAs and *elovl6* acting on saturated fatty acids (FA) and
316 monounsaturated fatty acids (MUFA) (Xie et al., 2021) showed a wide variety of patterns suggesting
317 an intricate level of regulation: *elovl5* expression is up-regulated so are the two *elovl6* genes,
318 whereas *elovl4* expression decreases. We also observed that *srebp1*, *pparγ*, *lxr* and *chreb*,
319 transcription factors known to be implicated in the regulation of *fads* and *elovl* genes in fish, are also
320 up-regulated during metamorphosis suggesting that a major coordinated change of gene expression
321 related to lipid biochemistry occurred at this stage (Fig. 5A) (Alves Martins et al., 2012; Pinto et al.,
322 2016; Zhang et al., 2016).

323 These data reveal that, while fatty acids are used as energy source during metamorphosis,
324 they are also metabolized to generate more complex lipids that are precursors for signaling
325 molecules. We therefore compared the lipids content between four post-embryonic stages: S3, S4
326 the pivotal stage, and two metamorphic stages, S5 and S6 (n=3 per stage; Fig. 5B). In accordance with
327 the increased expression of *fads2*, *elovl5* and *elovl6*, we observed a strong statistically significant
328 decrease of saturated lipids and, concomitantly, an increase in MUFA and PUFA. In contrast to those
329 two fatty acid classes, HUFA content decreased steadily during metamorphosis. Supplementary
330 Figure 8 show the complex changes of individual lipid molecules that we observed. Taken together,
331 these results suggest that the metamorphosis coincides also with a major change in fatty acid
332 biochemistry and the complexification of the molecules present that can be used as cell membranes
333 constituents, energy storage, fatty acid transport and as a source of signaling molecules (Hulbert,
334 2021).

335 To determine if these changes are under TH coordination, we analyzed the effects of TH on
336 the expression of key enzymes implicated in fatty acid desaturation and elongation and we studied if
337 TH treatment (10⁻⁷ and 10⁻⁸ M, 72 hours post treatment of stage 3 larvae) was effectively able to alter
338 the amount of specific lipids. At the gene expression level, we observed that TH exposure effectively
339 induces a change in the expression of key genes, increasing *fads2* expression and, surprisingly,
340 repressing *elovl5* gene expression (Fig. 5C). Interestingly, we noticed that TH also up regulate the
341 expression of *pparγ*, *srebp1*, *chreb* and *lxr*, major transcriptional regulators of desaturases and
342 elongases.

343 Concerning lipids, we observed a great variability from one fish to another, a situation
344 probably linked to the fact that it is almost impossible to control the metabolic status and feeding
345 time of such tiny aquatic organisms. This variability impacted the number of lipids for which we can

346 get statistically significant differences in level between TH treatment and controls (DMSO). However,
347 we observed that 37 lipids upon the 1323 studied show differences in levels after TH treatment
348 (Supp. Fig. 9) and we noticed a non-statistically significant trend for 197 others (data not shown). In
349 overall our data suggest that 144 lipids are increased after TH treatment whereas 137 are decreased
350 revealing a major implication of TH in lipid metabolism.

351 Taken together these data suggest that during metamorphosis, clownfish used free fatty
352 acids (saturated and HUFA) as a major energy source and, in addition, dietary lipids as substrates to
353 generate a pleiad of molecules, many of which served as fatty acid transport (acyl carnitine), energy
354 storage (triacylglycerol), membrane constituents (ceramide, sphingomyelin, phosphatidyl
355 ethanolamine) or signaling molecules (phosphatidylglycerol sphingomyelin). As vision and energy
356 metabolism, this transition in lipid biochemistry is also controlled by TH that appear as a major
357 conductor of the metamorphosis process.

358

359 *LXR modulation reveal an intimate link between metabolic transition and metamorphosis*

360 In the transcription factors regulating fatty acid biochemistry, LXR (liver X regulators, also
361 called NR1H3) is particularly interesting given its known pivotal role. This receptor is a major
362 regulator of lipid metabolism and also regulates fatty acid metabolism through, for example, the
363 control of *srebp* and elongases and desaturases genes (Wang and Tontonoz, 2018). Additionally, in
364 mouse *lxrβ* control TH signaling in the brain and adipose tissue and its knock-out resulted in
365 increased *dio2*, *nis* and TH transport genes (Ghaddab-Zroud et al., 2014; Miao et al., 2015). We used
366 SR9243, a selective LXR antagonist to test if inhibiting LXR action will affect clownfish metamorphosis
367 and its underlying gene regulatory program. Importantly, we verified that this compound specifically
368 inhibits fish LXR activity and is inactive on PPAR γ and PXR (Supp. Fig. 10).

369 As expected, after treating stage 3 larvae for 5 days with 10⁻⁷M SR9243, we observed a
370 dysregulation of lipid metabolism genes such as *acads*, *srebp*, *fads2* and *elovl5* genes (Fig. 6A)
371 confirming that in clownfish as in zebrafish, LXR is a regulator of lipid metabolism. We also observed
372 that glycolysis genes (*pfkma* and *pkmb*) are down regulated whereas citric acid cycle genes are up
373 regulated (Fig. 6A).

374 Interestingly, in this context, we also observed a clear acceleration of metamorphosis
375 revealed by white bar appearance (Salis et al., 2021; Figure 6B-C). By counting the number of
376 individuals harboring white bars we observed a significant difference in SR9243-treated fishes (66%
377 for head bar and 50% for trunk bar vs 16% and 16% in DMSO controls, respectively; Fig. 6C). This

378 shows that, as in mouse, LXR inhibition activate TH signaling and we effectively observed that *trβ*,
379 *dio2* and *dio3a* are up regulated after treatment (Fig. 6A).

380 Based on these results, we investigated if SR9243 exposure also affects the other processes
381 that are coordinated by TH and in particular vision. Interestingly, LXR antagonist also promoted the
382 opsin genes expression shift, decreasing short wavelength and increasing long wavelength opsin
383 expression (Fig. 6A). LXR antagonist also regulated the phototransduction gene *guca1a*, as observed
384 in zebrafish (Fig. 6A ; Pinto et al., 2016). Taken together, these results show that modulating lipid
385 homeostasis by inhibiting LXR activity affect TH signaling and vision, linking metabolic regulation with
386 the coordination of metamorphosis.

387

388 *Natural TH regulation in an ecological context, reveals metabolic and visual effect.*

389 We previously reported a natural situation in which endogenous TH level regulation occurs
390 (Salis et al., 2021). In Kimbe bay, Papua New Guinea *Amphiprion percula* can inhabit in two different
391 sea anemone hosts: the carpet sea anemone *Stichodactyla gigantea* and the magnificent sea
392 anemone *Heteractis magnifica*. In these two hosts, the new recruits exhibit differences in white bar
393 formation that are linked to a higher TH level (Fig. 7A) and higher *duox* expression in fish hosted by *S.*
394 *gigantea* (Salis et al., 2021). Given the results described above, we therefore measured the
395 expression levels of genes implicated in vision and metabolic regulation in this natural context.

396 We compared gene expression between new recruits found in *H. magnifica* (n = 3) or *S.*
397 *gigantea* (n = 3) by RNA sequencing of whole fish. On a PCA analysis based on vision and metabolic
398 genes, we observed a clear separation between both types of recruits (Fig. 7B). We noticed that, in
399 accordance with the higher TH level in *S. gigantea* recruits, the phototransduction gene *guca1a* is up
400 regulated whereas the short wavelength opsin *opnsw1* as well as the rhodopsin *rh1* and *rh2a* are
401 down regulated (Fig. 7D). We also detected a small down regulation of the long wavelength opsin
402 *opnlw*.

403 At the metabolic level we also observed a clear separation of recruits living in *S. gigantea* vs.
404 those in *H. magnifica* suggesting that effectively the metabolic status of both type is different (Fig.
405 7C). When individual genes are studied, we observed that *S. gigantea* recruits contain higher TH
406 level, a decrease in expression of the glycolytic *pkmb* gene and the lactic fermentation gene *ldhba*
407 and an increased expression of the TCA cycle gene *idh3* and the β -oxidation genes *acaa1* and *acads*
408 (Fig. 7E-F). Similarly, we observed a decrease in the expression level of the fatty acid synthase gene
409 *fasn*. These results are in accordance with the metabolic transition observed during metamorphosis

410 and demonstrate the relevance of these effects in natural populations living in their pristine
411 environment.

412

413 **Discussion**

414 In this paper we reveal how TH control and coordinate a major ecological transition, the
415 metamorphosis of a pelagic coral reef fish larvae into a benthic reef associated juvenile. Our data
416 therefore suggest a model, discussed below, according to which TH has a central coordinating
417 function to ensure the ecological success of the metamorphosed juvenile.

418 Three main functions were attributed to TH in vertebrates: (i) the triggering of
419 metamorphosis in amphibians and fish; (ii) the regulation of adult physiology and metabolism in
420 mammals and (iii) the adaptation to seasonality regulation in vertebrates (Dardente et al., 2014;
421 Laudet, 2011; Sayre and Lechleiter, 2012). The extends to which these functions are shared across
422 vertebrates remains unclear, but our data clearly suggest that the two first functions are active
423 during clownfish metamorphosis: TH promotes the morphological and behavioral transformation of
424 the larvae while also promoting a metabolic shift in energy source.

425 We have studied in detail the action of TH on vision and metabolism and in a recent report,
426 we uncovered their function for adult pigmentation pattern formation (Salis et al., 2021).
427 Interestingly, our transcriptomic data obtained on whole larvae reveal that several other important
428 biological processes such as bone mineralization and digestion are also changing during
429 metamorphosis. This, together with the detailed analysis of vision and metabolism that we have
430 performed, clearly illustrate the pleiotropic TH action during metamorphosis.

431

432 *A behavioral shift in vision controlled by TH*

433 We observed a TH-induced shift in between short wavelength and long wavelength opsin
434 gene expression at stage 4 during clownfish metamorphosis (Fig. 3). Interestingly, this shift occurs
435 concomitantly with a behavioral change, namely a change in color preference. Larval fish prefers a
436 blue background, in accordance with their natural pelagic environment, whereas juveniles prefer an
437 orange background in accordance with the shallow, colorful and chromatically dynamic environment
438 of the reef (Chiao et al., 2000). In addition, we showed that TH exposure decreases the preference
439 for blue background on treated fish. In addition, the TH-treated larvae, adopted a benthic behavior
440 characterized by low swimming activity close to the bottom of the experimental tank, which is in
441 accordance with the benthic live style of anemonefish juveniles. Our data do not unequivocally
442 demonstrate a direct effect of TH in color vision as many effects can also be elicited in neural circuit

443 mechanisms for color vision (Baden, 2021). However, they strongly suggest that TH globally controls
444 a visual shift that is ecologically critical for the recruitment success of juveniles.

445 Vision is one of the main sensory systems that has been studied by ecologists in the context
446 of fish recruitment (Job and Shand, 2001; Shand, 1997). A shift in opsin gene expression, and in
447 particular an increase of *opnlw* expression, has been observed in many fish species such as flatfishes,
448 black bream or cichlids (Carleton et al., 2008; Ferrarresso et al., 2013; Härer et al., 2017; Shand et al.,
449 2008; Shao et al., 2017). However, up to date, there is only a partial understanding of the
450 mechanistical control of this phenomenon and its integration with metamorphic transformation. In
451 zebrafish, TH signaling has been shown to regulate the *opnlw* and mutation of the *TR β* gene resulted
452 in an absence of red cones but no link with an ecological transition was established (Suzuki et al.,
453 2013). In surgeonfish, we have observed that shifts in retina structure and in the ability to visually
454 recognize predators are both controlled by TH but no data on gene expression have been obtained so
455 far, leaving the mechanism underpinning this recognition unclear (Besson et al., 2020).

456 The data we obtained here, are in accordance with what has been observed in mammals. In
457 the mouse, (Eldred et al., 2018) have shown that TH controls the temporal switch between S and L/M
458 cones which contains short and medium/long wavelength opsins, respectively. This suggests that the
459 TH action on opsin gene expression and more generally visual function is a general phenomenon in
460 vertebrates. By its ability to combine *in vitro* and field studies, the anemonefish model could allow to
461 integrate the TH action on retina formation and more generally on sensory systems maturation in an
462 ecologically relevant context (Roux et al., 2020).

463

464 *The metabolic transition is instrumental for metamorphosis completion*

465 We observed a major metabolic transition during metamorphosis, and we provided evidence
466 that TH is implicated in this transition. Namely, larvae preferentially use glycolysis and fermentation
467 to produce energy whereas juveniles rely on fatty acid β -oxidation and citric acid cycle.

468 Our data show that there is a clear shift from glycolysis during early larval life (stage 1-3) to β -
469 oxidation starting at stage 4 (Fig. 4). To put this finding in perspective we need to consider three
470 parameters: (i) the changes in the organism growth; (ii) the transition of the fish into a new
471 ecological life and (iii) the energy produced by each pathway. In glycolysis, one molecule of glucose
472 produces a net excess of only 2 ATP, 2 NADH and 2 molecules of pyruvate while oxidation of one
473 molecule of palmitic acid (C16:0) (one of the most common saturated fatty acid found in vertebrates),
474 produces a net gain of 129 ATP molecules by β -oxidation (Wang et al., 2020). Therefore, utilizing
475 fatty acid as a fuel source generates much more energy than sugar. During *A. ocellaris* post-
476 embryonic development, we observe a massive increase in size soon after stage 4. Growth in
477 vertebrates is highly demanding in energy and having the possibility for the larvae to utilize a fuel

478 source that produces a large number of ATP is certainly an advantage. Moreover, after stage 4, the
479 transforming fish must actively swim to find its juvenile habitat and once settled, will have to fight
480 with congeners to be accepted in the colony. All this will require high energy demand, hence high
481 ATP production explaining the transition to a more energy producing system.

482 In addition to this shift in energy production, we also reveal that TH also stimulates the
483 production of complex lipids suggesting that the biochemical landscape of the juvenile is more
484 elaborated than the larval one, because of TH action. These lipids can be used for fatty acid transport
485 (acyl carnitine), energy storage (triacylglycerol), membrane constituents (ceramide, sphingomyelin,
486 phosphatidyl ethanolamine) or signaling molecules (phosphatidylglycerol sphingomyelin) (Hulbert,
487 2021).

488 Interestingly, this metabolic transition is deeply linked to the morphological and behavioral
489 changes that we describe in this paper. Indeed, when we modify lipid metabolism by using a LXR
490 antagonist, we observed an acceleration of white bar appearance as well as a shift in opsin gene
491 expression, two of the most salient endpoints of metamorphosis. This suggests that LXR is active
492 during this period, in accordance with its expression peak at stage 4 and its up regulation by TH. LXR
493 might be involved in slowing-down the process, ensuring that it is progressing concomitantly with the
494 available energy production. In addition, by its known action in regulating elongases and desaturase
495 genes, LXR is likely playing an important role in the complexification of fatty acids that we observed
496 (Xie et al., 2021).

497

498 *The ecological function of thyroid hormone*

499 Collectively our data suggest that TH is instrumental in integrating the complex remodeling
500 occurring during metamorphosis with the available resources provided by the environment. This is
501 highly relevant for a marine fish that similarly to a pelagic larvae, is living in a very dynamic
502 environment characterized by patchy food resources (Seuront et al., 2001). It must be pointed out
503 that the challenges faced by a tiny pelagic fish larvae are impressive: It must combine energy
504 production from highly patchy food resources, mobilize its reserves in case of fasting and, at the
505 same time, mature sensory systems that can detect future juvenile habitat, all this while avoiding
506 predators! Our paper strongly suggests that TH plays a major role to orchestrate this complex
507 transformation.

508 Interestingly, the fact that metabolism must be coupled with developmental transition to
509 fulfill the energy requirements during organisms' life cycle, have been previously pointed out in the
510 context of insect metamorphosis. (Nishimura, 2020) have revealed that the programmed regulation of
511 metabolism by steroid hormones control the key steps during drosophila metamorphosis. This is
512 remarkably similar to the effects we observed here with the major difference that, in contrast to

513 insect pupa, the transforming fish larva rely on an external food source. However, in both cases, the
514 coordination of the larval transformation must integrate both environmental and internal conditions.
515 Adjusting metabolic regulation is a major tool for achieving this.

516 Our data are also relevant to consider in the light of previous work done on stickleback.
517 These fish can live in either marine or freshwater environment and the energy resource available in
518 these two situations are drastically different. Interestingly, Kitano et al., 2010 have shown that, in
519 freshwater sticklebacks there was a fine tuning of numerous physiological and metabolic traits that
520 ensure a reduced energy expenditure. These changes are driven by TH, as a genetic variant
521 expressing low level of TSH, the main hypothalamic peptide controlling TH production, has been fixed
522 in these populations (Kitano et al., 2010). In these freshwater sticklebacks in which TH level is low,
523 there is also evidence of a shift in opsin gene expression linked to local adaptation to different light
524 environment (Rennison et al., 2016). This suggests that, in stickleback as in anemonefish, there is a
525 tight integration of metabolic and sensory changes, and that TH may be instrumental in this process.
526 This case provides a genuinely nice illustration of a still unappreciated potential for TH regulation to
527 induce pleiotropic changes, allowing a multi-level regulation of a developmental transition (Laudet,
528 2010).

529 Our model has also a general significance regarding the effect of climate change and
530 pollution in animal populations. Indeed, the sensitivity of metamorphosis to environmental stressors
531 has been recently emphasized (Lowe et al., 2021). Metamorphosis being a life-history transition with
532 abrupt oncogenic changes tightly connecting to environmental conditions can make young life stages
533 vulnerable to stressors. Indeed, we demonstrated previously in convict surgeonfish, that
534 temperature and pollution both synergistically disrupt TH signaling affecting the ability of the young
535 juveniles to perform their ecological function (Besson et al., 2020). Our study therefore suggests that
536 more research is needed to decipher how stressors affects not only metamorphosis endpoints but
537 also the metabolic regulations that ensure its correct progression.

538

539

540 **Material and Methods**

541

542 ***Amphiprion ocellaris* maintenance and rearing**

543 *Amphiprion ocellaris* larvae were obtained from breeding paired maintained as described in Roux
544 et al. (2021) in a rearing structure. Reproductive pairs laid eggs every two weeks allowing us to rear
545 larvae regularly for the purpose of this study.

546 **Transcriptomic analysis**

547 **RNA extraction**

548 Three larvae per stage are sampled making a total of 21 samples. Larvae are euthanized in a
549 MS222 solution (200 mg/l), photographed for stage identification, and kept in RNAlater prior to RNA
550 extraction. Total RNA is extracted from whole individual larval body using a Maxwell®16 System
551 (Promega, Madison, USA) and following the manufacturer's instructions. RNA integrity and
552 concentration is verified with an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, USA)
553 and only samples with RIN values equal to, or above 8 are used. In our case, all samples had RIN
554 values above 9.

555 **RNA-Seq libraries preparation and sequencing**

556 RNA-Seq libraries and sequencing are performed by the IGBMC in Strasbourg on an Illumina
557 HiSeq4000 sequencer using a stranded protocol with paired-end 2x100bp.

558 **Software**

559 Transcriptomic analyses are performed by the Altrabio Company (Lyon, France) using the following
560 software: FasQCversion 0.11.8 and fastq_screen version 0.13.0, Salmon version 0.14.1, R version
561 3.6.1 (2019-07-05), x86_64-apple-darwin15.6.0.

562 **Pre-treatment (read quality, quantification, filtering, normalization)**

563 Quality of raw reads is assessed using the FastQC quality control tool1. Sample contamination is
564 assessed using the fastq_screen quality control tool2. The Bowtie2 indexes for *Amphiprion ocellaris*
565 are generated from Ensembl "Amphiprion_ocellaris.AmpOce1.0.dna.toplevel.fa" file. *Amphiprion*
566 *ocellaris* transcript sequences and annotations (Ensembl release 97) are downloaded from the
567 Ensembl website3 (files Amphiprion_ocellaris.AmpOce1.0.cdna.all.fa,
568 Amphiprion_ocellaris.AmpOce1.0.ncrna.fa and Amphiprion_ocellaris.AmpOce1.0.97.gtf). Some genes
569 which had no official symbols in Ensembl are given the following symbols: ENSAOCG00000023317
570 =tg, ENSAOCG00000008531 = mct8, ENSAOCG00000005709 = dio3a, ENSAOCG00000017526 = edn3b,

571 and ENSAOCG00000019494 = bnc2. Transcript expression quantification is performed from the raw
572 read data (FASTQ files) using Salmon4 (with parameters --gcBias, --seqBias, --validateMappings, --
573 discardOrphansQuasi, and --consistentHits). They are then summarized as gene counts using function
574 tximport from package tximport5 (with parameters type="salmon" and
575 countsFromAbundance="lengthScaledTPM"). Genes that did not have more than 0.502 count per
576 million counts in at least three samples are filtered out. To generate the normalized signals, the
577 effective library sizes are first computed using function estimateSizeFactors from package DESeq26.
578 The raw count values are then transformed using a variance stabilizing transformation (function
579 VarianceStabilizingTransformation from package DESeq2 with parameter blind=TRUE).

580 **Hierarchical clustering**

581 Hierarchical clusterings of samples are performed using the Wards agglomerative method,
582 passing the euclidean distances between samples to function hclust from package stats (with
583 parameter method="ward.D2"). Cluster stability is estimated by multiscale bootstrap resampling
584 using function pvclust from package pvclust7 (with parameter nboot=10000). Hierarchical clusterings
585 of genes are performed using the complete agglomerative method, passing the Euclidean distances
586 between centered (for expression data) or uncentered (for fold change data) and scaled gene signals
587 with function hclust from package stats.

588

589 **Principal component analysis**

590 Principal Component Analyses (PCA) of sample expression levels are performed with gene
591 signals centered but not scaled (using function prcomp from package stats). When displayed, gene
592 coordinates correspond to these genes' correlations with the presented components. Only the genes
593 that contribute most to the components are displayed.

594

595 **TH dosage**

596 To determine at which stage TH are surging and marking the beginning of metamorphosis, pools
597 of five clownfish larvae are sampled in triplicate at each of the seven developmental stages identified
598 in Roux et al. (2019). THs are extracted from dry-frozen larvae (previously euthanized in a 200 mg/l
599 solution of MS-222) following the protocol developed by Holzer et al. (2017) and adapted from
600 previous TH extractions in teleost fishes (Einarsdóttir et al., 2006b; Kawakami et al., 2008; Tagawa
601 and Hirano, 1989). TH concentrations are measured by a medical laboratory in Perpignan (Médipole)
602 using an ELISA kit (Access Free T3, T4, Beckman Coulter).

603 ***In situ* hybridization**

604 Digoxigenin RNA probes were synthesized using the T3/T7 Transcription Kit (Roche; Supp. Table 1).
605 Larvae were collected, euthanized in MS222 at 200 mg/L and fixed 12 hours in 4% paraformaldehyde
606 diluted in PBS (phosphate-buffered saline). Samples were subsequently dehydrated stepwise in
607 PBS/ethanol, and then put three times 10 min in butanol 100% and finally in two bath of paraffin
608 (respectively 1 and 4 hours) before being embedded in block. Embedded larvae were sectioned
609 transversally at 7 μ m using Leica Biosystems RM2245 Microtome the day before starting ISH. The
610 samples were then treated as in Thisse, Thisse, Schilling, and Postlethwait (1993).

611 **Behavioral test for visual perception**

612 Choice experiments are conducted on stage 2 and stage 5 to determine if there is a shift in visual
613 perception before and after metamorphosis. Thus, a dual choice aquarium (measuring 25x10x10 cm)
614 is built with white opaque comassel, fitted with transparent plexiglass on the sides (Fig. 3C). The
615 aquarium is divided into three equal compartments (Fig. 3C). The larvae are given the choice
616 between a short wavelength color: blue and a long wavelength color: orange. Each color is placed
617 close to the plexiglass sides. Behavioral experiments are conducted in a dark room where the choice
618 chamber is installed under a light ramp to ensure homogenous distribution of the light over the
619 device. All the larvae are tested individually. The larvae are introduced carefully in the choice
620 chamber without the colored panels and left for acclimation for 2 min. They are free to explore the
621 three compartments. After acclimation and once the larvae are localized in the central compartment,
622 blue and orange panels are installed, and the time spent by the larvae in each compartment is
623 recorded during a 5 min period. We established that a larva preferred a color when it is spending
624 most of its time in the compartment of the given color (Fig. 3C). Color panels are inverted after each
625 tested larva to ensure that the choice is due to the color and not to a defect in the experimental
626 device. Results are expressed in the mean percentage of time spent in each compartment. A Kruskal-
627 Wallis test is performed to determine if the difference in the amount of time spent between larvae
628 (stage 2 vs stage 5, and DMSO vs T3) is significantly different in each compartment.

629

630 **T3 treatments**

631 To test the effects of THs on the metamorphosis of clownfish *A. ocellaris*, larvae were exposed to
632 various concentration of T3 using the low-rearing volume protocol developed by Roux et al., (2021).
633 Briefly, larvae were exposed to three different concentrations of a mix of T3 and iopanoic acid (IOP)
634 to induce metamorphosis. IOP was added to block the action of the deiodinases and avoid the
635 degradation of the added T3 (Holzer et al., 2017). Larvae were exposed to increasing doses of T3: 10⁻

636 8M , 10^{-7}M , 10^{-6}M (the IOP concentration remained constant at 10^{-7}M), and to DMSO (as control)
637 diluted at 1:1000. Larvae were sampled at 12h, 24h, 48h and 72h for gene expression analysis using
638 nCounter technology from Nanostring. Exposition started at stage 3 before the beginning of the
639 metamorphosis and lasted for 12h, 24h, 48h and 72h. Larvae were introduced by groups of 10 in 800
640 ml beakers placed in a water bath to maintain the temperature at 27°C . Larvae were fed 3 times a
641 day with rotifers at a final concentration of 10 rotifers/ml and once a day with nauplii of artemia
642 (Roux et al., 2021). The algae *N. oculata* was added ($500\mu\text{l}/\text{beaker}$) at each feeding to create a green
643 environment and improve survival rates. During the experiment, water changes of 100 ml (with
644 addition of each treatment to maintain constant concentrations) were done daily to ensure water
645 quality. Larvae were sampled after 12h, 24h, 48h, and 72h of exposition, euthanized in a MS222
646 solution ($200\text{mg}/\text{l}$) and photographed under a Zeiss stereomicroscope (V20 discovery Plan S)
647 equipped with an Axiocam 105 camera for morphological analysis. Three replicates composed of
648 pools of two larvae were then kept in RNA later at -20°C for gene analysis using nCounter technology
649 from Nanostring.

650

651 **Gene expression measurements using nCounter technology**

652 **Probe synthesis**

653 Probes of 100 nucleotides were designed by Integrated DNA Technology for all the genes
654 investigated in this study (Supp. Table 2). However, no probes could be designed that specifically
655 targeted *TRaa*, *TRab*, *rh2a-1* and *rh2a-2* as sequences were too similar. For this reason, only one pair
656 of probes has been designed and targeting both *TRa* and *rh2a* genes. Seven reference genes were
657 chosen for normalization and expression analysis: *Tuba1*, *PolD2*, *g6pd*, *elf4a3*, *tbp*, *rpl7* and *rpl32*.

658

659 **Sample processing**

660 Samples of each experiment were processed in multiplexed reaction including, in each case,
661 six negative probes (to determine the background) and six positive control probes. Hybridization
662 reactions lasted 16 hours at 67°C . Data were then imported into the nSolver analysis software
663 (version2.5) for quality checking and normalization of data according to NanoString analysis
664 guidelines, using positive probes and 7 housekeeping genes (*Tuba1*, *PolD2*, *g6pd*, *elf4a3*, *tbp*, *rpl7*
665 and *rpl32*) (Kulkarni, 2011).

666

667 **Statistical analysis**

668 After normalization, analysis of the difference between treatments for each experiment was
669 conducted using ANOVA (R software 3.2.3 version) and fold change calculated on a \log_2 scale. As

670 multiple comparison was used, p-value was adjusted using the Benjamini & Hochberg method
671 (Ferreira and Zwinderman, 2006).

672

673 **Lipid composition measurements and analysis**

674 Larvae were sampled at stage 3, 4, 5 and 6 and starved for one hour before being flash frozen
675 in liquid nitrogen and stored at -80 C° until analysis. Three individuals were collected for each
676 condition and lipid extraction was done on each individual.

677 Samples were analyzed as previously described (Weir et al., 2013), briefly, samples were
678 thawed and lyophilized to remove all liquid. Samples were then reconstituted prior to extraction in
679 10 µl of water and 10 µl of internal standard mix (ISTD) was added to each sample. Lipids were
680 extracted by adding 200 µl chloroform/methanol (2:1) and sonicated in a bath for 30 minutes before
681 the supernatant was transferred to a 96-well plate and dried under vacuum in a SpeedVac
682 Concentrator (Savant). Samples were then reconstituted with 50 µl water saturated butanol and 50
683 µl methanol with 10 mM ammonium formate and analysed by LC ESI-MS/MS using an Agilent 1200
684 LC system and an ABSciex 4000 Qtrap mass spectrometer. Data was processed using MultiQuant 2.1
685 software. Lipids are presented as pmol/mg protein. The lipid standards used in this analysis were as
686 previously described (Weir et al., 2013).

687 Statistical analysis on developmental stage was performed using one way ANOVA with R
688 software for each lipid followed by a multiple pairwise comparison performed by pairwise t-test in
689 case of significant differences (p-value<0.05) (Fraher et al., 2016).

690 Similar analysis was performed on larvae treated for 72hours at 10⁻⁸ M and 10⁻⁷ M T3(mixed
691 with 10⁻⁷ M IOP). Sampling was performed as explained previously. Statistical analyses were
692 performed on comparing each T3 concentration with DMSO control condition using the test of
693 Student with R software.

694

695 **LXR experiments**

696 Specificity of a human LXR antagonist SR9243 was tested in reporter cell lines expressing the
697 ligand binding domains of zebrafish receptors LXR, PPARγ and PXR. Briefly, SR9243 was tested alone
698 for its agonistic activity in HG5LN-zfLXR, -zfPPARγ and zf-PXR cells (Creusot et al., 2020; Garoche et al.,
699 2021). For its antagonistic activity on -zfLXR, -zfPPARγ and zf-PXR cells, SR9243 was tested in
700 combination with T091317 30 nM, GW3965 3 µM and clotrimazole 0.1 µM respectively (Supp. Fig.
701 10).

702 Larvae of *A. ocellaris* were sampled at stage 3 and treated for 5 days with DMSO (control
703 condition) and LXR antagonist SR9243 at 10^{-7} M. Experiment was conducted following the protocol
704 described in Roux et al., 2021 and detailed previously. Gene expressions was obtained by RT-qPCR
705 (PrimeScript transcriptase, Takara, SYBRgreen) and normalized with Pfaffle equation using two
706 housekeeping genes (*rpl7*, *rpl32*) (Stahlberg et al., 2004). Specific primers were designed using
707 Primer3 software (Untergasser et al., 2012). Primers sequenced are summarized in Supp. Table 1.
708 Significant difference of gene expression level between DMSO control and SR9243 conditions was
709 assessed by using the test of Student with R software. Difference between the number of individuals
710 displaying white bars in both conditions was assessed by a Chi^2 test performed with R software.

711 ***Amphiprion percula* RNA sequencing**

712 *Amphiprion percula* new recruit were sampled in Kimbe, Papua New Guinea in both
713 *Heteractis magnifica* and *Stichodactyla gigantea*. A total of three new recruits per sea anemone were
714 euthanized in MS222 solution and stored in RNAlater until RNA extraction and RNA sequencing of the
715 whole fish individually (Salis et al. 2021). Potential adapter contaminations were removed and
716 trimmed to obtain raw reads with cutadapt (v.1.13; (Martin, 2011)) and sickle (v1.29, (Joshi and Fass,
717 2011)), respectively. The processed reads were mapped against *A. percula* reference genome
718 (Ensembl ID: GCA_003047355.1; (Lehmann et al., 2019)) using HiSat2 (v.2.1.0; (Kim et al., 2015)). Raw
719 counts for each gene were obtained with HTSeq (htseq-count, v.0.9.1; (Anders et al., 2015)), using
720 the available gene annotation of the *A. percula* reference genome (Salis et al. 2021). Raw counts
721 were then normalized as transcript per million to assess the difference in expression levels for gene
722 involved in vision and metabolic processes using the test of student. Principal component analysis
723 was also performed on each biological process separately.

724

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734

735 **Author contribution**

736 NR and VL wrote the manuscript with contributions from YG, KG, DL and LB. NR, VL, DL, LB
737 designed the whole study with the help of YG and KG for the metabolic aspects. Transcriptome
738 assembly was performed by SB and analysis were performed by SB and NR. NR performed all the
739 pharmacological treatments with the help of SM, MD, YT and FL. NR performed gene expression and
740 behavioral experiments linked to visual perception and metabolism (with the help of SM and YT). YG
741 and KG brought their expertise on metabolic regulation and lipid metabolism. YG helped in the lipid
742 analysis. NR, SM and YT conducted experiments on lipid metabolism gene expression. PS generated
743 the transcriptomic data set of *Amphiprion percula* recruits sampled in Kimbe, Papua New Guinea.
744 ABa and NR analyzed *A. percula* transcriptome. NR and MR conducted thyroid hormone
745 measurements. Abo performed LXR antagonist activity experiment on zebrafish.

746

747 **Ethics Approval**

748 All experiments conducted in this study were done under the approval from the C2EA-36 Ethics
749 Committee for Animal Experiment Languedoc-Roussillon (CEEA-LR - approval N°A6601601) as well as
750 following the Animal Experiment Regulations at Okinawa Institute of Science and Technology
751 Graduate University (approval N°20052605).

752

753 **Competing interests**

754 The authors declare no competing interests.

755 **References**

756 Alves Martins, D., Rocha, F., Martínez-Rodríguez, G., Bell, G., Morais, S., Castanheira, F., Bandarra, N.,
757 Coutinho, J., Yúfera, M., and Conceição, L.E.C. (2012). Teleost fish larvae adapt to dietary arachidonic
758 acid supply through modulation of the expression of lipid metabolism and stress response genes. *Br J*
759 *Nutr* *108*, 864–874.

760 Anders, S., Pyl, P.T., and Huber, W. (2015). HTSeq--a Python framework to work with high-throughput
761 sequencing data. *Bioinformatics* *31*, 166–169.

762 Baden, T. (2021). Circuit mechanisms for colour vision in zebrafish. *Current Biology* *31*, R807–R820.

763 Barth, P., Berenshtein, I., Besson, M., Roux, N., Parmentier, E., Banaigs, B., and Lecchini, D. (2015).
764 From the ocean to a reef habitat: how do the larvae of coral reef fishes find their way home. *VIE ET*
765 *MILIEU-LIFE AND ENVIRONMENT* *95*, 91–100.

766 Besson, M., Feeney, W.E., Moniz, I., François, L., Brooker, R.M., Holzer, G., Metian, M., Roux, N.,

- 767 Laudet, V., and Lecchini, D. (2020). Anthropogenic stressors impact fish sensory development and
768 survival via thyroid disruption. *Nat Commun* *11*, 3614.
- 769 Bianco, A.C., and Kim, B.W. (2006). Deiodinases: implications of the local control of thyroid hormone
770 action. *J Clin Invest* *116*, 2571–2579.
- 771 Buchholz, D.R. (2015). More similar than you think: Frog metamorphosis as a model of human
772 perinatal endocrinology. *Dev Biol* *408*, 188–195.
- 773 Buston, P. (2003). Social hierarchies: size and growth modification in clownfish. *Nature* *424*, 145–146.
- 774 Campinho, M.A. (2019). Teleost Metamorphosis: The Role of Thyroid Hormone. *Frontiers in*
775 *Endocrinology* *10*, 383.
- 776 Carleton, K.L., Spady, T.C., Strelman, J.T., Kidd, M.R., McFarland, W.N., and Loew, E.R. (2008). Visual
777 sensitivities tuned by heterochronic shifts in opsin gene expression. *BMC Biol* *6*, 22.
- 778 Chang, J., Wang, M., Gui, W., Zhao, Y., Yu, L., and Zhu, G. (2012). Changes in thyroid hormone levels
779 during zebrafish development. *Zoolog Sci* *29*, 181–184.
- 780 Charalambous, M., and Hernandez, A. (2013). Genomic imprinting of the type 3 thyroid hormone
781 deiodinase gene: Regulation and developmental implications. *Biochimica et Biophysica Acta (BBA) -*
782 *General Subjects* *1830*, 3946–3955.
- 783 Chiao, C.-C., Cronin, T.W., and Osorio, D. (2000). Color signals in natural scenes: characteristics of
784 reflectance spectra and effects of natural illuminants. *J. Opt. Soc. Am. A, JOSAA* *17*, 218–224.
- 785 Cortesi, F., Musilová, Z., Stieb, S.M., Hart, N.S., Siebeck, U.E., Cheney, K.L., Salzburger, W., and
786 Marshall, N.J. (2016). From crypsis to mimicry: changes in colour and the configuration of the visual
787 system during ontogenetic habitat transitions in a coral reef fish. *J Exp Biol* *219*, 2545–2558.
- 788 Creusot, N., Gassiot, M., Alaterre, E., Chiavarina, B., Grimaldi, M., Boulahtouf, A., Toporova, L., Gerbal-
789 Chaloin, S., Daujat-Chavanieu, M., Matheux, A., et al. (2020). The Anti-Cancer Drug Dabrafenib Is a
790 Potent Activator of the Human Pregnane X Receptor. *Cells* *9*.
- 791 Dardente, H., Hazlerigg, D., and Ebling, F. (2014). Thyroid Hormone and Seasonal Rhythmicity.
792 *Frontiers in Endocrinology* *5*.
- 793 Darias, M.J., Zambonino-Infante, J.L., Hugot, K., Cahu, C.L., and Mazurais, D. (2008). Gene expression
794 patterns during the larval development of European sea bass (*Dicentrarchus labrax*) by microarray
795 analysis. *Marine Biotechnology* *10*, 416–428.
- 796 Darras, V.M., and Van Herck, S.L.J. (2012). Iodothyronine deiodinase structure and function: from
797 ascidians to humans. *J Endocrinol* *215*, 189–206.
- 798 Deal, C.K., and Volkoff, H. (2020). The Role of the Thyroid Axis in Fish. *Frontiers in Endocrinology* *11*,
799 861.
- 800 Einarsdóttir, I.E., Silva, N., Power, D.M., Smáradóttir, H., and Björnsson, B.T. (2006a). Thyroid and
801 pituitary gland development from hatching through metamorphosis of a teleost flatfish, the Atlantic
802 halibut. *Anat Embryol* *211*, 47–60.
- 803 Einarsdóttir, I.E., Silva, N., Power, D.M., Smáradóttir, H., and Björnsson, B.T. (2006b). Thyroid and

- 804 pituitary gland development from hatching through metamorphosis of a teleost flatfish, the Atlantic
805 halibut. *Anat Embryol* 211, 47–60.
- 806 Eldred, K.C., Hadyniak, S.E., Hussey, K.A., Brennerman, B., Zhang, P.-W., Chamling, X., Sluch, V.M.,
807 Welsbie, D.S., Hattar, S., Taylor, J., et al. (2018). Thyroid hormone signaling specifies cone subtypes in
808 human retinal organoids. *Science* 362, eaau6348.
- 809 Ferrareso, S., Bonaldo, A., Parma, L., Cinotti, S., Massi, P., Bargelloni, L., and Gatta, P.P. (2013).
810 Exploring the larval transcriptome of the common sole (*Solea solea* L.). *BMC Genomics* 14, 315.
- 811 Ferreira, J.A., and Zwinderman, A.H. (2006). On the Benjamini–Hochberg method. *Ann. Statist.* 34,
812 1827–1849.
- 813 Fraher, D., Sanigorski, A., Mellett, N.A., Meikle, P.J., Sinclair, A.J., and Gibert, Y. (2016). Zebrafish
814 Embryonic Lipidomic Analysis Reveals that the Yolk Cell Is Metabolically Active in Processing Lipid. *Cell*
815 *Reports* 14, 1317–1329.
- 816 Garoche, C., Boulahtouf, A., Grimaldi, M., Chiavarina, B., Toporova, L., den Broeder, M.J., Legler, J.,
817 Bourguet, W., and Balaguer, P. (2021). Interspecies Differences in Activation of Peroxisome
818 Proliferator-Activated Receptor γ by Pharmaceutical and Environmental Chemicals. *Environ. Sci.*
819 *Technol.* 55, 16489–16501.
- 820 Ghaddab-Zroud, R., Seugnet, I., Steffensen, K.R., Demeneix, B.A., and Clerget-Froidevaux, M.-S. (2014).
821 Liver X receptor regulation of thyrotropin-releasing hormone transcription in mouse hypothalamus is
822 dependent on thyroid status. *PLoS One* 9, e106983.
- 823 Grimaldi, A., Buisine, N., Miller, T., Shi, Y.-B., and Sachs, L.M. (2013). Mechanisms of thyroid hormone
824 receptor action during development: Lessons from amphibian studies. *Biochimica et Biophysica Acta*
825 (BBA) - General Subjects 1830, 3882–3892.
- 826 Härer, A., Torres-Dowdall, J., and Meyer, A. (2017). Rapid adaptation to a novel light environment: The
827 importance of ontogeny and phenotypic plasticity in shaping the visual system of Nicaraguan Midas
828 cichlid fish (*Amphilophus citrinellus* spp.). *Mol Ecol* 26, 5582–5593.
- 829 Holzer, G., and Laudet, V. (2013). Thyroid Hormones and Postembryonic Development in Amniotes. In
830 *Current Topics in Developmental Biology*, (Elsevier), pp. 397–425.
- 831 Holzer, G., Besson, M., Lambert, A., François, L., Barth, P., Gillet, B., Hughes, S., Piganeau, G., Leulier, F.,
832 and Viriot, L. (2017). Fish larval recruitment to reefs is a thyroid hormone-mediated metamorphosis
833 sensitive to the pesticide chlorpyrifos. *ELife* 6.
- 834 Hulbert, A.J. (2021). The under-appreciated fats of life: the two types of polyunsaturated fats. *Journal*
835 *of Experimental Biology* 224, jeb232538.
- 836 Hulbert, A.J., and Else, P.L. (2000). Mechanisms Underlying the Cost of Living in Animals. *Annual*
837 *Review of Physiology* 62, 207–235.
- 838 Job, S., and Shand, J. (2001). Spectral sensitivity of larval and juvenile coral reef fishes: implications
839 for feeding in a variable light environment. *Mar. Ecol. Prog. Ser.* 214, 267–277.
- 840 Joshi, N., and Fass, J. (2011). Sickle: A sliding-window, adaptive, quality-based trimming tool for FastQ
841 files (Version 1.33) [Software]. Available at <https://github.com/Najoshi/Sickle>.

- 842 Kawakami, Y., Yokoi, K., Kumai, H., and Ohta, H. (2008). The role of thyroid hormones during the
843 development of eye pigmentation in the Pacific bluefin tuna (*Thunnus orientalis*). *Comparative*
844 *Biochemistry and Physiology Part B: Biochemistry and Molecular Biology* *150*, 112–116.
- 845 Kim, D., Langmead, B., and Salzberg, S.L. (2015). HISAT: a fast spliced aligner with low memory
846 requirements. *Nature Methods* *12*, 357–360.
- 847 Kulkarni, M.M. (2011). Digital Multiplexed Gene Expression Analysis Using the NanoString nCounter
848 System. *Current Protocols in Molecular Biology* *94*, 25B.10.1-25B.10.17.
- 849 Laudet, V. (2011). The Origins and Evolution of Vertebrate Metamorphosis. *Current Biology* *21*, R726–
850 R737.
- 851 Lehmann, R., Lightfoot, D.J., Schunter, C., Michell, C.T., Ohyanagi, H., Mineta, K., Foret, S., Berumen,
852 M.L., Miller, D.J., Aranda, M., et al. (2019). Finding nemo's genes: A chromosome-scale reference
853 assembly of the genome of the orange clownfish *Amphiprion percula*. *Molecular Ecology Resources*
854 *19*, 570–585.
- 855 Lowe, W.H., Martin, T.E., Skelly, D.K., and Woods, H.A. (2021). Metamorphosis in an Era of Increasing
856 Climate Variability. *Trends in Ecology & Evolution* *36*, 360–375.
- 857 Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads.
858 *EMBnet.Journal* *17*, 10.
- 859 Mazurais, D., Darias, M., Zambonino-Infante, J.L., and Cahu, C.L. (2011). Transcriptomics for
860 understanding marine fish larval development. *Canadian Journal of Zoology* *89*, 599–611.
- 861 McMenamin, S.K., and Parichy, D.M. (2013). Metamorphosis in Teleosts. In *Current Topics in*
862 *Developmental Biology*, (Elsevier), pp. 127–165.
- 863 Miao, Y., Wu, W., Dai, Y., Maneix, L., Huang, B., Warner, M., and Gustafsson, J.-Å. (2015). Liver X
864 receptor β controls thyroid hormone feedback in the brain and regulates browning of subcutaneous
865 white adipose tissue. *Proc Natl Acad Sci U S A* *112*, 14006–14011.
- 866 Mitchell, L.J., Cheney, K.L., Lührmann, M., Marshall, J., Michie, K., and Cortesi, F. (2021). Molecular
867 Evolution of Ultraviolet Visual Opsins and Spectral Tuning of Photoreceptors in Anemonefishes
868 (*Amphiprioninae*). *Genome Biology and Evolution* *13*, evab184.
- 869 Mullur, R., Liu, Y.-Y., and Brent, G.A. (2014). Thyroid hormone regulation of metabolism. *Physiol Rev* *94*,
870 355–382.
- 871 Nishimura, T. (2020). Feedforward Regulation of Glucose Metabolism by Steroid Hormones Drives a
872 Developmental Transition in *Drosophila*. *Current Biology* *30*, 3624-3632.e5.
- 873 Pinto, C.L., Kalasekar, S.M., McCollum, C.W., Riu, A., Jonsson, P., Lopez, J., Swindell, E.C., Bouhlatouf,
874 A., Balaguer, P., Bondesson, M., et al. (2016). Lxr regulates lipid metabolic and visual perception
875 pathways during zebrafish development. *Molecular and Cellular Endocrinology* *419*, 29–43.
- 876 Power, D.M., Einarsdóttir, I.E., Pittman, K., Sweeney, G.E., Hildahl, J., Campinho, M.A., Silva, N., Sæle,
877 Ø., Galay-Burgos, M., Smáradóttir, H., et al. (2008). The Molecular and Endocrine Basis of Flatfish
878 Metamorphosis. *Reviews in Fisheries Science* *16*, 95–111.
- 879 Rennison, D.J., Owens, G.L., Heckman, N., Schluter, D., and Veen, T. (2016). Rapid adaptive evolution

- 880 of colour vision in the threespine stickleback radiation. *Proc. R. Soc. B.* 283, 20160242.
- 881 Roux, N., Salis, P., Lambert, A., Logeux, V., Soulat, O., Romans, P., Frédérick, B., Lecchini, D., and
882 Laudet, V. (2019). Staging and normal table of postembryonic development of the clownfish
883 (*Amphiprion ocellaris*). *Developmental Dynamics* 248, 545–568.
- 884 Roux, N., Salis, P., Lee, S.-H., Besseau, L., and Laudet, V. (2020). Anemonefish, a model for Eco-Evo-
885 Devo. *EvoDevo* 11, 20.
- 886 Roux, N., Logeux, V., Trouillard, N., Pillot, R., Magré, K., Salis, P., Lecchini, D., Besseau, L., Laudet, V.,
887 and Romans, P. (2021). A star is born again: Methods for larval rearing of an emerging model
888 organism, the False clownfish *Amphiprion ocellaris*. *J Exp Zool (Mol Dev Evol)* jez.b.23028.
- 889 Russo, S.C., Salas-Lucia, F., and Bianco, A.C. (2021). Deiodinases and the Metabolic Code for Thyroid
890 Hormone Action. *Endocrinology* 162, bqab059.
- 891 Sachs, L.M., and Buchholz, D.R. (2017). Frogs model man: *In vivo* thyroid hormone signaling during
892 development: SACHS and BUCHHOLZ. *Genesis* 55, e23000.
- 893 Salis, P., Roux, N., Huang, D., Marcionetti, A., Mouginot, P., Reynaud, M., Salles, O., Salamin, N., Pujol,
894 B., Parichy, D.M., et al. (2021). Thyroid hormones regulate the formation and environmental plasticity
895 of white bars in clownfishes. *Proc Natl Acad Sci USA* 118, e2101634118.
- 896 Sayre, N.L., and Lechleiter, J.D. (2012). Fatty acid metabolism and thyroid hormones. *Current Trends*
897 *Endocrinology* 1, 65–76.
- 898 Seuront, L., Schmitt, F., and Lagadeuc, Y. (2001). Turbulence intermittency, small-scale phytoplankton
899 patchiness and encounter rates in plankton: where do we go from here? *Deep Sea Research Part I:*
900 *Oceanographic Research Papers* 48, 1199–1215.
- 901 Shand, J. (1997). Ontogenetic changes in retinal structure and visual acuity: a comparative study of
902 coral-reef teleosts with differing post-settlement lifestyles. *Environmental Biology of Fishes* 49, 307–
903 322.
- 904 Shand, J., Davies, W.L., Thomas, N., Balmer, L., Cowing, J.A., Pointer, M., Carvalho, L.S., Trezise, A.E.O.,
905 Collin, S.P., Beazley, L.D., et al. (2008). The influence of ontogeny and light environment on the
906 expression of visual pigment opsins in the retina of the black bream, *Acanthopagrus butcheri*. *Journal*
907 *of Experimental Biology* 211, 1495–1503.
- 908 Shao, C., Bao, B., Xie, Z., Chen, X., Li, B., Jia, X., Yao, Q., Ortí, G., Li, W., and Li, X. (2017). The genome
909 and transcriptome of Japanese flounder provide insights into flatfish asymmetry. *Nature Genetics* 49,
910 119.
- 911 Sheridan, Mark.A., and Kao, Y.-H. (1998). Regulation of Metamorphosis-Associated Changes in the
912 Lipid Metabolism of Selected Vertebrates. *Am Zool* 38, 350–368.
- 913 Sinha, R.A., Singh, B.K., and Yen, P.M. (2018). Direct effects of thyroid hormones on hepatic lipid
914 metabolism. *Nat Rev Endocrinol* 14, 259–269.
- 915 Stahlberg, A., Kubista, M., and Pfaffl, M. (2004). Comparison of Reverse Transcriptase in Gene
916 Expression Analysis. *Clinical Chemistry* 50, 1678–1679.
- 917 Suzuki, S.C., Bleckert, A., Williams, P.R., Takechi, M., Kawamura, S., and Wong, R.O.L. (2013). Cone

- 918 photoreceptor types in zebrafish are generated by symmetric terminal divisions of dedicated
919 precursors. *Proceedings of the National Academy of Sciences* *110*, 15109–15114.
- 920 Tagawa, M., and Hirano, T. (1989). Changes in tissue and blood concentrations of thyroid hormones in
921 developing chum salmon. *General and Comparative Endocrinology* *76*, 437–443.
- 922 Tata, J.R. (2006). Amphibian metamorphosis as a model for the developmental actions of thyroid
923 hormone. *Molecular and Cellular Endocrinology* *246*, 10–20.
- 924 Tocher, D.R. (2010). Fatty acid requirements in ontogeny of marine and freshwater fish. *Aquaculture*
925 *Research* *41*, 717–732.
- 926 Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B.C., Remm, M., and Rozen, S.G. (2012).
927 Primer3—new capabilities and interfaces. *Nucleic Acids Res* *40*, e115.
- 928 Wang, B., and Tontonoz, P. (2018). Liver X receptors in lipid signalling and membrane homeostasis.
929 *Nat Rev Endocrinol* *14*, 452–463.
- 930 Wang, Z., Guan, D., Wang, S., Chai, L.Y.A., Xu, S., and Lam, K.-P. (2020). Glycolysis and Oxidative
931 Phosphorylation Play Critical Roles in Natural Killer Cell Receptor-Mediated Natural Killer Cell
932 Functions. *Frontiers in Immunology* *11*.
- 933 Xie, D., Chen, C., Dong, Y., You, C., Wang, S., Monroig, Ó., Tocher, D.R., and Li, Y. (2021). Regulation of
934 long-chain polyunsaturated fatty acid biosynthesis in teleost fish. *Progress in Lipid Research* *82*,
935 101095.
- 936 Zhang, Q., You, C., Liu, F., Zhu, W., Wang, S., Xie, D., Monroig, Ó., Tocher, D.R., and Li, Y. (2016).
937 Cloning and Characterization of Lxr and Srebp1, and Their Potential Roles in Regulation of LC-PUFA
938 Biosynthesis in Rabbitfish *Siganus canaliculatus*. *Lipids* *51*, 1051–1063.

939

940 **Figure legends**

941 **Figure 1: Three distinct periods during clownfish post-embryonic development.** (A) Principal
942 component analysis (PCA) according to the 1000 genes with the highest variance showing three
943 major groups: early developmental stages, stage 4, and late developmental stages. (B) Heatmap of
944 the 100 genes with the highest variance. Colors represent the intensity of the centered (but
945 unscaled) signal that, for each gene, ranges from low (blue), to medium (white) and to high (red). (C)
946 PCA of the morphological transformation according to body depth (BD), head length (HL) and Snout
947 vent length (SVL) over Standard length (SL, in red). (D) Variation in TH levels during the seven post
948 embryonic stages of *A. ocellaris*. (E) Number of genes differentially expressed between contiguous
949 developmental stages.

950

951 **Figure 2: TH pathway is active during metamorphosis.** Expression levels of TH signaling pathway
952 genes extracted from the transcriptomic data, depicted in front of each step of the pathway. TRH:

953 thyroid releasing hormone, TSH: thyroid stimulating hormone, DUOX: dual oxidase, TG: thyroglobulin,
954 TPO: thyroperoxidase, NIS: sodium iodine symporter, DIO: deiodinase, TR: thyroid hormone receptor.

955

956 **Figure 3: TH control visual perception shift by regulating opsin gene expression.** (A) Heat map of the
957 eight opsin genes involved in visual perception organized from top to bottom according to short
958 (*opnsw2B*, *opnsw1α*, *opnsw1β*), medium (*rh2B*, *rh2A-1*, *rh2A-2*, *rh1*) and long (*opnlw*) wavelength
959 sensitivity. (B) Behavioral test for color preference. Mean percentage of time spent by stages 2
960 (n=20) and 5 (n=20) larvae in each compartment of a dual choice chamber. Statistical differences
961 (indicated by a star (*)) between each stage in each compartment were calculated by a chi² test. (C)
962 *In situ* hybridization of sectioned stage 2, 4 and 6 larval retina using probes for *opnsw2b* and *opnlw*.
963 Black arrowhead indicates expression signals in the photoreceptors (external nuclear layer). (D)
964 Regulation of *opnsw2B* and *opnlw* expression after 48 hours of treatment at 10⁻⁸, 10⁻⁷, 10⁻⁶ M T3 +
965 IOP (n=3 pools of 2 larvae per condition). Expression levels are expressed in fold change and
966 statistical differences between treatment and DMSO control are indicated by a star (*). (E) T3 affect
967 the color preference. Effects of T3 at 10⁻⁶M (n=19) compared to DMSO control (n=14) after 48 hours
968 treatment on the mean percentage of time spent by larvae in each compartment. Statistical
969 differences (indicated by a star (*)) between each condition in each compartment were calculated by
970 a chi² test.

971 **Figure 4: TH induce a major metabolic shift during metamorphosis.** (A) Expression levels of genes
972 involved in glycolysis, lactic fermentation, fatty acid β oxidation, and citric acid cycle at each
973 developmental stage (S1: stage 1 ; S2: stage 2 ; S3: stage 3 ; S4: stage 4 ; S5: Stage 5 ; S6: stage 6 and
974 S7: stage 7) extracted from transcriptomic data clearly revealing a change of expression at stage 4
975 (indicated on each graph by a green rectangle) for each pathway. (B) Effects of T3 (10⁻⁸, 10⁻⁷, 10⁻⁶ M,
976 n=3 pools of 2 larvae per condition) after 12-, 24-, 48- and 72-hours post treatment (hpt) on the
977 expression levels of the representative genes for glycolysis (*pkmb*, *pfkma* and *pfkmb*), β oxidation
978 (*acads*), citric acid cycle (*idh3a*), lactic fermentation (*ldhba*, *ldhbb*).

979 **Figure 5: TH action on lipid metabolism regulation during *A. ocellaris* metamorphosis.** (A)
980 expression levels of genes involved in *de novo* lipogenesis and lipid desaturation and elongation at
981 various stages (S1: stage 1; S2: stage 2; S3: stage 3; S4: stage 4; S5: Stage 5; S6: stage 6 and S7: stage
982 7). (B) Fatty acid content of *A. ocellaris* at stage 3, 4 5 and 6 illustrated with an heat map (left) and
983 box plots (right). Significant differences between each fatty acid class are indicated by different
984 letters. (Sat: saturated, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids,
985 HUFA: highly unsaturated fatty acids). (C) Effects of T3 (10⁻⁸, 10⁻⁷, 10⁻⁶ M, n=3 pool of 2 larvae per
986 condition) after 12, 24, 48 and 72 hours post treatment (hpt) on the expression levels of

987 representative genes for fatty acid synthesis (*fasn*) desaturation (*fads2*), elongation (*elovl5*) and for
988 the transcription factors involved in lipid metabolism regulation (*pparg*, *srebp1*, *chreb*, *lxr*).

989 **Figure 6: Link between metabolic changes and metamorphosis progression revealed by LXR**
990 **antagonist treatment.** (A) Effect of LXR antagonist at 10^{-7} M after 5 days of treatment on the
991 expression levels of genes involved in TH signaling (*trb*, *dio2*, *dio3a*), glycolysis (*pfkma*, *pkmb*), citric
992 acid cycle (TCA) (*idh3a*), lipid metabolism (*lxr*, *acads*, *srebp1*, *fads2*, *elovl5*) and vision (*guca1a*,
993 *opnsw2B* and *opnlw*). Significant differences are indicated by a star (*). (B) Pictures of *A. ocellaris*
994 larvae after 5 days of treatment (dpt) with LXR antagonist (SR9243) at 10^{-7} M (bottom) compared to
995 DMSO control (top). Grey and blue arrowhead indicates white bar appearance in controls and LXR
996 antagonist treated fish, respectively. (C) Quantitative analysis showing the percentage of individuals
997 presenting white bar on the head and trunk (n=6 per condition). Significant differences are indicated
998 by a star (*).

999 **Figure 7: Natural variations in TH levels reveal metabolic and visual effect.** (A) Thyroid hormone
1000 level (T3) of new recruits sampled in the sea anemone *Heteractis magnifica* (blue, n=5) versus
1001 *Stichodactyla gigantea* (orange, n=5) (from Salis et al., 2021). (B-C) Principal component analysis of
1002 genes involved in vision and metabolism showing the separation between *A. percula* recruits
1003 sampled in *H. magnifica* vs *S. gigantea*. (D-E-F) Expression levels of genes involved in vision (D) and
1004 metabolism (E and F), obtained after RNA sequencing of *A. percula* recruit sampled in both sea
1005 anemone species, n=3 per condition.

1006

1007 **Supplementary Figure legends**

1008 **Supplementary Figure 1:** (A) Principal component analysis generated using TH signaling pathway
1009 gene expression (obtained by RNA sequencing, n=3 per stage) showing a clear separation before and
1010 after metamorphosis. (B) Table summarizing the TH signaling genes significantly differentially
1011 expressed between each stage.

1012 **Supplementary Figure 2:** Volcano plots showing the number of gene significantly differentially
1013 expressed between each contiguous developmental stage (S1: stage 1; S2: stage 2; S3: stage 3; S4:
1014 stage 4; S5: Stage 5; S6: stage 6 and S7: stage 7).

1015

1016 **Supplementary Figure 3:** Graphics showing the effect of thyroid hormone (T3 at 10^{-8} , 10^{-7} , 10^{-6} M)
1017 after 12, 24, 48 and 72hours of treatment on the expression levels (fold change) for genes involved in

1018 (A) thyroid hormone receptors (*TR α* , *TR β*), (B) genes involved in TH metabolism (*dio1*, *dio2*, *dio3a*,
1019 *dio3b*) and (C) TH synthesis (*tg*, *tpo*, *sis*),

1020 **Supplementary Figure 4:** (A) Graphics showing the expression levels of *opnlw1*, *opnsw1*, *opnsw2B*,
1021 *Rh1*, *Rh2A* and *Rh2B* when larvae were treated for 48 hours to increasing concentrations of T3 (10^{-8} ,
1022 10^{-7} , 10^{-6} M). Stars (*) Indicate the significant differences between treatment and control DMSO. (B)
1023 Graphic showing the percentage of time spent by five 30 days post hatching (dph) clownfish juveniles
1024 in the dual choice chamber when given the choice between orange and blue colors.

1025
1026 **Supplementary Figure 5:** Figure showing the expression levels of all the genes encoding for the
1027 enzyme involved in glycolysis. Each color indicates the organs in which these genes are known to be
1028 preferentially expressed in zebrafish. (S1: stage 1; S2: stage 2; S3: stage 3; S4: stage 4; S5: Stage 5; S6:
1029 stage 6 and S7: stage 7).

1030
1031
1032 **Supplementary Figure 6:** Figure showing the expression levels of all the genes encoding for the
1033 enzyme involved in citric acid cycle during *A. ocellaris* development (S1: stage 1; S2: stage 2; S3: stage
1034 3; S4: stage 4; S5: Stage 5; S6: stage 6 and S7: stage 7).

1035
1036 **Supplementary Figure 7:** (A) the expression levels (obtained by RNA sequencing) during larval
1037 development of genes encoding for enzymes of the pyruvate dehydrogenase complex (catalyzing the
1038 transformation of pyruvate in acetyl coA).

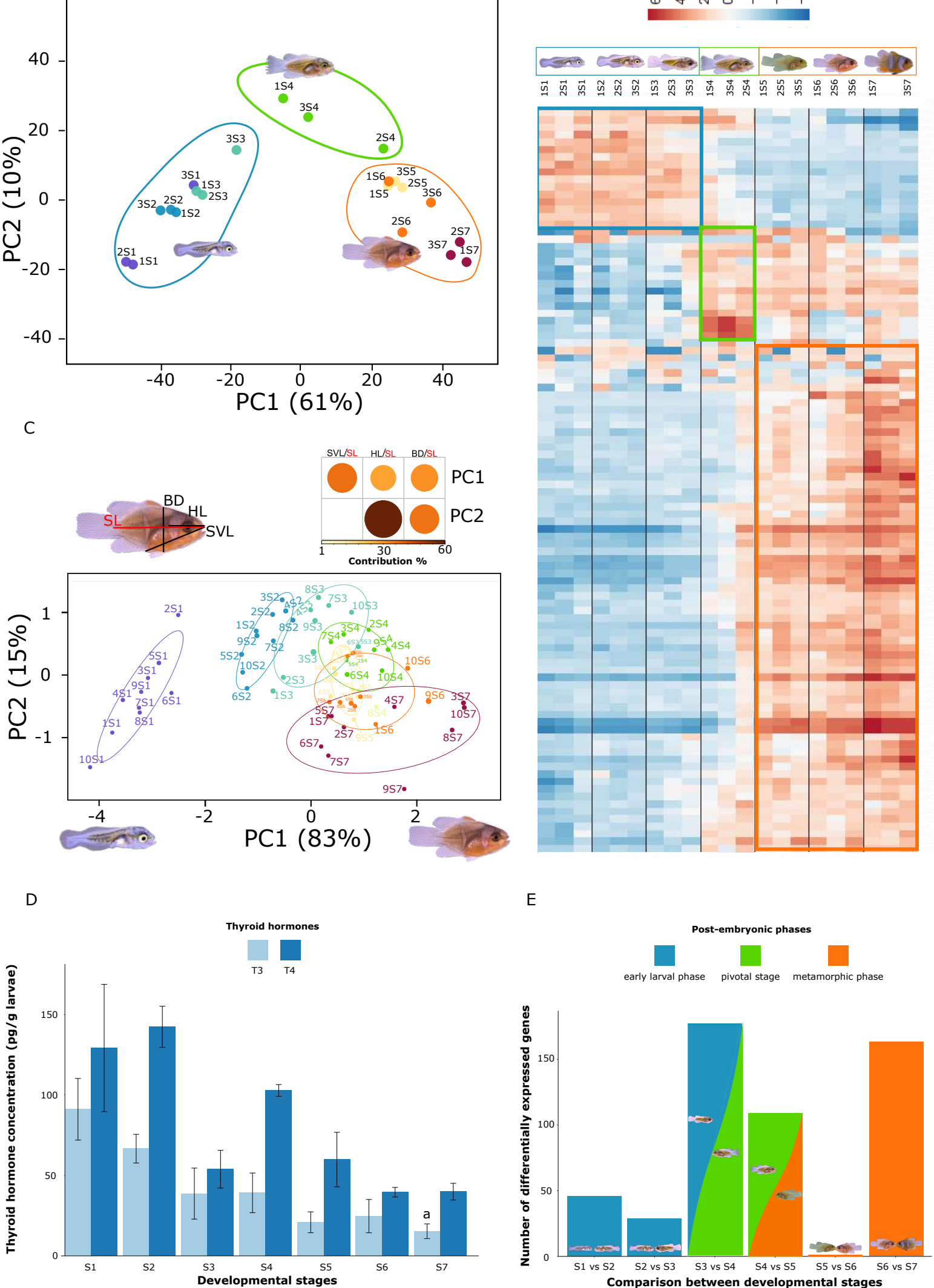
1039
1040 **Supplementary Figure 8:** Fatty acid concentration measured at stage 3, 4, 5 and 6 (n=3 per condition).
1041 Fatty acid with concentration significantly different between stages are highlighted in yellow and
1042 different letters indicate significant differences between stages.

1043
1044 **Supplementary Figure 9:** Complex fatty acid concentration whose differences are significantly
1045 different (*) between DMSO control larvae and T3 treated larvae (10^{-8} , 10^{-7} M, n=3 per condition).

1046
1047 **Supplementary Figure 10:** Antagonist Activities of zebrafish nuclear receptors (PXR, PPAR γ , LXR)
1048 against SR9243 confirming that SR9243 is a specific LXR antagonist.

1049
1050 **Supplementary Table 1:** Primers sequence for RT-qPCR analysis of the LXR antagonist experiment.

1051



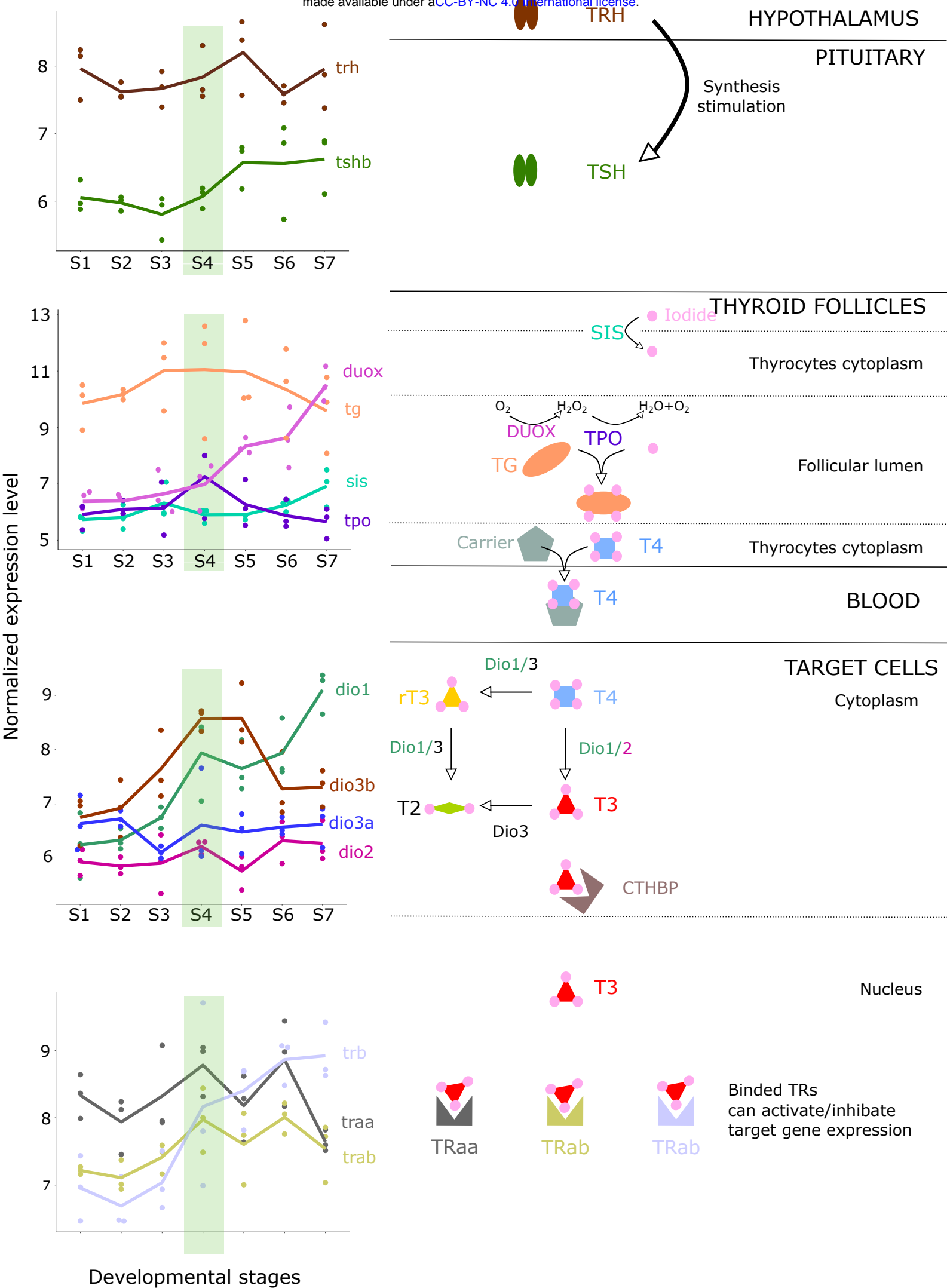
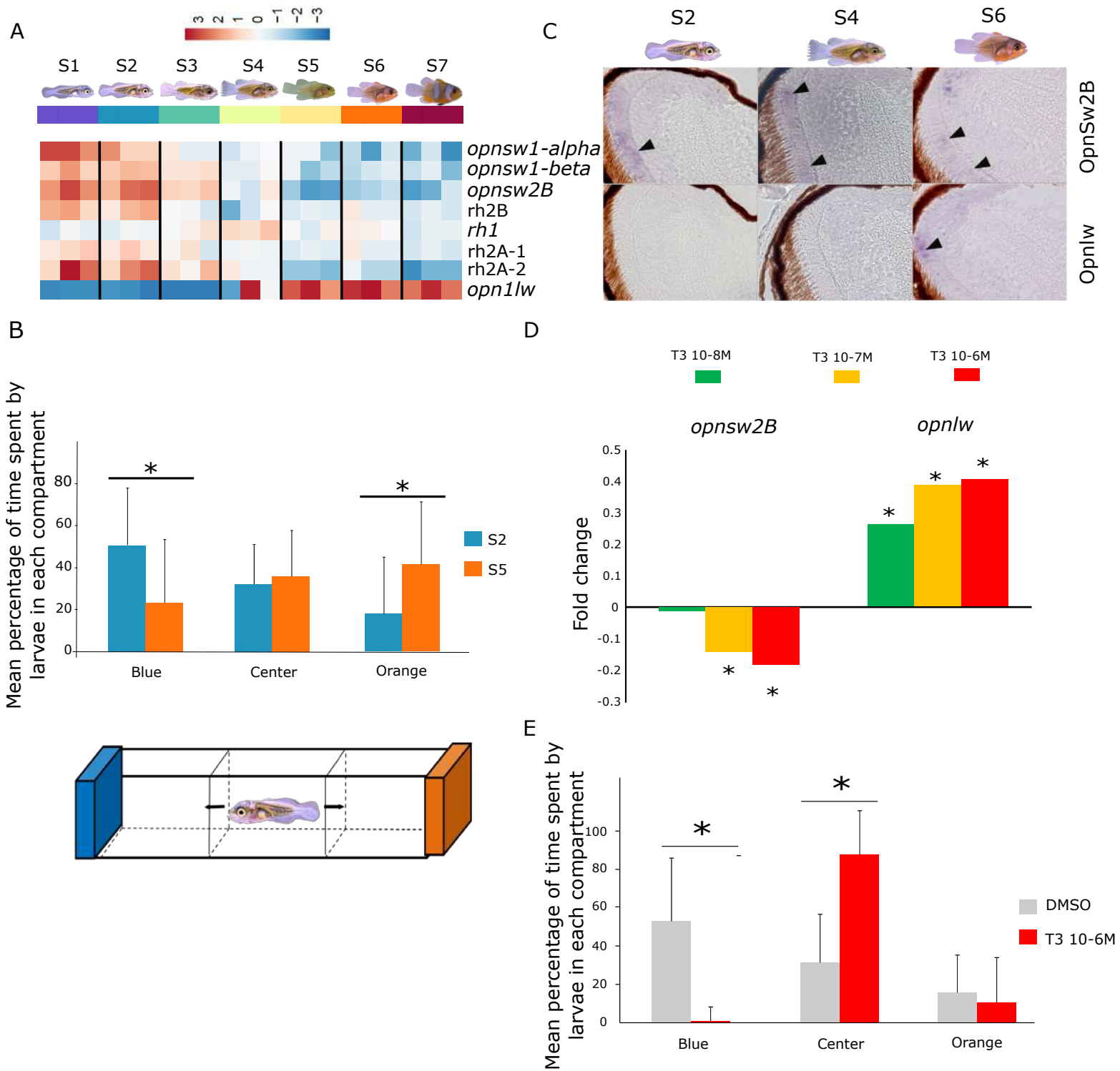
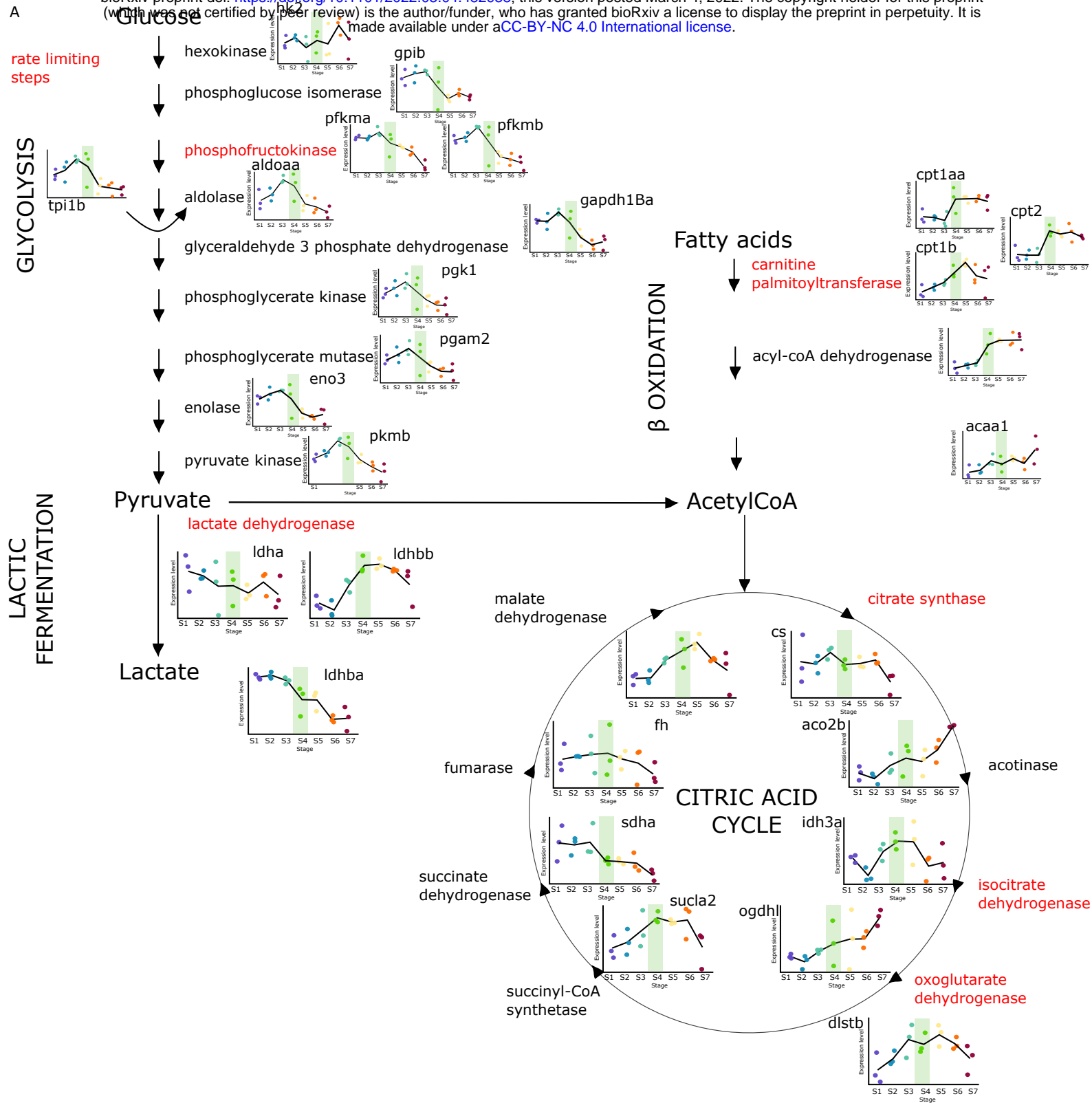
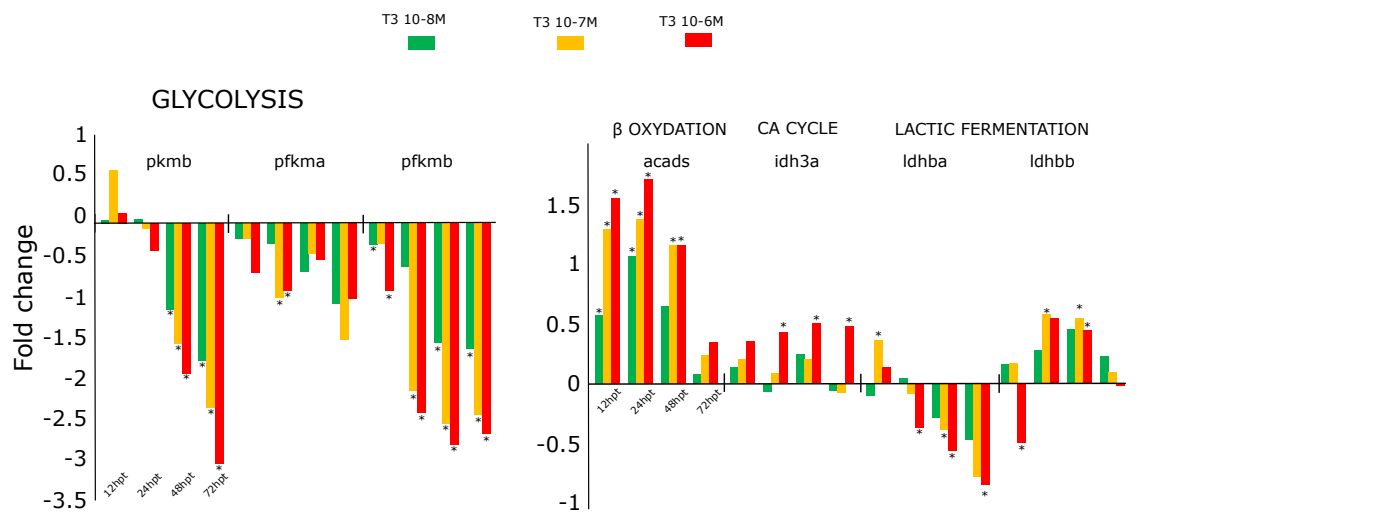


Figure 3, Roux et al.



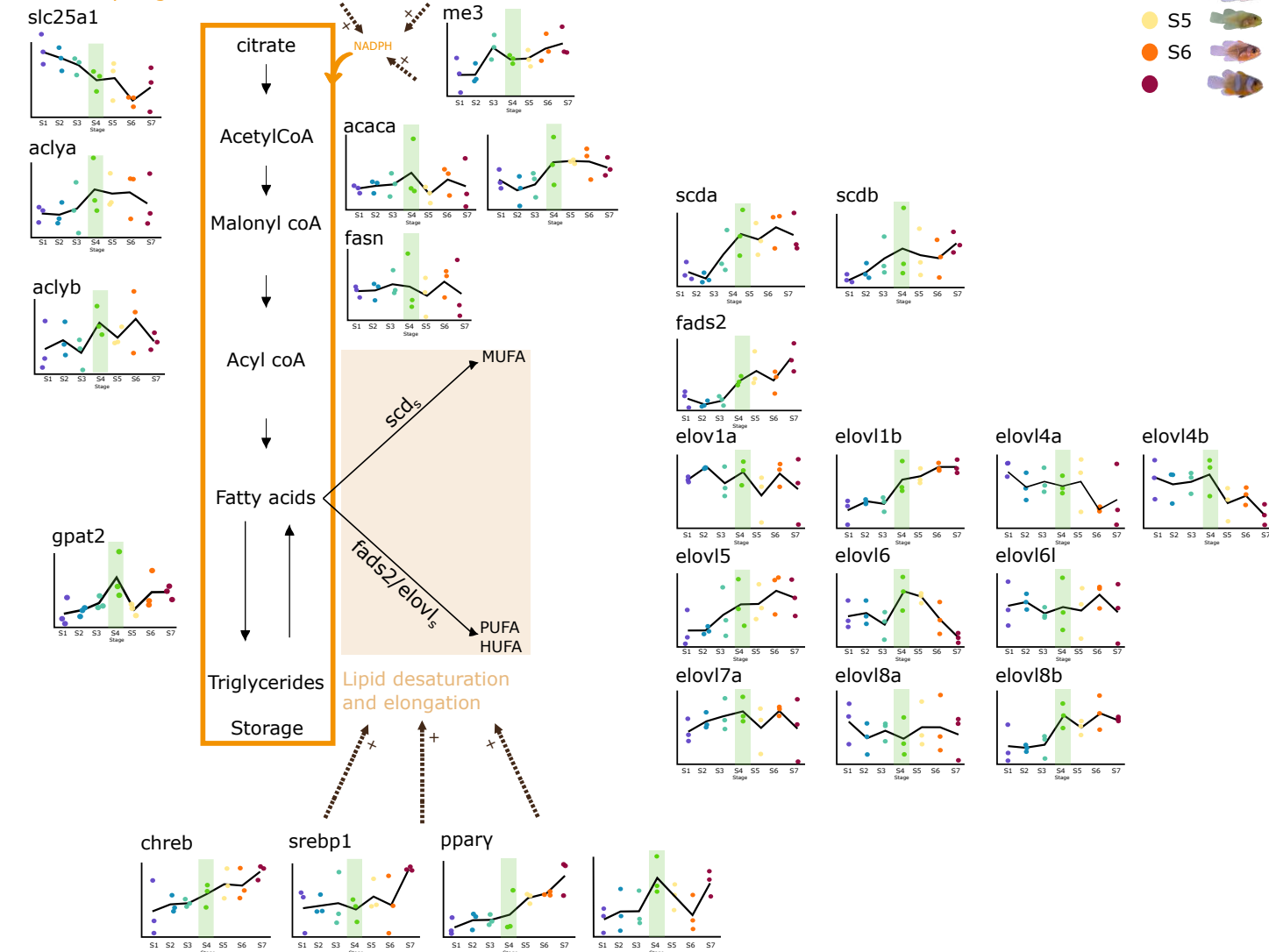


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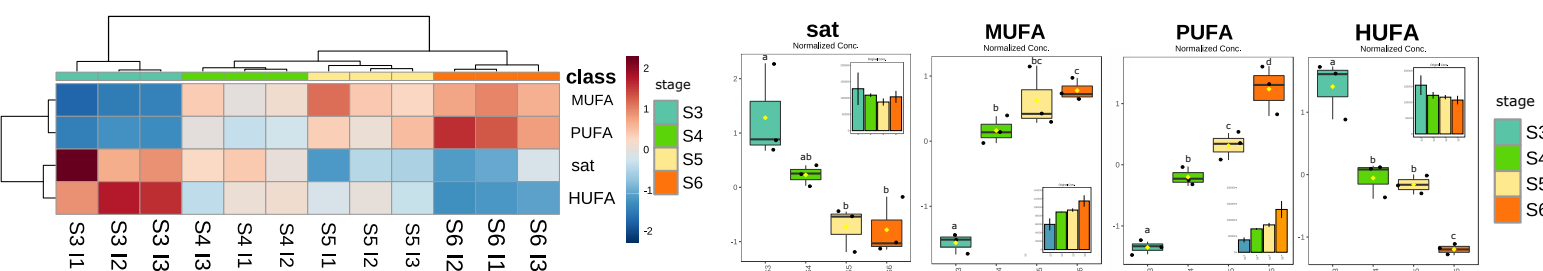


A

De novo lipidogenesis



B



C

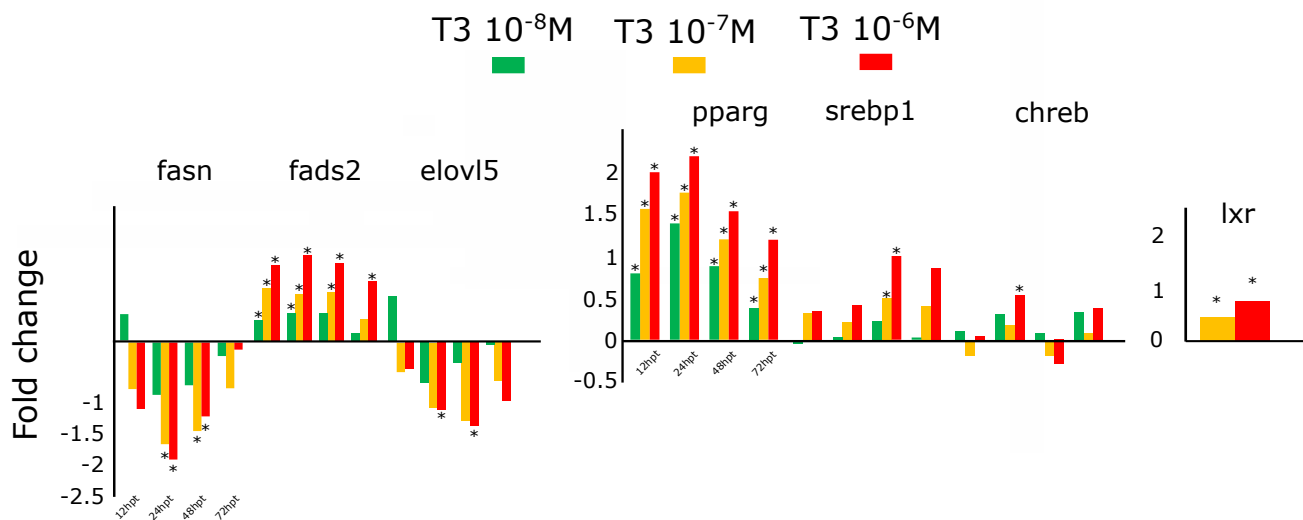
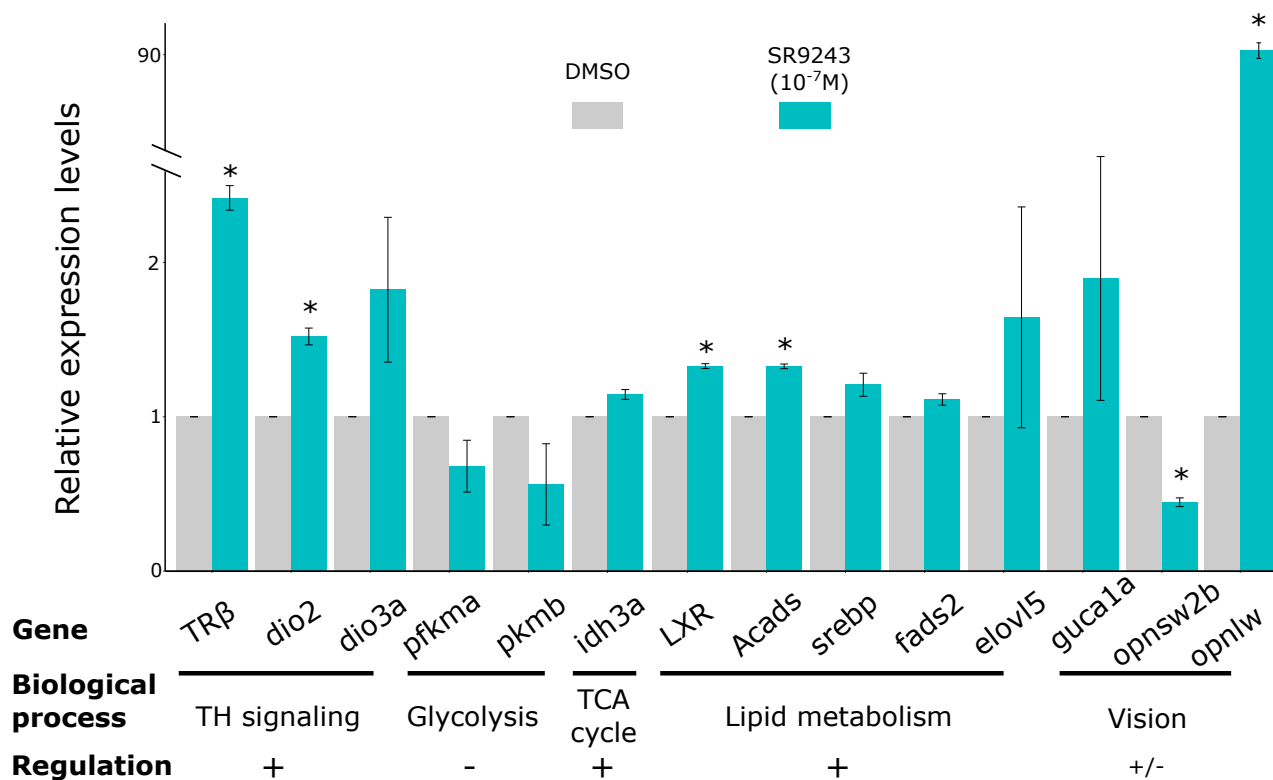
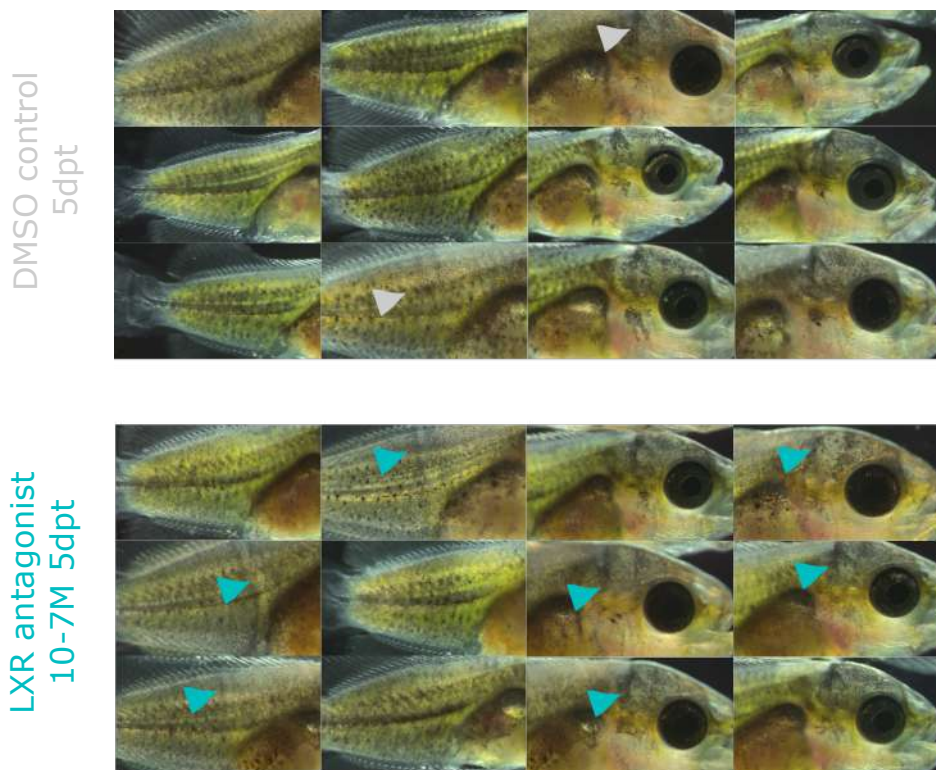


Figure 6, Roux et al.

A



B



C

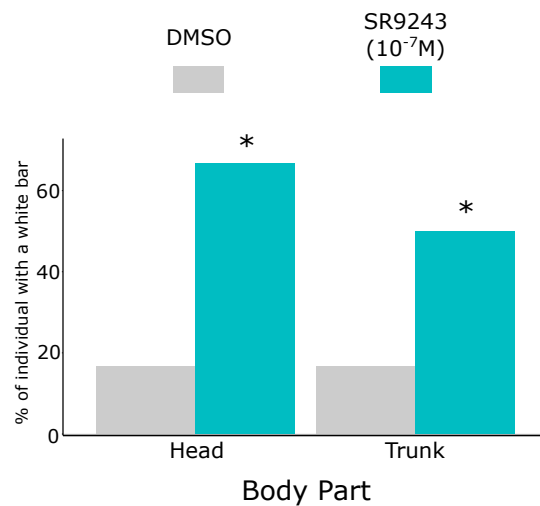
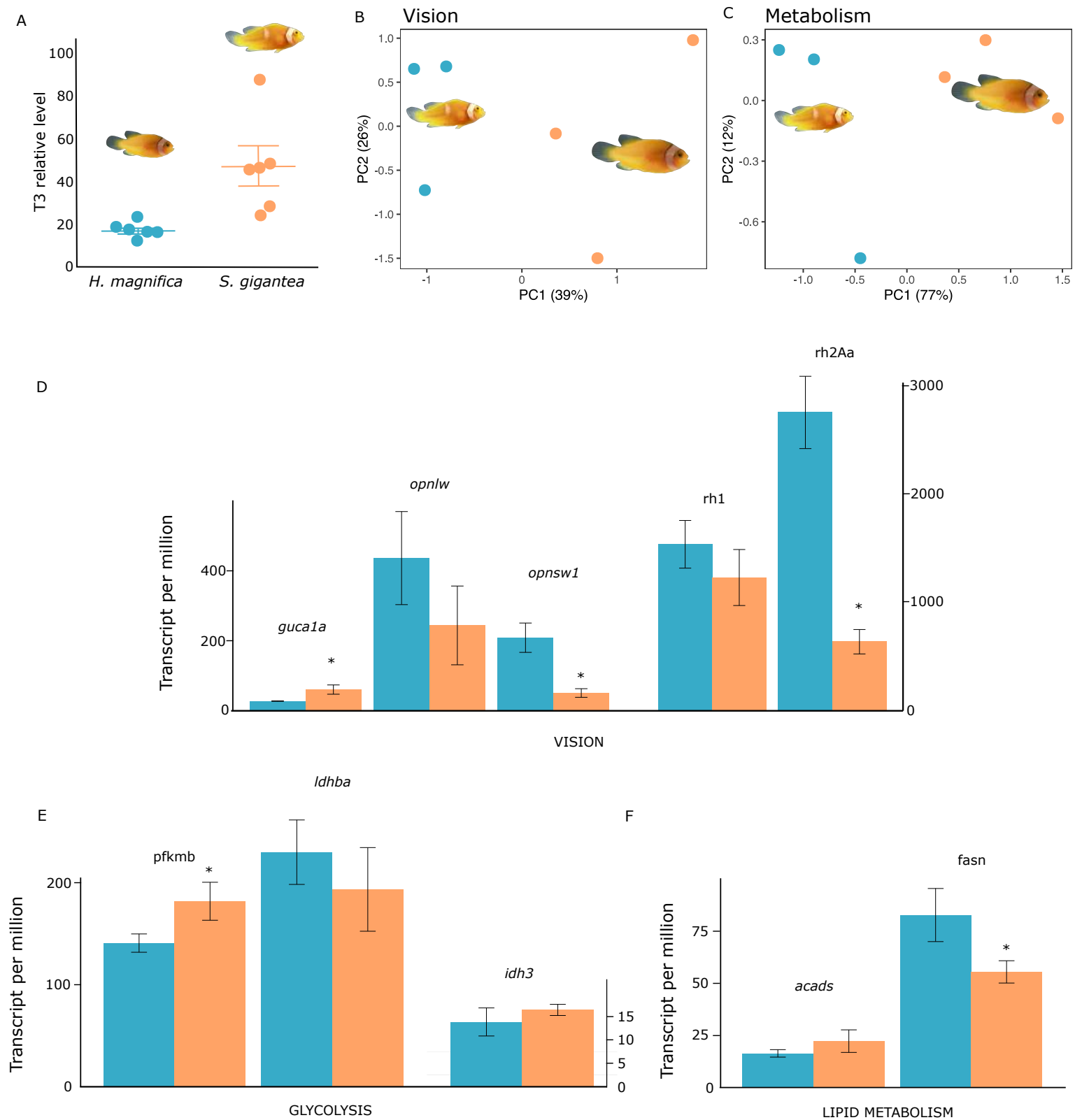
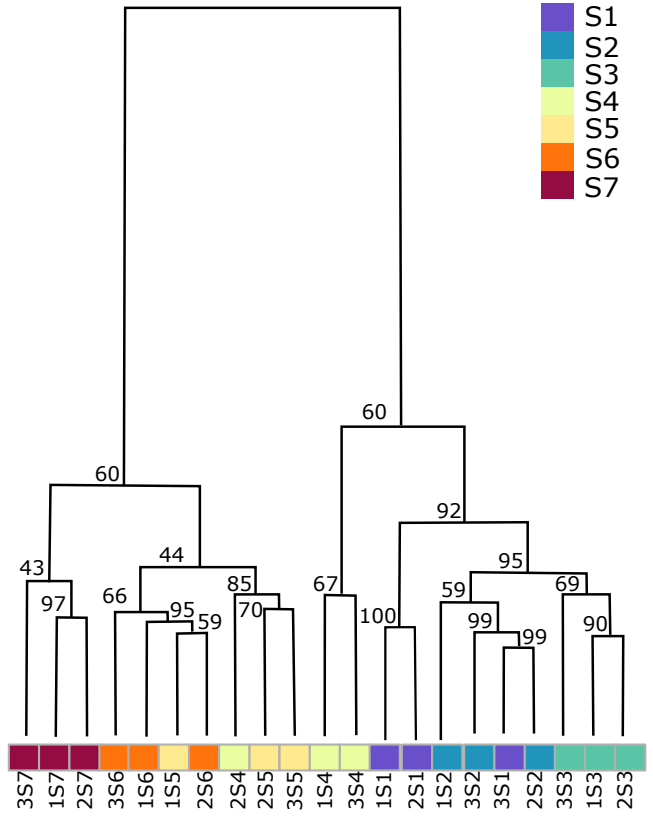


Figure 7, Roux et al.



Supp figure 1 Roux et al.

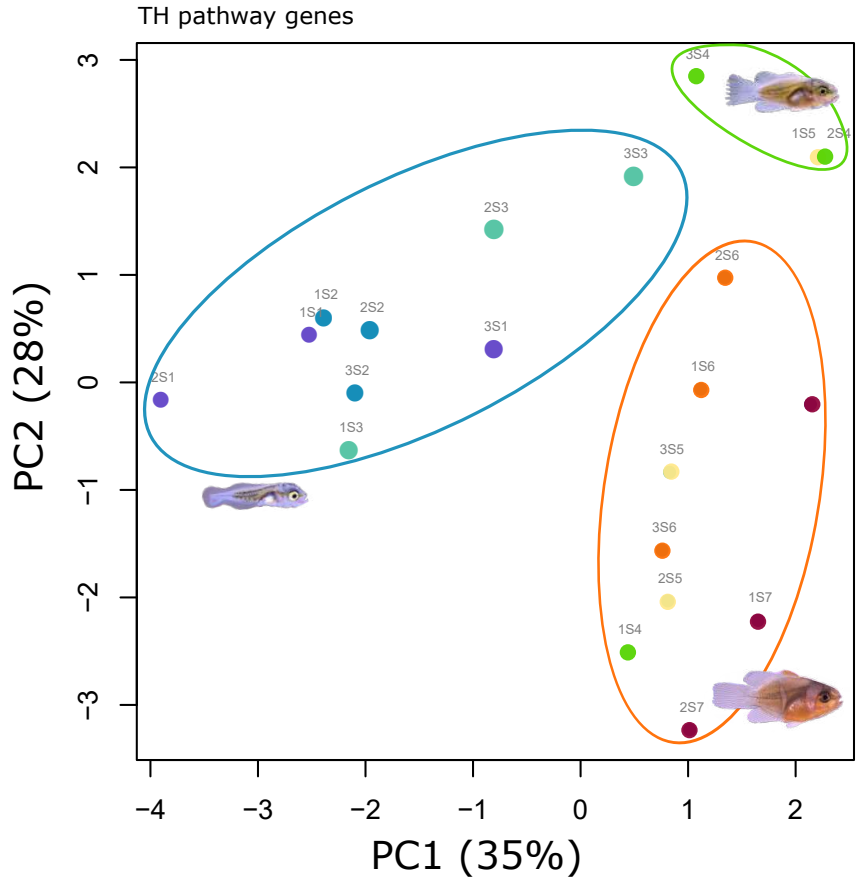
A

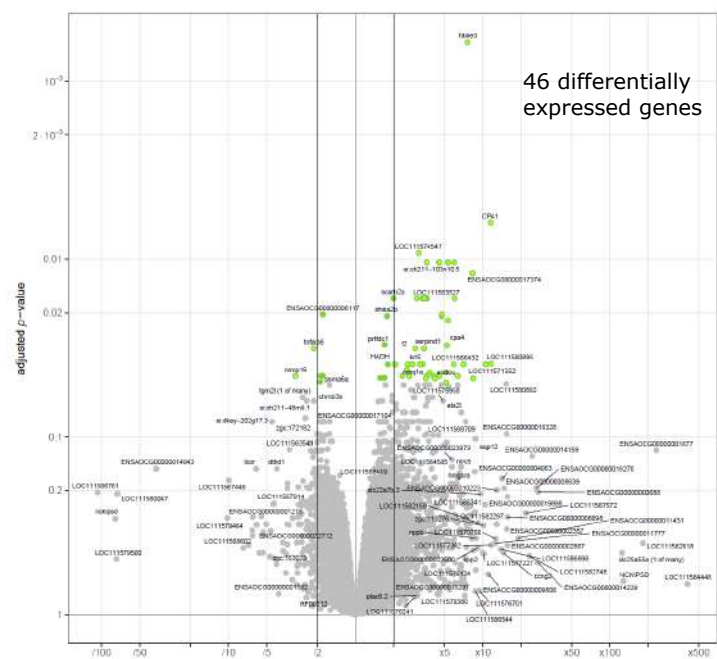


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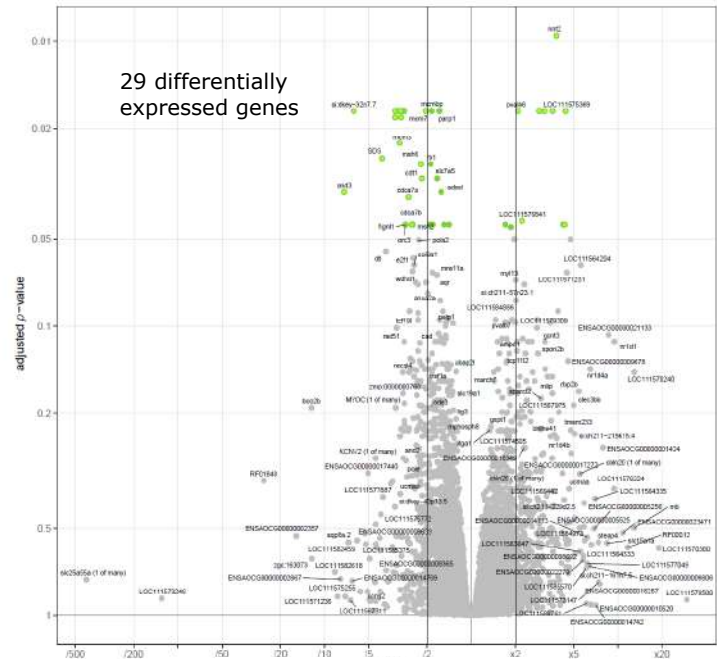
Gene	S2 vs S1	S3 vs S1	S4 vs S1	S5 vs S1	S6 vs S1	S7 vs S1	S3 vs S2	S4 vs S2	S5 vs S2	S6 vs S2	S7 vs S2	S4 vs S3	S5 vs S3	S6 vs S3	S7 vs S3	S5 vs S4	S6 vs S4	S7 vs S4	S6 vs S5	S7 vs S5	S7 vs S6
<i>trh</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>tshb</i>	-	-	-	-	-	-	-	-	-	-	*	-	-	-	-	-	-	-	-	-	-
<i>sis</i>	-	-	-	-	-	*	-	-	-	-	*	-	-	-	-	-	-	-	-	-	-
<i>duox</i>	-	-	-	*	**	-	-	-	*	*	***	-	-	*	***	-	-	**	-	*	*
<i>tg</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>tpo</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>dio1</i>	-	-	*	**	**	-	-	-	*	*	-	-	-	-	***	-	-	-	-	-	-
<i>dio2</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>dio3a</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>dio3b</i>	-	-	-	*	-	-	-	-	*	-	-	-	-	-	-	-	-	-	-	-	-
<i>Trax</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<i>Trab</i>	-	-	-	-	**	**	-	-	-	*	-	-	-	-	-	-	-	-	-	-	-
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C

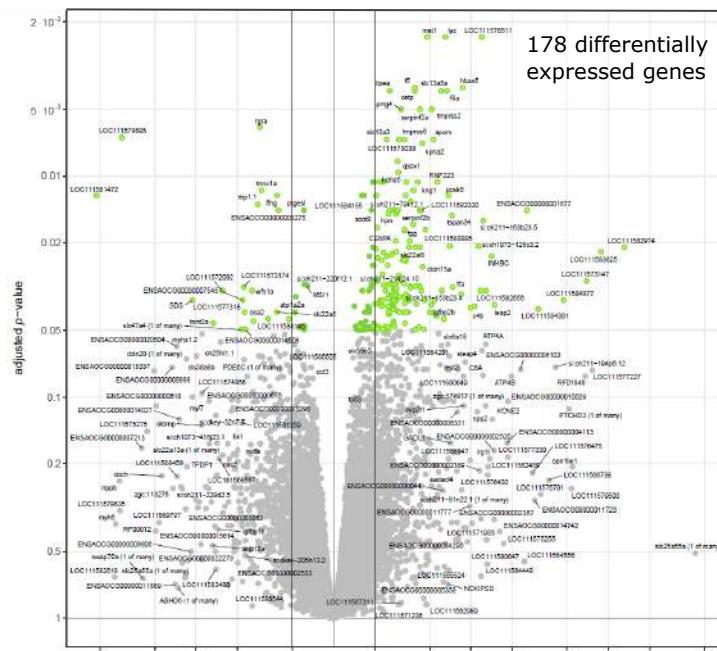




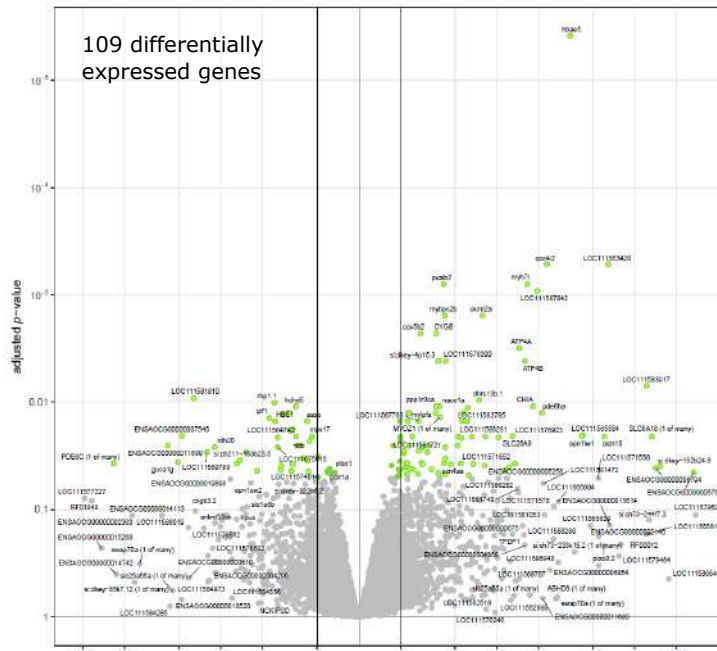
S3 vs S4



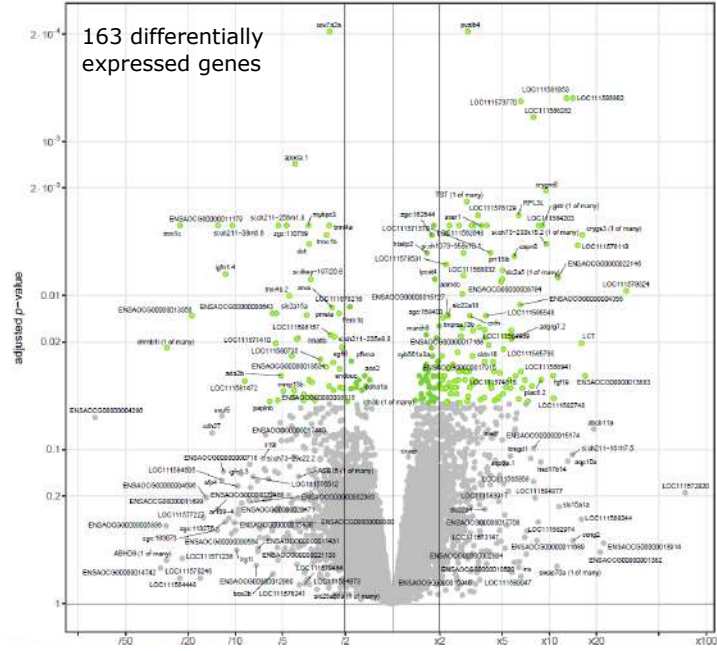
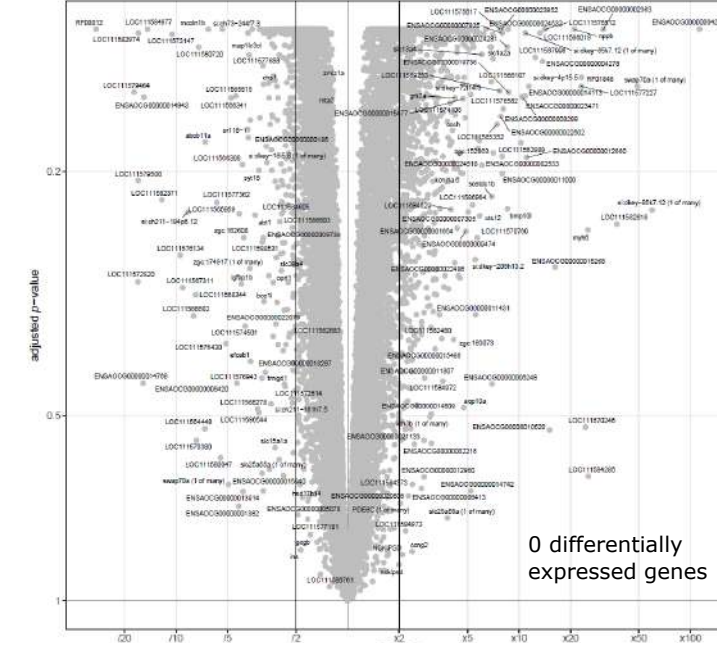
S4 vs S5

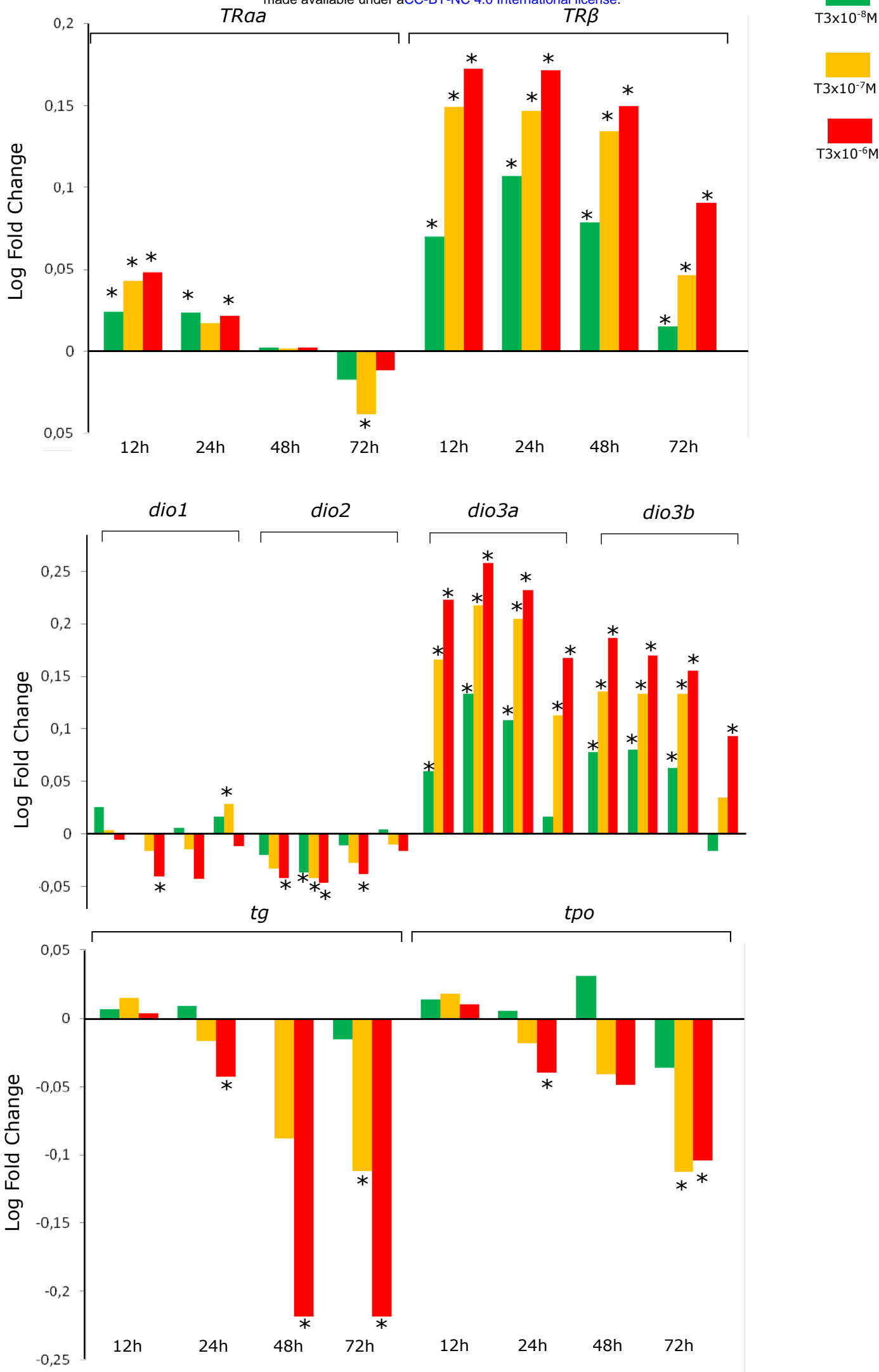


S5 vs S6



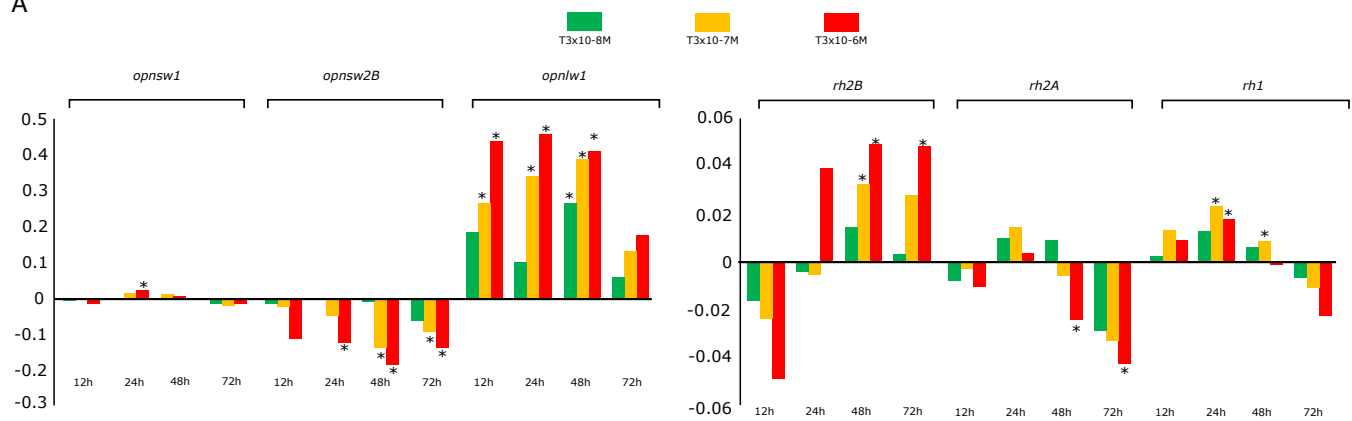
S6 vs S7



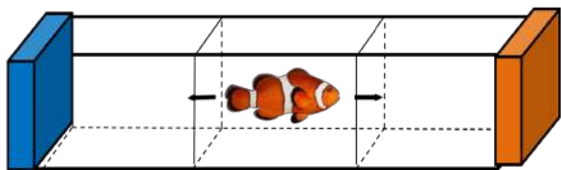
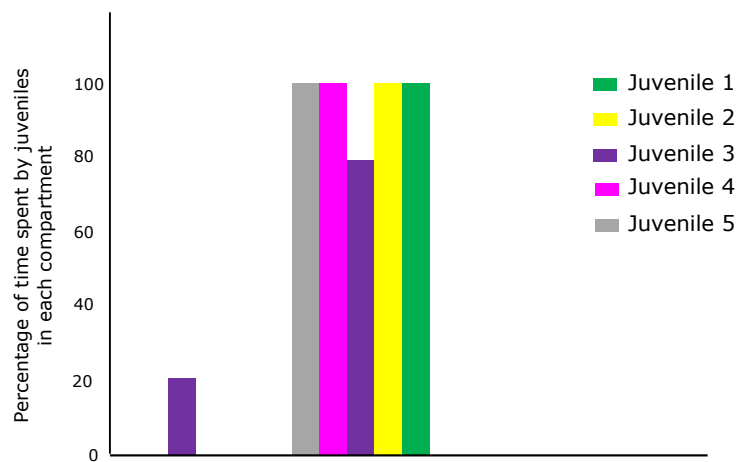


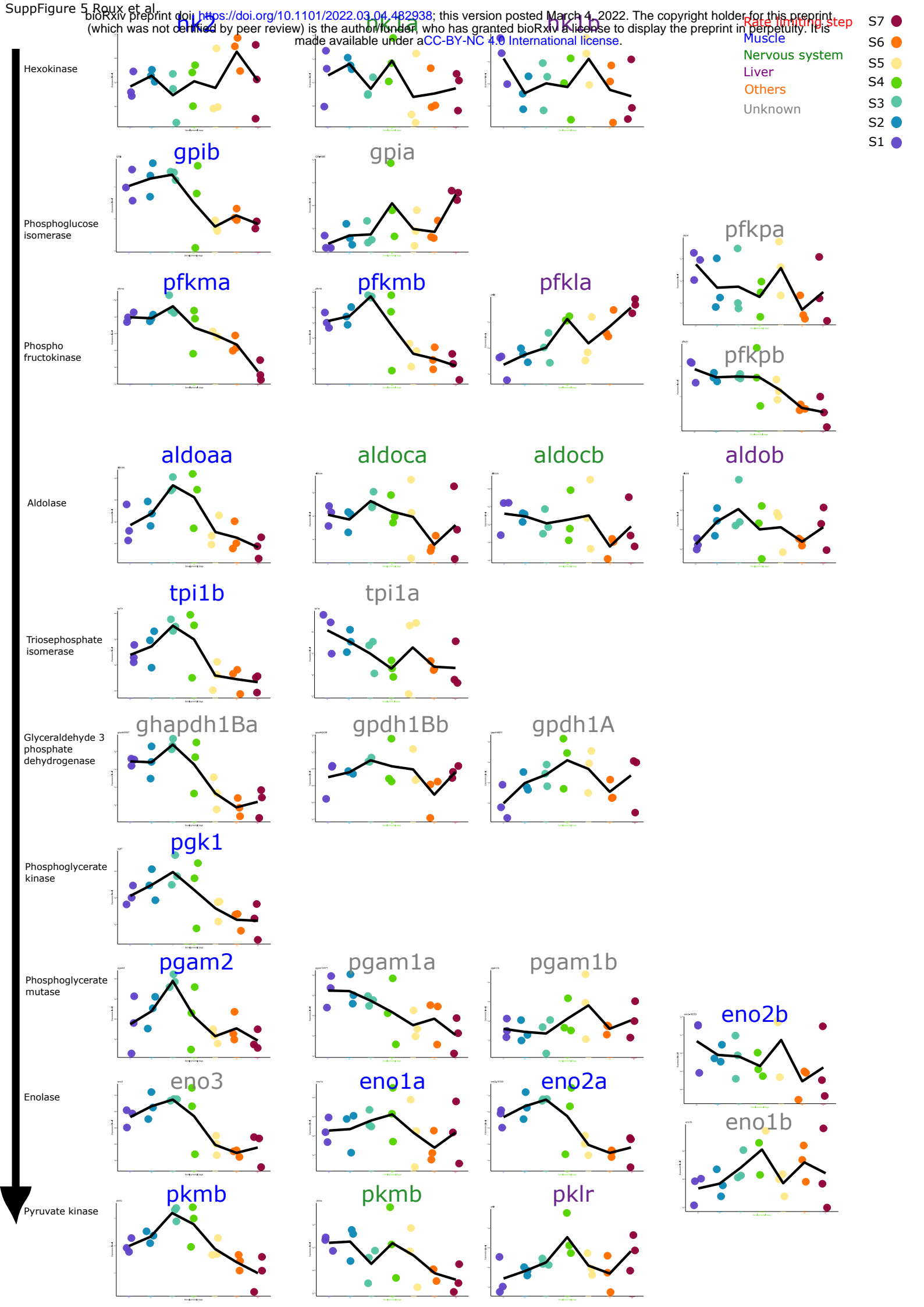
SuppFigure 4 Roux et al.

A

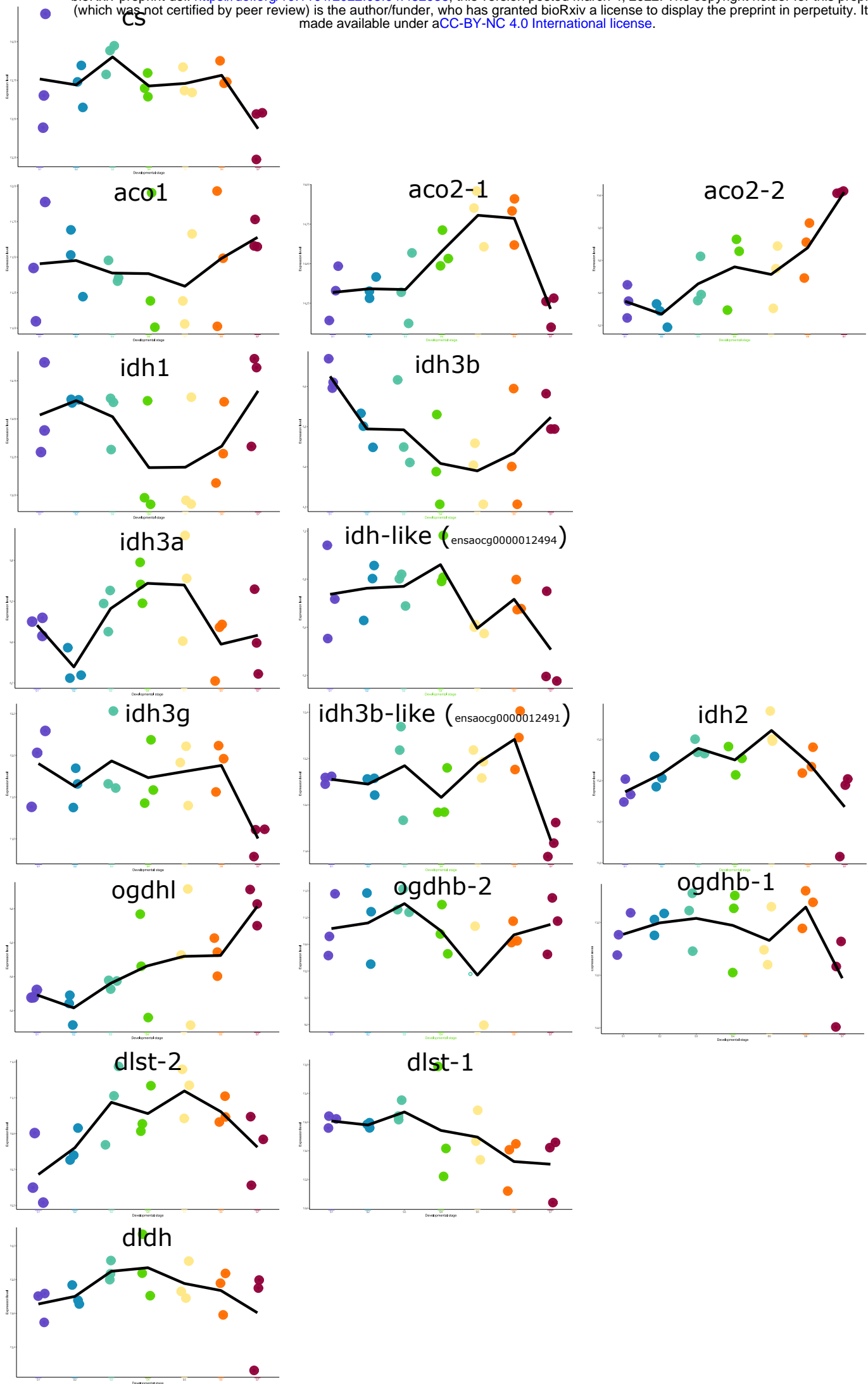


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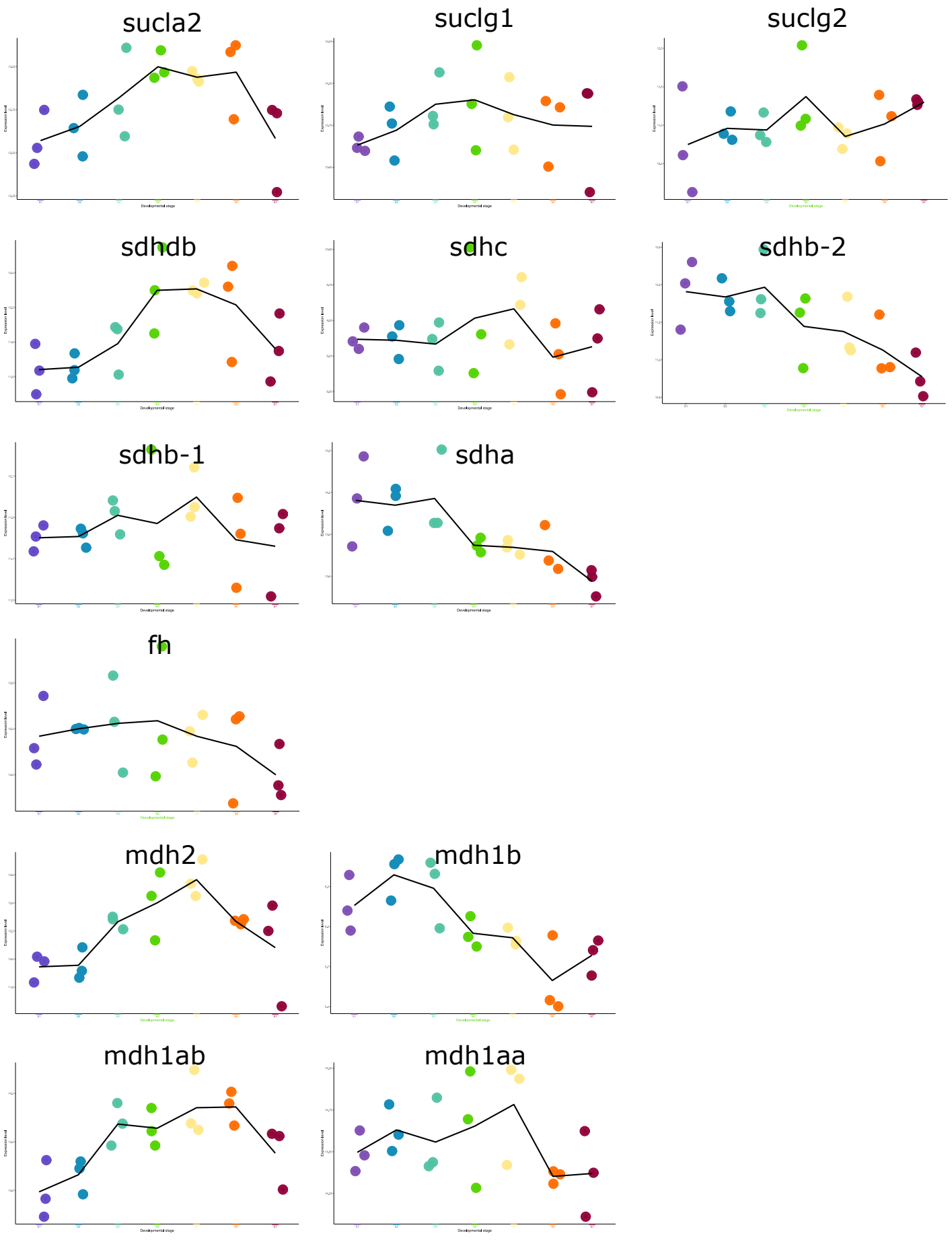




- S1
- S2
- S3
- S4
- S5
- S6
- S7



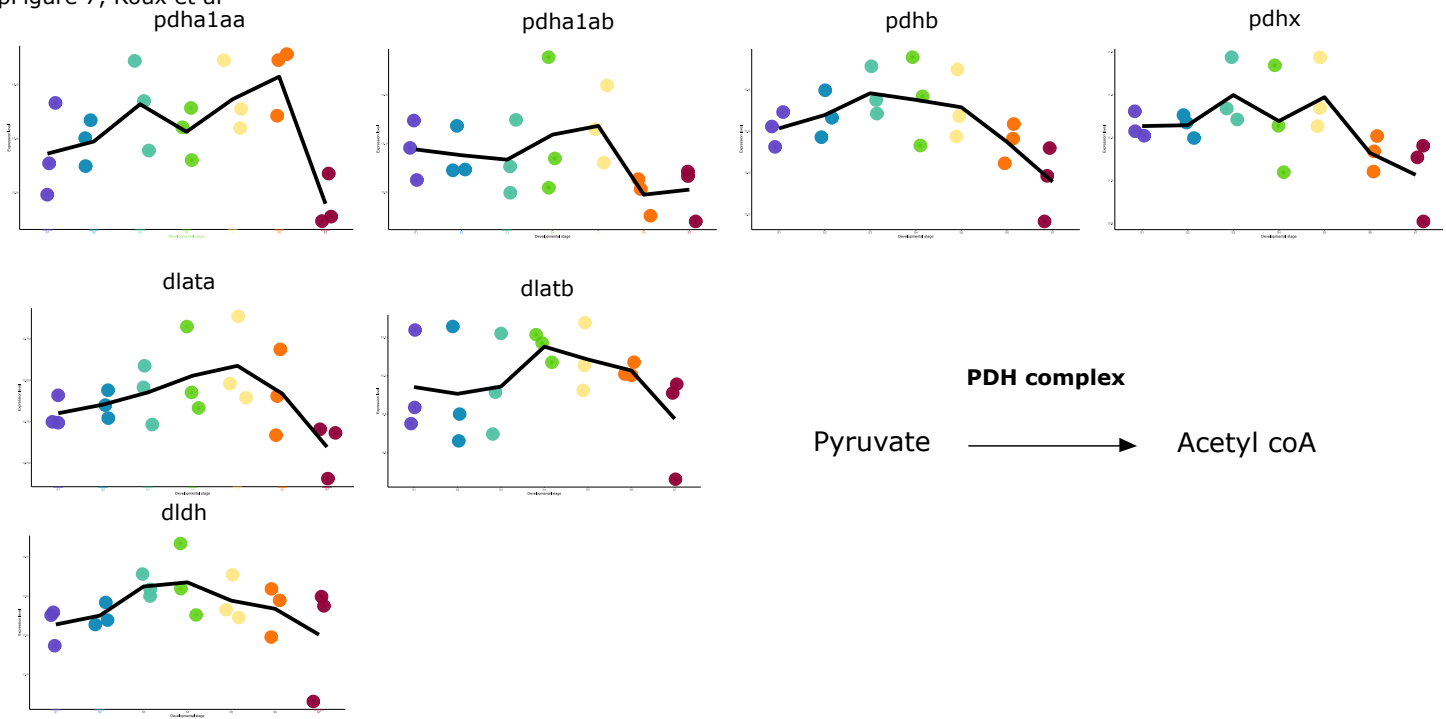
- S7
- S6
- S5
- S4
- S3
- S2
- S1



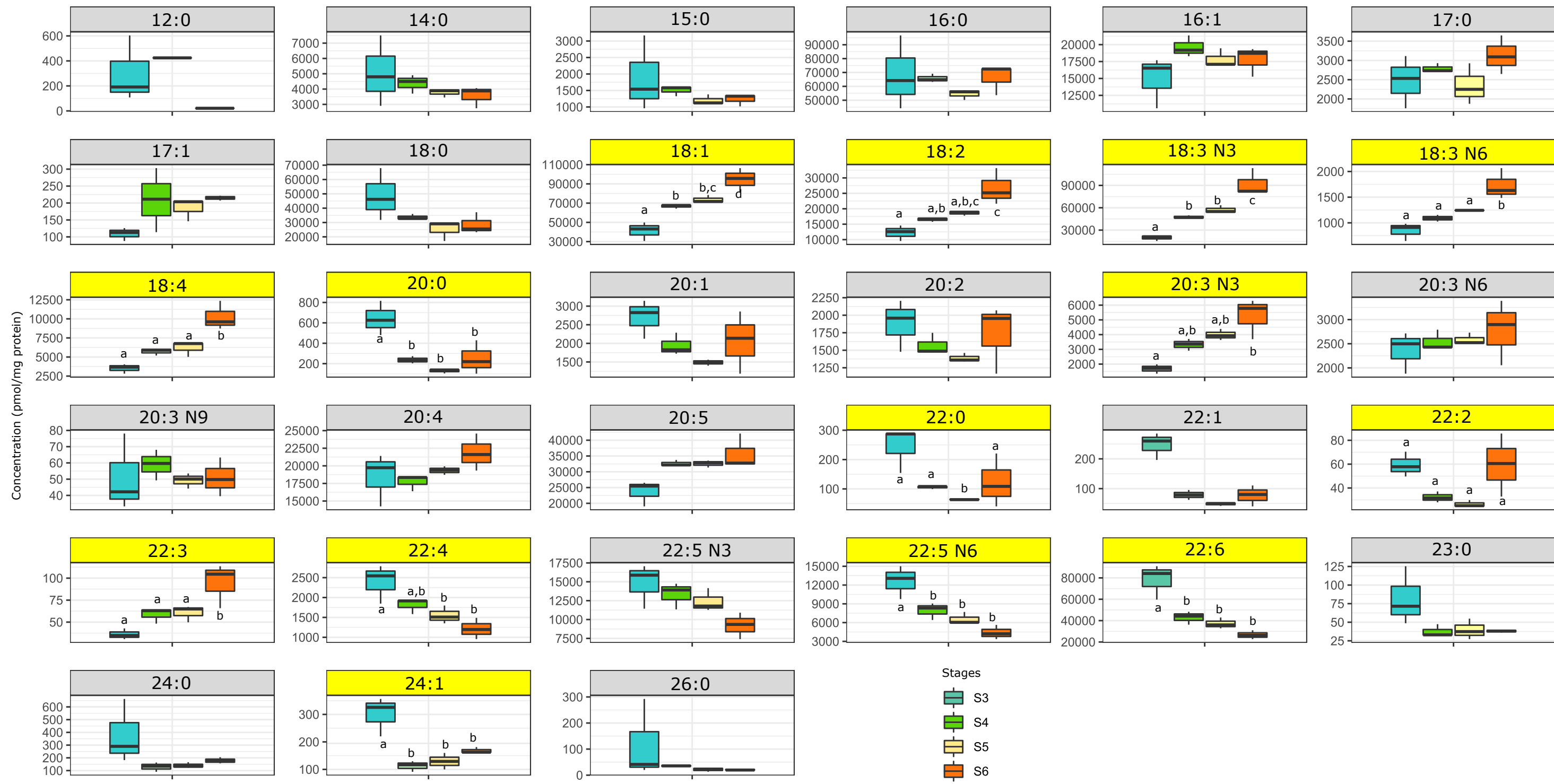
SuppFigure 7, Roux et al

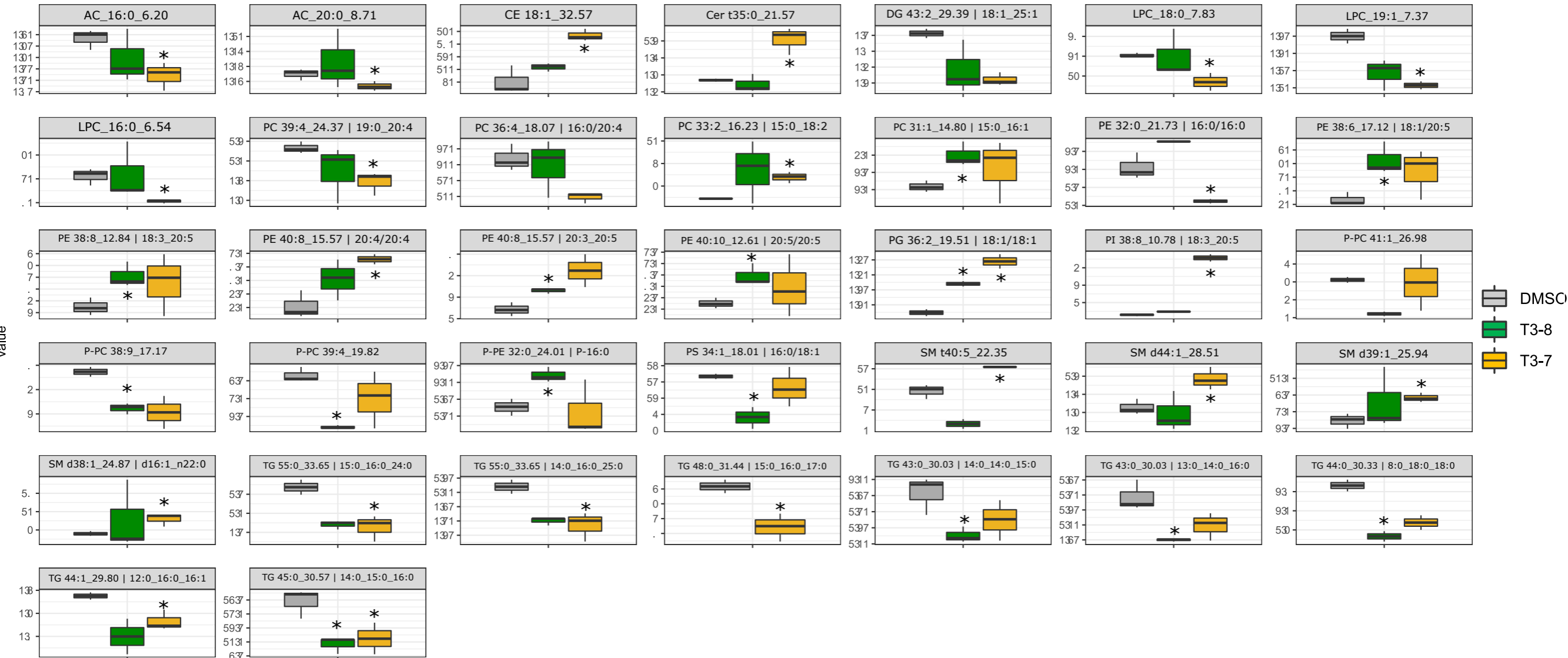
A

Pyruvate dehydrogenase complex



Supp Figure 8. Roux et al





AC: Acylcarnitine , allow fatty acids to cross mitochondrial membrane to be oxidized

CE: Cholesterol ester, store and transport cholesterol

Cer: Ceramid, involved in cell programmes (cell differentiation, cell death, cell proliferation), and membrane constituents

DG: Diacylglycerole, act as secondary messenger in lipid signalisation

LPC: Lysophosphatidylcholine, involved in phosphatidylcholine synthesis and byproducts of lipogenesis

PC: Phosphatidylcholine, involved in fat dissolution during digestion, component of cell membranes.

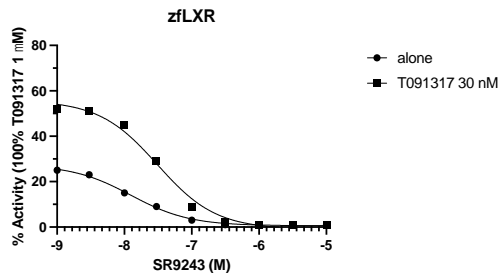
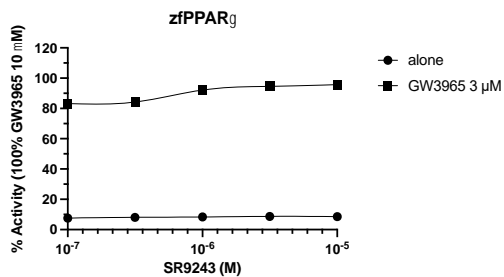
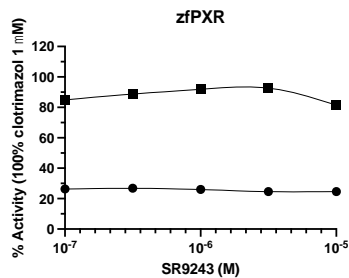
PE: Phosphatidylethanolamine, component of cell membranes

PG: Phosphatidylglycerol, component of cell membranes, and involved in the synthesis of numerous lipids

SM: Sphingomyelin, nerve myelin constituents and involved in transduction of extracellular signals

TG: Triacylglycerol, Fatty acid storage for energetic purposes through oxidation

Supp Figure 10, Roux et al.



Gene	Protein	Sens	Sequence	Amplicon size(pb)	qPCR working
<i>opnlw</i>	Opsin long wave length	Forward (5'– 3')	GAGGTGGGTAGTTGTGTGC	121	63
		Reverse (3'-5')	TCCAAACATGGGAGGAGCG		
<i>opnsw2</i>	Opsin short wave length	Forward (5'– 3')	CCAGTGGGGTGCAAGGTC	101	63
		Reverse (3'-5')	CAGACAACCAGCCATCTCT		
<i>guca1a</i>	Guanylate Cyclase Activator 1A	Forward (5'– 3')	GGAAACTCATCAGGCAGCAC	245	63
		Reverse (3'-5')	ATCACCAGACTCAGAGCAGC		
<i>TRβ</i>	Thyroid hormone receptor β	Forward (5'– 3')	GTGAGCTGCCTTGTGAAGAC	102	63
		Reverse (3'-5')	GCGTCTCACTCTGGATCA		
<i>Dio2</i>	Deiodinase 2	Forward (5'– 3')	AGGTCAAACCTGGGCTGTGAG	376	63
		Reverse (3'-5')	GCTCCCATCCTCTCCTCAA		
<i>Dio3a</i>	Deiodinase 3a	Forward (5'– 3')	GGGGTCGTGCAGCAGAAC	139	63
		Reverse (3'-5')	CGCTCAGCCTGTCCTCCA		
<i>acads</i>	Acyl-CoA dehydrogenase short chain	Forward (5'– 3')	AAGGGTATCAGTGCCTTTCTGG	271	63
		Reverse (3'-5')	TTCGTTTGTGTGCGTAGTCG		
<i>pfkma</i>	Phosphofructokinase, muscle	Forward (5'– 3')	TGATCATCGTTGCTGAAGGC	292	63
		Reverse (3'-5')	TTGGTCACTTGACACACTC		
<i>pkmb</i>	Pyruvate kinase	Forward (5'– 3')	CCATCATTGTGCTACCAAGTC	229	63
		Reverse (3'-5')	AAGAATTTGCGGTGCTTGCC		
<i>idh3a</i>	Hydrogenase [NAD] subunit alpha, n	Forward (5'– 3')	AAAGGACCTGGTGGCAAATG	136	63
		Reverse (3'-5')	TTTTCTCAGCAGCAGGTTT		
<i>srebp</i>	sterol regulatory element-binding protein	Forward (5'– 3')	CTCCTCCACCCAGACAAG	394	63
		Reverse (3'-5')	ACTTGGAGGCGAAGAGGAC		
<i>fads2</i>	Fatty acid desaturase	Forward (5'– 3')	CAGGAGGGTTTCGGGTCAT	408	63
		Reverse (3'-5')	TCTTGAAGACAGACAGATGACCA		
<i>elovl5</i>	Elongase	Forward (5'– 3')	GACTTACAAGAAGCACAGTGGT	124	63
		Reverse (3'-5')	CACCCTCAGTTTCTTGGCTG		
<i>rpl32</i>	L ribosomal protein-7 (HKG)	Forward (5'– 3')	AACCAAGAAGTTCATTGCGCATCAG	116	63
		Reverse (3'-5')	TGAGCATCTGACCCTTGAACC		
<i>rpl7</i>	L ribosomal protein-32 (HKG)	Forward (5'– 3')	GAACAAGGCTTCCATCAACATGCTG	265	63
		Reverse (3'-5')	CAGCTTGAAGGGCCACAGGAA		