Hierarchical transcriptional control regulates *Plasmodium falciparum* sexual differentiation

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Abstract

Malaria pathogenesis relies on sexual gametocyte forms of the malaria parasite to be transmitted between the infected human and the mosquito host but the molecular mechanisms controlling gametocytogenesis remains poorly understood. Here we provide a high-resolution transcriptome of *Plasmodium falciparum* as it commits to and develops through gametocytogenesis. The gametocyte-associated transcriptome is significantly different from that of the asexual parasites, with dynamic gene expression shifts characterizing early, intermediate and late-stage gametocyte development and results in differential timing for sex-specific transcripts. The striking transcriptional dynamics suggest strict transcriptional control during gametocytogenesis in *P. falciparum,* which we propose is mediated by putative regulators including epigenetic mechanisms (driving active repression of proliferation-associated processes) and a cascade-like expression of ApiAP2 transcription factors. The gametocyte transcriptome serves as the blueprint for sexual differentiation and will be a rich resource for future functional studies on this critical stage of *Plasmodium* development, as the intraerythrocytic transcriptome has been for our understanding of the asexual cycle.

Keywords:

Malaria, *Plasmodium*, gametocyte, gametocytogenesis, transcriptome, gene expression regulation, differentiation, sexual development

42 ## Introduction

43 Sustained malaria prevalence is ensured through continued human-to-mosquito
44 transmission of *Plasmodium* parasites with *Plasmodium falciparum* being the
45 causative agent of the most severe form of the disease in humans (1). The complex
46 life cycle of *P. falciparum* encompasses development in the liver and erythrocytes of
47 its human host and transmission by the female *Anopheline* mosquito. Two distinct
48 developmental phases characterize intraerythrocytic development: rapid, cyclic
49 asexual cell division manifesting in pathology, and the stochastic (<10%) sexual
50 differentiation into gametocytes (2,3), which produces the non-replicative, mature,
51 transmissible forms of the parasite. Whilst the intraerythrocytic developmental cycle
52 (IDC) is relatively rapid (~48 h) and results in massive cell number expansion, sexual
53 differentiation and development (gametocytogenesis) is a prolonged process (~10
54 days) in *P. falciparum* and is characterized by the development of the parasite through
55 five morphologically distinct gametocyte stages (stages I-V) (4).

56

57 The processes of asexual replication and sexual differentiation in *Plasmodium* are
58 associated with distinct patterns of gene expression that are tightly controlled through
59 complex regulatory systems (5). These patterns have been investigated to some
60 extent for asexual replication where *P. falciparum* parasites use both transcriptional
61 (6–8) and post-transcriptional processes (9,10) to effect a cascade of coordinated,
62 stage-specific gene expression (11,12). Despite the identification of some putative
63 regulators of gene expression, including the Apicomplexan-specific ApiAP2 family of
64 transcription factors (13–15) and epigenetic regulation of particular gene families
65 (16,17), the specific mechanisms controlling transcriptional activation in the parasite

are incompletely understood, with recent data clearly showing mRNA dynamics are also influenced by additional post-transcriptional mechanisms (18,19).

The mechanisms regulating commitment to gametocytogenesis have been somewhat clarified recently, with the discovery of host LysoPC restriction acting as an environmental factor driving gametocyte commitment (20). The AP2-G transcription factor acts as a molecular master switch of sexual commitment (21–24) and results in the expression of genes that drive entry into gametocytogenesis (22–26). The *ap2-g* gene is released from an epigenetically silenced state (27,28) through the antagonism of heterochromatin protein 1 (HP1) epigenetic silencing of the *ap2-g* locus by the gametocyte development protein 1 (GDV1). (29). Commitment to gametocytogenesis further requires stabilization of a subset of gametocyte-specific transcripts (18).

Despite these advances toward unravelling the mechanisms of commitment, the molecular functions governing subsequent gametocyte development and maturation remain poorly understood. Previously, deletion of certain ApiAP2 proteins has been shown to prevent progression of gametocyte development in the rodent parasite *P. berghei* (30) and *P. falciparum* (31). Further, a subset of transcripts are translationally repressed by RNA binding proteins such as the Pumilio family protein (PUF2) during gametocytogenesis and ATP-dependent RNA helicase DDX6 (DOZI) and trailer hitch homolog (CITH) that repress female gametocyte transcripts needed to complete gametogenesis (32,33). However, systematic exploration of gene expression for *P. falciparum* gametocytogenesis has been limited to evaluation of the transcriptome (34–38) and proteome (35,39–41) at specific developmental timepoints. This includes

90 the bifurcation in committing asexual parasites to gametocytogenesis (18,20,25), and
91 evaluation of mature gametocytes in preparation for transmission (35,40,41). Current
92 datasets that evaluate the complete gametocyte development process are sparse (36)
93 and preclude dynamic evaluation of the transcriptomic profile associated with the
94 extended gametocyte development process of *P. falciparum* parasites. Therefore, a
95 time-resolved, high-resolution dataset capturing the transcriptome of each stage of
96 gametocyte development would greatly enhance our ability to compare gene
97 expression levels throughout the 10 days of gametocyte development and maturation.

98

99 Here we describe a comprehensive transcriptome analysis of *P. falciparum* parasites
100 during all stages of sexual development at daily resolution. By measuring transcript
101 abundance pre- and post-commitment, the transcriptional profile of gametocytes can
102 be completely distinguished from that of asexual parasites. The data show marked
103 shifts in transcript abundance associated with morphological stage transitions,
104 indicating that gene expression occurs on a time scale consistent with developmental
105 decisions underlying gametocyte development. We also show that post-commitment,
106 the gametocyte transcriptome is shaped by specific epigenetic marks and ApiAP2
107 transcription factors. The gametocyte transcriptome provides a quantitative baseline
108 of gene expression throughout sexual development and constitutes a highly valuable
109 resource that could be exploited to further understand the molecular mechanisms
110 governing sexual differentiation and maturation of the malaria parasite.

111

112 **Results**

113 **Transition between sexual and asexual stages of development defined by**
114 **transcriptome**

115 *P. falciparum* NF54-*pfs16*-GFP-Luc (42) parasites were induced to form gametocytes
116 after 1.5 cycles of asexual development (3 days) and monitored for the next 13 days
117 to capture gametocyte commitment and development to mature stage V gametocytes
118 (Fig 1A). Tight synchronization of the asexual parasites ensured coordinated
119 gametocyte development, and gametocytes (stage I) were observed in culture from
120 day 0 onwards (Fig 1B). Morphological evaluation showed a shift from a predominantly
121 asexual parasite population to >60% gametocytes by day 3 of gametocytogenesis
122 following the removal of asexual stages (Fig 1B).

123

124 We measured mRNA abundance genome-wide using DNA microarrays at daily
125 intervals, capturing expression values for 96-99% of the 5443 genes measured per
126 timepoint (P<0.01, Supplementary File 1). Overall, the transcriptome of gametocytes
127 is distinct from asexual parasites, as is evidenced by a clear shift in Pearson
128 correlation between the transcriptomes of asexual parasites (day -2 to 2) and
129 gametocytes (day 3 onward) (Fig 1C). Populations containing predominantly asexual
130 parasites (days -2 to 2) were highly correlated across the first two 48 h cycles (r^2=0.54-
131 0.86, Supplementary File 2) and were characterized by periodic gene expression
132 changes between the asexual ring and trophozoite stages (Fig 1C). From Day 3
133 onward, the transcriptional profiles diverged indicating a switch from asexual to sexual
134 development, evidenced by a loss of the 48 h correlation pattern (Fig 1C). During
135 subsequent days of gametocytogenesis, peak correlations were associated with

136 developmental progression through stage I-II (days 3-5, r^2=0.56-0.73), stage III-IV
137 (days 6-9, r^2=0.51-0.92), and mature stage V gametocytes (days 10-13, r^2=0.50-0.84)
138 (Fig 1C, Supplementary File 2).

139

140 Progression from asexual to sexual development was also clearly detectable in the
141 expression profiles of individual genes required during asexual development (*e.g.*
142 *kahrp (pf3d7_0202000)*) while sexual genes were only expressed during gametocyte
143 development from Day 3 (43) (Fig 1D). The genes restricted to expression during
144 sexual development include downstream targets of PfAP2-G (23) and markers
145 associated with mature gametocyte sex-specificity (Fig 1D) (35) and 24 novel
146 gametocyte-associated transcripts (Supplementary File 2). Among these transcripts
147 were a putative ncRNA, three rRNAs and two tRNAs, suggesting that the expression
148 of non-coding RNAs may not only play a role during gametocyte commitment (18) but
149 also in gametocyte development and maturation in *P. falciparum.* Together, these data
150 comprise a high-resolution *P. falciparum* blood stage developmental transcriptome
151 that allows for the temporal evaluation of transcriptional abundance patterns
152 associated with gametocyte commitment, development and maturation.

153

154 **The gametocyte-specific transcriptional program reflects the molecular**
155 **landscape of gametocyte development**

156 To associate temporal gene expression to particular events during gametocyte
157 commitment and stage transitions throughout development, the full 16-day
158 transcriptome dataset was K-means clustered revealing 2763 transcripts with overall
159 decreased abundance (clusters 1-5) and 2425 with increased abundance during

160 gametocytogenesis (clusters 6-10, Fig 2A). Therefore, gametocytogenesis relies on a
161 more specialized program of gene expression compared to asexual development, with
162 only 45% of transcripts showing increased abundance during gametocyte
163 development (Fig 2A) compared to the 80-95% of transcripts increased during specific
164 phases of asexual development (11,19,44). Interestingly, individual clusters showed
165 specific patterns of gene expression throughout gametocyte development (Fig 2A),
166 with transcript abundance during gametocytogenesis either decreased following
167 asexual development (clusters 1-3, 1042 transcripts); maintained (clusters 4-5, 1721
168 transcripts) or increased (cluster 6-7, 1571 transcripts). Three clusters (clusters 8-10)
169 show transcripts with specific peaks in expression evident during development,
170 indicative of developmental regulation.

171

172 Cluster 1 is predominantly comprised of critical asexual stage transcripts which
173 showed a decline in abundance during gametocytogenesis, with up to 5 $\log_2$ fold
174 decreases in the expression of these transcripts between the ring and early
175 gametocyte stages (Fig 2A). These transcripts include Maurer's cleft proteins *e.g. rex1*
176 *(pf3d7_0935900)* and *rex2 (pf3d7_0936000)* as well as knob associated proteins that
177 form part of the cytoadherence complex *(kahrp (pf3d7_0202000), kahsp40*
178 *(pf3d7_0201800))*, supporting earlier observations that the gametocytes mediate
179 sequestration via different mechanisms than asexual parasites (45). Many of these
180 cytoadherence associated transcripts are associated with heterochromatin protein 1
181 (HP1) occupancy during gametocyte development (46), and other HP1 (46,47) and
182 H3K9me3 (17) repressed genes are also significantly enriched in cluster 1 (P<0.0001,
183 Fisher's exact test, genes listed in Supplementary File 3). This suggests asexual
184 development-specific genes are actively repressed by epigenetic regulation

185 throughout gametocyte development. Clusters 1-3 also contain transcripts involved in
186 metabolic processes that are not critical to gametocyte development including genes
187 encoding for enzymes of heme metabolism and glycolysis (Fig 2B, cluster 3,
188 Supplementary File 1) as well as regulators of egress (*pkg (pf3d7_1436600)*) and
189 invasion (*bdp1 (pf3d7_1033700)* and *ap2-i* (*pf3d7_1007700)*), all processes that are
190 not necessary for gametocyte maturation (Fig 2A, cluster 2). Beyond these examples,
191 clusters 1-3 also contain 214 unannotated genes that could be specifically required for
192 asexual development only (Fig 2B).

193

194 Some transcripts show low abundance throughout gametocytogenesis (Fig 2A,
195 clusters 4 and 5, average expression <0.1 $\log_2$(Cy5/Cy3), with amplitude change <0.5
196 $\log_2$(Cy5/Cy3)). These clusters include regulators of proliferation (*e.g.* origin of
197 replication complex protein *mcm4 (pf3d7_1317100),* proliferating cell antigen 1
198 (*pf3d7_1361900)* and cyclin dependent kinase *crk4 (pf3d7_0317200*)). By
199 comparison, clusters with transcripts maintained at increased levels throughout
200 commitment and development (Fig 2A, clusters 6 and 7, average $\log_2$(Cy5/Cy3) >0.31)
201 included expected gene sets involved in the constitutive processes of macromolecular
202 metabolism (*e.g.* DNA replication, protein modification and RNA metabolism Fig 2B,
203 Supplementary File 1) (36,38). Interestingly, cluster 6 (and cluster 2) showed a high
204 degree of cyclic oscillation in transcript abundance (Fig 2A). Many of these transcripts
205 relate to transport, general cellular metabolism and homeostasis, functions in which
206 fluctuation would not be unexpected (Fig 2B, Supplementary File 1). Importantly,
207 cluster 7 also contained transcripts classified by gene ontology as involved in cellular
208 differentiation *(caf40 (pf3d7_0507600)*, *pf3d7_0918400, pf3d7_0926800* and *speld*
209 *(pf3d7_1137800))* (GO:0030154, *P*=0.026, Fig 2B, S1 Table).

210

211 A significant proportion (15%) of the transcriptome associated with peak expression
212 during specific stage-transitions in gametocyte development (Fig 2A, clusters 8-10),
213 reminiscent of the phased expression typical of the asexual transcriptome (11,12).
214 Transcripts involved in early-stage development increased from stage I-II in cluster 8
215 in a transcriptional profile often associated with targets of AP2-G (22,23,25).
216 Transcripts in cluster 9 increased in abundance in the intermediate phase of
217 development (stage III-IV) before the expression of transcripts required for
218 development in mosquitoes in cluster 10 (stage V, *gamer* (*pf3d7_0805200*), *mtrap*
219 (*pf3d7_1028700*), *cht1* (*pf3d7_1252200*), Fig 2A &B). The transcripts in clusters 8-10
220 are thus markers of biological transitions during gametocyte development. Clusters 6
221 & 8 are enriched for genes that contribute to the metabolic shift to mitochondrial
222 metabolism (*e.g.* malate dehydrogenase *(mdh, pf3d7_0618500))* and fatty acid
223 biosynthesis (*e.g.* β-ketoacyl-ACP synthase III (*kasIII, pf3d7_0618500))* (48,49) in
224 gametocytes, followed by the emergence of processes related to cytoskeletal
225 formation (clusters 8 & 9, Fig 2A &B, Table S1) that lead to the construction of a rigid
226 subpellicular microtubule array during the sequestering stages (stages I-IV) of
227 gametocytes (50). The microtubule array results in the characteristic crescent shape
228 of the intermediate stages before the complex depolymerizes in stage V that is
229 accompanied by the increased transcript abundance of actin depolymerization factors
230 1 and 2 (Fig 2A, cluster 10, *pf3d7_0503400, pf3d7_1361400*) to allow for a more
231 deformable erythrocyte that can re-enter circulation (50). This cluster also includes the
232 genes encoding the serine repeat antigens (*sera*) 3 and 5 *(pf3d7_0207800,*
233 *pf3d7_0207600)* that play a role in egress in asexual parasites (51,52), implying that
234 they may retain this role during gametocyte egress from the erythrocyte in the

235 mosquito midgut. The striking temporal patterns of transcript abundance in clusters 8-
236 10 suggests strict transcriptional regulation of these genes to ensure the timing of
237 gametocyte sequestration, circulation and egress. Interestingly, these patterns are
238 exhibited by parasites that need not fulfil any of these functions when grown *in vitro* in
239 the absence of host-interactions, suggesting that transcription of these genes is hard-
240 wired.

241

242 **Different regulatory modules enable sexual commitment and development**

243 The time-resolved gametocyte transcriptome also allows interrogation of the
244 expression of genes involved in sexual commitment throughout gametocyte
245 development (18,20,25) (Fig 3). In total, previous reports produced a set of 1075
246 unique genes proposed to function as an "on switch" that characterizes gametocyte
247 commitment (18,20,25). Of these, 680 genes (63%) also have increased transcript
248 abundance during gametocyte development (Fig 3). These increased transcripts
249 include those encoding epigenetic regulators involved in cell cycle control such as
250 SIR2A (PF3D7_1328800) and SAP18 (PF3D7_0711400) that contribute to decreased
251 DNA synthesis and a block in proliferation (53,54) necessary for the parasite to
252 differentiate (Fig 3A). The remaining 395 transcripts are not increased in abundance
253 during gametocyte development, suggesting that these transcripts are short lived and
254 possibly only essential during gametocyte commitment. These short lived transcripts
255 include *gdv1,* whose protein product prevents epigenetic repression of *ap2-g* during
256 commitment (29), and *iswi* and *sn2fl*, which encode chromatin remodeling proteins
257 (Fig 3A), that are expressed in sexually committed cells downstream of *ap2-g* (25).
258 From our data we also identified a specific 5' *cis*-regulatory motif (AGACA) that is

259 enriched upstream of 539 genes with increased transcript abundance throughout
260 gametocytogenesis (Fig 3B, Supplementary File 3), suggesting that transcriptional
261 regulation of genes containing this motif could be important for gametocyte
262 development. This AGACA motif has been associated with sexual commitment and
263 development in previous datasets (18,55). In addition, a second motif (ATGTGTA) was
264 also highly over-represented in the transcripts with increased abundance throughout
265 gametocytogenesis (Fig 3B, S3 Table). This motif has been correlated with genes
266 involved in DNA replication (56) and the significance of this enrichment in genes
267 associated with differentiation is unclear and their *trans*-acting factors have not been
268 identified (15,57).

269

270 **Transcriptional patterns characterize distinct transitions in gametocyte**
271 **development**

272 Apart from commitment to sexual development, the parasite also undergoes distinct
273 developmental and transcriptional transitions during gametocyte development. The
274 initial transition occurring in stage I gametocytes and regulating immature gametocyte
275 development is characterised by increased transcript abundance in cluster 8 (Fig 4A),
276 which showed a significant enrichment for genes involved in regulation of transcription
277 (GO:0010468, 11 transcripts, P=0.029) including the specific ApiAP2 transcription
278 factors *pf3d7_0404100, pf3d7_0516800, pf3d7_1429200* and the *myb1* transcription
279 factor (*pf3d7_1315800)* (Fig 4A). Other genes with potential regulatory functions
280 include a possible novel transcription factor, *pf3d7_0603600*, which contains an AT-
281 rich interaction domain (IPR001606: ARID) and an uncharacterized RNA binding
282 protein *(pf3d7_1241400)*. Proteins expressed by these two genes have been detected

283 previously during gametocyte development (Fig 4A) (34,35,40,41). These proteins,
284 along with the C-Myc binding protein MYCBP (PF3D7_0715100), are of interest for
285 further study to determine their role in controlling gene expression during gametocyte
286 development.

287

288 A second outcome of the initial transition into gametocytogenesis is the determination
289 of sex differentiation in *P. falciparum* parasites, which is proposed to be an PfAP2-G
290 independent process that occurs at the very onset of commitment (18,35,40,41,58).
291 However, sexually dimorphic gametocytes are only morphologically detectable by
292 microscopy from stage III onwards (59). Our data indicate that the male-enriched
293 transcripts from Lasonder *et al.* 2016 (35) show increased abundance earlier in
294 development (stage I-II; 27% of cluster 8, $P<0.0001$, two-tailed Fisher's exact test, Fig
295 4B, Supplementary File 3) compared to female transcripts. This observation is
296 particularly interesting given the low ratio of male to female gametocytes in mixed
297 populations (60), which suggests the rapid increase in male-enriched transcript
298 abundance may be important for promoting male gametocyte development. These 98
299 male-enriched transcripts include 3 genes containing putative RNA binding motifs
300 (*pf3d7_1126800, pf3d7_0414500, pf3d7_1438800*) that are highly abundant from
301 stage I onwards, suggesting that these transcripts may be good biomarkers of early
302 male differentiation as an alternative to α-tubulin II, which is expressed promiscuously
303 in early gametocyte populations (61). Other male transcripts code for a meiotic protein
304 (PF3D7_1311100), MGET (PF3D7_1469900), PF3D7_1010400 and P25A
305 (PF3D7_1236600), which increase in abundance following the initial transcripts.

306

307 Female-enriched transcripts (35) peak in abundance only after sexual dimorphism is
308 clearly discernible, from stage II-III onwards (Fig 4B) and are significantly
309 overrepresented in the intermediate development cluster 9 (Fig 4B, Supplementary
310 File 3, 76% of the cluster, $P<0.0001$, two-tailed Fisher's exact test). Overall, this trend
311 held true for the 158 female-enriched transcripts in cluster 9, including those encoding
312 canonical female markers, *e.g.* osmiophilic body protein *g377* (*pf3d7_1250100)*
313 (62,63), late-stage antigen *pfs25* (*pf3d7_1031000)* (35,63) and *ccp1-3*
314 *(pf3d7_1475500, pf3d7_1455800, pf3d7_1407000)* (35,63) and *ccp4*
315 *(pf3d7_0903800)* that was recently used to reliably type male and female
316 gametocytes in late-stage gametocytes (64). We also detect a small subset of female-
317 enriched transcripts (*pf3d7_0918700, imp2 (pf3d7_0730400), pf3d7_1007800,*
318 *pf3d7_1466800, pf3d7_1146800, obc13 (pf3d7_1214800))* that are expressed earlier
319 in gametocyte development (Fig 4B) and could potentially be important for female
320 development before morphological differences are apparent.

321

322 The second transcriptional transition we observed coincides with the onset of
323 gametocyte maturation from stage IV to V (Fig 4C). These transcripts show increased
324 abundance in sexually committed asexual parasites as well as mature stage V
325 gametocytes but have diminished abundance during the early and intermediate stages
326 of gametocytogenesis (cluster 10, Fig 4C). This cluster was significantly enriched for
327 transcripts stabilized during commitment (47% of transcripts, $P<0.0001$, two-tailed
328 Fisher's exact test) (18), as well as genes marked with H3K36me3 in asexual parasites
329 (49% $P<0.0001$, Fisher's exact test) (16). Interestingly, the epigenetic H3K36me3
330 mark is abundant during the intermediate stages of gametocyte development (65) and
331 genes overlapping in the three datasets encode transcripts associated with the

332 intracellular signalling machinery of the parasite (*cdpk1 (pf3d7_0217500), cdpk5*
333 *(pf3d7_1337800)* and adenylyl cyclase beta *(pf3d7_ 0802600,* (66)), along with cAMP-
334 dependent protein kinase A catalytic and regulatory subunits (*pkac (pf3d7_0934800),*
335 *pkar (pf3d7_1223100)* (Fig 4C). Of these, CDPK1 has been confirmed to function in
336 de-repressing female gametocyte transcripts during parasite development in
337 mosquitoes (67). Several of the genes in cluster 10 also have roles in invasion
338 including the merozoite proteins *msp1, pf3d7_0930300, msp2, pf3d7_0206800, rh4,*
339 *pf3d7_0424200, and rh5, pf3d7_0424100*, suggesting that invasion genes need to
340 again be expressed for transition to gametogenesis in the mosquito. Overall, the
341 gametocyte transcriptome reveals three major stages in gametocyte development
342 (differentiation (Fig 4A), intermediate development (Fig 4B), maturation (Fig 4C)) that
343 promote gametocyte development of *P. falciparum* parasites.

344

345 **ApiAP2 transcription factors are expressed at specific intervals during**
346 **gametocytogenesis**

347 To investigate the possible contribution of factors associated with transcriptional
348 regulation to the observed stage-progressions during gametocytogenesis, we
349 interrogated the expression of the genes encoding the ApiAP2 transcription factor
350 family (Fig 5). Of the 27 family members, 15 genes encoding ApiAP2 transcription
351 factors increased in transcript abundance during gametocyte development. Transcript
352 abundance for *pf3d7_0404100, pf3d7_1350900, pf3d7_1449500, pf3d7_0802100,*
353 *pf3d7_1429200* increased consistently throughout the time course (Figure S1).
354 However, most ApiAP2-encoding transcripts increased in abundance at discrete
355 intervals (Fig 5A) throughout gametocytogenesis. As expected, *ap2-g*

356 *(pf3d7_1222600)* transcript abundance increased before the appearance of
357 gametocytes (days -1 to 2). The target genes bound by AP2-G (23), peaked in
358 transcript abundance directly following AP2-G peak abundance as expected,
359 coinciding with stage I of gametocyte development (Supplementary Fig S2).
360 Thereafter, three transcription factors *pf3d7_1408200, pf3d7_1317200* and
361 *pf3d7_0611200* were increased during stage I to III of development (days 2-6). In the
362 rodent-infectious malaria parasites *P. berghei* and *P. yoelii*, orthologs of the first two
363 genes have been associated with gametocyte development through knockout studies
364 (21,30). Three ApiAP2-encoding transcripts for *pf3d7_0516800, pf3d7_1222400,*
365 *pf3d7_0934400* were increased in abundance from stage I to V of development (Fig
366 5A), following a pattern similar to the increased abundance of cluster 8 (Fig 4A). During
367 the later stages, *pf3d7_1143100, pf3d7_1239200* and *pf3d7_0613800* were increased
368 in abundance. Expression of PF3d7_1143100 is translationally repressed in *P. berghei*
369 gametocytes (32), indicating that these transcription factors may not contribute to gene
370 expression in *P. falciparum* gametocytes, but may instead have functional significance
371 in subsequent development in the mosquito.

372

373 To associate functional regulation of gene sets due to the cascade-like increased
374 abundance of the transcripts encoding the ApiAP2 transcription factors, the
375 gametocyte transcriptome was analysed using Gene Regulatory Network Inference
376 Using Time Series (GRENITS (68)) (Supplementary File 1) with the strongest
377 predicted regulators shown in Fig 5B. From this analysis, *pf3d7_0611200,* which
378 increased in abundance directly following *ap2-g,* coexpressed with 314 genes
379 (probability linkage >0.6), 223 of which were anti-correlated for expression and
380 functionally enriched for genes involved in host invasion (GO:0044409: entry into host,

381 *P*=2.97e^{-12}; Fig 5B). The large proportion of genes putatively repressed by this
382 transcription factor would point to *pf3d7_0611200* acting as a repressive ApiAP2
383 transcription factor during gametocyte development, either alongside or instead of the
384 *P. falciparum* ortholog of *pbap2-g2*, *pf3d7_1408200.* The second *apiap2* transcript
385 increased in abundance, is *pf3d7_1317200,* the *P. falciparum* ortholog of *pbap2-g3,*
386 coexpressed with 21 genes involved in cell cycle processes, including DNA replication
387 (GO:0044786, *P*=0.0061) and chromosome organization (GO:0051276 *P*=0.0046).
388 This ApiAP2 transcription factor has been previously associated with gametocyte
389 development (31,69) and could be an important driver of stage II-III development. The
390 two final ApiAP2 transcription factors are increased between stage I-V of development,
391 with the first, *pf3d7_0934400,* showing mostly negative co-expression with its target
392 genes (27/37 transcripts, including *pkg* and *ptex88 (pf3d7_1105600)*), suggesting this
393 ApiAP2 transcription factor might also act as repressor. Secondly, the transcript of
394 *ap2-o2* is increased in abundance throughout development but peaks at stage IV (day
395 8-9) of development and was predicted to regulate 22 target genes. Taken together,
396 this data supports the involvement of successive expression of ApiAP2 transcription
397 factors in a regulatory cascade during gametocyte development, as has been
398 proposed for *P. berghei* gametocytes (21) and shows that this subsequent expression
399 co-occurs with stage transition during *P. falciparum* gametocytogenesis.

400

401

402 **Discussion**

403 We describe a high-resolution gametocyte transcriptome of malaria parasite
404 differentiation from the asexual form through sexual commitment and all stages of
405 development to mature stage V gametocytes. We find that gametocytogenesis in *P.*
406 *falciparum* is a well-controlled process involving successive activation of regulatory
407 processes that mediate development during stage-transition, ultimately resulting in a
408 parasite poised for transmission. These observations emphasize that stage-specific
409 gene expression is an essential feature of regulation of gene expression in
410 *Plasmodium* spp. and is particularly true for the extended and morphologically diverse
411 gametocyte development of *P. falciparum* parasites. The dynamic evaluation of the
412 transcriptome allows for the construction of a more complete molecular roadmap for
413 gametocyte development (Fig 6).

414

415 We propose that multiple transition points are passed during sexual differentiation and
416 that mechanisms independent of initial sexual commitment during asexual proliferation
417 are needed to ultimately result in completion of gametocyte development. First,
418 committed parasites pass an initial transition point, whereby processes are initiated to
419 drive early gametocyte development. This includes repression of genes typically
420 associated with proliferation through epigenetic mechanisms (H3K9me3, HP1
421 occupancy), post-transcriptional regulation (18) and the activity of transcription factors
422 that repress asexual-specific transcription (Fig 6). This transition point is also
423 characterized by the increased transcript abundance of genes required during early
424 and intermediate gametocyte development. A portion of these transcripts are
425 expressed specifically in either sex, with an apparent delay between the peak

426 abundance of male-specific and female-specific transcripts (Fig 6). It is possible that
427 tracing the transcriptional dynamics within each sex separately would result in higher
428 resolution data for this observation and resolve whether this is a true delay or
429 underplayed by more complicated transcriptional dynamics that impact the RNA
430 biology of the disparate sexes. As the gametocyte then reaches the critical transition
431 for its pathology, the point of gametocyte maturation, a different set of genes increase
432 in abundance. These genes are active in important processes specific to maturation
433 into stage V gametocytes, including the switch from sequestration in the bone marrow
434 to re-entering circulation and readying for transmission to the mosquito by involving
435 the parasite's intracellular signalling machinery.

436

437 A particularly interesting observation is the decreased abundance of important
438 regulators of commitment, *ap2-g* and *gdv1,* as the parasite enters the early
439 gametocyte stages (Fig 6). It is possible that the limited activity of these regulators
440 might be essential for gametocytogenesis to occur normally, to allow the distinct
441 patterns of gene expression we see here. It would be of interest to test what the effect
442 of overexpression of one or both of these factors would be on gametocyte
443 development. We also add to data on the transcriptional regulation in *P. falciparum* by
444 the ApiAP2 transcription factor family downstream of AP2-G, affirming the presence
445 of a transcription factor cascade enabling passage through gametocytogenesis as
446 postulated for *P. berghei* (21). The involvement of *ap2-g2* and *pf3d7_0611200* in
447 repressing transcription of asexual genes during gametocyte development (Fig 6) is
448 also of particular interest for investigation, bringing into question if one or both of these
449 factors fulfil this role in *P. falciparum* gametocytes. However, the possibility of novel
450 regulators of transcription in early gametocyte development cannot be overlooked,

451 with RNA binding proteins and the possible ARID transcription factor (Fig 6) good
452 candidates for functional characterization.

453

454 The high-resolution transcriptome profile of *P. falciparum* gametocytes offers a
455 complete molecular landscape of parasite differentiation. We identify putative
456 regulators of mRNA dynamics facilitating a well-timed transcriptional program that
457 prepares the parasite for transmission. The profile provides molecular identity to
458 differences and similarities in asexual and sexual development that can be exploitable
459 for pharmaceutical intervention. Finally, the stage-specific events that complicate
460 transmission-blocking drug discovery are highlighted, 1) the immediate divergence of
461 the gametocyte's molecular profile from asexual development, 2) the later sexual
462 dimorphism in intermediate stage development and 3) the apparent transcriptional
463 divergence between immature and mature gametocytes. The gametocyte
464 transcriptome further provides a valuable resource for further interrogation of the
465 function of gene products and regulatory mechanisms important for
466 gametocytogenesis in *P. falciparum.*

467

468 **Material and Methods**

469 **Parasite culturing and sampling**

470 *In vitro* cultivation of intraerythrocytic *P. falciparum* parasites and volunteer blood
471 donation for human erythrocytes holds ethics approval from the University of Pretoria
472 (EC120821-077). Asexual *P. falciparum* NF54 parasite cultures (NF54-*pfs16*-GFP-
473 Luc,(42)) were maintained at 5-8% parasitemia 37°C in human erythrocytes at 5%
474 hematocrit in RPMI 1640 medium supplemented with 25 mM HEPES, 0.2 % D-
475 glucose, 200 µM hypoxanthine, 0.2% sodium bicarbonate, 24 µg/ml gentamicin with
476 0.5 % AlbuMAX® II and incubated under hypoxic conditions (90% N_2, 5% O_2, and 5%
477 CO_2)(70). Synchronous asexual cultures (>95% synchronized ring-stage parasites)
478 were obtained by three consecutive cycles of treatment with 5% D-sorbitol, each 6-8
479 h apart.

480 Gametocytogenesis was induced by employing a strategy of concurrent nutrient
481 starvation and a decrease of hematocrit(70). Ring-stage parasite cultures were
482 adjusted to a 0.5% parasitemia, 6% hematocrit in RPMI 1640 medium prepared as for
483 growth of asexual parasites without additional glucose supplementation (day -3) and
484 maintained under the same hypoxic conditions at 37°C without shaking. After 72 h,
485 the hematocrit was adjusted to 3% (day 0). After a further 24 h, induction medium was
486 replaced with medium containing 0.2% (w/v) D-glucose as the asexual parasites were
487 removed daily with 5% D-sorbitol treatment for 15 minutes at 37°C and/or *N*-
488 acetylglucosamine included in the culture medium for duration of the sampling.

489 All cultures were maintained with daily medium changes and monitored with Giemsa-
490 stained thin smear microscopy and parasite stage distribution determined by counting
491 ≥ 100 parasites per day. Parasite samples (30 ml of 2-3% gametocytemia, 4-6%

492 hematocrit) were harvested daily for microarray analysis on days -2 to 13 following
493 gametocyte induction. The samples harvested on days -2 to 7 were isolated from
494 uninfected erythrocytes via 0.01% w/v saponin treatment for 3 minutes at 22°C while
495 samples from day 8 to 13 were enriched for late stage gametocytes via density
496 centrifugation using Nycoprep 1.077 cushions (Axis-Shield). Late stage gametocyte
497 samples were centrifuged for 20 min at 800x*g* and the gametocyte containing bands
498 collected(70). All parasite samples were washed with phosphate-buffered saline
499 before storage at -80˚C until RNA was isolated.

500 **RNA isolation, cDNA synthesis and microarray hybridization and scanning**

501 Total RNA was isolated from each parasite pellet with a combination of TRIzol (Sigma
502 Aldrich, USA) treatment and using a Qiagen RNeasy kit (Qiagen, Germany) as per
503 manufacturer's instructions. The quantity, purity and integrity of the RNA were
504 evaluated by agarose gel electrophoresis and on a ND-2000 spectrophotometer
505 (Thermo Scientific, USA). For each RNA sample, 3-12 µg total RNA was used to
506 reverse transcribe and dye couple cDNA as described previously (71). The reference
507 cDNA pool was constructed from a mixture of all the gametocyte samples used in the
508 experiment in a 1:4 ratio with cDNA from a 6-hourly time course of asexual *P.*
509 *falciparum* 3D7 parasites. For microarray hybridization, equal amounts of cDNA
510 between 150 and 500 ng of Cy5 labeled sample and Cy3 labeled reference pool were
511 prepared for hybridization as described previously (71). Arrays were scanned on an
512 Agilent G2600D Microarray Scanner (Agilent Technologies, USA) with 5 µm resolution
513 at wavelengths of 532 nm (Cy3) and 633 nm (Cy5). Linear lowess normalized signal
514 intensities were extracted using the Agilent Feature Extractor Software version
515 11.5.1.1 using the GE2_1100_Jul11_no_spikein protocol and data was uploaded onto
516 the Princeton University Microarray Database (https://puma.princeton.edu/).

517 **Data analysis**

518 Signal intensities loaded on the Princeton University Microarray Database were filtered
519 to remove background and unsatisfactory spots were flagged for removal using spot
520 filters $P<0.01$ and $\log_2$ (Cy5/Cy3) expression values were used for further analysis.
521 Euclidean distance clustered heatmaps were generated using TIGR MeV software
522 version 4.9.0 (http://www.tm4.org/mev.html). The R statistical package (version 3.3.2)
523 was used to calculate Pearson correlation coefficients and these were visualized using
524 the Corrplot package. Data were divided into 10 clusters using K-means analysis
525 following a within sum of squares test to determine the optimal number of clusters.

526 For functional analysis of genes, gene ontology enrichments were obtained for
527 biological processes with $P<0.05$ using curated evidence using PlasmoDB Release v
528 33 (http://www.plasmodb.org/) and supplemented with annotation from MPMP (66)
529 and Interpro (https://www.ebi.ac.uk/interpro/). Additional supplementary datasets for
530 translationally repressed genes (33,35), transcripts involved in commitment (18,20,25)
531 and gametocyte transcriptomes and proteomes (35,39–41) were probed for significant
532 association with clusters of expression using a two-tailed Fisher's exact test to
533 calculate significant association between the datasets. For comparison between
534 transcript abundance and histone post-translation modifications, supplementary
535 information was sourced for ChIP-seq or ChIP-chip experiments from the Gene
536 Expression Omnibus (GEO) datasets for H3K56ac, H4K5/8/12ac (72) as well as
537 Salcedo-Amaya *et al.* for H3K9me3 (17), Jiang *et al.* 2014 for H3K36me3 (16) and
538 Flueck *et al.* 2009 and Fraschka *et al.* for HP1 occupancy in *P. falciparum* parasites
539 (46,47). The genes associated with the specific histone marks in each of the
540 publications were then probed for association with specific clusters of expression using
541 two-tailed Fisher's exact tests and increased presence of the post-translational

542 modification in gametocytes (65). To determine the involvement of ApiAP2
543 transcription factors in gametocyte development, the Gene Regulation Network
544 Inference Using Time Series (GRENITS) package in R was applied (probability
545 threshold >0.7) using the total transcriptome as possible regulated genes (68). The
546 package uses Dynamic Bayesian Networks and Gibbs Variable Selection to construct
547 a linear interaction model between gene expression profiles of putative "regulators"
548 and "regulatees" over time-correlated data. Following the identification of 5 ApiAP2
549 transcription factors (*ap2-g* was not included in further predictive analysis) with
550 putative regulatory activity, these transcription factors were re-probed as regulators,
551 using genes containing the transcription factor's binding site as possible regulated
552 genes if the binding site had been determined (13). The number of links per model,
553 per threshold was evaluated to determine the set probability threshold for the regulated
554 genes of each transcription factor. The online FIRE algorithm (56) was used to identify
555 enriched regulatory motifs in genes of interest.

556 **Data availability**

557 The microarray data has been submitted to the GEO with accession number
558 GSE104889 (www.ncbi.nlm.nih.gov/geo/).

559

560 **Author Contributions**

561 RvB and LMB conceived the study. RvB, LO conducted experiments, HP contributed
562 data, RvW, RvB, JR performed the analyses. RvB, RvW, JN, HP, ML and LMB
563 interpreted results. RvB, ML, LMB wrote the paper with inputs from the other authors.
564 All co-authors approved the final version of the paper.

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Competing interests

The authors declare that they have no competing interests

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802

803

804 **Figure Legends**

805 **Fig 1: The developmental and associated transcriptomic profile of *P. falciparum***
806 **NF54 gametocytes from commitment to maturity. (A)** Sampling and culturing
807 strategy and stage distribution of parasites on each day of the time course. Colored
808 lines indicate the presence of specific stages at different time points. Abbreviations
809 indicate parasitemia (P) and hematocrit (HC) at induction, * indicate the addition of N-
810 acetyl glucosamine (NAG) or 5% D-sorbitol. Parasite drawings were modified from
811 freely available images (https://smart.servier.com/), under a Creative Commons
812 Attribution 3.0 Unported Licence. **(B)** Morphological development was monitored from
813 induction (day -2) over 16 days of development using Giemsa-stained thin-smear
814 microscopy. The stage distribution for each day was calculated by counting ≥100
815 parasites on each day of monitoring. Legend: I-V indicates different stages of
816 gametocyte development, R=ring and T=trophozoite stage asexual parasites. **(C)**
817 Pearson correlation coefficients of the total transcriptomes obtained for each day of
818 development. Red boxes indicate localized phases of increased correlation. **(D)**
819 Expression of "gold standard" asexual and gametocyte genes (43) are shown for the
820 gametocyte time course in heatmaps. **(A-D)** Area plot designates the timing of
821 appearance and abundance of specific stages throughout the time course.

822 **Fig 2: Distinct clusters of expression link to biological development of the *P.***
823 ***falciparum* gametocyte.** Clusters of genes expressed during gametocyte
824 development following K10 clustering of the total transcriptome. **(A)** The 10 clusters
825 were grouped into phases of decreased, maintained, increased or developmentally
826 regulated transcript abundance with number of transcripts per cluster indicated in
827 brackets and genes of interest from specific clusters highlighted next to heatmaps.
828 Area plot designates the timing of appearance and abundance of specific stages

829 throughout the time course. **(B)** Biological processes of interest were selected from
830 GO enrichment (Supplementary File 1) of each of the clusters (P<0.05) with the
831 number of genes related to these functions shown for the groups of clusters in bar
832 graphs with generic descriptions of the gene sets used to describe their function.

833

834 **Fig 3: Commitment and development are distinctly regulated processes. (A)** The
835 genes increased in expression during commitment (18,20,25) were compared to
836 transcripts increased in abundance during gametocytogenesis (Clusters 6-10, 2425
837 transcripts) with overlapping genes of interest: *sap18 (pf3d7_0711400), sir2a*
838 *(pf3d7_1328800)* and genes only increased during commitment *gdv1*
839 *(pf3d7_0935400)*, *iswi (pf3d7_0624600), sn2fl (pf3d7_1104200)* highlighted in
840 heatmaps. **(B)** The 2429 genes expressed during gametocytogenesis also contained
841 significantly enriched regulatory 5' motifs identified using the FIRE algorithm (56).

842 **Fig 4: Stage-specific increases in gene expression contribute to the extended**
843 **differentiation of *P. falciparum* gametocytes. (A)** During stage I-III of development
844 genes in cluster 8 sharply increased in expression (indicated with dotted line) with the
845 abundance of these transcripts indicated by ribbon plot with mean±SD. GO enrichment
846 of genes involved in regulation of transcription (GO:0010468, 11 transcripts, P=0.029)
847 is present in this cluster, with presence of protein for these genes in stage I/II and V
848 indicated in black (35,39–41) and the corresponding Interpro domains
849 (https://www.ebi.ac.uk/interpro/) of proteins with unknown function indicated on the
850 right. **(B)** The timing of sexually dimorphic transcript profiles (35) are shown in line
851 graphs while the association of male-and female-enriched transcripts with specific
852 clusters (6-10) are shown as standardized residuals and significance of these

853 associations indicated (*P*<0.05*,0.001**,0.0001***, Fisher's exact test). Genes of
854 interest for each sex are highlighted in heatmaps next to male and female symbols.
855 **(C)** The genes expressed during maturation (cluster 10) showed a significant
856 association (*P*<0.0001, two-tailed Fisher's exact test) with genes stabilized post-
857 transcriptionally during commitment (18) and H3K36me3-associated genes in asexual
858 development (16,73) before a sharp increase at stage IV-V of development (dashed
859 line). Blocks indicate the timing of stabilization of the transcripts (18) or abundance of
860 the H3K36me3 mark (65) and the overlap between the 3 datasets are indicated in the
861 Venn diagram. Genes of interest within the three functional datasets are highlighted in
862 heatmap. **(A-C)** Area plot designates the timing of appearance and abundance of
863 specific stages throughout the time course.

864 **Fig 5: ApiAP2 transcription factors act as regulatory elements during**
865 **gametocytogenesis. (A)** ApiAP2 transcription factors increased in transcript
866 abundance during gametocytogenesis were evaluated for their expression throughout
867 gametocyte development with blocks indicating periods of increased abundance. Area
868 plot designates the timing of appearance and abundance of specific stages throughout
869 the time course. **(B)** The transcription factors were also probed for regulatory activity
870 using coexpression analysis by GRENITS. Transcription factors with known binding
871 sites (13), were probed against genes containing the transcription factor binding sites
872 indicated or the total transcriptome if their binding site was unknown. The targets of
873 each transcription factor are shown by shaded ribbons, with correlated transcripts
874 indicated in red and anticorrelated transcripts indicated in blue. Generic functional
875 terms describing enriched gene ontology terms or individual gene products are
876 indicated in red (increased transcripts) or blue (decreased transcripts).

877 **Fig 6: Molecular model of regulatory modules that shape cellular differentiation**
878 **during gametocytogenesis.** Specific regulatory events are mapped out over the
879 extended gametocyte development of *P. falciparum* parasites. Molecular regulators
880 are highlighted in red while specific events or epigenetic marks are shown in black.
881 Colored blocks indicate the span of specific phases of transcript abundance, with
882 dotted lines indicating transition points in gametocyte development and grey triangles
883 indicate the timing of repressive mechanisms in gametocyte development. Parasite
884 drawings were modified from freely available images (https://smart.servier.com/),
885 under a Creative Commons Attribution 3.0 Unported Licence.

886 **Supporting information captions**

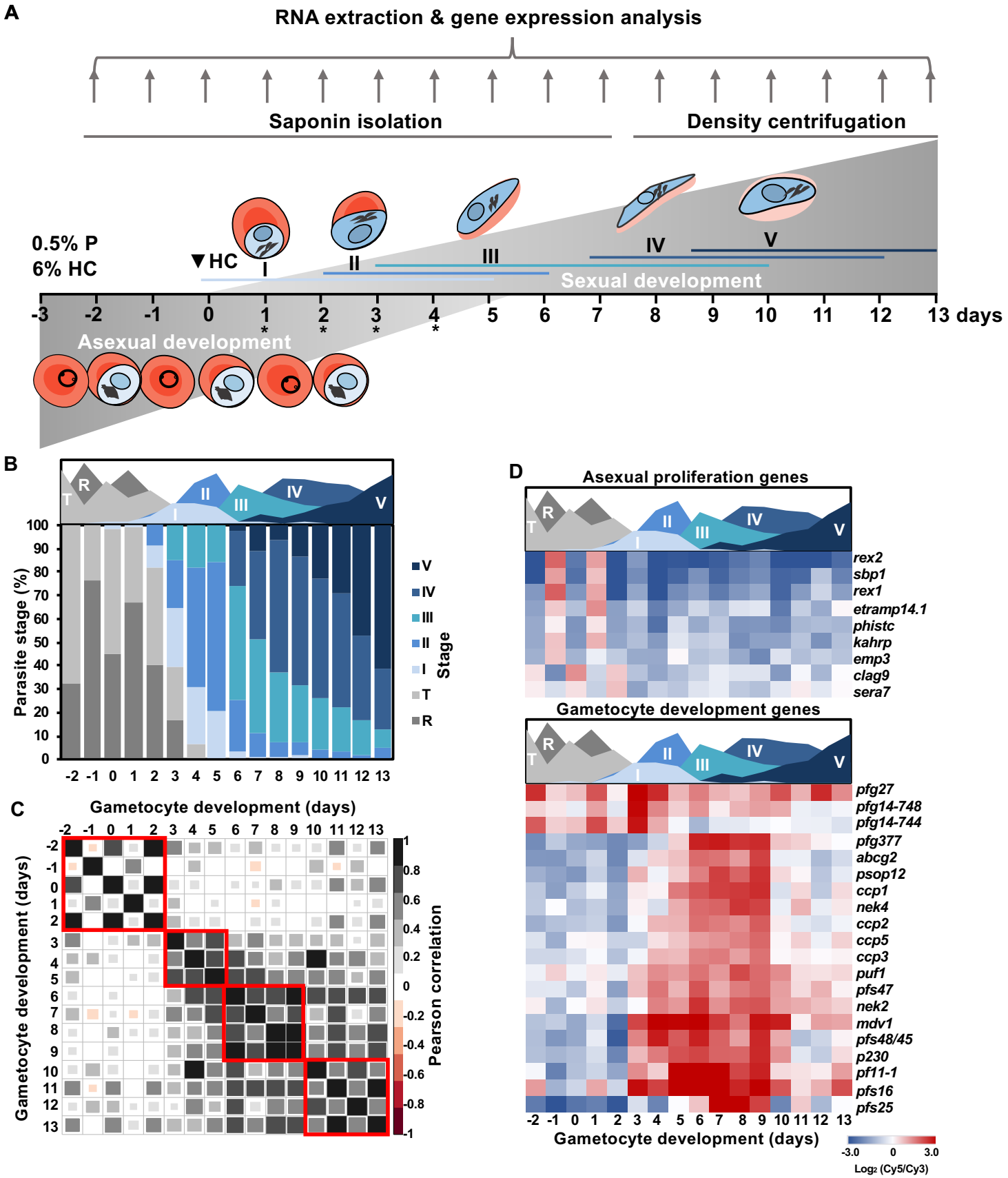
887 **Supplementary Fig S1. Transcript abundance of ApiAP2 transcription factors**
888 **during *P. falciparum* gametocyte development**

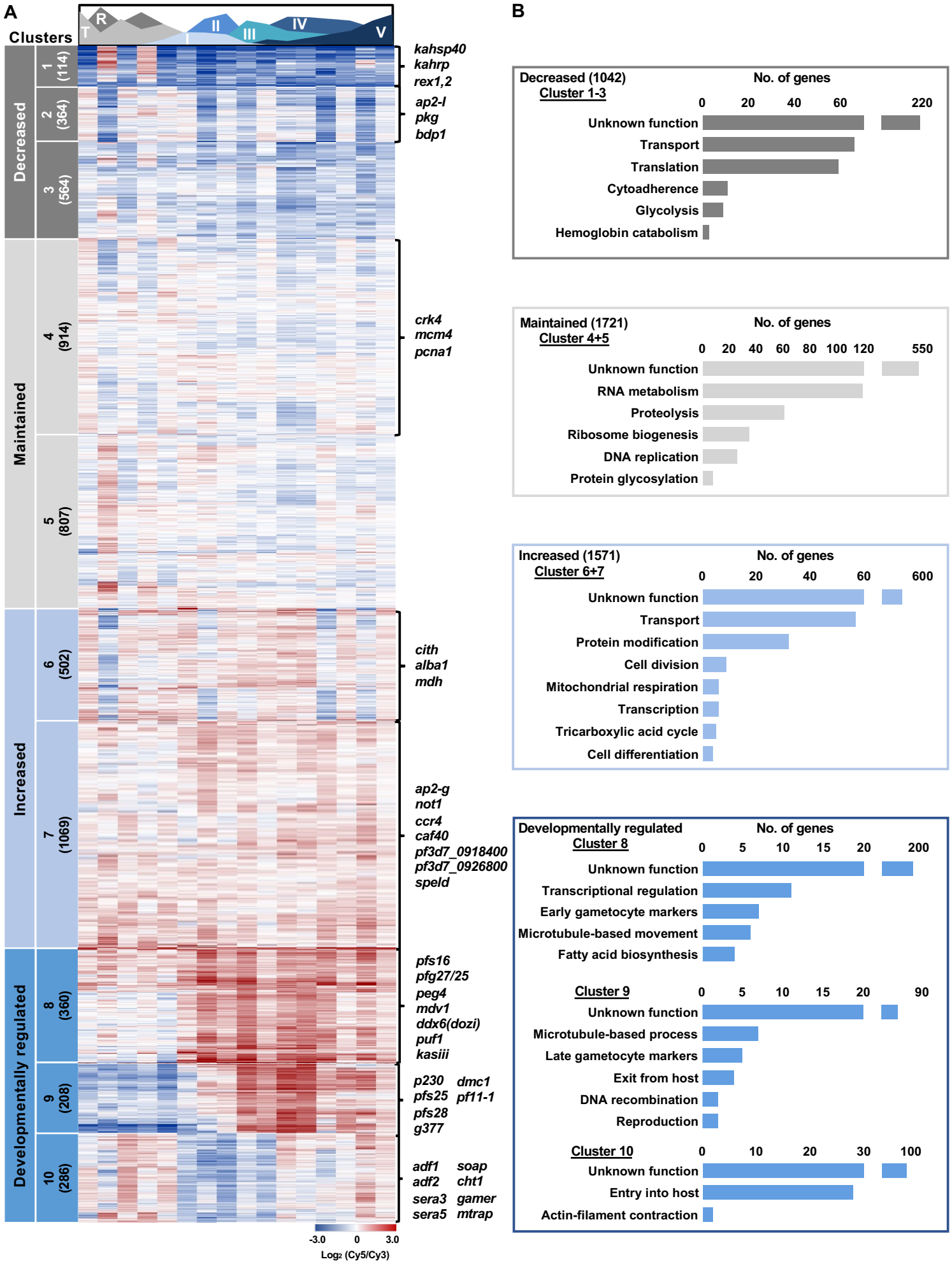
889 **Supplementary Fig S2. Transcript abundance of *ap2-g* and downstream genes**
890 **(identified in Josling *et al.* 2019)**

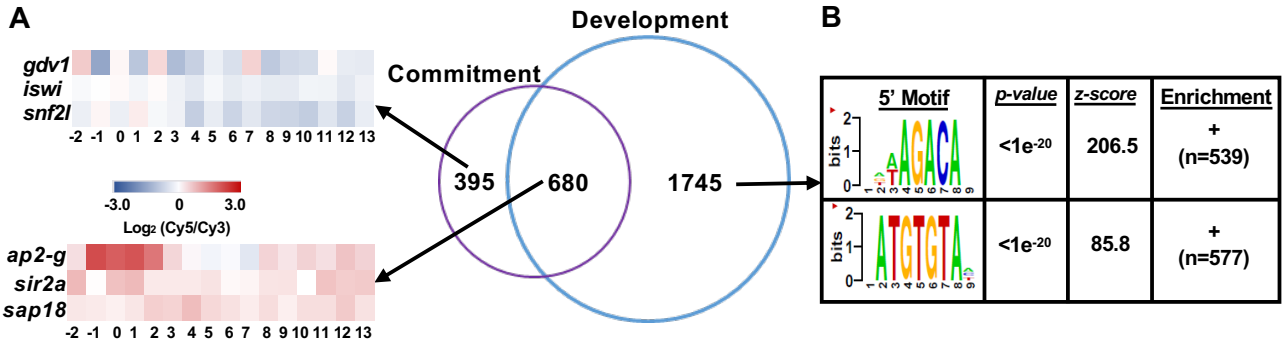
891 **Supplementary File 1. Total microarray data with GO enrichment pertaining to**
892 **Figure 1&2**

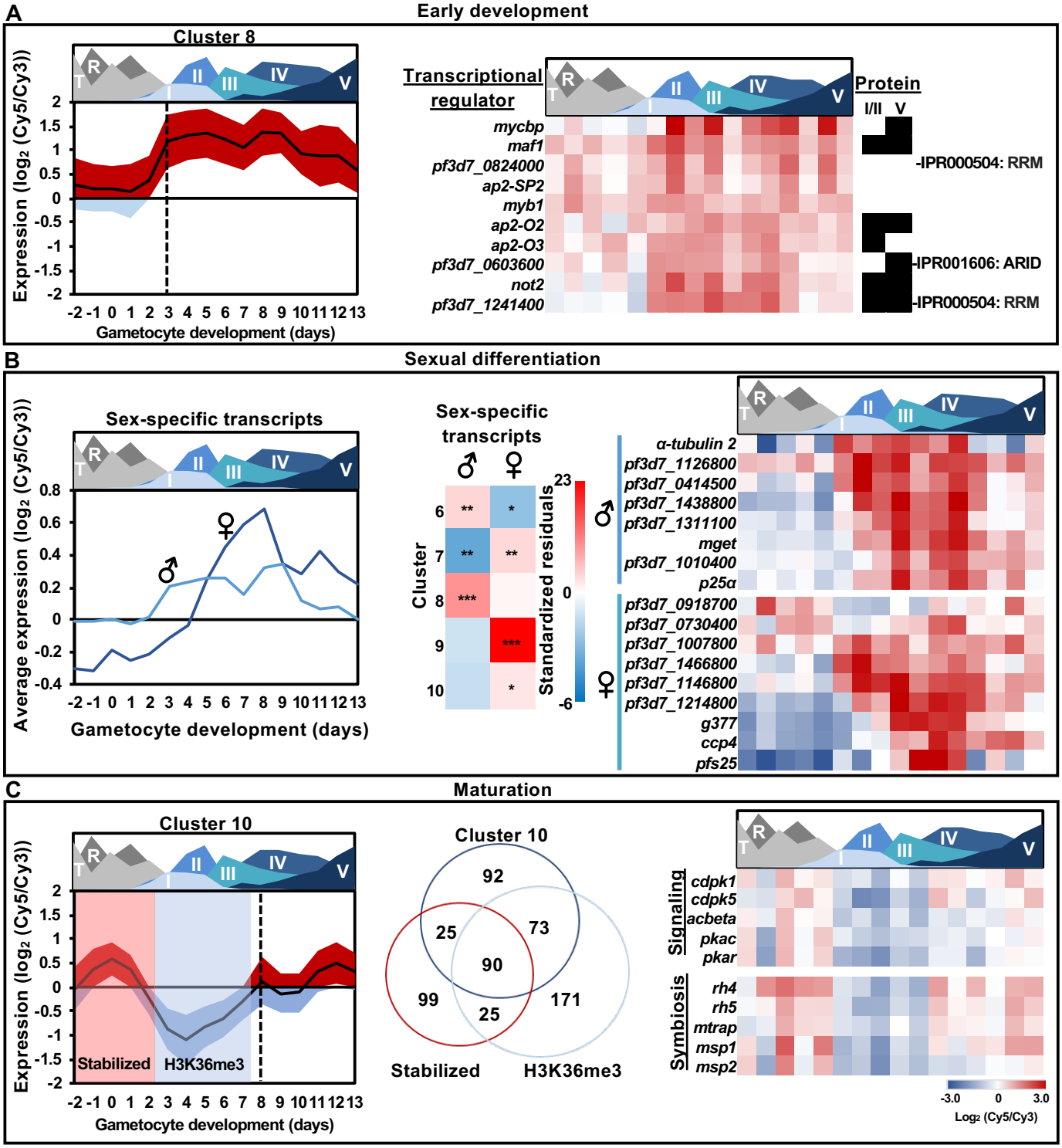
893 **Supplementary File 2. Correlation of microarray time points and gametocyte**
894 **markers pertaining to Figure 1**

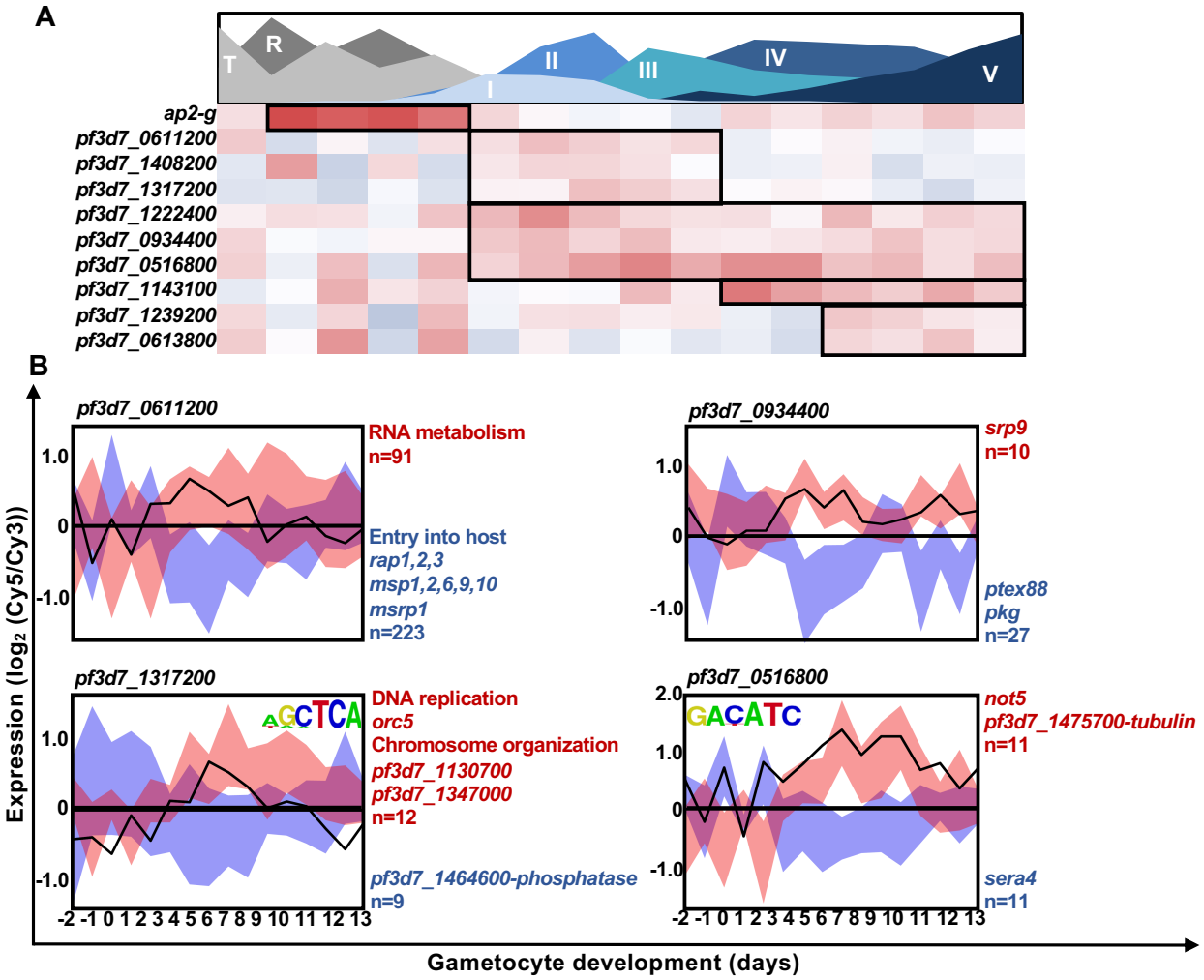
895 **Supplementary File 3. Cross-dataset comparison and functional enrichment**
896 **pertaining to Figures 2-5**

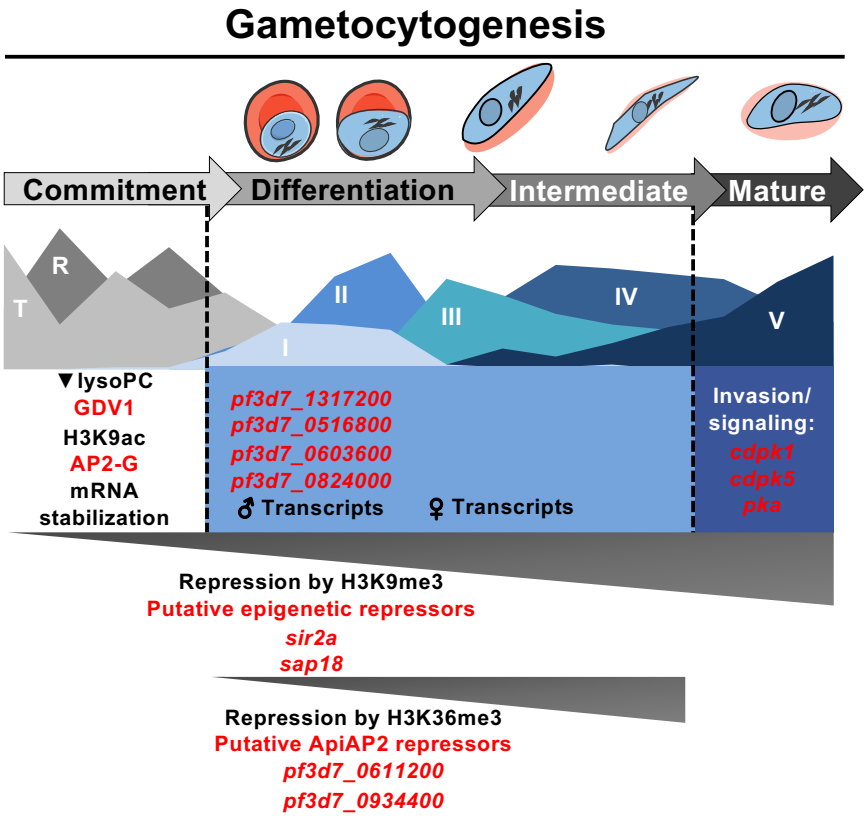

A
RNA extraction & gene expression analysis
Saponin isolation
Density centrifugation
0.5% P
6% HC
HC
I
II
III
IV
V
Sexual development
Asexual development
-3 -2 -1 0 1 2 3 4 5 6 7 8 9 10 11 12 13 days
B
Parasite stage (%)
Stage
C
Gametocyte development (days)
Pearson correlation
D
Asexual proliferation genes
rex2
sbp1
rex1
etramp14.1
phistc
kahrp
emp3
clag9
sera7
Gametocyte development genes
pfg27
pfg14-748
pfg14-744
pfg377
abcg2
psop12
ccp1
nek4
ccp2
ccp5
ccp3
puf1
pfs47
nek2
mdv1
pfs48/45
p230
pf11-1
pfs16
pfs25
Gametocyte development (days)
Log2 (Cy5/Cy3)


A
Clusters
R
T
I
II
III
IV
V
Decreased
Maintained
Increased
Developmentally regulated
1 (114)
2 (364)
3 (564)
4 (914)
5 (807)
6 (502)
7 (1069)
8 (360)
9 (208)
10 (286)
kahsp40
kahrp
rex1,2
ap2-I
pkg
bdp1
crk4
mcm4
pcna1
cith
alba1
mdh
ap2-g
not1
ccr4
caf40
pf3d7_0918400
pf3d7_0926800
speld
pfs16
pfg27/25
peg4
mdv1
ddx6(dozi)
puf1
kasiii
p230
pfs25
pfs28
g377
dmc1
pf11-1
adf1
adf2
sera3
sera5
soap
cht1
gamer
mtrap
-3.0
0
3.0
Log2 (Cy5/Cy3)
B
Decreased (1042)
Cluster 1-3
No. of genes
0 20 40 60 220
Unknown function
Transport
Translation
Cytoadherence
Glycolysis
Hemoglobin catabolism
Maintained (1721)
Cluster 4+5
No. of genes
0 20 40 60 80 100 120 550
Unknown function
RNA metabolism
Proteolysis
Ribosome biogenesis
DNA replication
Protein glycosylation
Increased (1571)
Cluster 6+7
No. of genes
0 20 40 60 600
Unknown function
Transport
Protein modification
Cell division
Mitochondrial respiration
Transcription
Tricarboxylic acid cycle
Cell differentiation
Developmentally regulated
Cluster 8
No. of genes
0 5 10 15 20 200
Unknown function
Transcriptional regulation
Early gametocyte markers
Microtubule-based movement
Fatty acid biosynthesis
Cluster 9
0 5 10 15 20 90
Unknown function
Microtubule-based process
Late gametocyte markers
Exit from host
DNA recombination
Reproduction
Cluster 10
0 10 20 30 100
Unknown function
Entry into host
Actin-filament contraction


A
gdv1
iswi
snf2l
-2 -1 0 1 2 3 4 5 6 7 8 9 10 11 12 13
-3.0 0 3.0
Log2 (Cy5/Cy3)
ap2-g
sir2a
sap18
-2 -1 0 1 2 3 4 5 6 7 8 9 10 11 12 13
Commitment
Development
395
680
1745
B
5' Motif
p-value
z-score
Enrichment
bits
<1e-20
206.5
+
(n=539)
<1e-20
85.8
+
(n=577)


A
Early development
Cluster 8
Expression (log2 (Cy5/Cy3))
Gametocyte development (days)
Transcriptional regulator
Protein
I/II
V
mycbp
maf1
pf3d7_0824000
ap2-SP2
myb1
ap2-O2
ap2-O3
pf3d7_0603600
not2
pf3d7_1241400
-IPR000504: RRM
-IPR001606: ARID
-IPR000504: RRM
B
Sexual differentiation
Sex-specific transcripts
Average expression (log2 (Cy5/Cy3))
Gametocyte development (days)
Sex-specific transcripts
Cluster
Standardized residuals
α-tubulin 2
pf3d7_1126800
pf3d7_0414500
pf3d7_1438800
pf3d7_1311100
mget
pf3d7_1010400
p25α
pf3d7_0918700
pf3d7_0730400
pf3d7_1007800
pf3d7_1466800
pf3d7_1146800
pf3d7_1214800
g377
ccp4
pfs25
C
Maturation
Cluster 10
Expression (log2 (Cy5/Cy3))
Stabilized
H3K36me3
Gametocyte development (days)
Cluster 10
92
25
73
90
99
25
171
Stabilized
H3K36me3
Signaling
cdpk1
cdpk5
acbeta
pkac
pkar
Symbiosis
rh4
rh5
mtrap
msp1
msp2
Log2 (Cy5/Cy3)


A
T
R
I
II
III
IV
V
ap2-g
pf3d7_0611200
pf3d7_1408200
pf3d7_1317200
pf3d7_1222400
pf3d7_0934400
pf3d7_0516800
pf3d7_1143100
pf3d7_1239200
pf3d7_0613800
B
Expression (log2 (Cy5/Cy3))
pf3d7_0611200
RNA metabolism
n=91
Entry into host
rap1,2,3
msp1,2,6,9,10
msrp1
n=223
pf3d7_0934400
srp9
n=10
ptex88
pkg
n=27
pf3d7_1317200
DNA replication
orc5
Chromosome organization
pf3d7_1130700
pf3d7_1347000
n=12
pf3d7_1464600-phosphatase
n=9
pf3d7_0516800
not5
pf3d7_1475700-tubulin
n=11
sera4
n=11
Gametocyte development (days)


Gametocytogenesis
Commitment
Differentiation
Intermediate
Mature
R
T
I
II
III
IV
V
▼lysoPC
GDV1
H3K9ac
AP2-G
mRNA
stabilization
pf3d7_1317200
pf3d7_0516800
pf3d7_0603600
pf3d7_0824000
♂ Transcripts
♀ Transcripts
Invasion/
signaling:
cdpk1
cdpk5
pka
Repression by H3K9me3
Putative epigenetic repressors
sir2a
sap18
Repression by H3K36me3
Putative ApiAP2 repressors
pf3d7_0611200
pf3d7_0934400