Developmental Glucocorticoid exposure in zebrafish primes expression of genes linked to human psychiatric disorders

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Abstract

Chronic or severe stress exposure during development primes an individual for altered stress sensitivity in adulthood and increases psychiatric disease susceptibility. However, the identity and function of genes affected by developmental stress exposure are poorly understood. Here, we used an optogenetic zebrafish model to identify brain-wide molecular alterations caused by chronic Glucocorticoid (GC) exposure during development and following subsequent exposure to an acute stressor in adulthood. We show that developmental GC-exposed fish display wide-ranging behavioral changes affecting social behavior, food consumption, and associative learning. The brain transcriptome of GC-exposed fish showed alteration of genes associated with DNA metabolism, axon development, and social behavior-related genes such as oxytocin across the life course. Strikingly, when GC-exposed fish were subjected to a stress challenge in adulthood, they showed exaggerated endocrine responses and a great number of brain-wide transcriptional changes, including gene sets associated with axon development, neuronal signaling, and epigenetic modulators. These altered gene sets showed an overrepresentation of genes linked to human psychiatric disorders. In short, our study provides a unique resource of long-term, time-dependent gene expression changes induced by developmental GC-exposure and identifies novel GC-primed genes which may shape adult responses of early life stress-exposed individuals.

Introduction

Chronic or severe stress exposure in early life has long-term consequences affecting the development of an individual[1]. In the brain, early life stress (ELS) can lead to long-term alterations in neural plasticity, functional connectivity, epigenetic dynamics, and behavior[2-6]. Reflecting these far-reaching effects, ELS is a strong risk factor for developing psychiatric disorders in humans[7-9]. The molecular response to stress in vertebrates is primarily mediated by a highly conserved neuroendocrine stress regulatory system called the Hypothalamo-Pituitary-Adrenal axis (HPA-axis) in humans[10] and the Hypothalamic-Pituitary-Interrenal axis (HPI-axis) in fish[11]. Like the mammalian adrenal gland, the fish interrenal gland is composed of two cell types: aminergic chromaffin cells and steroidogenic interrenal cells, the latter of which predominantly produce glucocorticoids (GCs), effector stress hormones that are highly conserved across the vertebrate kingdom[10]. While GCs have pleiotropic effects on many aspects of animal physiology, exposure to elevated GCs during brain development is known to cause long-term alteration of behavior, physiology, and stress regulation[1]. However, how high GC exposure during development causes alteration of adult phenotypes is poorly understood. One potential mechanism is long-lasting epigenetic changes, which modify how animals respond to stress in adulthood. For example, a recent study identified differentially expressed genes (DEGs) "primed" by ELS in specific regions of ELS-exposed mouse brains[12]. These genes showed enhanced differential expression in adulthood upon re-exposure to stress and may be associated with behavioral changes in adulthood. Another study identified GC-primed molecular alterations using a GC (dexamethasone)-exposed human hippocampal progenitor cell line and identified long-lasting DNA methylation alterations[13]. The above studies show that ELS or developmental GC exposure results in differential responses to stress exposure later in life by long-term maintained alteration and priming of specific genes. However, so far, ELS or GC-primed genes have only been identified in specific tissues or cell lines and brain-wide GC-primed molecular alterations have not been investigated.

In this study, we utilized optogenetic manipulation of steroidogenic interrenal cells in a zebrafish model to increase the endogenous GC level during development and identified subsequent behavioral and molecular alterations using whole-brain transcriptome analyses in both larval and adult brains. We found changes in adult behavior and brain-wide transcriptomic alteration of GC-exposed fish across the life course. Strikingly, when subjected to acute stress in adulthood, GC-exposed fish show highly exaggerated endocrine and transcriptional responses. Interestingly, the DEGs primed by GC-exposure were enriched in gene sets associated with human neuropsychiatric disorders. In short, our study reports brain-wide

transcriptional changes following the elevation of endogenous GC and identifies for the first time GC-primed DEGs in the whole brain, a gene set likely to play an important role in modifying the adult stress response.

Methods and materials

The detailed protocol for all the procedures used in this study is available at the step-wise online methods[14].

Zebrafish husbandry and maintenance

The tübingen (TU) strain was kept at 28°C on a 12:12 hour light/dark cycle and housed at a maximum density of 5 fish/L. For experiments with adult fish, only females were used. All animal procedures were carried out in compliance with the ethical guidelines of the national animal welfare law and approved by authorities (Landesuntersuchugsamt Rheinland-Pfalz, Germany, Project number 23 177-07/G20-1-033 or UK Home Office PPL number PEF291C4D).

Generation of transgenic line

Linearized pT2-2kbStAR:bPAC-2A-tdTomato[15](ZFIN ID: ZDB-GENE-001120-3) recombinant plasmids were injected into 1-cell stage wild-type embryos of TU strain at 10-50 ng/mL in the presence of 10-50 ng/mL Tol2 transpose mRNA and 0.05% phenol red. The progenies of injected fish were screened for interrenal-specific tdTomato expression and the stable transgenic line, *Tg(star:bPAC-2A-tdTomato)*^{uex300}, was established.

Sample collection

All samples were collected within a 2-hour window during the morning (08:30 to 10:30) without feeding.

Whole-body Cortisol assay

The competitive cortisol assay (Cisbio HTRF® Cortisol Kit, 62CRTPEG) was performed following the manufacturer's protocol. ELISA signal was detected by CLARIOstar plate reader (BMG Labtech, Ortenberg, Germany).

Behavior tests

Social behavior tests and LD (looming dot) presentation were carried out as described in Cook et al., 2023[16]. For all behavioral tests, female adult zebrafish (6-9 months old) were moved into a designated behavior experimental room one week prior to testing and housed in groups of 10 fish in a 3L tank. Experiments were carried out between 09:00 and 14:00 and fish were not fed on the morning of testing. Fish were not used in any repeat experiments. The trials were recorded with cameras at 30 FPS. A camera was positioned at the front and/or top side of the tank. The test fish was tracked live using Ethovision (EthoVision XT 13, Noldus Information Technology), which generated X and Y coordinates for each frame.

RNA preparation and quantification

Each sample for the larval stage (6 and 13 days post fertilization (dpf)) contained 25-30 larval whole brains whilst for the adult stage (120 dpf) three whole brains constituted one sample. Transcripts were quantified using real-time qPCR and next-generation mRNA sequencing. Primer information for qPCR is described in Supplementary Table 13. A total of 60 samples was sequenced, consisting of 5 biological replicates at four different time points (6, 13, 120 dpf, and acute-stressed at 120 dpf) for each genotype (wild type, bPAC+ and bPAC-).

Immunohistochemistry

Immunohistochemistry was performed as described with minor modifications[17]. Custom-made primary rabbit anti-oxt antibodies in 1:200 dilution[17] and Alexa 488 anti-rabbit antibodies (A-11008, Invitrogen, USA) as secondary antibodies (in 1:1000 dilution) were used for the visualization of oxytocinergic neurons.

Imaging and analysis

Confocal stacks through the larval head were recorded using a Zeiss LSM 880 with Airyscan FAST with PApo 10X/0.45 – dry objective with Airyscan mode. Stacks were analyzed using ImageJ (FIJI, 1.53c)[18]. Corrected Total Cell Fluorescence (CTCF) was calculated as integrated density − (Area of cells × Mean fluorescence of background reading).

Statistics

Statistical analyses were performed using R and Prism 9 (Graphpad Software Inc, San Diego, CA, USA). A detailed description of the statistical analyses is provided in online methods[14]

Data availability

All sequenced reads for RNA-seq were deposited in European Nucleotide Archive (ENA, PRJEB53713).

Code availability

The detailed data processing and R scripts for bioinformatic analysis are available at the GitHub repository (https://github.com/minkechoi/tx_star-bPAC_brain).

Results

Optogenetic elevation of endogenous GC during development

We have previously reported that elevation of endogenous GC is induced in a transgenic zebrafish line by blue light present in ambient light which activates *beggiatoa* Photoactivated Adenylate Cyclase (bPAC), expressed specifically in steroidogenic interrenal cells[15, 19]. For the present study, we generated *Tg(star:bPAC-2A-tdTomato)*^{uex300} using the same construct in a different strain to obtain a higher cortisol level than what was previously feasible and raised the fish under ambient light (hitherto referred to as bPAC+) (Fig. 1a,b). Indeed, we observed a strong cortisol increase at 5 days post fertilization (dpf) in bPAC+ compared to bPAC-, siblings of bPAC+ which do not carry the transgene (Fig. 1c). bPAC+ larvae raised under a filter, which restricts blue light penetration, did not show a cortisol increase, supporting that the cortisol elevation was due to optogenetic activation of bPAC (Fig. 1c). Moreover, the expression of *fkbp5*, which regulates the responsiveness of the glucocorticoid receptor (GR)[20], was increased in bPAC+ compared to bPAC- and to wild type, confirming the activation of the GR signaling cascade (Fig. 1d). Although bPAC- fish did not carry the transgene, since transgenerational inheritance of stress-induced phenotypes is reported in mammals and fish[21-23], we compared bPAC+ and bPAC- to each other, as well as to wild type in this study. Consistent with the reported onset of endogenous *star* gene expression in an Expression Atlas[24], an increased cortisol level was observed starting around 2 dpf (Fig. 1e). The high cortisol level was sustained during larval and juvenile stages in bPAC+ fish, however, by adulthood (three months post-fertilization (mpf)), no significant difference was detected between bPAC+ and wild type (Figs. 1e,f). These results indicate that using bPAC+ we can achieve a robust and persistent increase in endogenous cortisol levels during development.

Alteration of adult behavior in developmental GC-exposed fish

We assessed whether bPAC+ fish exhibited behavioral changes as adults using a battery of tests aimed at assessing goal-directed behaviors affected in neuropsychiatric diseases[25]. bPAC+

169 fish did not show significant differences in basal locomotor activity compared to wild type and
170 bPAC- fish (Supplementary Figs. 1a,b). To assess social behavior, we employed a new test that
171 we recently established, which allows assessment of different phases of social behavior,
172 namely a drive to approach a social cue, plus an ability to engage and maintain social
173 interaction (Fig. 2a)[16]. After the fish was placed in a test tank and before the presentation of
174 conspecifics, wild type, bPAC- and bPAC+ all displayed an immediate and spontaneous
175 preference for the blue-colored bottom zone with no significant genotype differences
176 (Supplementary Figs. 1d-f). When the social cue was revealed (Supplementary Fig. 1c), within
177 10 seconds the vast majority of wild type, bPAC-, and bPAC+ entered the social interaction
178 zone, a C-shaped zone (shaded green in Fig. 2a) adjacent to the conspecifics, within which wild
179 type display a stereotypical pattern of swimming. No difference in social approach between the
180 groups was found using a log-rank test for trend (P=0.69), indicating that bPAC+ did not show
181 deficits in recognition or approach to social cues (Supplementary Fig. 1g). In contrast, we
182 found significant differences in the time spent in the interaction zone and the number of social
183 interactions performed between wild type and bPAC+ during the 2-minute social phase (Figs.
184 2b,c). We confirmed that bPAC- also spent less time in the interaction zone and performed
185 fewer interactions than the wild type (Supplementary Figs. 1h,i).
186 Next, we tested food consummatory behavior by counting the number of floating food pellets
187 eaten by a fish during a 10-minute interval (Fig. 2d). Wild-type adult fish consumed an average
188 of 14.5 pellets while bPAC+ fish consumed an average of 2.33 pellets out of a total of 25 (Figs.
189 2e,f). This was not due to the lack of recognition nor interest in the food, as bPAC+ fish
190 approached the food pellets and actually spent a longer time in the zone near the pellets (feeding
191 zone) than wild type (Fig. 2g).
192 To test associative learning, we employed a Pavlovian avoidance learning test where fish learn
193 to associate the color green with a mild electric shock in the testing chamber (Figs. 2h,i). Fish
194 were first habituated in the chamber for 30 minutes and tracked to determine the baseline
195 preference for green or blue color. Wild type and bPAC+ fish showed no significant difference
196 in baseline color preference (Fig. 2j). However, after the conditioning, wild-type fish spent an
197 average of 89.2% of their time in the blue zone, significantly more than bPAC+ fish who spent
198 62.68% of their time in the blue zone (Fig. 2k), suggesting a deficit in associative learning in
199 bPAC+ fish.
200 Lastly, we tested the behavior of bPAC+ fish in a novel environment using a novel tank test
201 (Supplementary Figs. 1j-p). In the novel tank test, wild-type fish first dive to the bottom and
202 demonstrate a sustained depth-preference whilst performing bouts of erratic high-speed

203 swimming[26, 27], meanwhile bPAC+ fish showed reduced depth-preference (Supplementary
204 Figs. 1k,n) and swam slower than wild- type controls (Supplementary Figs. 1l,o). Time spent
205 performing fast-swimming bouts was also significantly decreased in bPAC+ fish
206 (Supplementary Fig. 1m). However, there was no significant difference in freezing duration
207 between groups (Supplementary Fig. 1p).
208 Together these results show that social interaction, food consumption, associative learning, and
209 adaptation to a novel environment are altered in bPAC+ fish. Our results point to modification
210 of a broad range of goal-directed behavior performance without changes in basal locomotion
211 parameters in bPAC+.
212
213 **Whole-brain transcriptional alterations in bPAC+ fish across the life course**
214 Whole-brain RNA sequencing was carried out using larval (6 and 13 dpf) and adult (120 dpf)
215 brains to identify and compare transcriptomic differences in wild type, bPAC+, and bPAC-. A
216 large number of DEGs was identified in pairwise comparisons of bPAC+ and wild-type brains
217 at 6 dpf (up/down=602/1253), 13 dpf (up/down=777/1566), and 120 dpf (up/down=684/1078)
218 (Fig. 3, Supplementary Table 1). At all-time points, the number of downregulated genes was
219 greater than the number of upregulated genes, indicating that developmental GC-exposure
220 tends to repress rather than activate genes (Fig. 3a). Further, the number of DEGs consistently
221 downregulated from early life until adulthood (273 DEGs) was greater than the upregulated
222 DEGs (48 DEGs) (Fig. 3a). We identified fewer DEGs when comparing bPAC- to wild type,
223 with 571 (up/down=329/242), 1026 (up/down=372/654), and 74 (up/down=42/32) DEGs at 6,
224 13, and 120 dpf, respectively, and many of these DEGs overlapped with those identified in the
225 bPAC+/wild type comparison (Supplementary Fig. 2a). To compare the overall transcriptomic
226 landscape of all identified DEGs, we performed unsupervised hierarchical clustering, which
227 revealed large differences in the gene expression landscape between the bPAC+ and bPAC-
228 compared to wild type (Fig. 3b). At each time point, bPAC+ and bPAC- transcriptome profiles
229 clustered together separately from wild type. Next, to confirm an increase in GR signaling, we
230 measured the expression level of GR target genes, *nr3c1, nr3c2*, *tsc22d3*, *fkbp5*, and *hsd11b2*
231 at 6, 13, and 120 dpf. The expression levels of *nr3c1, tsc22d3, fkbp5,* and *hsd11b2* were altered
232 at multiple time points in bPAC+ (Fig. 3c). bPAC- also showed differences to wild type, albeit
233 generally to a lesser degree (Fig. 3c). Both the alteration of GR signaling and similarity of
234 DEGs identified in bPAC- and bPAC+ point to potential transgenerational effects present in
235 bPAC- resulting from high GC-exposure in the parental generation. Supporting this notion,
236 even though bPAC- was not exposed to a high level of GC, some of the DEGs at 6 dpf in

237 bPAC- are involved in processes that potentially regulate GC inactivation (Supplementary Fig.
238 2b, Supplementary Table 2). Therefore, overlapping GO terms for DEGs that were enriched in
239 both bPAC- and bPAC+ may reveal functions associated with transgenerational effects.
240

241 **Developmental GC exposure leads to long-lasting suppression of nervous system**
242 **development and adult-onset alteration of gene regulation**

243 To identify direct molecular consequences of developmental GC exposure, we characterized
244 DEGs in bPAC+ compared to wild type. Upregulated DEGs in bPAC+ fish brains at 6 dpf were
245 enriched in the GO terms that include potential regulatory enzymes for GC such as cytochrome
246 monooxygenases, 11β-hydroxysteroid dehydrogenase or sulfotransferases[28-30] (left panel of
247 Fig. 3d). At 13 dpf, the top-ranked enriched biological processes for upregulated DEGs were
248 cell cycle, chromosome segregation, and DNA metabolic process which can cause genomic
249 instability by replication stress[31] (left panel of Fig. 3d). At 120 dpf, bPAC+ brains showed
250 upregulation of genes involved in ribonucleoprotein complex biogenesis, peptide metabolic,
251 translation, protein modification, RNA processing and more, suggesting effects of
252 developmental GC exposure on gene expression regulation and post-transcriptional
253 modification in adulthood (left panel of Fig. 3d, Supplementary Table 2). In contrast,
254 downregulated DEGs across all time points were mainly enriched in processes related to cell
255 adhesion and cell migration, as well as nervous system development including neuron
256 differentiation, cell morphogenesis, and axon development (right panel of Fig. 3d,
257 Supplementary Table 2). During larval stages, downregulated DEGs were enriched in
258 anatomical structure formation, muscle structure development, vasculature development,
259 external encapsulating structure organization and more. We also identified functions associated
260 with time-point-specific downregulated DEGs (Supplementary Table 2). For example, DEGs
261 at 6 dpf were enriched in brain development while DEGs at 13 dpf were enriched in the cellular
262 response to organic substance (right panel of Fig. 3d). At 120 dpf, we identified downregulated
263 DEG enrichments in synaptic signaling, regulation of molecular function, and regulation of
264 GTPase activity and more (right panel of Fig. 3d).
265 These results highlight long-lasting downregulation of neuronal development and signaling
266 processes, and adult-onset alteration of transcription and translation regulation following
267 developmental GC exposure.
268

269 **Social behavior-associated genes are downregulated in bPAC+ fish**

270 To search for a potential link between the observed molecular changes and social behavior, we
271 analyzed the expression patterns of 101 known social behavior-associated genes from the Gene
272 Ontology Annotation database (henceforth referred to as social genes). Interestingly, 47 of
273 these social genes were differentially expressed in bPAC+ brains, where 37 were
274 downregulated at one time point at least (|FC|>1.5 and FDR<0.05) and only 9 were upregulated
275 (Supplementary Fig. 3a). Among the three-time points analyzed, 6 dpf brains showed the
276 largest change (up/down=2/31). The most significant downregulated social genes over time
277 were *nlgn2b*, *mapk8ip2*, *brinp1*, *slc6a4a,* as well as *oxytocin* (*oxt)*, well-known social gene
278 (Supplementary Fig. 3a). We confirmed the downregulation of *oxt* both in larval and adult
279 brains using qPCR and immunohistochemistry (Supplementary Fig. 3b,c).
280 Using differentially expressed social genes at 6 dpf, we performed network-based analyzes
281 using the String database[32] (Supplementary Fig. 3d). The identified protein network was
282 enriched in modulation of chemical synaptic transmission, synaptic signaling, learning and
283 memory, pre-synapse assembly and social behavior. Notably, *oxt* regulators including *myt1la*,
284 *med12*, and *thrab* were downregulated at 6 dpf and two of them (*myt1la*, *med12*) remained
285 downregulated until adulthood (Supplementary Fig. 3e). Further, transcription factor (TF)
286 enrichment analysis of human orthologues of the social genes identified *myt1la* as one of the
287 top regulators of 47 differentially expressed social genes, based on the ChEA3 database[33]
288 (Supplementary Fig. 3f). These results suggest that downregulation of *myt1la* in bPAC+ may
289 result in the altered expression of social genes including *oxt*.
290
291 **bPAC+ fish show an enhanced endocrine response to acute stress in adulthood**
292 To assess whether high GC exposure during development affects the stress response in
293 adulthood, we subjected wild-type, bPAC+, and bPAC- fish to a looming dot stimulus (LD),
294 which mimics an approaching predator[16] (Fig. 4a, Supplementary Fig. 4a). Interestingly,
295 bPAC+ adults exhibited a much greater cortisol increase after LD exposure compared to wild-
296 type adults (Fig. 4b), indicative of a sensitized endocrine response to acute stress in bPAC+.
297 Similarly, we found an altered transcriptional response of stress-associated genes in bPAC+
298 after LD exposure (Fig. 4c). For example, the expression of *nr3c2*, the gene coding for the
299 mineralocorticoid receptor (MR), was further downregulated only in bPAC+ adults following
300 LD exposure, although basal expression of *nr3c2* was already decreased in bPAC+ compared
301 to wild type. In contrast, the GC-inactivating enzyme coding gene, *hsd11b2,* was significantly
302 upregulated in the wild type after LD exposure, but upregulated to a lesser degree in bPAC+
303 following LD exposure. Together, the significant increase in LD-induced cortisol level and

304 altered expression of *nr3c2*, *fkbp5,* and *hsd11b2* indicate an enhanced endocrine response
305 following acute stress exposure in adult bPAC+.
306
307 **Developmental GC exposure primes large-scale differential gene expression following**
308 **acute stress in adulthood**
309 To identify the transcriptional response to acute stress following developmental GC exposure,
310 we carried out whole-brain RNA sequencing of wild type, bPAC+ and bPAC- that had been
311 exposed to LD in adulthood and made three separate comparisons (Figs. 4d,e). First, we
312 compared the wild-type transcriptome at basal (pre-LD) and after LD exposure (post-LD), to
313 identify the normal acute stress response in wild-type fish (referred to as DEG1). Secondly, we
314 compared pre-LD and post-LD in bPAC+ (referred to as DEG2), and finally the post-LD
315 transcriptome of wild type and bPAC+ was also compared (referred to as DEG3). DEG3
316 includes LD-induced DEGs (overlapping with DEG1 and DEG2) as well as those DEGs that
317 were already altered in bPAC+ prior to LD exposure and therefore the number of DEG3 genes
318 (2034 genes) was greater than that of DEG2 (Fig. 4f). Strikingly, bPAC+ showed more than
319 20-fold greater number of DEGs in response to LD exposure (DEG2, 1617 genes) compared
320 to wild type (DEG1, 64 genes) (Fig. 4f). Altogether we identified 2515 genes
321 (DEG1+DEG2+DEG3) that appeared to be altered in the adult brain by developmental GC
322 exposure in bPAC+, and refer to them collectively as adult bPAC+ DEGs.
323 The exaggerated transcriptional LD response in bPAC+ indicates that developmental GC
324 exposure in zebrafish primes enhanced alteration of gene expression in adulthood, similar to
325 observations in previous mammalian ELS or GC-primed gene studies[12, 13]. To characterize the
326 GC-primed genes, we combined the 18 genes in DEG1 indicating altered stress responses in
327 bPAC+ that did not overlap with DEG2, with the DEG2 genes excluding the 44 genes
328 representing normal stress responses in bPAC+ that overlapped with DEG1 (Fig. 4f,
329 Supplementary Table 3). This set of 1591 genes is collectively referred to as GC-primed genes
330 here and represents those genes that are primed by developmental GC exposure to produce an
331 altered transcriptional response to acute stress in adulthood. Among the 1591 GC-primed genes,
332 1337 downregulated genes were enriched in neuron projection development, synaptic signaling,
333 receptor complex, synapse, metal ion transmembrane transport, and more (Fig. 4g,
334 Supplementary Table 4). A subset of social genes was also part of the GC-primed genes
335 (Supplementary Fig. 4b). Next, we performed a TF enrichment test of GC-primed genes using
336 the ChEA3 platform[33] and identified common potential upstream regulators of GC-primed
337 genes (Supplementary Table 5, Supplementary Fig. 5). For the upregulated GC-primed genes,

338 immediate early genes were top-ranked TFs. Among the top-ranked TFs for downregulated
339 GC-primed genes, *camta2* and *myt1l* may be involved in the regulatory process of 149 and 139
340 downregulated genes, respectively. Expression of *myt1l* was consistently downregulated in
341 bPAC+ adults at both pre-LD and post-LD condition (Supplementary Fig. 5c).
342 Lastly, we carried out a comparative analysis with a recent study that reported 702 GC-primed
343 DEGs linked with long-lasting differentially methylated sites following re-exposure to GC
344 (dexamethasone)[13]. These DEGs were identified in GC-treated mature neurons which were
345 exposed to GC during neurogenesis in a human hippocampal progenitor cell (hpc) line.
346 Through the comparison between 702 DEGs (hpc_ELS-primed genes, up:368, down:334)[13]
347 and our DEG3, which represents a similar GC re-exposed condition, we found 163 overlapping
348 genes (up/down=99/64). Among them, 21 overlapping DEGs were also classified as GC-
349 primed DEGs and they were linked to functions such as generation of neurons (*AUTS2, IRX5,*
350 *SPOCK1, POU3F2*) (Supplementary Fig. 6, Supplementary Table 6).
351 Together, these results reveal for the first time a brain-wide set of genes primed by
352 developmental GC exposure and identify their potential upstream regulators. Further, we found
353 a significant overlap between subsets of GC-primed genes identified in zebrafish with those
354 from mammalian studies suggesting a high degree of evolutionary conservation of molecular
355 mechanisms underlying epigenetic reprogramming by GC.
356
357 **Adult bPAC+ DEGs are associated with neuropsychiatric disorders in humans**
358 Since stress exposure during development is a risk factor for the development of psychiatric
359 disorders in humans[7-9], GC-primed genes were compared with known psychiatric disease risk
360 factors using the curated DisGeNET database[34]. Strikingly, human homologs of GC-primed
361 genes exhibit a significant enrichment of known psychiatric disease-associated genes
362 (Supplementary Table 7). Top-ranked psychiatric disorders were schizophrenia, bipolar
363 disorder, and autistic disorder (Supplementary Fig. 7). Further, the entire adult bPAC+ DEGs
364 set was strongly enriched in known psychiatric disease risk factors and in this case depressive
365 disorder was also included in the top four ranked diseases (Figs. 5a,b, Supplementary Table 8).
366 Upon LD exposure, the difference in expression of schizophrenia-associated genes in bPAC+
367 was greatly enhanced as represented by the size of nodes in Fig. 5c. Further, schizophrenia-
368 associated adult bPAC+ DEGs were enriched in either of two KEGG pathways: neuroactive
369 ligand-receptor interaction (FDR=2.01e-08), or MAPK signaling pathway (FDR=9.74e-08).
370 Overall, across all four disease categories, the top-ranked disease-associated with adult bPAC+

371 DEGs were mainly enriched in cell communication, nervous system development, response to
372 endogenous stimulus, and neuroactive ligand-receptor interaction (Supplementary Fig. 8).
373 Adult bPAC+ DEGs associated with schizophrenia, bipolar disorder, depressive disorders and
374 autistic disorders were predominantly downregulated (Fig. 5d). Interestingly, we noted that
375 some of the disease-associated adult bPAC+ DEGs were already differentially expressed from
376 the larval stage (trans-temporal, 60 genes) while some others showed alteration in their
377 expression only upon exposure to acute stress in adulthood (adult-onset, 65 genes) (Figs. 5e).
378 Other non-disease-associated adult bPAC+ DEGs maintained differential expression from the
379 larval stage (540 genes) or exhibited an enhanced alteration upon adulthood stress (adult-onset,
380 463 genes) (Supplementary Table 9). Trans-temporal adult bPAC+ DEGs were mostly
381 enriched in axon development (FDR= 1.33e-08, 31 DEGs), whilst adult-onset adult bPAC+
382 DEGs were enriched in ion transport (FDR= 7.26eE-05, 44 DEGs) (Supplementary Table 10).
383 Interestingly, a cell-type enrichment test using the human homologs of adult bPAC+ DEGs
384 showed that 24 trans-temporal adult bPAC+ DEGs were associated with non-microglial
385 oligodendrocyte lineages. In contrast, the downregulated adult-onset adult bPAC+ DEG set
386 was associated with non-microglial neuron-enriched genes including GABAergic receptor-
387 associated genes (*GABRA1*, *GABRG2*), other ion channel activity genes (*SLC12A5*, *CACNA1E*,
388 *CACNA1I*, *UNC80*, *KCNH1*, *SCN2B*), and genes associated with synapse and neuronal cell
389 signaling (*DNM1*, *DLGAP2*, *RPH3A*, *GPR158*) (Supplementary Table 11). More than half of
390 those genes were GC-primed genes. These results indicate that oligodendrocyte lineage cells
391 are consistently suppressed following developmental GC-exposure, and developmental GC
392 primes suppression of neuronal signaling primarily in adulthood when encountering stressful
393 stimuli.
394
395 **Altered gene expression associated with epigenetic modifications and RNA processing in**
396 **bPAC+ fish**
397 To identify underlying mechanisms by which developmental GC exposure regulates gene
398 expression in the brain, we analyzed the expression of epigenetic modifiers. We identified 70
399 adult bPAC+ DEGs associated with epigenetic modifications (Figs. 6a,b, Supplementary Table
400 12). These genes are associated with RNA modification (up/down=3/3), RNA processing
401 including mRNA splicing (up/down=27/17), histone modification (up/down=4/18), and DNA
402 modification (up/down=0/3). Among them, the DNA methyltransferase (DNMT) 3aa gene and
403 the histone modification-associated genes, *chd3*, *hdac4*, *hdac5*, *kdm2ba*, *kdm4aa*, *mta3*, *nsd3*,
404 *padi2*, *suv39h1b*, and *yeats2,* showed consistent downregulation across time-points.

405 We also found significant differences in the expression of 4 out of 7 *DNMTs* in the zebrafish
406 genome including *dnmt1*, *dnmt3aa*, *dnmt3ab,* and *dnmt3bb.3* across time points and conditions
407 in bPAC+ compared to the wild type (Fig. 6c). In the wild type, *dnmt1* is highly expressed at
408 6 dpf and expression then gradually decreases over time, but in bPAC+ expression was
409 significantly reduced at 6 dpf. Expression patterns of *dnmt3aa* and *dnmt3ab*, orthologous to
410 human *DNMT3A,* known as one of the writers of *de novo* methylation, mainly showed
411 differences in bPAC+ adult brains. Further, low expression of *dnmt3aa* and upregulation of
412 *dnmt3ab* was observed upon LD exposure in adulthood in bPAC+. To identify whether
413 alteration of the top epigenetic modulators (labeled with * in Fig. 6a) is long-lasting throughout
414 adulthood, we analyzed their expression at 8 mpf, with and without LD exposure.
415 Downregulated expression of those genes was continuously observed in the later adult stage (8
416 mpf) mainly upon acute stress in bPAC+ adults (Fig. 6d). These results suggest that
417 developmental GC exposure leads to pervasive, long-lasting changes to epigenetic modulators.
418

419 **Discussion**

420 This study examines the effects of chronic developmental GC exposure in shaping adult
421 phenotypes at both behavioral and molecular levels. We report altered goal-directed behaviors
422 in adults and brain-wide molecular consequences of GC-exposure in both larvae and adults.
423 Further, this study represents the first comprehensive brain-wide identification of genes
424 differentially expressed in adult bPAC+, many of which correspond to genes associated with
425 neuropsychiatric disorders in humans.
426

427 Our bPAC+ model involves the optogenetic activation of *star*-expressing interrenal cells,
428 leading to an increase in endogenous cortisol levels in transgenic animals. The mammalian
429 counterpart of the fish interrenal, the adrenal gland, is known to produce three types of steroid
430 hormones, GCs, mineralocorticoids (MCs), and androgens[35]. However, cortisol/GC is believed
431 to be the primary output of the zebrafish interrenal gland. Previous studies in fish using LC-
432 MS have not detected MCs in any significant quantities in either larval or adult samples[36-39],
433 suggesting that the zebrafish interrenal produces no/negligible amounts of MC. Androgens are
434 not detectable in zebrafish larvae[36], but are detectable in adult male whole-body samples [37-39],
435 suggesting that androgens are most likely only produced in the testes in zebrafish. As such, we
436 believe that the phenotypes observed in our bPAC+ model are the effects of GC exposure,
437 rather than of exposure to additional non-GC interrenal hormones.
438

One of our interesting findings is the enhanced endocrine sensitivity of adult bPAC+ fish in response to acute stress. Further, we observed a significant decrease in the expression of *nr3c2,* which encodes the high GC-affinity MC receptor (MR), both at pre- and post-LD exposure (Fig. 4c). Given the higher affinity of MR to GC, MR can be a regulator of GR signaling in MR and GR co-expressing cells in basal conditions[40, 41]. Moreover, reduced transcriptional reactivity of *hsd11b2,* a key enzyme for cortisol inactivation[42, 43], in bPAC+ after LD (Fig. 4C) may lead to an increased accumulation of active cellular cortisol. These results suggest that developmental GC-exposure may lead to a sensitized response to stress by decreased MR expression at pre- and post-LD and an attenuated increase of *hsd11b2* expression. This blunted response to stress is reminiscent of the HPA axis dysregulation observed in humans exposed to ELS and in those suffering from psychiatric diseases[44, 45].

We show that goal-directed behaviors relevant to neuropsychiatric disorders are altered in bPAC+ fish. bPAC+ fish exhibit a reduction in the maintenance of social interactions in the absence of social recognition or approach deficits. These results are consistent with phenotypes observed in rodent ELS models in which deficits in social interaction in the absence of social recognition and approach have been observed[46]. Furthermore, social withdrawal is a common feature of autism[47] and schizophrenia[48] models in rodents. Similarly in previous zebrafish ELS studies, diminished shoaling behavior was observed following early-life social isolation and was associated with both motivational deficiencies linked to dopaminergic systems[49] and increased sensitivity to stimuli which was reversed by anxiolytic treatment[50]. Further, bPAC+ fish showed deficits in cognition and fear processing. This phenotype is also consistent with what has been reported of rodent ELS models, which showed deficits in fear learning, learning impairment, and decreased cognitive flexibility[51-53]. In zebrafish, fear-conditioned avoidance learning is a robust phenotype whereby fish exhibit freezing and erratic behavior upon re-exposure to the conditioned stimulus (CS)[54, 55]. Although one study found that chronic unpredictable stress did not have any effect on fear conditioning in adult zebrafish[56], a different ELS protocol found improvement of short-term memory by early life stress when testing in the unconditioned y-maze[57]. Our results demonstrate that developmental GC exposure results in far-reaching behavioral alterations in zebrafish, which manifests in a diminished response and engagement to both positive and negative stimuli, combined with cognitive deficits consistent with ELS-associated clinical outcomes.

472 We identified brain-wide GC-modulated alterations through whole-brain transcriptome
473 analyses using larval and adult zebrafish. The GC-modulated downregulated genes are mainly
474 enriched in cell morphogenesis, neuronal cellular signaling, and neuropsychiatric disorders-
475 associated gene sets. Despite the differences in species, stressor type, and brain region, results
476 of our GO enrichment test and observed phenotypes are broadly similar with previous findings
477 obtained from ELS rodent models which report alterations in axon development, synaptic
478 density and plasticity, altered hippocampal mossy fibres[5, 6, 58]. However, mammalian ELS
479 paradigms may represent a milder and shorter stress exposure when compared to the chronic
480 high GC elevation in the model described here.
481
482 Interestingly, we identified a large set of adult-onset upregulated DEGs which were enriched
483 in DNA metabolism, various RNA processing processes, and posttranslational modifications.
484 This may indicate that developmental GC-exposure can lead to genome instability by DNA
485 replication stress and altered gene expression regulation via alternative splicing, non-coding
486 RNAs, and posttranslational modifications in adulthood. Moreover, in adult bPAC+, we
487 identified oligodendrocyte lineage cell markers in downregulated trans-temporal DEGs while
488 neuron-enriched genes were enriched in downregulated adult-onset DEGs. This result suggests
489 that developmental GC-exposure has a cell-type-specific impact.
490
491 Growing evidence indicates that GC signaling can lead to modifications to DNA methylation,
492 histone codes, and non-coding RNAs[59-61]. Similarly, we identified significantly altered
493 expression of epigenetic modulators. One of the most significantly altered epigenetic regulators
494 in bPAC+ brains are *DNMTs* (*dnmt1* and *dnmt3aa*) at the larval stage (6 dpf), which may result
495 in a global or local reduction in DNA methylation. *DNMT1* mainly plays a role in the
496 maintenance of DNA methylation[62] while accumulation or gain of DNA methylation is
497 achieved by *DNMT3a* and *-3b*[63]. Therefore, developmental GC exposure may lead to an
498 abnormal DNA methylation landscape by suppressing *dnmt1* and *dnmt3aa* expression during
499 development and altering expression of *dnmt3aa* and *dnmt3ab* in adult brains. Indeed, we
500 identified genes including *auts2b, dmbx1b, gadd45gb.1, isl2a*, *irx2a, irx5a, irx6a*, *neurod6b*,
501 *uncx4.1, and zgc*:*110843* that are shared between adult bPAC+ DEGs and the list of DEGs
502 associated with differentially methylated regions (DMR) in *dnmt3aa* knockout mutant
503 zebrafish[64]. Lack of *DNMTs* during development causes defects in neuronal development and
504 function[65, 66] as well as altered behaviors including social interest and anxiety-like behavior [67].

505 Given such far-reaching effects of each *DNMT*, alteration of *dnmt* levels that we observe in
506 bPAC+ are predicted to change the global DNA methylation landscape across the life course.
507
508 In our study, we identified consistently downregulated adult bPAC+ DEGs associated with
509 histone modifications across the life course (marked with light green bars in Fig. 6). Temporal
510 regulation of chromatin structure via the "histone code" in neurons has been regarded as a
511 crucial factor for dynamic plasticity in the mature nervous system and long-lasting neuronal
512 responses[68]. Among the histone modulators, *hdac4*, a consistently downregulated gene in
513 bPAC+ fish, has been implicated in depression, fear learning, PTSD, eating disorder (Anorexia
514 nervosa), and schizophrenia[69-73]. Furthermore, many epigenetic-associated adult bPAC+ DEGs
515 are involved in RNA processing and RNA editing. Among them, we found a downregulation
516 of *adarb2* and *pum2* in bPAC+ fish after exposure to LD. Chen et al. suggest that ADARB2
517 has a regulatory role in RNA editing by inhibiting the binding of other adenosine deaminases
518 acting on RNA (ADARs)[74] and ADARs are associated with the development of epileptic
519 seizures in mice and depressive phenotypes[75, 76]. Further, *pum2* is involved in axon growth and
520 branching[77]. However, further investigation is required to reveal direct mechanisms between
521 the identified adult bPAC+ DEGs and alterations to epigenetic modulators, as well as the
522 potential link between the adult bPAC+ DEGs and the phenotypes observed in human
523 neuropsychiatric disorders.
524
525 In conclusion, we show that developmental GC exposure leads to altered behaviors and brain-
526 wide transcriptional changes that were enriched in the processes of DNA metabolism, nervous
527 system development and neuronal signaling across the life course. We identified brain-wide
528 adult bPAC+ DEGs that are overrepresented in gene sets associated with neuropsychiatric
529 disorders and are enriched in epigenetic modulators. Further, developmental GC exposure led
530 to exaggerated endocrine responses and primed a set of genes to exhibit altered transcriptomic
531 responses to subsequent challenge in adult fish. We propose that the brain-wide DEG set
532 identified here will be useful for guiding future studies dissecting the mechanism underlying
533 developmental GC-exposure and behavioral dysfunction and neuropsychiatric disease
534 susceptibility in adulthood.
535
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Author contributions

SR conceived and MC designed the project. MC acquired, analyzed and interpreted data. AC acquired, analyzed, and interpreted behavior data. SK, HE, AT and SK contributed to reagent generation or data acquisition. MC, SR, and AC drafted the article. MC, HE, and SR critically revised the manuscript. All authors approved the submission.

Competing interests

SR holds a patent, European patent number 2928288 and US patent number 10,080,355: “A novel inducible model of stress.”. The remaining authors declare no known competing interests.

557 **Figure legends**

558 **Figure 1. Ambient light-exposed bPAC+ fish show elevation of cortisol until the juvenile**
559 **stage.** (a) An image of *Tg(star:bPAC-2A-tdTomato)*$^{+/-}$ larva at 5 dpf. The transgene is
560 expressed solely in the interrenal gland (arrow). The schematic presents the intracellular events
561 in the steroidogenic interrenal cells of transgenic fish (dotted box). Endogenous GC production
562 is initiated by Adrenocorticotropic Hormone (ACTH) signaling with adenylyl cyclase (AC).
563 Likewise, bPAC mimics ACTH signaling by synthesizing Cyclic adenosine monophosphate
564 (cAMP) when exposed to blue light. Scale bar indicates 1.5 mm. (b) Fish were raised under
565 conventional aquarium lighting conditions with white fluorescent light (12-hour light and 12-
566 hour dark). Elevation of basal GC level was observed at larval and juvenile stages. (c) White
567 ambient light (blue+) elevates the basal GC level of 6 dpf bPAC+ larvae. ***=P<0.001, two-
568 tailed unpaired t-test. However, there was no significant difference in GC level after filtering
569 out the blue light spectrum from the ambient light (blue-). (d) One of the well-known GR
570 signaling markers, *fkbp5*, was significantly upregulated in bPAC+ larvae (P = 0.016) compared
571 to wild-type control, two-tailed unpaired t-test. * = P<0.05. Basal GC level at larval stages (e)
572 and from juvenile to adult (f).
573
574 **Figure 2. Reduced behavioral response to a novel environment, social interaction, feeding,**
575 **and fear conditioning in adult bPAC+ fish.** (a) Schematic figures for the social approach and
576 maintenance test. Representative traces of fish during the social phase are shown on the right
577 side. Green shade indicates the interaction zone. (b-c) bPAC+ showed a significantly decreased
578 duration and frequency of social interactions compared to wild type. Dunnett's multiple
579 comparisons test followed one-way ANOVA; b: (F (2, 98) = 6.273, P=0.0027), c: (F (2, 98) =
580 10.93, P<0.0001). (d) Schematic for the feeding behavior test. (e-g) bPAC+ fish consumed
581 significantly fewer pellets within 10 min, despite spending more time near the food. Mann-
582 Whitney test, n=16. (h) Chambers for the fear conditioning test. (i) For fear conditioning, an
583 electric shock was given to subject fish when they swim onto the green color. After fear
584 conditioning, time spent in each color zone was measured. (j) No significant difference was
585 observed in color preference prior to fear conditioning. Mann-Whitney test, n=6. (k) bPAC+
586 showed less preference for the safe zone color (blue) after fear conditioning using an electric
587 shock. Mann-Whitney test, n=18. Error bars on bar graphs indicate the mean±SD, and shading
588 in line graphs represents the mean±SEM.
589
590 **Figure 3. Developmental GC exposure leads to differential gene expressions across the**
591 **life course** (a) Venn Diagram for up- and down-regulated DEGs between wild type and bPAC+;
592 criteria for DEG identification: |FC|>2 and FDR <0.01. However, a weaker FC threshold,
593 |FC|>1.5, was applied for samples at 120 dpf. (b) Average read counts of genes by genotype
594 and time points are represented by z-score in the heat map. Bars on the right side indicate the
595 paternity of fish. (c) Expression profiles of GC-associated genes. *=P<0.05, **=P<0.01,
596 ***=P<0.001, ****=P<0.0001. Dunnett's multiple comparisons test followed two-way
597 ANOVA. (d) Top enriched biological processes for up- and down-regulated DEGs in bPAC+.
598 d6: 6 dpf, d13: 13 dpf, d120: 120 dpf, wt: wild type, FC: fold change, GC: glucocorticoid, *adj.p*:
599 adjusted P-value, ratio: identified DEGs/the number of genes included in the term. Complete
600 DEG-enriched GO terms are described in Supplementary Table 2.
601
602 **Figure 4. Developmental GC exposure primes differential gene expression following acute**
603 **stress in bPAC+ adults.** (a) Schematic for acute-stressed and control sample collection
604 (arrows). Acute stress is induced by looming dot (LD)-exposure in adults (120 dpf). post-LD
605 samples were collected after 15 min of LD exposure. Yellow striped box: bPAC+ activation;
606 yellow stars: LD. (b) bPAC+ showed significantly higher GC levels in post-LD conditions,

compared with basal. One-way ANOVA; F(5, 54) = 19.31, $P<0.0001$. (c) Expression profiles of GC-induced genes following LD exposure. *= $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$ from multiple two-tailed unpaired t-tests. Error bars indicate mean+SEM. (d) Schematic for three comparisons to identify transcriptional changes in response to LD exposure in adults. DEGs were determined by FC and FDR. |FC|>1.5 and FDR<0.01 for DEG1 and 2; |FC|>2 and FDR<0.01 for DEG3. Methods for LD exposure are described in Supplementary Fig. 4a. Yellow striped box: bPAC+ activation; yellow stars: LD. (e) z-score for mean CPM of adult bPAC+ DEGs is visualized in a heatmap. (f) Venn diagram for adult bPAC+ DEGs. The list of DEGs is described in Supplementary Table 3. GC-primed genes = DEG1 + DEG2 - (DEG1 ∩ DEG2). There is an unlabelled overlap (2 genes) between DEG1 and DEG3 due to lack of space. (g) The top 3 GO terms enriched in downregulated GC-primed genes. The enrichment score = $-\log_{10}$(adjusted *P*-value). The complete results of the functional enrichment test are described in Supplementary Table 4.

Figure 5. Adult bPAC+ DEGs **are enriched in gene sets associated with human neuropsychiatric disorders.** (a) Result of human disorders enrichment test based on the DisGeNET database. The ratio represents overlapping/input genes. Top ranked disorders are schizophrenia (FDR= 1.61e-04, ratio (adult bPAC+ DEGs/disease genes)=170/883), bipolar disorder (FDR= 7.48e-04, ratio=100/477), depressive disorder (FDR= 2.89e-03, ratio=63/289), mental depression (FDR= 2.87e-03, ratio=59/254) and autistic disorder (FDR= 1.61e-04, ratio=65/261). (b) Venn diagram for adult bPAC+ DEGs that are associated with the top five human psychiatric disorders in panel a. Depressive disorder and Mental Depression-associated genes (as shown in a), are combined and referred to as depressive disorders in b and d. (c) STRING protein-protein interaction (PPI) networks of schizophrenia-associated adult bPAC+ DEGs genes in pre- and post-LD conditions indicate LD-sensitive gene expression. The size and color of the node represent comparative fold change, and gene expression; up (orange) or downregulation (blue), respectively. DEGs enriched in KEGG pathways Neuroactive ligand-receptor interaction (FDR = 2.01e-08) are labelled in yellow whilst those enriched in MAPK signaling pathway (FDR=9.74e-08) are labelled in green. (d) The heatmap depicts the $\log_2$FC of GC-altered genes associated with the top five human psychiatric disorders in panel a (Depressive disorder and mental depression are merged). The numbers in the brackets represent the number of adult bPAC+ DEGs. 6, 13, and 120 denote dpf, and LD denotes post-LD at 120 dpf. (e) Subsets of adult bPAC+ DEGs associated with the top five diseases in a show consistent differential expression across the life course (significant at all timepoints, labelled trans-temporal) or only in adulthood (significant only in adulthood, labelled adult-onset). Genes that exhibited expression patterns outside of these conditions were excluded.

Figure 6. Adult bPAC+ DEGs gene expression changes associated with epigenetic modifications. (a) Heatmap represents $\log_2$FC of known epigenetic regulators associated with DNA (dark purple), RNA (yellow), and histone (green) modification or process among adult bPAC+ DEGs. The asteria mark in each cell refers to FDR < 0.05. (b) The number of adult bPAC+ DEGs in the three different categories. (c) Expression trajectories for differentially expressed DNMTs in bPAC+ brains, unpaired t-test with Welch correction. (d) real-time qPCR results of 5 selected genes from panel a labeled with asteria at 6, 13 dpf and 8 mpf. Gray: wild type, Pink: bPAC+, unpaired t-test. dpf: day post fertilization, mpf: month post fertilization. *=$P<0.05$, **=$P<0.01$, ***=$P<0.001$, ****=$P<0.0001$.

References

1. Lupien SJ, McEwen BS, Gunnar MR, Heim C. Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nat Rev Neurosci* 2009; **10**(6)**:** 434-445.

2. Chen Y, Baram TZ. Toward Understanding How Early-Life Stress Reprograms Cognitive and Emotional Brain Networks. *Neuropsychopharmacology* 2016; **41**(1)**:** 197-206.

3. Kaiser RH, Clegg R, Goer F, Pechtel P, Beltzer M, Vitaliano G *et al.* Childhood stress, grown-up brain networks: corticolimbic correlates of threat-related early life stress and adult stress response. *Psychol Med* 2018; **48**(7)**:** 1157-1166.

4. Matrisciano F, Tueting P, Dalal I, Kadriu B, Grayson DR, Davis JM *et al.* Epigenetic modifications of GABAergic interneurons are associated with the schizophrenia-like phenotype induced by prenatal stress in mice. *Neuropharmacology* 2013; **68:** 184-194.

5. Sandi C, Haller J. Stress and the social brain: behavioural effects and neurobiological mechanisms. *Nat Rev Neurosci* 2015; **16**(5)**:** 290-304.

6. Brunson KL, Kramár E, Lin B, Chen Y, Colgin LL, Yanagihara TK *et al.* Mechanisms of late-onset cognitive decline after early-life stress. *J Neurosci* 2005; **25**(41)**:** 9328-9338.

7. Kessler RC, McLaughlin KA, Green JG, Gruber MJ, Sampson NA, Zaslavsky AM *et al.* Childhood adversities and adult psychopathology in the WHO World Mental Health Surveys. *Br J Psychiatry* 2010; **197**(5)**:** 378-385.

8. McEwen BS, Gianaros PJ. Central role of the brain in stress and adaptation: links to socioeconomic status, health, and disease. *Ann N Y Acad Sci* 2010; **1186:** 190-222.

9. Heim C, Nemeroff CB. The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. *Biol Psychiatry* 2001; **49**(12)**:** 1023-1039.

10. Charmandari E, Tsigos C, Chrousos G. Endocrinology of the stress response. *Annu Rev Physiol* 2005; **67:** 259-284.

11. Wendelaar Bonga SE. The stress response in fish. *Physiol Rev* 1997; **77**(3)**:** 591-625.

12. Peña CJ, Smith M, Ramakrishnan A, Cates HM, Bagot RC, Kronman HG *et al.* Early life stress alters transcriptomic patterning across reward circuitry in male and female mice. *Nat Commun* 2019; **10**(1)**:** 5098.

13. Provençal N, Arloth J, Cattaneo A, Anacker C, Cattane N, Wiechmann T *et al.* Glucocorticoid exposure during hippocampal neurogenesis primes future stress

response by inducing changes in DNA methylation. *Proc Natl Acad Sci U S A* 2020; **117**(38)**:** 23280-23285.

14. Choi M, Cook A, Eachus H, Tochwin A, Kuntz S, Ryu S. Comprehensive analysis methods for developmental GC exposed zebrafish. *protocolsio* 2023.

15. Gutierrez-Triana JA, Herget U, Castillo-Ramirez LA, Lutz M, Yeh CM, De Marco RJ *et al.* Manipulation of Interrenal Cell Function in Developing Zebrafish Using Genetically Targeted Ablation and an Optogenetic Tool. *Endocrinology* 2015; **156**(9)**:** 3394-3401.

16. Cook A, Beckmann H, Azap R, Ryu S. Acute stress modulates social approach and social maintenance in adult zebrafish. *bioRxiv* 2023**:** 2023.2002.2010.528021.

17. Herget U, Wolf A, Wullimann MF, Ryu S. Molecular neuroanatomy and chemoarchitecture of the neurosecretory preoptic-hypothalamic area in zebrafish larvae. *J Comp Neurol* 2014; **522**(7)**:** 1542-1564.

18. Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T *et al.* Fiji: an open-source platform for biological-image analysis. *Nature Methods* 2012; **9**(7)**:** 676-682.

19. Nagpal J, Eachus H, Lityagina O, Ryu S. Optogenetic induction of chronic glucocorticoid exposure in early-life impairs stress-response in larval zebrafish. *bioRxiv* 2022**:** 2022.2009.2009.507267.

20. Binder EB. The role of FKBP5, a co-chaperone of the glucocorticoid receptor in the pathogenesis and therapy of affective and anxiety disorders. *Psychoneuroendocrinology* 2009; **34 Suppl 1:** S186-195.

21. Meaney MJ. Maternal Care, Gene Expression, and the Transmission of Individual Differences in Stress Reactivity Across Generations. *Annual Review of Neuroscience* 2001; **24**(1)**:** 1161-1192.

22. Vera-Chang MN, St-Jacques AD, Gagné R, Martyniuk CJ, Yauk CL, Moon TW *et al.* Transgenerational hypocortisolism and behavioral disruption are induced by the antidepressant fluoxetine in male zebrafish Danio rerio. *Proceedings of the National Academy of Sciences* 2018; **115**(52)**:** E12435-E12442.

23. Ord J, Heath PR, Fazeli A, Watt PJ. Paternal effects in a wild-type zebrafish implicate a role of sperm-derived small RNAs. *Molecular Ecology* 2020; **29**(14)**:** 2722-2735.

24. White RJ, Collins JE, Sealy IM, Wali N, Dooley CM, Digby Z *et al.* A high-resolution mRNA expression time course of embryonic development in zebrafish. *Elife*, vol. 62017, p e30860.

25. Griffiths KR, Morris RW, Balleine BW. Translational studies of goal-directed action as a framework for classifying deficits across psychiatric disorders. *Frontiers in Systems Neuroscience* 2014; **8**.

26. Levin ED, Bencan Z, Cerutti DT. Anxiolytic effects of nicotine in zebrafish. *Physiology & Behavior* 2007; **90**(1)**:** 54-58.

27. Egan RJ, Bergner CL, Hart PC, Cachat JM, Canavello PR, Elegante MF *et al.* Understanding behavioral and physiological phenotypes of stress and anxiety in zebrafish. *Behavioural Brain Research* 2009; **205**(1)**:** 38-44.

28. Gulliver LS. Xenobiotics and the Glucocorticoid Receptor. *Toxicol Appl Pharmacol* 2017; **319:** 69-79.

29. Draper N, Stewart PM. 11beta-hydroxysteroid dehydrogenase and the pre-receptor regulation of corticosteroid hormone action. *J Endocrinol* 2005; **186**(2)**:** 251-271.

30. Balyan R, Cai M, Zhao W, Dai Z, Zhai Y, Chen G. Repeated restraint stress upregulates rat sulfotransferase 1A1. 2019; **30**(2)**:** 265-273.

31. Zheng D-Q, Zhang K, Wu X-C, Mieczkowski PA, Petes TD. Global analysis of genomic instability caused by DNA replication stress in Saccharomyces cerevisiae. *Proceedings of the National Academy of Sciences* 2016; **113**(50)**:** E8114-E8121.

32. Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J *et al.* STRING v11: protein–protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. *Nucleic Acids Research* 2019; **47**(D1)**:** D607-D613.

33. Keenan AB, Torre D, Lachmann A, Leong AK, Wojciechowicz ML, Utti V *et al.* ChEA3: transcription factor enrichment analysis by orthogonal omics integration. *Nucleic Acids Research* 2019; **47**(W1)**:** W212-W224.

34. Piñero J, Saüch J, Sanz F, Furlong LI. The DisGeNET cytoscape app: Exploring and visualizing disease genomics data. *Computational and Structural Biotechnology Journal* 2021; **19:** 2960-2967.

35. Bacila I, Cunliffe VT, Krone NP. Interrenal development and function in zebrafish. *Molecular and Cellular Endocrinology* 2021; **535:** 111372.

36. Eachus H, Zaucker A, Oakes JA, Griffin A, Weger M, Güran T *et al.* Genetic Disruption of 21-Hydroxylase in Zebrafish Causes Interrenal Hyperplasia. *Endocrinology* 2017; **158**(12)**:** 4165-4173.

37. Oakes JA, Li N, Wistow BRC, Griffin A, Barnard L, Storbeck K-H *et al.* Ferredoxin 1b Deficiency Leads to Testis Disorganization, Impaired Spermatogenesis, and Feminization in Zebrafish. *Endocrinology* 2019; **160**(10)**:** 2401-2416.

38. Li N, Oakes JA, Storbeck K-H, Cunliffe VT, Krone NP. The P450 side-chain cleavage enzyme Cyp11a2 facilitates steroidogenesis in zebrafish. *Journal of Endocrinology* 2020; **244**(2)**:** 309-321.

39. Oakes JA, Barnard L, Storbeck K-H, Cunliffe VT, Krone NP. 11β-Hydroxylase loss disrupts steroidogenesis and reproductive function in zebrafish. *Journal of Endocrinology* 2020; **247**(2)**:** 197-212.

40. de Kloet ER, Joëls M, Holsboer F. Stress and the brain: from adaptation to disease. *Nature Reviews Neuroscience* 2005; **6**(6)**:** 463-475.

41. Reul JM, van den Bosch FR, de Kloet ER. Differential response of type I and type II corticosteroid receptors to changes in plasma steroid level and circadian rhythmicity. *Neuroendocrinology* 1987; **45**(5)**:** 407-412.

42. Seckl JR. 11beta-hydroxysteroid dehydrogenases: changing glucocorticoid action. *Curr Opin Pharmacol* 2004; **4**(6)**:** 597-602.

43. Wyrwoll CS, Holmes MC, Seckl JR. 11β-hydroxysteroid dehydrogenases and the brain: from zero to hero, a decade of progress. *Front Neuroendocrinol* 2011; **32**(3)**:** 265-286.

44. Rao U, Hammen C, Ortiz LR, Chen LA, Poland RE. Effects of early and recent adverse experiences on adrenal response to psychosocial stress in depressed adolescents. *Biol Psychiatry* 2008; **64**(6)**:** 521-526.

45. Ouellet-Morin I, Odgers CL, Danese A, Bowes L, Shakoor S, Papadopoulos AS *et al.* Blunted cortisol responses to stress signal social and behavioral problems among maltreated/bullied 12-year-old children. *Biol Psychiatry* 2011; **70**(11)**:** 1016-1023.

46. Todeschin AS, Winkelmann-Duarte EC, Jacob MHV, Aranda BCC, Jacobs S, Fernandes MC *et al.* Effects of neonatal handling on social memory, social interaction, and number of oxytocin and vasopressin neurons in rats. *Hormones and Behavior* 2009; **56**(1)**:** 93-100.

47. Brodkin ES. BALB/c mice: Low sociability and other phenotypes that may be relevant to autism. *Behavioural Brain Research* 2007; **176**(1)**:** 53-65.

48. Miyakawa T, Leiter LM, Gerber DJ, Gainetdinov RR, Sotnikova TD, Zeng H *et al.* Conditional calcineurin knockout mice exhibit multiple abnormal behaviors related to schizophrenia. *Proceedings of the National Academy of Sciences* 2003; **100**(15)**:** 8987-8992.

49. Shams S, Amlani S, Buske C, Chatterjee D, Gerlai R. Developmental social isolation affects adult behavior, social interaction, and dopamine metabolite levels in zebrafish. *Developmental Psychobiology* 2018; **60**(1)**:** 43-56.

50. Tunbak H, Vazquez-Prada M, Ryan TM, Kampff AR, Dreosti E. Whole-brain mapping of socially isolated zebrafish reveals that lonely fish are not loners. *Elife* 2020; **9:** e55863.

51. Lehmann J, Pryce CR, Bettschen D, Feldon J. The maternal separation paradigm and adult emotionality and cognition in male and female Wistar rats. *Pharmacol Biochem Behav* 1999; **64**(4)**:** 705-715.

52. Aisa B, Tordera R, Lasheras B, Del Río J, Ramírez MJ. Effects of maternal separation on hypothalamic-pituitary-adrenal responses, cognition and vulnerability to stress in adult female rats. *Neuroscience* 2008; **154**(4)**:** 1218-1226.

53. Thomas AW, Caporale N, Wu C, Wilbrecht L. Early maternal separation impacts cognitive flexibility at the age of first independence in mice. *Dev Cogn Neurosci* 2016; **18:** 49-56.

54. Maximino C, do Carmo Silva RX, Dos Santos Campos K, de Oliveira JS, Rocha SP, Pyterson MP *et al.* Sensory ecology of ostariophysan alarm substances. *J Fish Biol* 2019; **95**(1)**:** 274-286.

55. Blank M, Guerim LD, Cordeiro RF, Vianna MR. A one-trial inhibitory avoidance task to zebrafish: rapid acquisition of an NMDA-dependent long-term memory. *Neurobiol Learn Mem* 2009; **92**(4)**:** 529-534.

56. Fontana BD, Gibbon AJ, Cleal M, Norton WHJ, Parker MO. Chronic unpredictable early-life stress (CUELS) protocol: Early-life stress changes anxiety levels of adult zebrafish. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* 2021; **108:** 110087.

57. Fontana BD, Gibbon AJ, Cleal M, Sudwarts A, Pritchett D, Miletto Petrazzini ME *et al.* Moderate early life stress improves adult zebrafish (Danio rerio) working memory but does not affect social and anxiety-like responses. *Dev Psychobiol* 2021; **63**(1)**:** 54-64.

58. Wang XD, Rammes G, Kraev I, Wolf M, Liebl C, Scharf SH *et al.* Forebrain CRF_1 modulates early-life stress-programmed cognitive deficits. *J Neurosci* 2011; **31**(38)**:** 13625-13634.

59. Matthews SG, McGowan PO. Developmental programming of the HPA axis and related behaviours: epigenetic mechanisms. *Journal of Endocrinology* 2019; **242**(1)**:** T69-T79.

60. Wang HT, Huang FL, Hu ZL, Zhang WJ, Qiao XQ, Huang YQ *et al.* Early-Life Social Isolation-Induced Depressive-Like Behavior in Rats Results in Microglial Activation and Neuronal Histone Methylation that Are Mitigated by Minocycline. *Neurotox Res* 2017; **31**(4)**:** 505-520.

61. Thomassin H, Flavin M, Espinás M-L, Grange T. Glucocorticoid-induced DNA demethylation and gene memory during development. *The EMBO Journal* 2001; **20**(8)**:** 1974-1983.

62. Hermann A, Goyal R, Jeltsch A. The Dnmt1 DNA-(cytosine-C5)-methyltransferase Methylates DNA Processively with High Preference for Hemimethylated Target Sites. *Journal of Biological Chemistry* 2004; **279**(46)**:** 48350-48359.

63. Okano M, Bell DW, Haber DA, Li E. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell* 1999; **99**(3)**:** 247-257.

64. Lai YH, Audira G, Liang ST, Siregar P, Suryanto ME, Lin HC *et al.* Duplicated dnmt3aa and dnmt3ab DNA Methyltransferase Genes Play Essential and Non-Overlapped Functions on Modulating Behavioral Control in Zebrafish. *Genes (Basel)* 2020; **11**(11).

65. Hutnick LK, Golshani P, Namihira M, Xue Z, Matynia A, Yang XW *et al.* DNA hypomethylation restricted to the murine forebrain induces cortical degeneration and impairs postnatal neuronal maturation. *Hum Mol Genet* 2009; **18**(15)**:** 2875-2888.

66. Pensold D, Symmank J, Hahn A, Lingner T, Salinas-Riester G, Downie BR *et al.* The DNA Methyltransferase 1 (DNMT1) Controls the Shape and Dynamics of Migrating POA-Derived Interneurons Fated for the Murine Cerebral Cortex. *Cereb Cortex* 2017; **27**(12)**:** 5696-5714.

67. Christian DL, Wu DY, Martin JR, Moore JR, Liu YR, Clemens AW *et al.* DNMT3A Haploinsufficiency Results in Behavioral Deficits and Global Epigenomic Dysregulation Shared across Neurodevelopmental Disorders. *Cell Reports* 2020; **33**(8)**:** 108416.

68. Borrelli E, Nestler EJ, Allis CD, Sassone-Corsi P. Decoding the epigenetic language of neuronal plasticity. *Neuron* 2008; **60**(6)**:** 961-974.

69. Hobara T, Uchida S, Otsuki K, Matsubara T, Funato H, Matsuo K *et al.* Altered gene expression of histone deacetylases in mood disorder patients. *J Psychiatr Res* 2010; **44**(5)**:** 263-270.

70. Maddox SA, Kilaru V, Shin J, Jovanovic T, Almli LM, Dias BG *et al.* Estrogen-dependent association of HDAC4 with fear in female mice and women with PTSD. *Mol Psychiatry* 2018; **23**(3)**:** 658-665.

71. Saha P, Gupta R, Sen T, Sen N. Histone Deacetylase 4 Downregulation Elicits Post-Traumatic Psychiatric Disorders through Impairment of Neurogenesis. *Journal of Neurotrauma* 2019; **36**(23)**:** 3284-3296.

72. Cui H, Moore J, Ashimi SS, Mason BL, Drawbridge JN, Han S *et al.* Eating disorder predisposition is associated with ESRRA and HDAC4 mutations. *J Clin Invest* 2013; **123**(11)**:** 4706-4713.

73. Wu Y, Hou F, Wang X, Kong Q, Han X, Bai B. Aberrant Expression of Histone Deacetylases 4 in Cognitive Disorders: Molecular Mechanisms and a Potential Target. *Front Mol Neurosci* 2016; **9:** 114.

74. Chen CX, Cho DS, Wang Q, Lai F, Carter KC, Nishikura K. A third member of the RNA-specific adenosine deaminase gene family, ADAR3, contains both single- and double-stranded RNA binding domains. *Rna* 2000; **6**(5)**:** 755-767.

951 75. Higuchi M, Maas S, Single FN, Hartner J, Rozov A, Burnashev N *et al.* Point
952 mutation in an AMPA receptor gene rescues lethality in mice deficient in the RNA-
953 editing enzyme ADAR2. *Nature* 2000; **406**(6791)**:** 78-81.
954
955 76. Bombail V, Qing W, Chapman KE, Holmes MC. Prevention of 5-
956 hydroxytryptamine2C receptor RNA editing and alternate splicing in C57BL/6 mice
957 activates the hypothalamic-pituitary-adrenal axis and alters mood. *Eur J Neurosci*
958 2014; **40**(11)**:** 3663-3673.
959
960 77. Martínez JC, Randolph LK, Iascone DM, Pernice HF, Polleux F, Hengst U. Pum2
961 Shapes the Transcriptome in Developing Axons through Retention of Target mRNAs
962 in the Cell Body. *Neuron* 2019; **104**(5)**:** 931-946.e935.
963
964

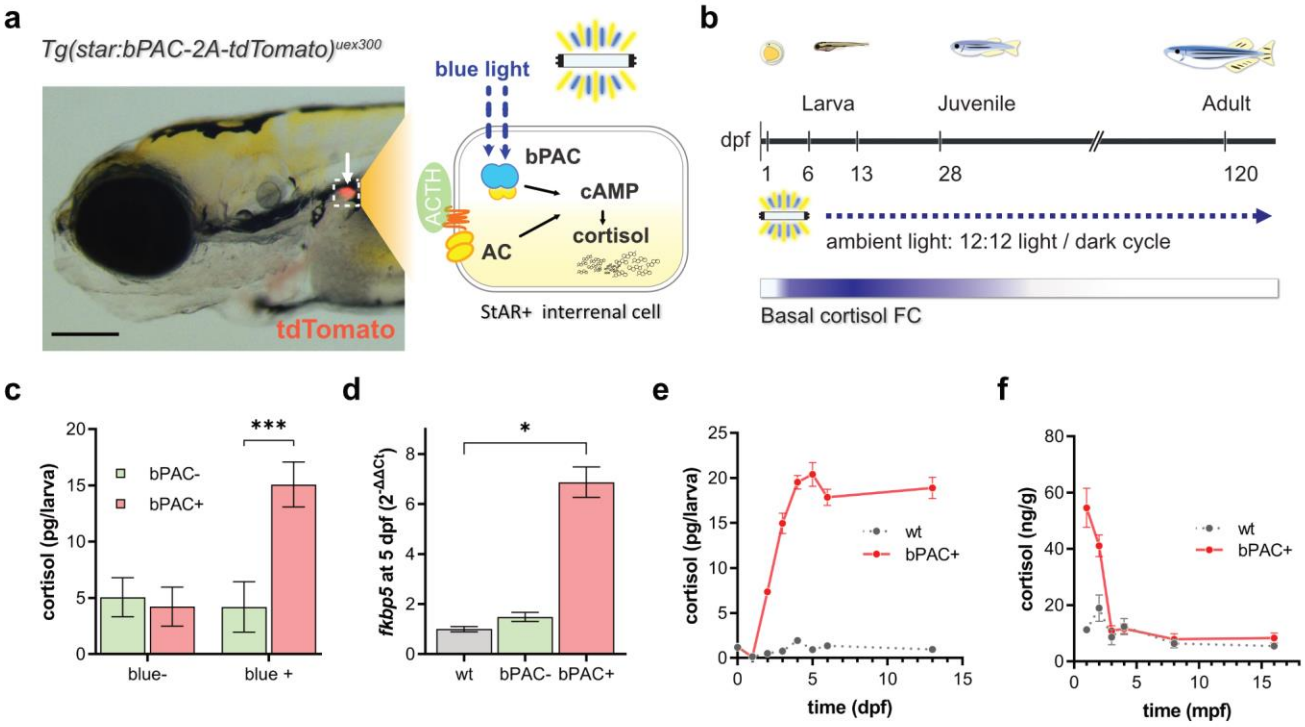
a
Tg(star:bPAC-2A-tdTomato)uex300
tdTomato
blue light
bPAC
cAMP
cortisol
AC
ACTH
StAR+ interrenal cell
b
Larva
Juvenile
Adult
dpf
1
6
13
28
120
ambient light: 12:12 light / dark cycle
Basal cortisol FC
c
cortisol (pg/larva)
bPAC-
bPAC+
***
blue-
blue +
d
fkbp5 at 5 dpf (2-ΔΔCt)
*
wt
bPAC-
bPAC+
e
cortisol (pg/larva)
wt
bPAC+
time (dpf)
f
cortisol (ng/g)
wt
bPAC+
time (mpf)

Figure 1

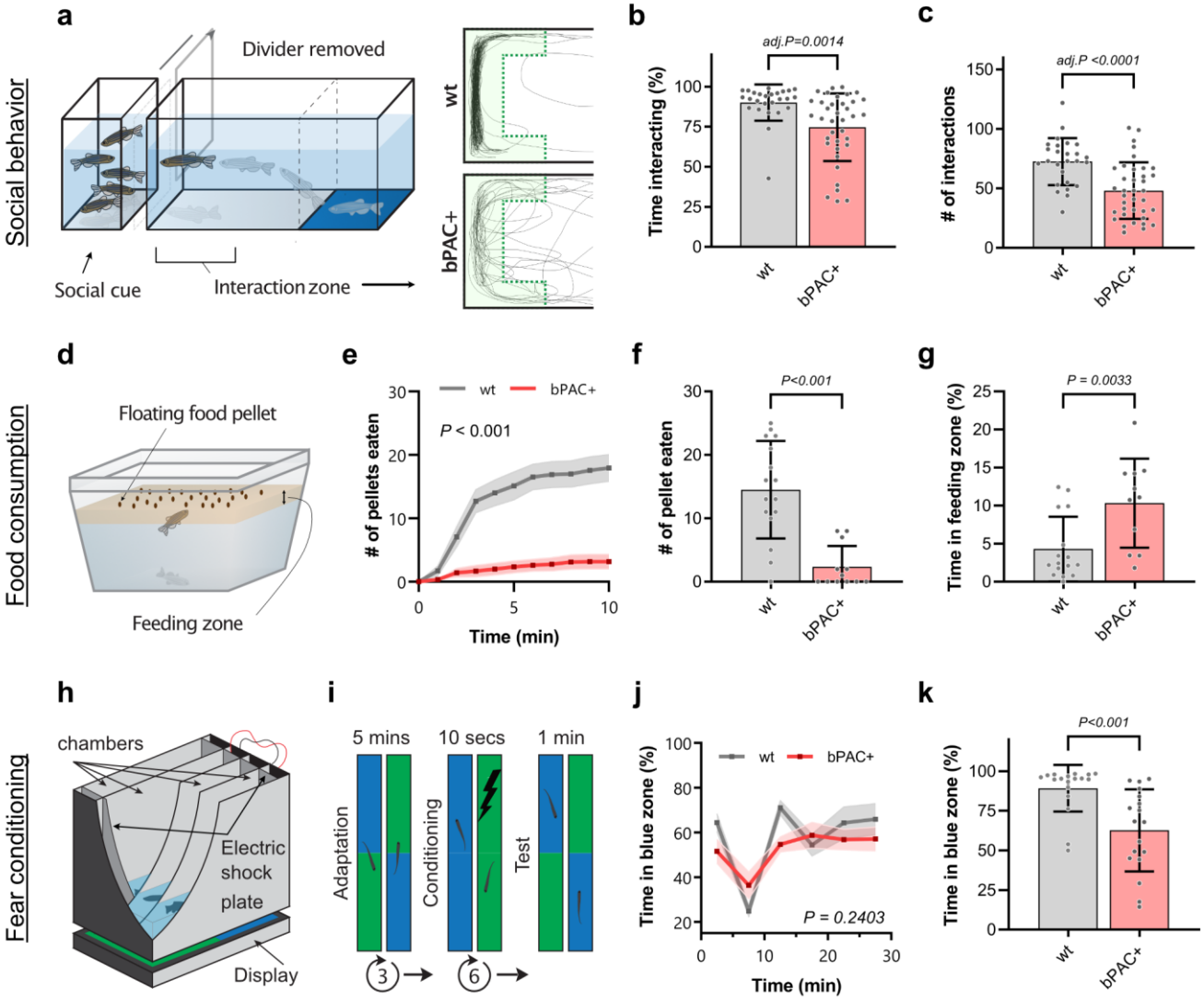
a
Social behavior
Divider removed
Social cue
Interaction zone
wt
bPAC+
b
adj.P=0.0014
Time interacting (%)
wt
bPAC+
c
adj.P <0.0001
# of interactions
wt
bPAC+
d
Food consumption
Floating food pellet
Feeding zone
e
wt
bPAC+
P < 0.001
# of pellets eaten
Time (min)
f
P<0.001
# of pellet eaten
wt
bPAC+
g
P = 0.0033
Time in feeding zone (%)
wt
bPAC+
h
Fear conditioning
chambers
Electric shock plate
Display
i
5 mins
10 secs
1 min
Adaptation
Conditioning
Test
3
6
j
wt
bPAC+
Time in blue zone (%)
P = 0.2403
Time (min)
k
P<0.001
Time in blue zone (%)
wt
bPAC+

Figure 2

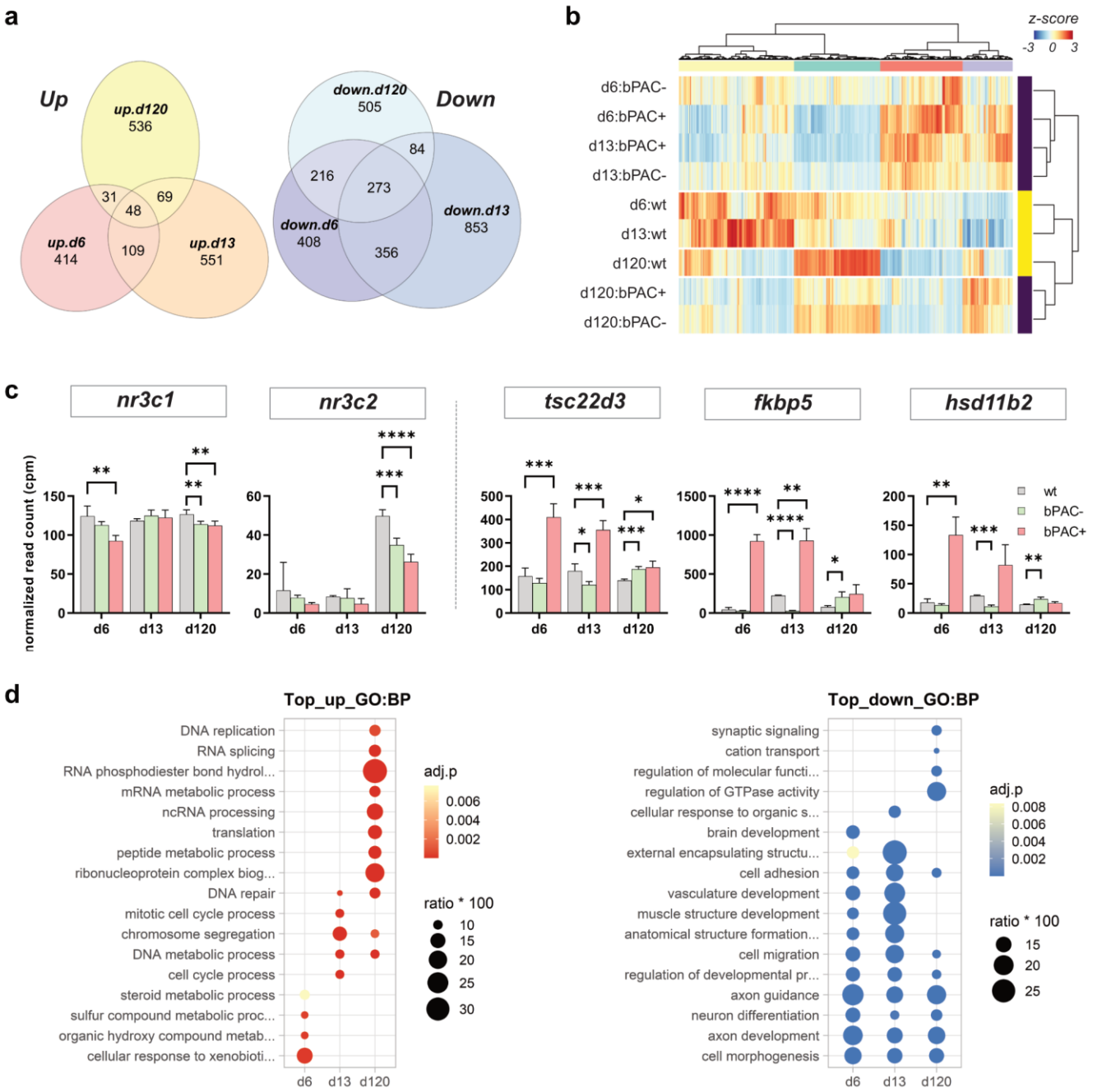
a
Up
up.d120
536
up.d6
414
up.d13
551
31
69
48
109
Down
down.d120
505
down.d6
408
down.d13
853
84
216
273
356
b
z-score
-3 0 3
d6:bPAC-
d6:bPAC+
d13:bPAC+
d13:bPAC-
d6:wt
d13:wt
d120:wt
d120:bPAC+
d120:bPAC-
c
nr3c1
nr3c2
tsc22d3
fkbp5
hsd11b2
normalized read count (cpm)
d6
d13
d120
wt
bPAC-
bPAC+
d
Top_up_GO:BP
DNA replication
RNA splicing
RNA phosphodiester bond hydrol...
mRNA metabolic process
ncRNA processing
translation
peptide metabolic process
ribonucleoprotein complex biog...
DNA repair
mitotic cell cycle process
chromosome segregation
DNA metabolic process
cell cycle process
steroid metabolic process
sulfur compound metabolic proc...
organic hydroxy compound metab...
cellular response to xenobioti...
adj.p
0.006
0.004
0.002
ratio * 100
10
15
20
25
30
Top_down_GO:BP
synaptic signaling
cation transport
regulation of molecular functi...
regulation of GTPase activity
cellular response to organic s...
brain development
external encapsulating structu...
cell adhesion
vasculature development
muscle structure development
anatomical structure formation...
cell migration
regulation of developmental pr...
axon guidance
neuron differentiation
axon development
cell morphogenesis
adj.p
0.008
0.006
0.004
0.002
ratio * 100
15
20
25

Figure 3

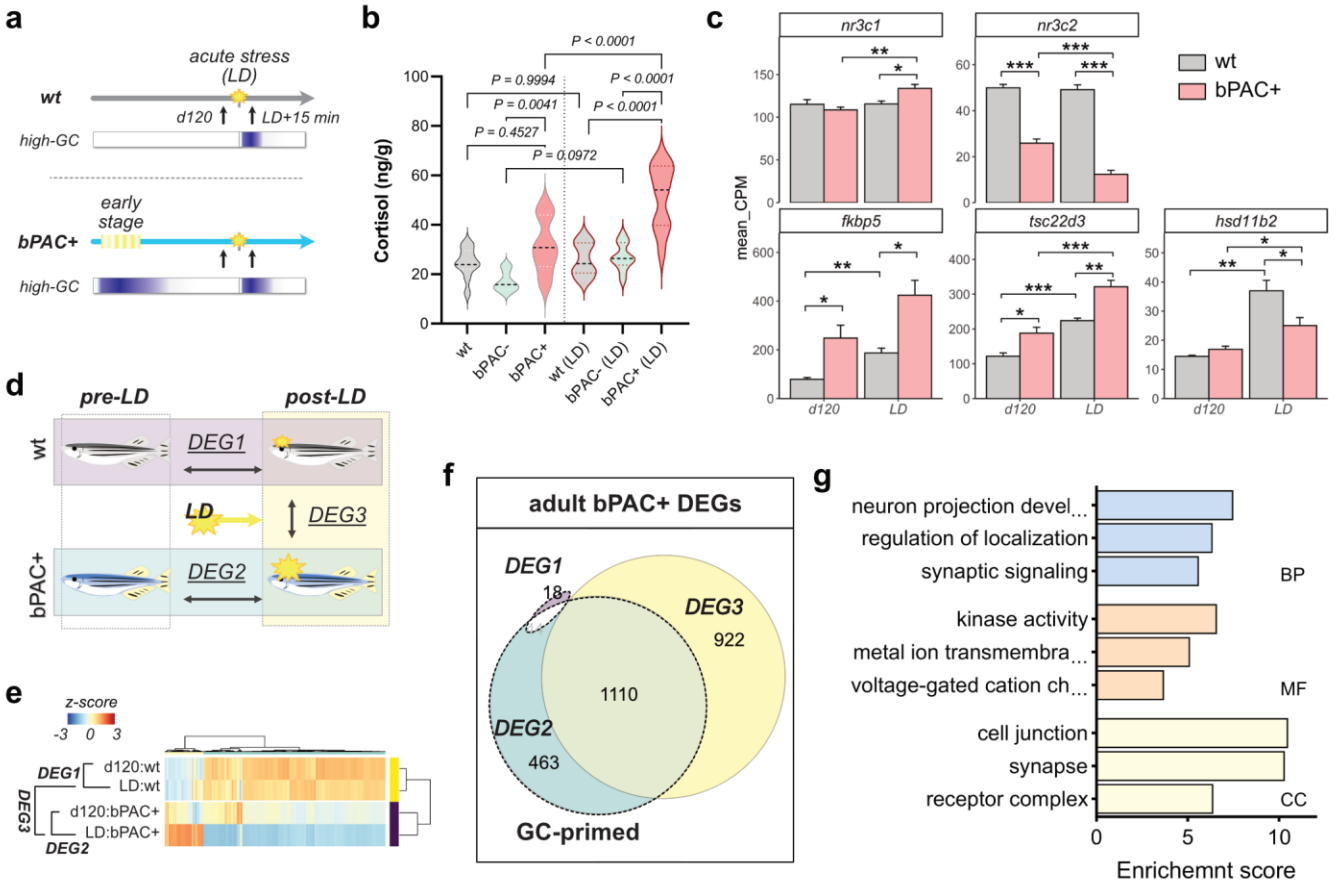
a
acute stress (LD)
wt
d120
LD+15 min
high-GC
early stage
bPAC+
high-GC
b
Cortisol (ng/g)
P < 0.0001
P = 0.9994
P < 0.0001
P = 0.0041
P < 0.0001
P = 0.4527
P = 0.0972
wt
bPAC-
bPAC+
wt (LD)
bPAC- (LD)
bPAC+ (LD)
c
nr3c1
nr3c2
fkbp5
tsc22d3
hsd11b2
mean_CPM
wt
bPAC+
d120
LD
d
pre-LD
post-LD
wt
DEG1
LD
DEG3
bPAC+
DEG2
e
z-score
-3 0 3
DEG1
d120:wt
LD:wt
DEG3
d120:bPAC+
LD:bPAC+
DEG2
f
adult bPAC+ DEGs
DEG1
18
DEG3
922
1110
DEG2
463
GC-primed
g
neuron projection devel...
regulation of localization
synaptic signaling
BP
kinase activity
metal ion transmembra...
voltage-gated cation ch...
MF
cell junction
synapse
receptor complex
CC
0
5
10
Enrichemnt score

Figure 4

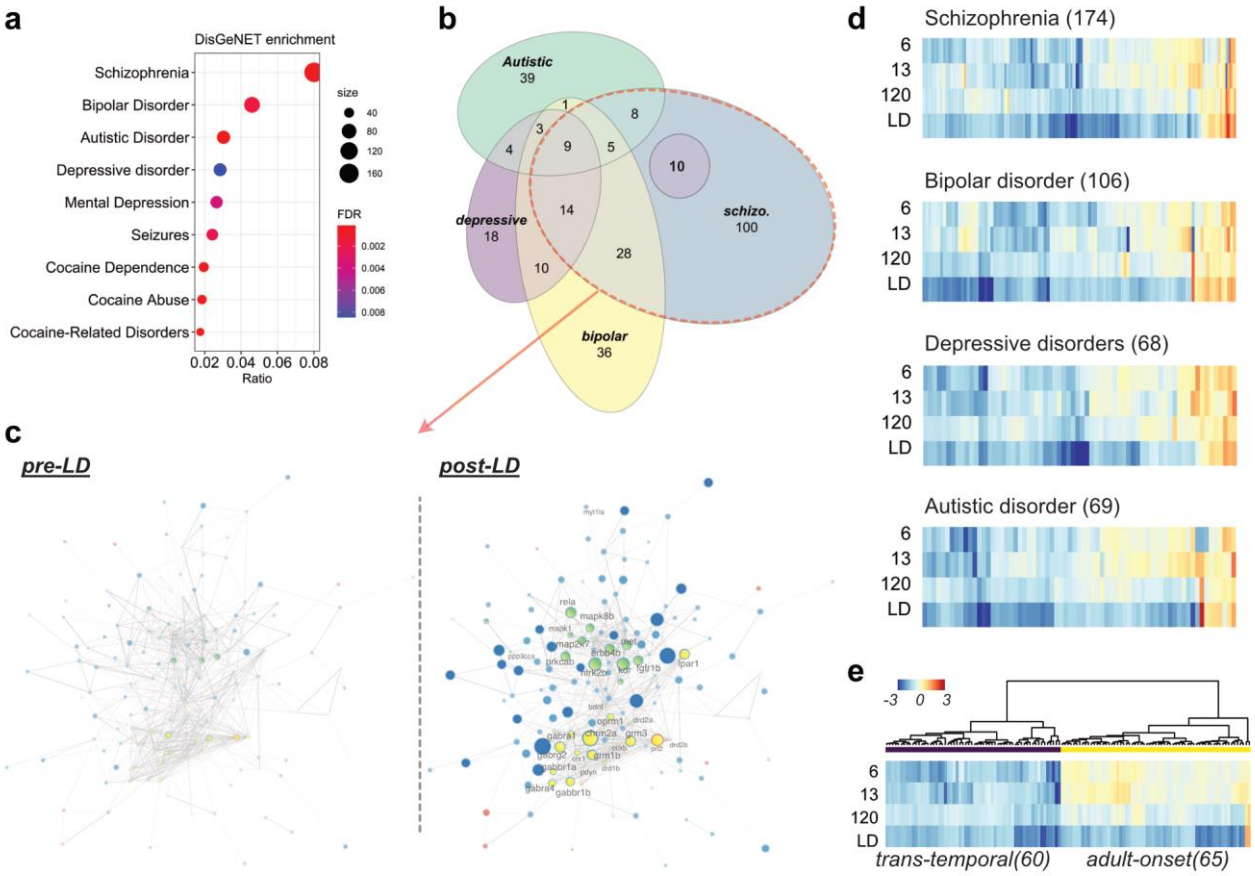
a
DisGeNET enrichment
Schizophrenia
Bipolar Disorder
Autistic Disorder
Depressive disorder
Mental Depression
Seizures
Cocaine Dependence
Cocaine Abuse
Cocaine-Related Disorders
0.02 0.04 0.06 0.08
Ratio
size
40
80
120
160
FDR
0.002
0.004
0.006
0.008
b
Autistic
39
depressive
18
schizo.
100
bipolar
36
1
3
8
4
9
5
10
14
28
10
c
pre-LD
post-LD
d
Schizophrenia (174)
Bipolar disorder (106)
Depressive disorders (68)
Autistic disorder (69)
6
13
120
LD
e
-3 0 3
6
13
120
LD
trans-temporal(60)
adult-onset(65)

Figure 5

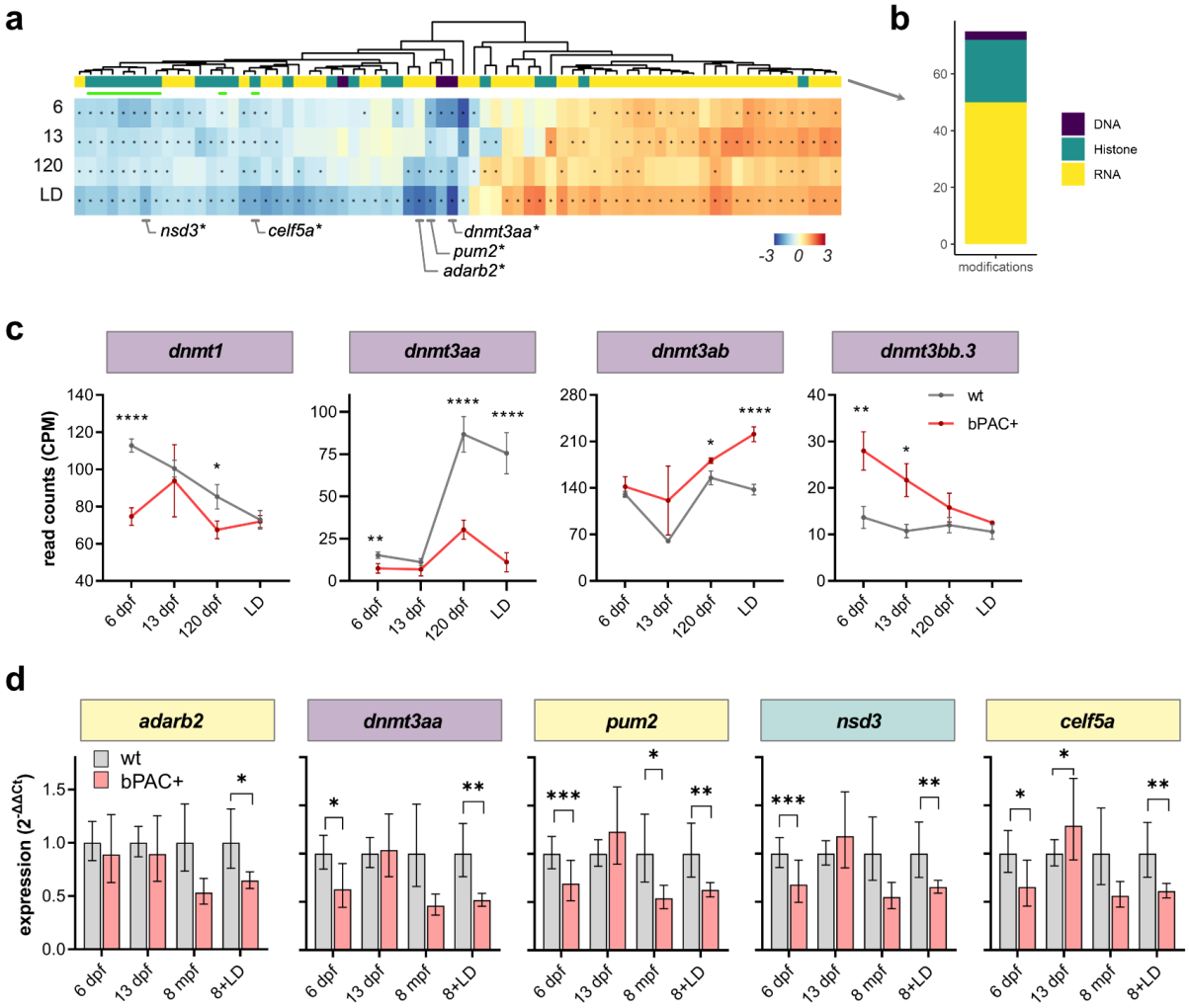
a
6
13
120
LD
nsd3*
celf5a*
dnmt3aa*
pum2*
adarb2*
-3 0 3
b
DNA
Histone
RNA
modifications
c
dnmt1
dnmt3aa
dnmt3ab
dnmt3bb.3
read counts (CPM)
wt
bPAC+
6 dpf
13 dpf
120 dpf
LD
d
adarb2
dnmt3aa
pum2
nsd3
celf5a
expression (2^-ΔΔCt)
wt
bPAC+
6 dpf
13 dpf
8 mpf
8+LD

Figure 6