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1 **Genome-wide analysis of DNA methylation reveals selection signatures of grass**
2 **carp during the domestication**
3
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29 **ABSTRACT**

30 With the rapid development of aquaculture, more and more fish species from wild environments
31 are artificially domesticated and cultured. In the process of domestication, the fish develop some
32 adaptations and phenotypic traits, namely selection signatures. However, it is still unclear about
33 the biological process underlying these selection signatures. Here, we used grass carp
34 (*Ctenopharyngodon idellus*), an aquaculture fish with the largest production worldwide, to detect
35 its selection signatures and investigate the roles of DNA methylation in the emergence of selection
36 signatures during domestication based on whole-genome bisulfite sequencing technology. Our
37 results showed that domesticated grass carp demonstrated four selection signatures, including
38 growth and metabolism, immunity, foraging and learning behaviors, and 38 candidate genes were
39 associated with these traits. 16 of candidate genes, such as IGF-1, GK, GYS1, etc., were found to
40 play major roles in the growth and metabolism. Immunity signature was related to 11 of candidate
41 genes, including MHCI, MHCII, C1QA, etc. The GRM1, TAS1R1 and TAS1R3 genes were
42 essential for the adaptation of domesticated grass carp to commercial feed in artificial rearing
43 condition. The C-FOS, POMC and CBP genes might be responsible for the acquisition of novel
44 feeding habits and contribute to faster growth indirectly by enhancing food intake. These findings
45 would provide new insights to expand our understanding on the role of DNA methylation in
46 shaping physiological phenotypes in fish, and also contribute to efficient breeding of aquaculture
47 stocks and restocking programs.

48

49 *Keywords:*

50 DNA methylation

51 Selection signatures

52 Grass carp

53 Domestication

54 Epigenetics

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58 **1. Introduction**

59 As an economically food source of humans, fish is one of the most important domesticated
60 species. In modern agricultural industry, fish have undergone strong long-term artificial selection
61 and developed a range of adaptations and phenotypic traits, namely selection signatures (Milla et
62 al., 2021). Fish selection signatures usually include the higher flexibility in diet, rapid growth, less
63 stress susceptibility, a more socially tolerant disposition and enhanced prolificacy (Pasquet et al.,
64 2018), and they distinguish the domesticated breeds from their wild counterparts. These
65 phenotypic differences make fish highly suitable for animal agriculture and comparative studies.

66 Epigenetic changes is increasingly recognized to contribute to the emergence of phenotypic
67 differences (Goldberg et al., 2007). DNA methylation, the most widely studied epigenetic
68 regulatory mechanisms, can respond to environmental changes and, at the same time, be stable
69 enough to be maintained throughout lifetime, even across generations (Anastasiadi et al., 2019).
70 DNA methylation has been associated with many biological process, including gene expression
71 regulation, genomic imprinting, X chromosome inactivation, embryonic development, the
72 alteration of chromatin structure and transposon inactivation, etc. (Jones et al., 2012). Compared
73 to other approaches which have been developed to analyze DNA methylation profiles at the
74 genome-wide levels, whole-genome bisulfite sequencing (WGBS) has two major advantages of
75 assessing the methylation state of nearly every CpG site and determining absolute DNA
76 methylation levels (Yong et al., 2016). WGBS has been broadly employed to analyze the
77 genome-wide methylation profiles of many animals, including sea cucumber (Yang et al., 2020)
78 and Chinese perch (Pan et al., 2021).

79 In recent years, some studies explore the roles of DNA methylation regulation on fish during
80 domestication. However, these studies mainly focus on the role of DNA methylation in the
81 changes of one particular phenotypic characteristics, such as growth (Li et al., 2017), reproduction
82 (Podgorniak et al., 2019), morphological changes (Zhang et al., 2017), and learning behaviors
83 (Dou et al., 2018). But, until now, no systematic studies have investigated the roles of DNA
84 methylation in the emergence of different selection signatures in the process of fish domestication,
85 such as growth and metabolism, immunity, foraging and learning behaviors.

86 Grass carp (*Ctenopharyngodon idellus*) is one of the most important freshwater aquaculture

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87 species, and its global production reaches 5.704 million tons in 2018, the highest in fish
88 production worldwide, providing low-cost, high-quality animal protein especially for developing
89 and underdeveloped regions (FAO, 2020). Although some farmers have carried out breeding for
90 several generations, many farmers still rely on wild-caught fish for broodstock to maintain genetic
91 diversity (Fu et al., 2015). Furthermore, farmed grass carp shares some of the characteristics of the
92 domestication syndrome, including the changes in morphological features (Zhao et al., 2020),
93 rapid growth (Ashraf et al., 2011) and anti-predator behavior (Tang et al, 2017), indicating that
94 this species could be a suitable model species for studying the roles of DNA methylation in the
95 formation of selection signatures in fish species.

96 This study used WGBS (whole-genome bisulfite sequencing) to carry out genome-wide DNA
97 methylation analysis of the domesticated and wild grass carp. We obtained comprehensive DNA
98 methylation profiles for the two groups and identified differentially methylated genes (DMGs) that
99 might contribute to the emergence of four selection signatures differences: growth and metabolism,
100 immunity, foraging and learning behaviors. These findings would provide new insights to expand
101 our understanding about the role of epigenetic modifications in shaping physiological phenotypes
102 in fish.

103

104 2. Materials and Methods

105 *2.1 Domesticated trial*

106 A total of 20 wild sub-adult grass carp at the approximately 18-month old determined by
107 annulus characteristics of scale (Cai et al., 2020), were captured using traps and gill nets along the
108 Xijiang river in Zhaoqing City, Guangdong Province, China, within a 1000 m radius from the
109 following coordinates (latitude: 23.1034.8 °N; longitude: 112.4554.0 °E). Subsequently, the fish
110 were transported to breeding base and randomly allocated into two ponds (1.5 m × 1.5 m × 1 m),
111 10 individuals in each pond for subsequent domesticated experiments in the Pearl River Fisheries
112 Research Institute. The fish were fed commercial feed at 8:00 am and 4:00 pm each day for 180
113 days. The water temperature was kept at 22~27 °C, pH was 6.5~7.5, and dissolved oxygen was
114 above 5.0 mg/L.

115 At the first week, the food intake of wild sub-adult grass carp was extremely low.

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116 Subsequently, some wild grass carp started to snatch food during feeding with the feed amount for
117 each day was 0.5~2% of fish weight. After about two month training, the behavior of competition
118 for food was obvious, and the feed amount for each day remained 2~3% of fish weight.

119 *2.2 Sample Collection and Preparation*

120 Three largest domesticated grass carp after six months of domestication were collected from
121 ponds (with a body weight of 680 ± 25 g). Wild grass carp at the approximately 24-month old
122 were collected from same place where wild sub-adult grass carp were captured and weighed to get
123 a body weight of 550 ± 41 g. Then, whole blood was collected from were collected from the
124 domesticated grass carp (sample DGC1-DGC3) and wild grass carp (sample WGC1-WGC3)
125 groups. Subsequently, blood samples were treated with EDTAK2 and then centrifuged at 12,000
126 rpms for 3 min to separate red blood cells (RBCs) from serum.

127 The experimental protocols used in this study were approved by the Laboratory Animal
128 Ethics Committee of Pearl River Fisheries Research Institute, CAFS, China, under permit number
129 LAEC-PRFRI-2021-06-03.

130 *2.3 Serum immune parameter analysis*

131 Blood samples were centrifuged at 3000 g for 15 min at 4 °C and serum was stored at -80 °C
132 for analysis. The activities of lysozyme was determined by the Ultra-Sensitive Fish ELISA Kits
133 (Sino, China) (Kit No. YX-E21980F). Besides, the Ultra-Sensitive Fish ELISA Kits were used to
134 measure the contents of complement C3 (Kit No. YX-E21980F) and total protein (Kit No.
135 YX-E21980F).

136 *2.4 Genomic DNA Extraction and Whole-Genome Bisulfite Sequencing*

137 Blood is the most common source of biomarkers and materials for genetic studies, because it
138 interacts with all organs and is easily collected. Global analysis of methylation profiles using
139 blood DNA has been broadly used to explain phenotypic differences in growth, metabolism,
140 immunity, learning and memory in human and other animals (Wang et al., 2017; Desrivieres et al.,
141 2021). Thus, in this present study, genomic DNA was extracted from RBCs of domesticated grass
142 carp and wild grass carp groups and sent to BGI (BGI Tech Co., Ltd., Shenzhen, China) for
143 whole-genome bisulfite sequencing. For normal WGBS library constructing, the DNA was
144 fragmented by sonication using a Bioruptor (Diagenode, Belgium) to a mean size of

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145 approximately 250 bp, followed by the blunt-ending, dA addition to 3'-end, finally, adaptor
146 ligation(in this case of methylated adaptors to protect from bisulfite conversion), essentially
147 according to the manufacturer's instructions. Ligated DNA was bisulfite converted using the EZ
148 DNA Methylation-Gold kit (ZYMO). Different Insertsize fragments were excised from the same
149 lane of a 2% TAE agarose gel. Products were purified by using QIAquick Gel Extraction kit
150 (Qiagen) and amplified by PCR. At last, Sequencing was performed using the HighSeq4000 or
151 other Illumina platforms.

152 *2.5 Data Filtering and Reads Alignment*

153 After sequencing data was delivered, the raw reads were filtered by removing adaptor
154 sequences, contamination and low-quality reads from raw reads. Low-quality reads include two
155 types, and the reads meet any one of the two conditions will be removed: (1) Unknown bases are
156 more than10%; (2) The ratio of bases whose quality was less than 20was over 10%. After filtering,
157 the Clean data was then mapped to the reference genome of grass carp (Wang et al., 2015) by
158 BSMAP, and then remove the duplication reads and merge the mapping results according to each
159 library. The BSMAP script was BSMAP -a filename_1.clean.fq.gz -b filename_2.clean.fq.gz -o
160 filename.sam - d ref.fa -u -v 8 -z 33 -p4 -n 0 -w20 -s 16 -f 10 -L 100. The sam files were converted
161 to bam files using scripts (samtools view -S-b-o filename.bam filename.sam; samtools sort-m
162 2000000000 filename.bam filename.sort; samtools index filename.sort.bam). The mapping rate
163 and bisulfite conversion rate of each sample were calculated.

164 *2.6 Identification of Differentially Methylated Regions*

165 The methylation level was determined by dividing the number of reads covering each mC by
166 the total reads covering that cytosine (Lister, 2009), which was also equal the mC/C ratio at each
167 reference cytosine (Xiang et al., 2010). The formula is Rmaverge = Nmall/Nmall+Nnmall. Nm
168 represents the reads number of mC, while Nnm represents the reads number of non-methylation
169 reads. Putative DMRs were identified by comparison of the sample DGC and sample WGC
170 methylomes using windows that contained at least 5 CpG (CHG or CHH) sites with a 2- fold
171 change in methylation level and Fisher test p value < = 0.05.

172 *2.7 Gene Ontology and Pathway Enrichment of DMRs*

173 GO enrichment analysis provides all GO terms that significantly enriched in a list of

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174 differentially methylated genes, comparing to a genome background, and filter the differentially
175 methylated genes that correspond to specific biological functions. This method firstly maps all
176 differentially methylated genes to GO terms in the database (http://www.geneontology.org/),
177 calculating gene numbers for every term, then uses hypergeometric test to find significantly
178 enriched GO terms in the input list of differentially methylated genes, based on ' GO ::
179 TermFinder' (http://www.yeastgenome.org/help/analyze/go-term-finder). KEGG pathway
180 enrichment analysis helps to identify significantly enriched metabolic pathways or signal
181 transduction pathways in differentially methylated genes comparing with the whole genome
182 background. The calculating formula is the same as that in GO analysis.

183 *2.8 Protein-protein interaction network construction based on differentially expressed mRNAs*

184 Differentially methylated genes were selected for the construction of protein-protein
185 interaction network. Briefly, potential or confirmed protein interactions were generated and
186 analyzed using the STRING Version 11.0 (https://string-db.org/) based on the zebrafish database,
187 with combined score > 0.4. The PPI network was then visualized using Cytoscape v. 3.8.2
188 software.

189 *2.9. Data analysis*

190 SPSS software (version 20.0) was applied for statistical analysis. All values were expressed
191 as mean ± SE. Data were analyzed by using the Student *t*-test. The *P* value less than 0.05 was
192 considered to be statistically significant.

193

194 **3. Results**

195 *3.1 Serum immune parameters*

196 The levels of serum lysozyme, total protein in domesticated grass carp group were significantly
197 lower than those of wild grass carp group, indicated decreased immunity in domesticated grass
198 carp group (Table 1). The content of serum complement C3 also exhibited a downward tendency
199 in the domesticated grass carp group, but no significant difference was observed between two
200 groups (Table 1).

201 *3.2 Global mapping and statistical analysis of the WGBS reads*

202 We conducted whole-genome bisulfite sequencing of grass carp blood from domesticated grass

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203 carp group and wild grass carp group. After quality control of filtering, a total of 2.36 billion clean
204 reads were generated, consisting of 378.12 million, 387.63 million, and 321.7 million reads for
205 each domesticated grass carp sample and 371.48 million, 493.82 million, and 410.42 million reads
206 for each wild grass carp sample (Table 2). The bisulfite conversion rate (%) of all sequencing
207 libraries ranges from 99.16% to 99.28%. After read alignment, clean reads were mapped to the
208 reference genome of grass carp with mapping rates ranging from 88.03 % to 92.11 % (Table 2).

209 *3.3 Global DNA methylation patterns of domesticated and wild grass carp*

210 The methylation levels of the whole genom were listed in Table 3. In each group, approximately
211 9% of all genomic C sites were methylated. Methylation in grass carp was found to exist in three
212 sequence contexts: CG, CHG (where H is A, C, or T), and CHH. The average methylation levels
213 of CG, CHG, and CHH at the whole genome levels were 80.3%, 1.09%, and 1.08% in the
214 domesticated grass carp group, and 75.04%, 1.07%, and 1.13% in the wild grass carp group. The
215 average methylation of CG showed a significantly increased within genome in the domesticated
216 grass carp group compared with the wild grass carp group ($p < 0.05$). The methylation level of
217 CHG and CHH showed no differences between domesticated grass carp and wild grass carp
218 groups ($p > 0.05$).

219 Methylation status of CG, CHG and CHH of grass carp genome showed the methylome's
220 overall characteristics (Fig.1). The methylation levels of approximately 25% of all mCG were
221 hypermethylated (methylation level > 90%). However, only about 3% of mCHH and mCHG were
222 hypermethylated (methylation level > 90%) compared with mCG. The methylated Cs mostly
223 occur in the form of mCG, followed by mCHH and mCHG. The methylation level distribution of
224 mC and mCG were alike. The methylated Cs mostly occur in the form of mCG; approximately 96%
225 of all detected mCs (Table 4). Proportion of mCHG range from 0.75% to 1.15%, and proportion of
226 mCHH range from 2.7% to 3.05% (Table 4). The proportion of mCG, mCHG and mCHH showed
227 no significant difference between domesticated grass carp group and wild grass carp group ($p >$
228 0.05).

229 *3.4 DNA methylation levels of different genomic features*

230 The heatmap presents the methylation landscape in different genomic features (whole
231 genome, CGI, downstream 2 kb, upstream 2 kb, mRNA, repeat, CDS, exon), providing additional

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232 information as well as a global assessment of some of these components (Fig.2). CpG islands
233 contained the highest numbers of CpG sites (approximately 10 - 20 CpG sites in a 200 bp window)
234 compared with other genomic features. About 60% of CpG sites in CpG islands were
235 hypermethylated (methylation levels >90%) in the heatmap (Figure2 and Supplementary Material
236 Figure S2). The other genomic features generally contained 0 - 10 CpG sites in the 200 bp window.
237 A lower proportion of CpG sites within whole genome, mRNA and repeat were hypomethylated
238 (methylation levels < 10%) than CpG sites within remaining genomic features (CGI, downstream
239 2 kb, upstream 2 kb, CDS, exon).

240 *3.5 DNA methylation patterns across the entire transcriptional units at whole genome level*

241 In order to reveal the relationship between DNA methylation profiles and genes expression in
242 detail, Canonical DNA methylation profiles of the entire transcriptional units were divided into
243 distinct functional elements to study the changes of methylation levels in different features (Fig.3).
244 Methylation differences between CG and non-CpG methylation (CHG and CHH) are visible
245 (Fig.3), as methylation levels of CG are higher than those of CHG and CHH across the entire
246 transcriptional units. Another feature is a modest elevation in methylation level at internal exons
247 and internal intron during transcriptional unit scanning. The lowest methylation level occurs in the
248 first intron, followed by the first exon and downstream.

249 *3.6 Identification and enrichment analysis of differential methylated regions*

250 To characterize the differences of genome methylation levels between domesticated grass
251 carp and wild grass carp groups, DMRs and differentially methylated genes (DMGs) were
252 detected. For CG context methylation, a total of 533,235 DMRs were identified between the two
253 groups, which corresponded to 20,010 DMGs in promoter regions and 27,016 DMGs in gene
254 body.

255 GO enrichment analysis of DMGs was performed to provide significantly enriched GO terms
256 corresponding to specific biological process, cellular component, and molecular function (Fig.4).
257 In promoter regions, the over-represented GO terms in the biological process are cellular process,
258 metabolic process, and biological regulation. The top enriched GO terms in the cellular
259 component are cellular anatomical entity, intracellular and protein−containing complex. In terms

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260 of molecular function, the top enriched GO terms are binding, catalytic activity, and molecular
261 transducer activity. In gene body region, the over-represented GO terms in the biological process
262 are cellular process, metabolic process and biological regulation. The top enriched GO terms in
263 the cellular component are cellular anatomical entity, intracellular and protein-containing complex.
264 In terms of molecular function, the top enriched GO terms are binding, catalytic activity and
265 transporter activity. KEGG pathway analysis, which is an alternative approach to categorize gene
266 functions, was also conducted for the DMGs in promoter and gene body regions. In promoter
267 region, DMGs were significantly enriched in pathways in cancer, neuroactive ligand−receptor
268 interaction and PI3K−Akt signaling pathway. In gene body region, DMGs were significantly
269 enriched in metabolic pathways, pathways in cancer and MAPK signaling pathway (Fig.5).

270 *3.7 Candidate DMGs associated with selection signatures.*

271 Many transcriptome and association studies have explored the molecular mechanisms
272 underlying selection signatures in fish and other species, laying a foundation for our investigation
273 of the involvement of DMGs in selection signatures. Besides, it is reported that DNA methylation,
274 especially in the promoter regions, usually affects gene expression by different modes (Moore et
275 al., 2012). Thus, 38 candidate DMGs in promoter region associated with the four selection
276 signatures (growth and metabolism, immunity, foraging and learning behaviors), were identified
277 according to the following criteria: (1) genes were differentially methylated in grass carp and wild
278 grass carp groups; (2) genes were enriched in pathways related to selection signatures; (3) genes
279 were differentially expressed in fish and other species or related with selection traits reported by
280 previous studies. The 38 DMGs were used to construct the protein-protein interaction network
281 using STRING database. We first obtained 107 pairs of interaction between 38 DEGs, and then
282 these DMGs were functionally grouped according to KEGG pathway information (Fig.6). The
283 network was divided into four parts, including foraging behaviors, learning behaviors, immunity
284 and growth and metabolism. Among them, the network of learning behaviors was core and
285 connected to the three other networks.

286

287 **4. Discussion**

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288 Domestication is a process by which humans select some phenotypes of wild animal species
289 (i.e., morphological traits or growth). During the domestication process, some phenotypic traits of
290 animal could be altered by the artificial selection to help domesticated animal species adapt to new
291 environmental conditions (Sylvain et al., 2021). DNA methylation, one of the most important and
292 stable epigenetic modifications in eukaryotes, can lead to heritable phenotypic and transcriptomic
293 changes (Höglund et al., 2020). However, in aquatic species, there is still limited research that
294 illustrate the effects of DNA methylation on selection signatures by comparing genome-wide
295 methylation profiles between domesticated and wild fish species. Our study first systematically
296 compared the genome-wide methylation profiles from domesticated and wild grass carp and
297 identified some key differentially methylated genes related to selection signatures (growth and
298 metabolism, immunity, foraging and learning behaviors) to uncover its genetic characters.

299 *4.1 DNA methylation profiles in domesticated and wild grass carp*

300 In the present study, we reported a genome-wide examination of DNA methylation in
301 domesticated grass carp and wild grass carp. The mapping rates of clean reads ranged from 86.25%
302 to 92.11% (Table 2), which is above the average value reported in other fish species (Pan et al.,
303 2021). However, the WGBS results in this study were still consistent because bisulfite conversion
304 reduces the complexity of the genomic sequence and the ability of most computational programs
305 to align sequences onto the reference genome (Yang et al., 2020).

306 Further DNA methylation profile analysis was also conducted in our study across the distinct
307 genomic features and entire transcriptional units. Our results showed that the genome-wide
308 methylation patterns were similar between two groups. Approximately 9% of all total cytosines
309 and 80% of CG were methylated and only a small proportions of methylation at non-CpG
310 methylation (CHG and CHH) was observed within all regions, which is consistent with previous
311 study (Cai et al., 2020). The high level of DNA methylation in CG content was a specific
312 characteristic of animals. DNA methylation in CHH and CHG patterns is a major characteristic of
313 plant methylomes and largely absent in animal methylomes (Zemach et al., 2010).

314 A modest elevation in methylation level at internal exons and intron was observed during
315 transcriptional unit scanning. The lowest methylation level occurs in the first exons, followed by
316 the first intron and last exon. These results was consistent with previous findings in grass carp

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317 (Cai et al., 2020), suggesting that mutagenic effects appear to occur at the first exons and internal
318 exon and intron tend to be influenced by the regulatory impact of DNA methylation.

319 *4.2 Key differentially methylated genes related to growth and metabolism*

320 Domestication is a long process which forces animals to adapt to captivity by modifying an
321 animal' s growth and metabolism, immune response, foraging and social behaviors. In the process
322 of domestication, fish undergo great changes in the growth and metabolism due to the difference
323 of environmental conditions and food resource (Teletchea, 2016), which is closely associated with
324 the differential expression of genes involved in growth and metabolism (Shen et al., 2021). In this
325 study, several genes involved in growth and metabolism, such as IGF-1 (Insulin-like growth
326 factors -1), GK (Glycerol kinase), GYS1 (Glycogen synthase 1), FASN (Fatty acid synthase,
327 animal type), etc., exhibited hypomethylation in domesticated grass carp compared to wild grass
328 carp. On the other hand, some genes related to growth and metabolism exhibited hypermethylation
329 in domesticated grass carp, including G6PC (Glucose-6-phosphatase), PCK1
330 (Phosphoenolpyruvate carboxykinase 1), FBP1 (Fructose-1,6-bisphosphatase 1), FOXO1
331 (Forkhead box protein O1), ACAA1 (Acetyl-Coenzyme A acyltransferase 1) and CPT1 (Carnitine
332 palmitoyltransferase I).

333 IGF-1, an important growth hormone, mediates the anabolic and linear growth and is thought
334 to be a candidate gene for regulating muscle growth (Laron et al., 2001). GK is a key enzyme that
335 catalyzes the first step in glycolysis (Enes et al., 2009). FOXO1, G6PC, FBP1 and PCK1 are key
336 enzymes of gluconeogenesis (Lu et al., 2018). Our results indicated that, compared to wild grass
337 carp, the glycolytic capacity was enhanced and the capacity for gluconeogenesis was inhibited in
338 domesticated grass carp feeding commercial diet. These results was consistent with the previous
339 findings that high-carbohydrate diets induce glycolysis and inhibit gluconeogenesis (Liu et al.,
340 2021). GSY1 is a rate-limiting enzyme in glycogen synthesis and plays an important role in the
341 synthesis of glycogen in the muscle (Martins et al., 2013). These results suggested that the
342 capacity for muscle glycogen storage in domesticated grass carp possibly increased compared to
343 that of wild grass carp.

344 FASN is believed to be the central enzyme of hepatic lipid accumulation (Dorn et al., 2010).
345 ACAA1 and CPT1 play a certain role in the lipid degradation (Song et al., 2019). In the present

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346 study, our results suggested that, in domesticated grass carp, the ability to synthesize fat was
347 enhanced and lipid degradation was inhibited compared to wild grass carp, which may account for
348 elevated lipid levels of muscle in domesticated grass carp feeding commercial feed (Ashraf et al.,
349 2011). According to the results, an explanation for the enhanced fat deposition in fish might be
350 excessive energy intake in commercial diet (Wu et al., 2021). Overall, DNA methylation was
351 likely to play an important role in regulating growth and metabolism of domesticated grass carp
352 by influencing these gene expression.

353 *4.3 Key differentially methylated genes related to immunity*

354 To maximize profitability, domesticated fish were cultured in intensive farming conditions
355 with limited space, high density and other stressors, which influence fish immune response
356 negatively and even result in large-scale disease (Lin et al., 2018). It was reported that, in the
357 process of domestication, the immune status of domesticated fish is affected negatively due to
358 increased chronic stress by confinement, and makes fish more susceptible to pathogens and
359 ultimately impair fish survival (Mandiki et al., 2011).

360 In this study, several immune related genes exhibited hypermethylation in domesticated grass
361 carp compared to wild grass carp, including MHCI (MHC class I), MHCII (MHC class II), C1QA
362 (Complement C1q subcomponent subunit A), C3 (Complement component 3), C4A (Complement
363 component 4), C5 (Complement component 5), IFN-γ (Interferon gamma), etc. These genes
364 suggested the expression of these genes might be downregulated. MHCI and MHCII are two cell
365 surface proteins essential for the acquired immune system for antigen presentation to recognize
366 foreign molecules in vertebrates and can promote the development and expansion of T cells
367 (Huang et al., 2005). The complement system, an essential part of both innate and adaptive
368 immunity in teleosts, is initiated by one or a combination of three pathways, the alternative, lectin
369 and classical (Yang et al., 2016). IFN-γ, one of critical antiviral cytokines, modulate functions of
370 the immune system by up-regulate major histocompatibility complex molecules (MHC I and
371 MHC II) and directly activate other immune cells, such as macrophages and natural killer cells
372 (Borden et al., 2007). In our results, these immune related genes mentioned above identified in
373 domesticated grass carp exhibited hypermethylation, indicating a poor immune performance of
374 grass carp during long-term domestication, which was consistent with the results of immune

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375 parameters that decreased levels of serum lysozyme, total protein in domesticated grass carp group
376 reflected a reduction tendency in immune response (Lin et al., 2018).

377 *4.4 Key differentially methylated genes related to foraging behaviors*

378 During the domestication process, fish change their foraging habits to obtain food in captive
379 conditions (Pasquet et al., 2018). In this study, foraging behaviors-related genes were also found to
380 be under strong selection in domesticated carp. The genes of GRM1 (Metabotropic glutamate
381 receptor 1), TAS1R1 (Taste receptor type 1 member 1) and TAS1R3 (Taste receptor type 1
382 member 3) exhibited hypomethylation in domesticated grass carp group compared to wild grass
383 carp group, suggesting the expression of these genes might be upregulated. The TAS1R1 and
384 TAS1R3 heterodimer receptor functions as an umami receptor, responding to L-amino acid
385 binding, especially L-glutamate (Nelson et al., 2001). GRM1, which is widely expressed
386 throughout the central nervous system and regulates synaptic signaling, is another L-glutamate
387 receptor (Gabriel et al., 2009). Generally, these taste receptors play an important roles in
388 perception of L-amino acids and feeding behavior (Cai et al., 2021). Thus, these genes with
389 hypomethylation were likely to be upregulated in domesticated grass carp, facilitating the
390 adaptation of domesticated grass carp to commercial feed by enhancing taste perception of
391 nutrients in commercial diet in artificial rearing condition.

392 *4.5 Key differentially methylated genes related to learning behaviors*

393 Learning and memory enable the organism to plastically respond to the changing
394 environment. Increasing research has investigated the learning (cognitive) and memory
395 characteristics of fish in the past few decades, including spatial cognition, learned recognition,
396 social learning, foraging activity, etc. (Dou et al., 2018). In this study, through domestication,
397 social learning-related genes have also been under selection pressure. The gene POMC
398 (Pro-opiomelanocortin) exhibited hypermethylation. In contrast, the genes of C-FOS
399 (Proto-oncogene C-FOS) and CBP (CREB-binding protein) exhibited hypomethylation in
400 domesticated grass carp group compared to wild grass carp group, suggesting the expression of
401 these genes might be upregulated. C-FOS is necessary for consolidation of non-spatial
402 hippocampal-dependent memory (Countryman et al., 2005), and C-FOS mRNA expressions are
403 up-regulated in response to a variety of neuronal activation protocols, including behavioral

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404 training (Smulders et al., 2000) and long-term protentiation (Alberini et al., 2009). CBP is a
405 coactivator of transcription that play an essential role in memory consolidation (Korzus et al.,
406 2004). It was reported that gene expression of C-FOS and CBP facilitated the acquisition of novel
407 feeding habits in fish through the memory formation (Peng et al., 2019; Dou et al., 2018). Besides,
408 the C-FOS gene might be an important transcriptional factor to inhibit the expression of the
409 anorexigenic gene POMC, resulting in an increase of the food intake of dead prey fish in mandarin
410 fish (Peng et al., 2019). Therefore, it tempts us to speculate that the learning-related genes of
411 C-FOS and CBP may be responsible for the acquisition of novel feeding habits by reinforcing
412 memory in domesticated grass carp, which considerably increase the rate of domestication.
413 Furthermore, individual food intake is enhanced through the interaction between the learning gene
414 C-FOS and the appetite control gene POMC, contributing to the faster growth of domesticated
415 grass carp indirectly.

416

417 **Declaration of competing interest**

418 The authors declare that they have no known competing financial interests or personal
419 relationships that could have appeared to influence the work reported in this paper.

420

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428 ## References

429 Alberini, C. M., 2009. Transcription factors in long-term memory and synaptic plasticity. Physiol
430 Rev. 89(1), 121-145.
431 Ashraf, M., Zafa, A., Rauf, A., Mehboob, S., Qureshi, N. A., 2011. Nutritional values of wild and
432 cultivated silver carp (Hypophthalmichthys molitrix) and grass carp (*Ctenopharyngodon*

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433 *idella*). Int J Agric Biol. 13(2), 1560-8530.

434 Borden, E. C., Sen, G. C., Uze, G., Silverman, R. H., Ransohoff, R. M., Foster, G. R., 2007.
435 Interferons at age 50: past, current and future impact on biomedicine. Nat Rev Drug Discov.
436 6(12), 975-990.

437 Cai, Z. L., Duan, Y. J., Xu, J. C., He, Y., Geng J. Z., Liu, X., Han, M. X., Liu, Y. H., 2020
438 Comparison of five structure materials in age determination of Amur ide Leuciscus waleckii
439 in Dali Lake. Journal of Dalian Ocean University. 35(1), 2019-183

440 Cai, W. J., Liang, X. F., Yuan, X. C., Li, A. X., He, S., 2020. Changes of DNA methylation pattern
441 in metabolic pathways induced by high-carbohydrate diet contribute to hyperglycemia and fat
442 deposition in grass carp (*Ctenopharyngodon idellus*). Front Endocrinol. 11, 398.

443 Cai, W. J., Li, J., Li, L., Chen, X., Liang, X. F., 2021. Knockout of tlrl gene in zebrafish (*Danio*
444 *rerio*) by crispr/cas9 reveals its roles in regulating feeding behavior. Aquaculture. 545,
445 737189.

446 Christoph, D., Marc-Oliver, R., Georgi, K., Michael, S., Kathrin, S., Weiss, T. S., 2010.
447 Expression of fatty acid synthase in nonalcoholic fatty liver disease. Int J Clin Exp Patho.
448 3(5), 505.

449 Countryman, R.A., Kaban. N.L., Colombo, P.J. 2005. Hippocampal c-fos is necessary for
450 long-term memory of a socially transmitted food preference. Neurobiol Learn Mem. 84(3),
451 175-183.

452 Dafni, A., Francesc, P., 2019. Epimutations in developmental genes underlie the onset of
453 domestication in farmed European sea bass. Mol Biol Evol. 10, 10.

454 Desrivières S., Jia T. Y., Chu C. Y., Liu Y., 2021. Epigenome-wide meta-analysis of blood DNA
455 methylation and its association with subcortical volumes: findings from the ENIGMA
456 Epigenetics Working Group. Mol Psychiatr. 26, 3884-3895

457 Dou, Y., He, S., Liang, X. F., Cai, W., Wang, J., Shi, L., 2018. Memory function in feeding habit
458 transformation of mandarin fish (*Siniperca chuatsi*). Int J Mol Sci. 19(4), 1254.

459 Doi, A., Park, I. H., Wen, B., Murakami, P., Aryee, M. J., Irizarry, R., 2009. Differential
460 methylation of tissue- and cancer-specific CpG island shores distinguishes human induced

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461 pluripotent stem cells, embryonic stem cells and fibroblasts. Nat Genet. 41(12), 1350.
462 Enes, P., Panserat, S., Kaushik, S., Oliva-Teles, A. A., 2008. Nutritional regulation of hepatic
463 glucose metabolism in fish. Fish Physiol Biochem. 35(3), 519-539.
464 FAO, 2020. The State of World Fisheries and Aquaculture 2020. Sustainability in Action. Rome.
465 Fu, J., Shen, Y., Xu, X., Liu, C., Li, J., 2015. Genetic parameter estimates and genotype by
466 environment interaction analyses for early growth traits in grass carp (*Ctenopharyngodon*
467 *idella*). Aquacult Int. 23(6), 1427-1441.
468 Gabriel, A. S., Maekawa, T., Uneyama, H., Torii, K., 2009. Metabotropic glutamate receptor type
469 1 in taste tissue. Am J Clin Nutr. 90(3), 743S-746S.
470 Goldberg, A. D., Allis, C, D., Bernstein, E., 2007. Epigenetics: a landscape takes shape. Cell.
471 128(4), 635-638.
472 Pasquet, A., 2018. Effects of domestication on fish behavior. Intechopen.78752.
473 Höglund, A., Henriksen, R., Fogelholm, J., Churcher, A. M., Guerrero-Bosagna, C. M.,
474 Martinez-Barrio, A., 2020. The methylation landscape and its role in domestication and gene
475 regulation in the chicken. Nat Ecol Evol. 4, 1713-1724.
476 Huang, S., Gilfillan, S., Cella, M., Miley, M. J., Lantz, O., Lybarger, L., Fremont, D., Hansen, T.
477 H., 2005. Evidence for mr1 antigen presentation to mucosal-associated invariant T cells. J
478 Biol Chem. 280(22), 21183-21193.
479 Jones, P. A., 2012. Functions of DNA methylation: islands, start sites, gene bodies and beyond.
480 Nat Rev Genet. 13(7), 484-492.
481 Korzus, E., Rosenfeld, M. G., Mayford, M., 2004. CBP histone acetyltransferase activity is a
482 critical component of memory consolidation. Neuron. 42(6), 961-972.
483 Laron, Z., 2001. Insulin-like growth factor 1 (igf-1): a growth hormone. Mol Pathol MP. 54 (5),
484 311.
485 Lister, R., Pelizzola, M., Dowen, R. H., Hawkins, R. D., Hon, G., Tonti-Filippini, J., 2009. Human
486 DNA methylomes at base resolution show widespread epigenomic differences. Nature.
487 462(7271), 315-22.
488 Li, S. P., He, F., Wen, H. S., Li. J. F., Si, Y. F., Liu, M. Y., He. H. W., Huang, Z. J., 2017. Analysis
489 of DNA methylation level by methylation-sensitive amplification polymorphism in half

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490 smooth tongue sole (*Cynoglossus semilaevis*) subjected to salinity stress. J. Ocean Univ.
491 China. 16(2), 269-278.
492 Li, C, L., Zuo, W. D., Tong, X. L., Han, M. J., Gao, R., Hu, H., Lu, K. P., Luan, Y., Zhang, B, L.,
493 Liu, Y. Y., Dai, F. Y., 2020. Whole-genome resequencing reveals loci under selection during
494 silkworm improvement. 138, 278-290.
495 Lin, W., Li, L., Chen, J., Li, D. P., Hou, J., Guo, H. H., Shen, J. Z., 2018. Long-term crowding
496 stress causes compromised nonspecific immunity and increases apoptosis of spleen in grass
497 carp (*Ctenopharyngodon idella*). 80, 540-545.
498 Fang, L., Guo, X.Z., Liang, X.F., 2021. First feeding of grass carp (*Ctenopharyngodon idellus*)
499 with a high-carbohydrate diet: the effect on glucose metabolism in juveniles. Aquacult Rep.
500 21, 100830.
501 Lu, S., Wu, X., Gao, Y., Gatlin, D. M., Wu, M., Yao, W., 2018. Effects of dietary carbohydrate
502 sources on growth, digestive enzyme activity, gene expression of hepatic gluts and key
503 enzymes involved in glycolysis-gluconeogenesis of giant grouper *Epinephelus lanceolatus*
504 larvae. Aquaculture. 343-350.
505 Martins, F. O., Rito, J., Jarak, I., Viegas, I., Jones, J. G., 2013. Disposition of [U-2H7] glucose into
506 hepatic glycogen in rat and in seabass. CBPA. 166 (2).
507 Mandiki, S., Henrotte E., Milla S., Douxfils J., Wang N., Rougeot, C., 2011. How physiological
508 status and immune defense are affected by photo-thermal regimes and domestication process
509 in captive Eurasian perch? ISEE. 4(S8), 298–299.
510 Milla, S., Pasquet, A., Mohajer, L. E., Fontaine, P., 2021. How domestication alters fish
511 phenotypes. Rev Aquacult. 13, 388-405.
512 Moore, L. D., Le, T., Fan, G., 2012. DNA methylation and its basic function.
513 Neuropsychopharmacology. 38(1), 23-38.
514 Nelson, G., Hoon, M. A., Chandrashekar, J., Zhang, Y. F., Nicholas, J. P. R., Zuke, C. S., 2001.
515 Mammalian sweet taste receptors. Cell. 106, 381-390.
516 Pan, Y. X., Chen, L., Cheng, J., Zhu, X., Wu, P., Bao, L. S., Chu, W. Y., He, S., Liang, X. F.,
517 Zhang, J. S., 2021. Genome-wide DNA methylation profiles provide insight into epigenetic
518 regulation of red and white muscle development in Chinese perch *Siniperca chuatsi.* Comp

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519 Biochem Phys B. 256, 110647
520 Pasquet, A., 2018. Effects of domestication on fish behaviour. DOI: 10.5772/intechopen.78752.
521 Song, M., Zhong, Y., Guo, J., Chen, Y., Luo, L., Lin, S., 2019. Effects of dietary lipid levels on the
522 expression of cpt1 in *Micropterus salmoides*. Journal of Fisheries of China. 43, 2166-2174.
523 Smulders, T. V., Devoogd, T. J., 2000. Expression of immediate early genes in the hippocampal
524 formation of the black-capped chickadee (*Poecile atricapillus*) during a food-hoarding task.
525 Behav Brain Res. 114(1), 39-49.
526 Shen, Y. W., Li, H. Y., Zhao, J. L., Tang, S. J., Chen, X. W., 2021. The digestive system of
527 mandarin fish (*Siniperca chuatsi*) can adapt to domestication by feeding with artificial diet.
528 Aquaculture. 538, 736546.
529 Tang, L. J., Jacquin, L., Lek, S., Liu, H. Z., Li, Z. J., Liu, J. S., Zhang, T. L., 2017. Differences in
530 anti-predator behavior and survival rate between hatchery-reared and wild grass carp
531 (*Ctenopharyngodon idellus*). Ann Limnol Int. 53, 361-367.
532 Teletchea, F., 2016. Is fish domestication going too fast? Nat Resour. 07(6), 399-404.
533 Podgorniak, T., Brockmann, S., Konstantinidis, I., Fernandes, J. M. O., 2019. Differences in the
534 fast muscle methylome provide insight into sex-specific epigenetic regulation of growth in
535 Nile tilapia during early stages of domestication. Epigenetics. 14(8), 818-836.
536 Wang, H. F., Wang, J. Y., Ning. C., Zheng, X. R., Fu, J. L., 2017. Genome-wide DNA methylation
537 and transcriptome analyses reveal genes involved in immune responses of pig peripheral
538 blood mononuclear cells to poly I: C. Sci Rep. 7(1), 9709.
539 Wu, B.L., Huang, L., Chen, X. J., Zhang, Y., Chen, X. J., Wu, C. C. Deng, X. J., Gao, J., He, J. X.,
540 2021. Effects of feeding frequency on growth performance, feed intake, metabolism and
541 expression of fgf21 in grass carp (*Ctenopharyngodon idellus*). Aquaculture. 545 (15),
542 737196.
543 Wang, Y. P., Lu, Y., Zhang, Y., Ning, Z. M., Li, Y., Zhao, Q., Lu, H. Y., 2015. The draft genome of
544 the grass carp (*Ctenopharyngodon idellus*) provides insights into its evolution and vegetarian
545 adaptation. Nat Genet. 47, 625-631.
546 Xiang, H., Zhu, J. D., Chen, Q., Dai, F. Y., Li, X., Wang, J., 2010. Single base-resolution
547 methylome of the silkworm reveals a sparse epigenomic map. Nat Biotechnol. 28 (5),

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548 516-20.

549 Yang, Y. J., Zheng, Y. Q., Sun, L. N., Chen, M. Y., 2020. Genome-wide DNA methylation
550 signatures of sea cucumber *Apostichopus japonicus* during environmental induced aestivation.
551 Genes. 11 (9), 1020.

552 Yang, Y., Yu, H., Li, H., Wang, A, L., Yu, H. Y., 2016. Effect of high temperature on immune
553 response of grass carp (*Ctenopharyngodon idellus*) by transcriptome analysis. Fish Shellfish
554 Immun. 58, 89-95.

555 Yong, W. S., Hsu, F. M., Chen, P. Y., 2016. Profiling genome-wide DNA methylation. Epigenet
556 Chromatin. 9 (1), 26.

557 Zemach, A., McDaniel, I. E, Silva, P., Zilberman, D., 2010. Genome-wide evolutionary analysis of
558 eukaryotic DNA methylation. Science. 328, 916.

559 Zhang, Y. Q., Liu, J. H., Fu, W., Xu, W. T., Zhang, H. Q., Chen, S, J., Xiao, Y. M., 2017.
560 Comparative transcriptome and DNA methylation analyses of the molecular mechanisms
561 underlying skin color variations in Crucian carp (*Carassius carassius* L). BMC Genet. 18 (1),
562 95.

563 Zhao, Y. M., Zhang, L., Wang, C. F., Xie, C. X., 2020. Biology and ecology of grass carp in china:
564 a review and synthesis. N Am J Fish Manage. 40 (6), 1379-1399.

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566 **Figures Captions**

567 **Fig.1** Distribution of methylation levels of mC in each sequence context .The x-axis was defined
568 as the percentage of reads mC at a reference cytosine site. The y-axis indicated the fraction of total
569 mC calculated within bins of 10%. DGC, domesticated grass carp; WGC, wild grass carp.

570 **Fig.2** Heat maps of distinct methylation and CpG density patterns. CpG density (x-axis) was
571 defined as numbers of CpG dinucleotides in 200bp windows. Methylation level (y- axis) was
572 defined as average methylation level of cytosines in CpGs. The thin black lines within each heat
573 map denoted the median methylation level of CpGs at the given local density. The red color

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574 gradient indicated abundance of CpGs that falled into bins of given methylation levels and CpG
575 densities. The blue bar charts above each heat map showed the distribution of CpG densities,
576 projected onto the x-axis of the heat maps. The green bar charts to the right of the heat maps show
577 the distribution of methylation levels, projected onto the y-axis of the heat maps. (A) domesticated
578 grass carp (DGC) sample 1; (B) sample DGC2; (C) sample DGC3; (D) wild grass carp (WGC)
579 sample 1; (E) sample WGC2; (F) sample WGC3.

580 **Fig.3** DNA methylation patterns across the entire transcriptional units at whole genome level. The
581 canonical gene structure was defined by 7 different features, denoted by the x-axis. The length of
582 each feature was normalized and divided into equal numbers of bins. Each dot denoted the mean
583 methylation level per bin and the respective lines denoted the 5-bin moving average. Each feature
584 was analyzed separately for the numbers listed in the table below the figure. The green vertical
585 line indicated the mean location of the transcription start sites. DGC, domesticated grass carp;
586 WGC, wild grass carp.

587 **Fig.4** GO analysis of differentially methylated regions (DMRs)-related genes. The x-axis
588 represented three domains of GO and the y-axis represented the gene number in each pathway and
589 process. (A) GO analysis of DMRs-related genes in promoter region; (B) GO analysis of
590 DMRs-related genes in gene body region.

591 **Fig.5** Pathway analysis of differentially methylated regions (DMRs)-related genes. The abscissa
592 represented the richness factor, and the ordinate represented the enriched pathway terms. Q-value
593 represented the corrected P, and a small Q-value indicated high significance. (A) Pathway analysis
594 of DMRs-related genes in promoter region; (B) Pathway analysis of DMRs-related genes in gene
595 body region.

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596 **Fig.6** Protein-protein interaction networks of differentially methylated regions in different
597 selection signatures.
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609 **Table 1**. The four selection signatures of domesticated and wild grass cap

Selection signatures		Domesticated grass carp	Wild grass carp
Growth and metabolism	Initial weight (g)	250 ± 18	
	Final weight (g)	680 ± 27[a]	550 ± 41[b]
Immunity	Lysozyme (U/ml)	85.3 ± 3.52[b]	97.26 ± 3.42[a]
	Total protein (g/L)	0.08 ± 0.01[a]	0.1 ± 0.01[a]
	Complement C3 (g/L)	32.18 ± 1.64[b]	37.14 ± 1.05[a]
Foraging behaviors and learning behaviors		At the first week of domestication, the food intake of wild grass carp was extremely low. Subsequently,	

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some wild grass carp started to snatch food during feeding with the feed amount for each day was 0.5~2% of fish weight. After two month training, the behavior of competition for food was obvious, and the feed amount for each day remained 2~3% of fish weight.

610 Note: Values are expressed as means ± SE (n = 6), and different letters in the same line are
611 significantly different ($P < 0.05$).
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618 **Table 2.** Alignment statistics with reference genome

Sample ID	Clean reads	Mapped reads	Mapping rate (%)	Uniquely mapped reads	Uniquely mapping rate (%)	Bisulfite conversion rate (%)
DGC1	378,121,760	347,944,087	92.02	329,583,800	87.16	99.28
DGC2	387,627,636	353,434,830	91.18	335,393,994	86.52	99.23
DGC3	321,704,534	296,306,210	92.11	281,433,956	87.48	99.16
WGC1	371,478,196	321,457,393	86.53	300,678,599	80.94	99.16
WGC2	493,819,092	425,920,306	86.25	398,434,532	80.68	99.24
WGC3	410,419,590	361,301,972	88.03	339,621,985	82.75	99.22

619 Note: DGC, domesticated grass carp; WGC, wild grass carp.
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625 **Table 3.** Average methylation levels of different genomic regions

Groups	Regions	C (%)	CG (%)	CHG (%)	CHH (%)
DGC1	Genome	9.76	80.38	0.99	1.01
DGC2	Genome	9.69	80.55	1.18	1.06
DGC3	Genome	9.68	79.98	1.11	1.17
WGC1	Genome	9.21	74.7	1.12	1.18
WGC2	Genome	9.19	75.04	1.02	1.09
WGC3	Genome	9.13	75.39	1.07	1.12

626 Note: DGC, domesticated grass carp; WGC, wild grass carp. CG, CHG, and CHH (Where H is A,
627 C, or T).
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629
630
631 **Table 4.** Proportions of CG, CHG and CHH in all Methyl-cytosine

		mCG	mCHG	mCHH
DGC1	mC number	26,890,172	208,311	750,652
	Proportions (%)	96.557	0.748	2.695
DGC2	mC number	26,953,558	323,600	784,048
	Proportions (%)	96.053	1.153	2.794
DGC3	mC number	26,520,365	219,138	811,594
	Proportions (%)	96.259	0.795	2.946
WGC1	mC number	26,700,829	228,299	848,145
	Proportions (%)	96.125	0.822	3.053
WGC2	mC number	27,258,571	220,189	831,299

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	Proportions (%)	96.286	0.778	2.936
WGC3	mC number	26,934,911	222,172	821,714
	Proportions (%)	96.269	0.794	2.937

632 Note: DGC, domesticated grass carp; WGC, wild grass carp. CG, CHG, and CHH (where H is A,

633 C, or T).

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Fig. 1 Distribution of methylation level of mC in each sequence context .

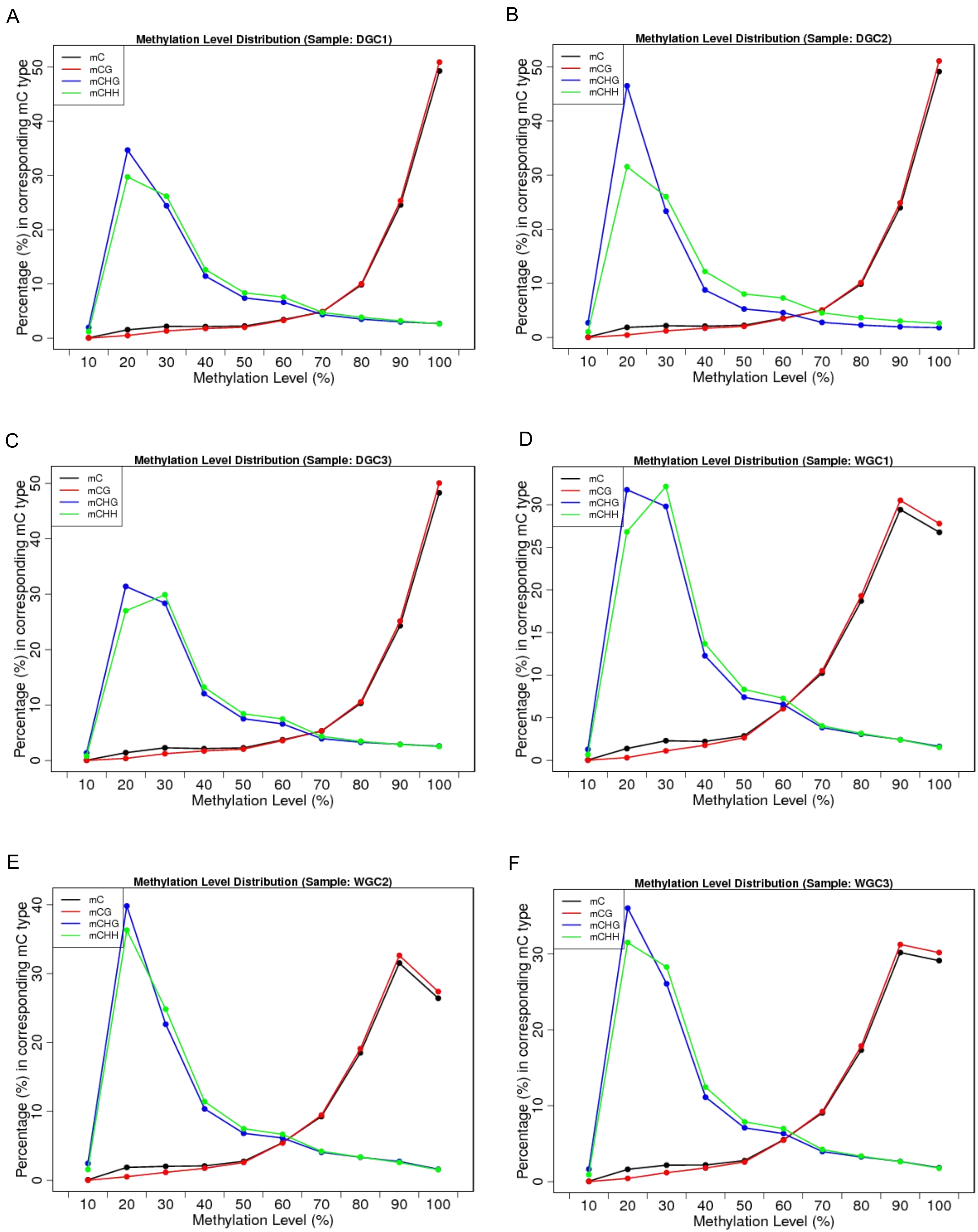
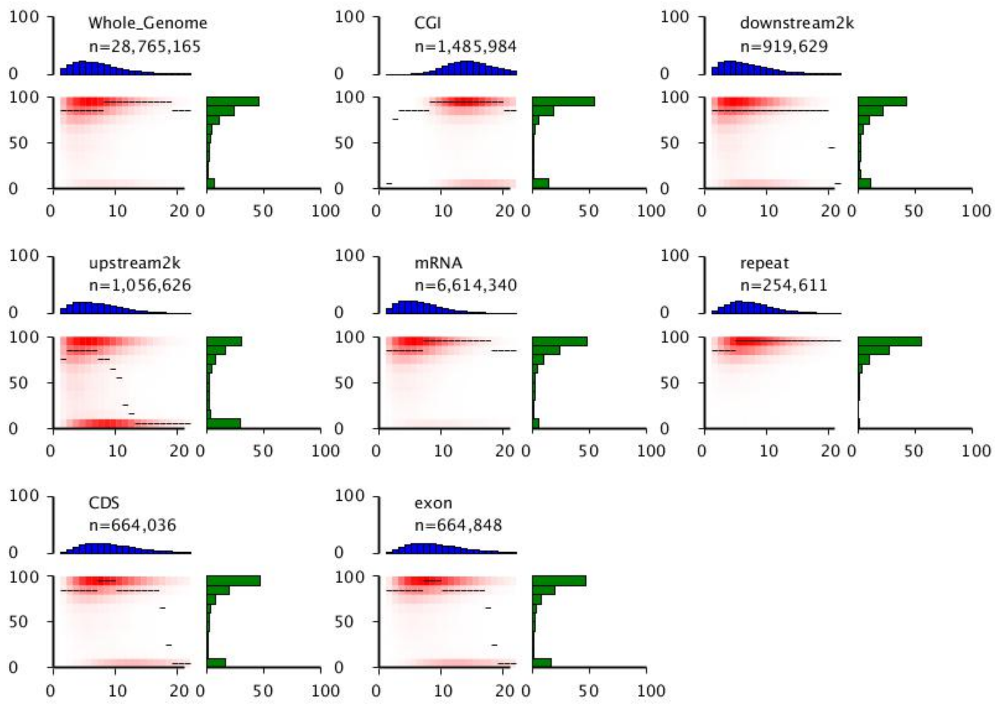
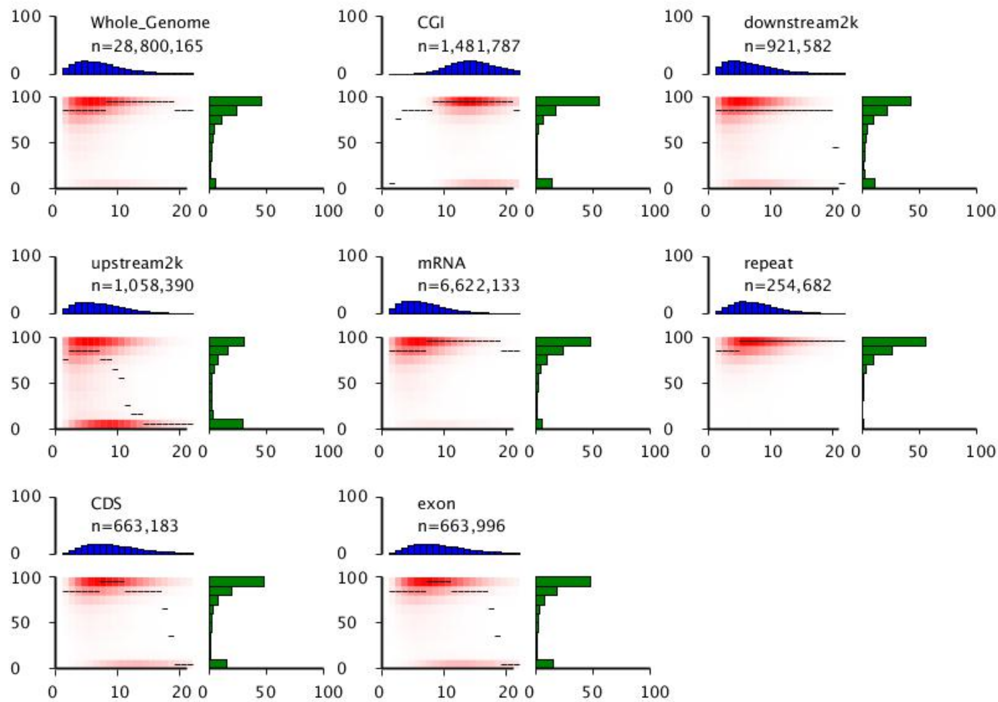


Figure 2. Heat maps show distinct methylation and CpG density patterns.

A



B



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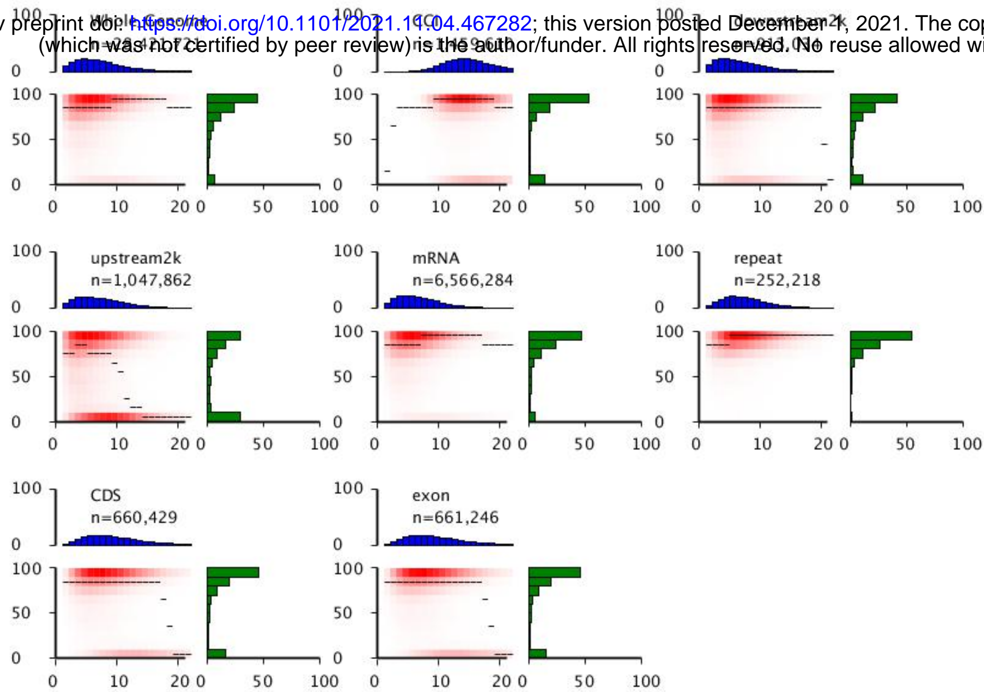
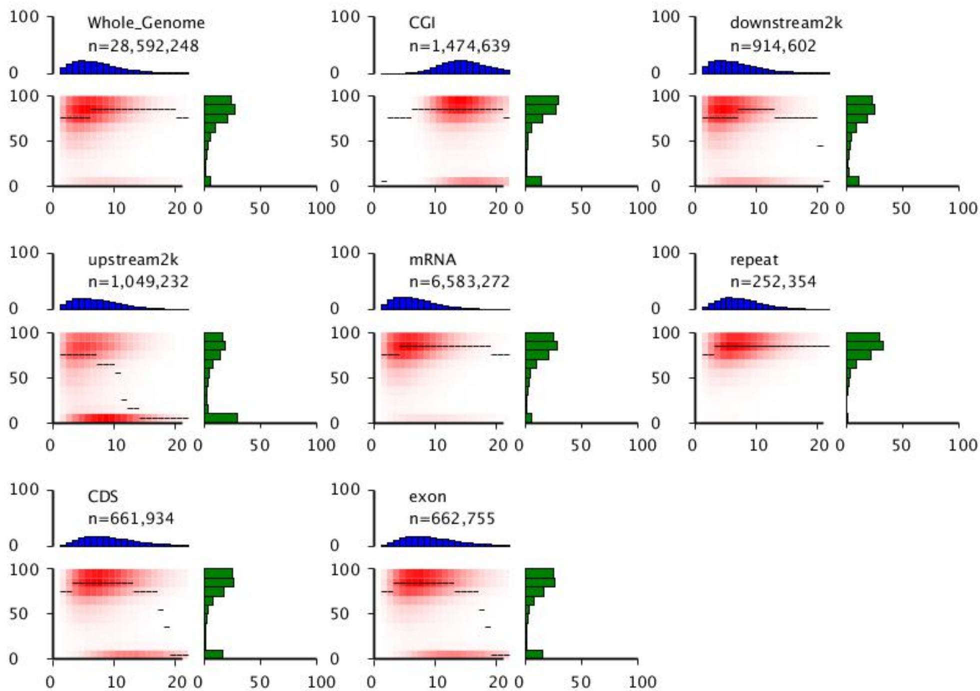
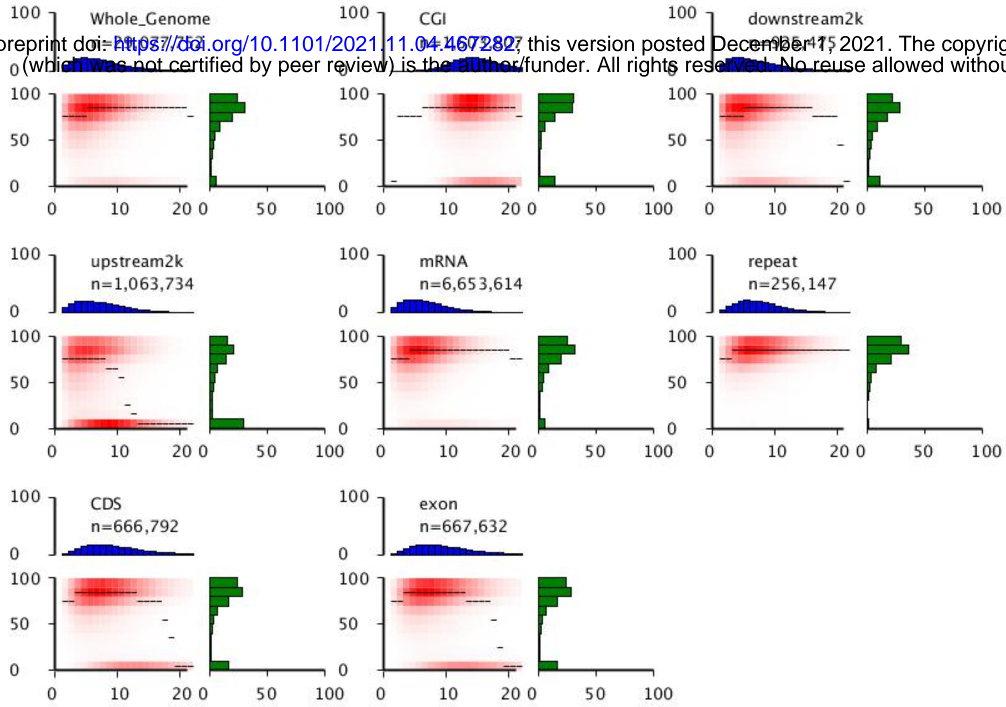


D



E



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F

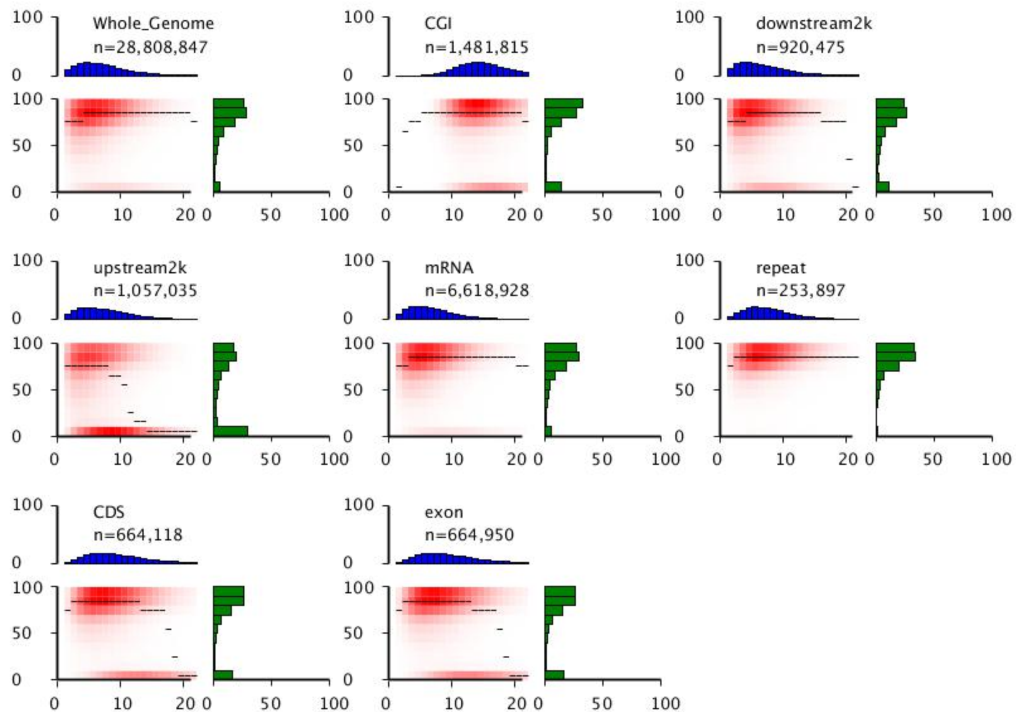

Figure 3. DNA Methylation Patterns Across the Entire Transcriptional Units at Whole Genome Level of sample DGC1.

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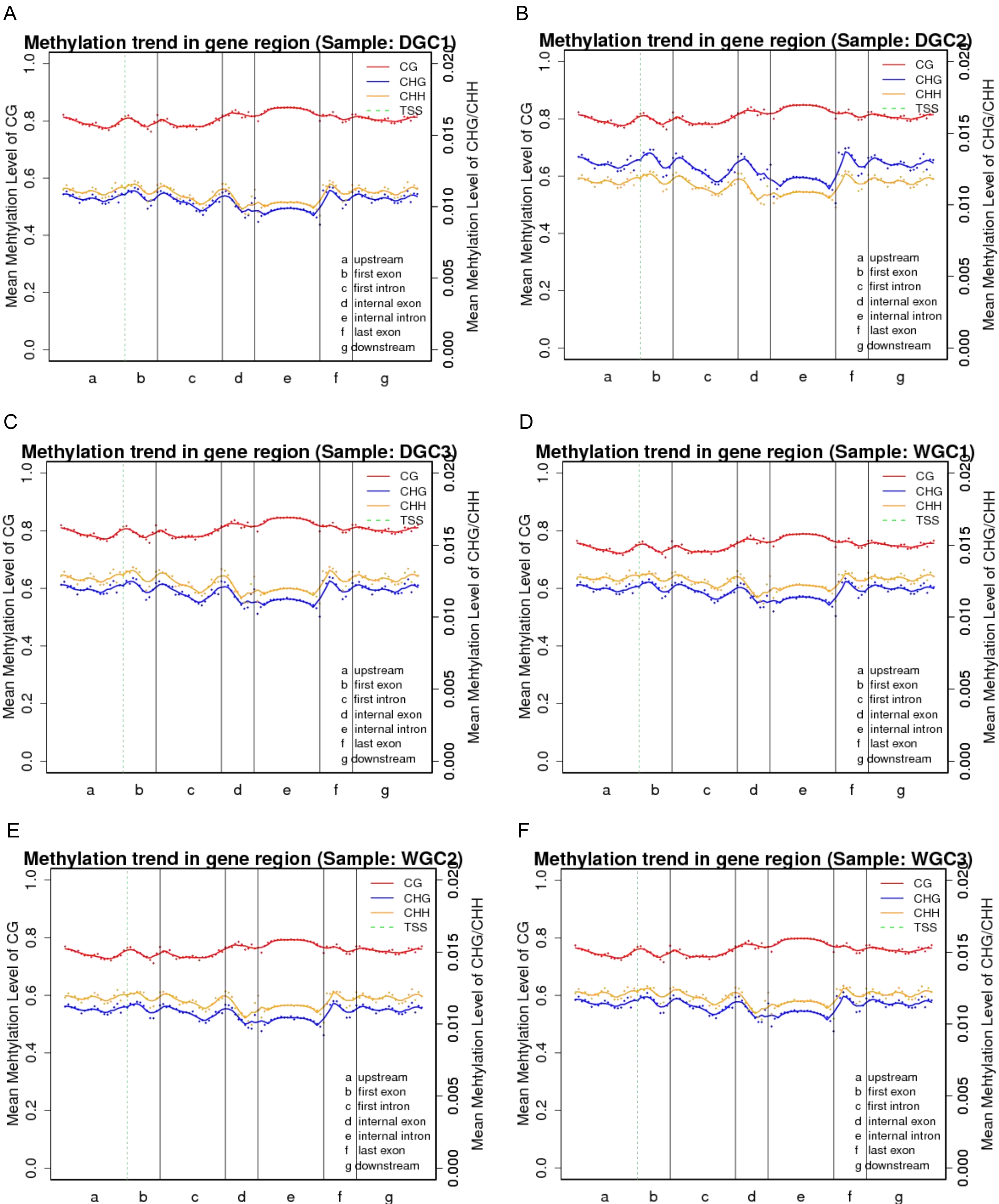

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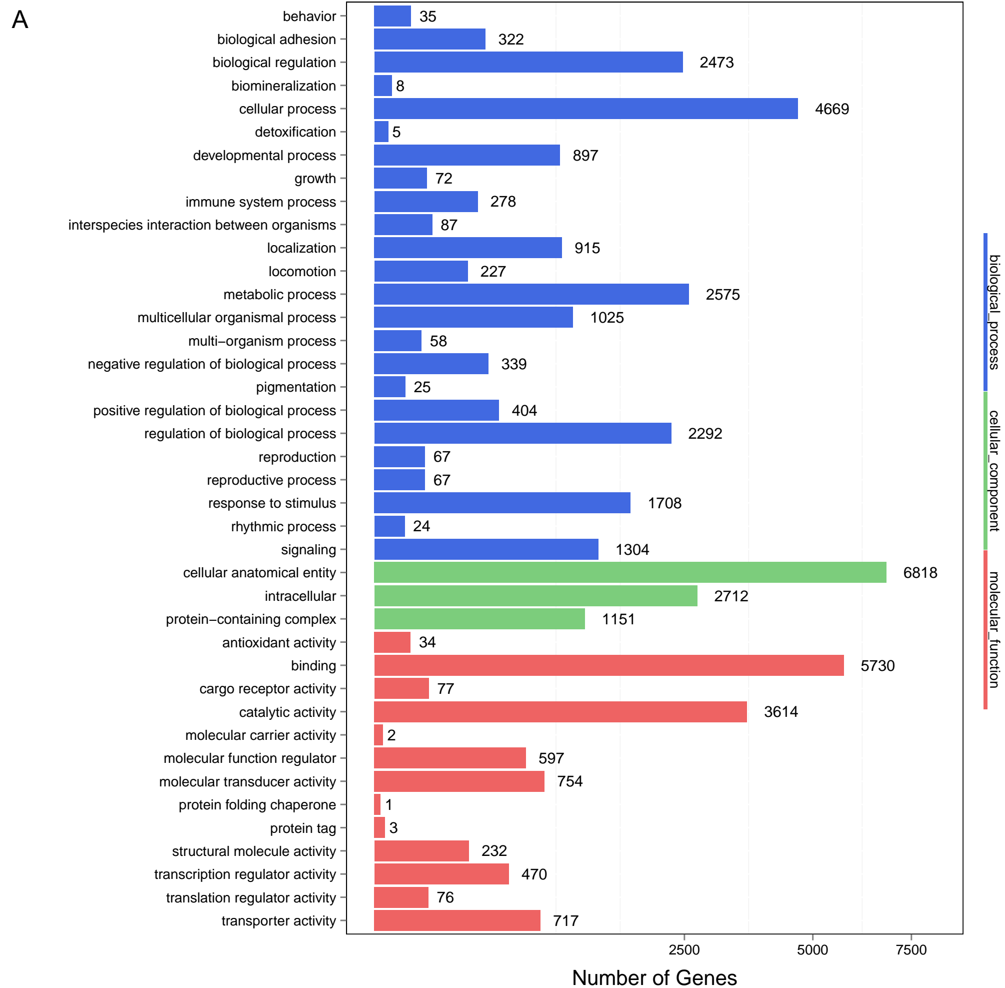
Figure 4. GO analysis of differentially methylated regions (DMRs)-related genes.



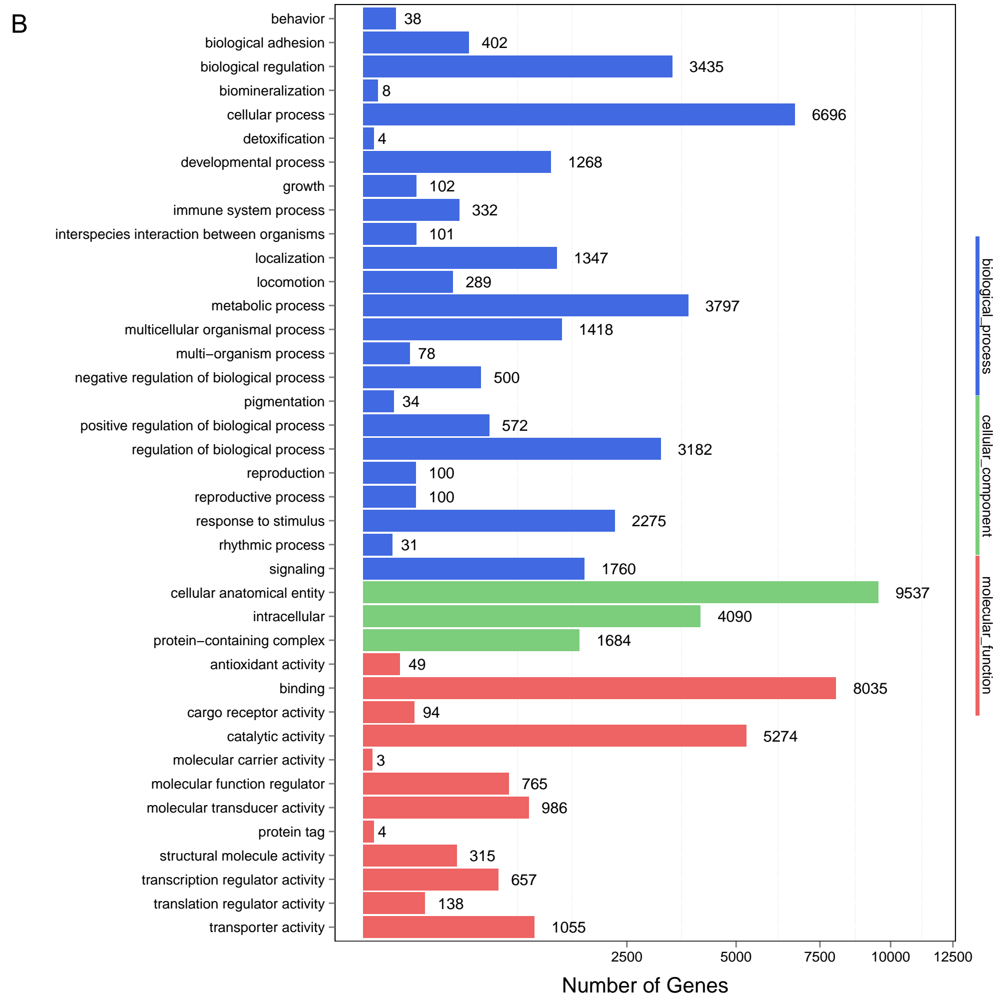


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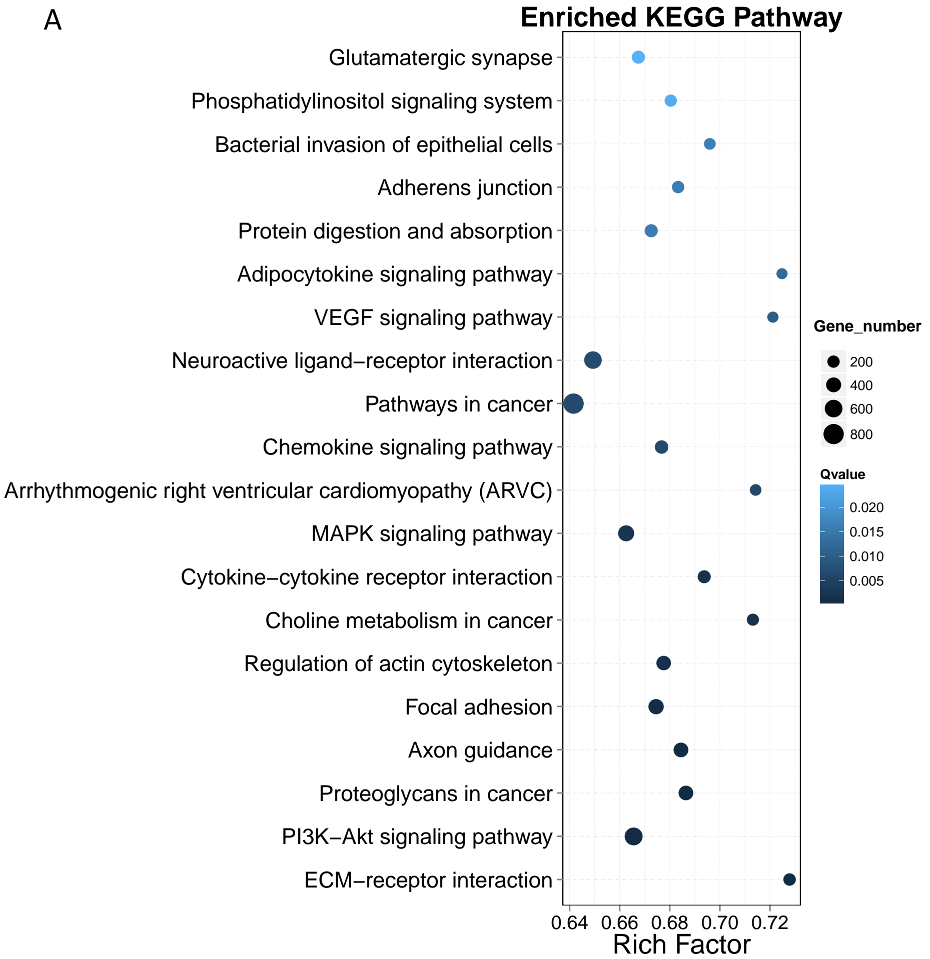
Figure 5. Pathway analysis of DMRs-related genes



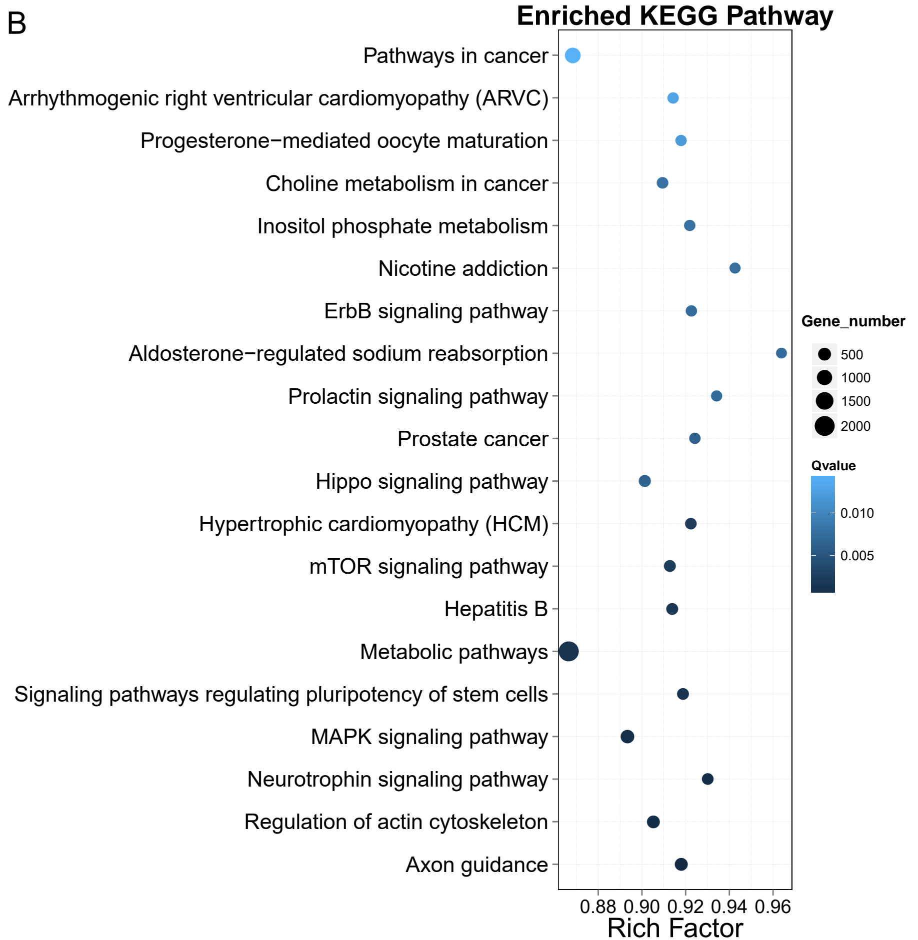


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Figure 6. Protein-protein interaction network analysis of DMGs in different selection signatures.