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1 **The *in vivo* transcriptome of *Schistosoma mansoni* in two prominent vector species,**
2 ***Biomphalaria pfeifferi* and *B. glabrata***

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13 **ABSTRACT**

14 **Background**

15 The full scope of the genes expressed by schistosomes during intramolluscan development
16 has yet to be characterized. Understanding the gene products deployed by larval schistosomes
17 in their snail hosts will provide insights into their establishment, maintenance, asexual
18 reproduction, ability to castrate their hosts, and their prolific production of human-infective
19 cercariae. Using the Illumina platform, the intramolluscan transcriptome of *Schistosoma*
20 *mansoni* was investigated in field-derived specimens of the prominent vector species
21 *Biomphalaria pfeifferi* at 1 and 3 days post infection (d) and from snails shedding cercariae.
22 These *S. mansoni* samples were derived from the same snails used in our complementary *B.*
23 *pfeifferi* transcriptomic study. We supplemented this view with microarray analyses of *S.*
24 *mansoni* from *B. glabrata* at 2d, 4d, 8d, 16d, and 32d.

25 **Principal Findings**

26 Transcripts representing at least 7,740 (66%) of known *S. mansoni* genes were expressed
27 during intramolluscan development, with the greatest number expressed in snails shedding
28 cercariae. Many transcripts were constitutively expressed throughout development featuring
29 membrane transporters, and metabolic enzymes involved in protein and nucleic acid synthesis
30 and cell division. Several proteases and protease inhibitors were expressed at all stages,
31 including some proteases usually associated with cercariae. Transcripts associated with G-
32 protein coupled receptors, germ cell perpetuation, and stress responses and defense were well
33 represented. We noted transcripts homologous to planarian anti-bacterial factors, several neural
34 development or neuropeptide transcripts including neuropeptide Y, and receptors that may be

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35 associated with schistosome germinal cell maintenance and that could also impact host
36 reproduction. In at least one snail the presence of larvae of another digenean species (an
37 amphistome) was associated with repressed *S. mansoni* transcriptional activity.

38 **Conclusions/Significance**

39 This *in vivo* study, particularly featuring field-derived snails and schistosomes, provides a
40 distinct view from previous studies of development of cultured intramolluscan stages from lab-
41 maintained organisms. We found many highly represented transcripts with suspected or
42 unknown functions, with connection to intramolluscan development yet to be elucidated.

43

44 **AUTHOR SUMMARY**

45 *Schistosoma mansoni* is one of the most important schistosome species causing the
46 neglected tropical disease human intestinal schistosomiasis. By focusing on *S. mansoni in vivo*
47 with its broadly distributed sub-Saharan African snail intermediate host, *Biomphalaria pfeifferi*,
48 we uncover new insights and basic knowledge of this host-parasite relationship that are critical
49 for understanding schistosomiasis transmission. We show that *in vivo* studies, particularly using
50 field-derived specimens, provides a distinct view from the uniformed transcriptional responses
51 traditionally seen from *in vitro* studies on *S. mansoni* and *Biomphalaria* snails. With the growing
52 consensus that we need to supplement chemotherapy with other control methods,
53 understanding how *S. mansoni* interacts with its obligatory snail host becomes integral for future
54 planning of control programs. The data provided within provides specific analysis on how the
55 schistosomes successfully protect themselves from host defenses and the necessary
56 transcriptional responses required for its amplifying asexual proliferation that result in human-
57 infective cercariae.

58

59 **INTRODUCTION**

60 The vast majority of the estimated 18,000 species of digenetic trematodes depend on a
61 molluscan host, usually a gastropod, in which to undertake the complex developmental program
62 characterized by extensive asexual reproduction and production of numerous cercariae [1-3].
63 The extent to which this large lineage of parasites has remained true to its dependence on
64 molluscs, and the evident success achieved by digeneans - including by some species
65 responsible for causing human disease - pose fundamental questions of interest for
66 parasitologists, evolutionary biologists, ecologists, developmental biologists and comparative
67 immunologists.

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68 There is much about digenean-gastropod associations worthy of study: the host specificity
69 often shown; the manner by which digeneans establish intimate infections without provoking
70 destructive host responses; the ability of digeneans to affect and manipulate the energy and
71 resource budgets of their hosts, including to achieve host castration; the intricate developmental
72 program featuring multiple stages, asexual reproduction and the perpetuation of the germinal
73 cell lineage; and finally the tendency for some infections to persist for long periods of time,
74 implying protection of the snail-digenean unit that might involve contributions by the parasite to
75 promote its perpetuation. These common and enduring relationships are also targeted and
76 exploited by other organisms, including competing digenean species. One way forward to gain a
77 deeper understanding of all these processes is to acquire a comprehensive overview of the
78 genes expressed by host snails and larval digeneans during the course of infection. This in turn
79 sets the stage for eventually learning more about these two sets of gene products interact (the
80 interactome) to influence the outcome of this interaction.
81 Because *S. mansoni* causes intestinal schistosomiasis in an estimated 166 million people in
82 the Neotropics, Africa and Southwest Asia, it has long been intensively studied, in part because
83 it is relatively easily maintained in a laboratory setting [4,5]. Many molecular tools, including the
84 current fifth version of an improving genome sequence and assembly are available for *S.*
85 *mansoni* [6,7]. Additionally, *B. glabrata*, the most important host for *S. mansoni* in the
86 Neotropics, has become a model gastropod host, including with a recently available genome
87 sequence [8]. In Africa, several *Biomphalaria* species play an important role in transmission,
88 with the most important being *B. pfeifferi*. The latter species occurs widely across sub-Saharan
89 Africa, where >90% of the world's cases of schistosomiasis now occur. *B. pfeifferi* is probably
90 responsible for transmitting more *S. mansoni* infections than any other snail species [9].
91 With respect to the intramolluscan development of *S. mansoni*, following penetration of
92 miracidia usually into the tentacles or head-foot of the snail, there is a 24 hour period of
93 transformation as miracidial ciliated epidermal plates, apical papilla, and sensory papillae are
94 shed and a syncytial tegument is formed around the developing mother (or primary) sporocyst
95 [10,11]. This early period can be thought of as one of parasite transition and establishment. The
96 miracidium has carried into the snail a series of germinal cells that are destined to give rise to
97 the daughter (or secondary) sporocysts [10]. Mitotic division of germinal cells begins as early as
98 24 hours and germinal cells proliferate notably in an enlarging mother sporocyst [12]. By 6 days
99 after infection, all mother sporocysts have germinal balls (daughter sporocyst embryos) which
100 occupy nearly the entire body cavity [10,13,14]. The embryonic daughter sporocysts, of which
101 there are an average of 23 produced per mother sporocyst [10], continue to grow and elongate.

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102 Daughter sporocysts exit mother sporocysts at 12-14 days and start their migration to the
103 digestive gland and ovotestis region of the snail [10,13,15]. Upon release of daughter
104 sporocysts, mother sporocysts collapse and typically do not continue to produce daughter
105 sporocysts, but they nonetheless persist in the head-foot of the snail. By 15-20 days, daughter
106 sporocysts undergo a remarkable transformation, losing their definitive vermiform shape to
107 become amorphous and are wedged between lobules of the digestive gland. Within them,
108 cercarial embryos begin to develop, passing through 10 characteristic developmental stages
109 culminating in the production of a muscular tail and a body dominated by gland cells that are
110 filled with lytic enzymes [10,16]. Once again, a separate allotment of germinal cells is
111 sequestered in the cercarial body and these are destined to become the gonads and
112 reproductive cell lineages of adult worms. Around 32 days post-exposure, cercariae exit from
113 daughter sporocysts, migrate through the snail's body and emerge into the water, usually
114 through hemorrhages in the mantle. Daughter sporocysts occupy a significant proportion of the
115 snail host's body; 65% of the snail's digestive gland can be occupied by daughter sporocysts in
116 a patent, cercariae-producing infection [17].

117 The timing of these events is dependent on temperature but cercarial shedding can occur as
118 early as 19 days post-miracidial penetration [18]. Also, in some situations, daughter sporocysts
119 will produce granddaughter sporocysts in lieu of cercariae [19]. So, beginning with the
120 penetration of a single miracidium and proceeding through at least two distinct phases of
121 asexual reproduction, thousands of cercariae can ultimately be produced [20]. A typical
122 consequence of infection is that snails are partially or totally castrated, the extent depending on
123 whether they were infected before or after achieving maturity [15,21]. This interaction is
124 remarkable in that some *Biomphalaria* snails can survive for over a year shedding cercariae
125 daily [20], although there is considerable variability in the duration of survival of infected snails.
126 The productivity of infections within snails has no doubt contributed greatly to the success of all
127 digenetic trematodes, and in the case of human-infecting schistosomes, is a major factor
128 complicating their control.

129 Despite the significant immunobiological, physiological, and reproductive changes inflicted
130 upon infected *Biomphalaria* snails [22]. we still lack a comprehensive picture of what the
131 parasite is producing to effect such changes. Verjovski-Almeida et al. [23] recovered 16,715
132 ESTs from early-developing cercarial germ balls derived from snails with patent *S. mansoni*
133 infections. These ESTs were distinctive from those noted for miracidia, cercariae,
134 schistosomulae, eggs, and adults. A first-generation *S. mansoni* microarray containing 7,335
135 features was used to monitor expression changes of miracidia and *in vitro*-cultured 4d mother

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136 sporocysts [24]. Of the 7,335 features, 273 (6%) of these were expressed only in sporocysts.
137 Gene products with antioxidant activity, oxidoreductases, and intermolecular binding activity
138 were represented in mother sporocyst-specific genes. Proteomic analyses of products released
139 *in vitro* by miracidia of *S. mansoni* transforming into mother sporocysts revealed 127 proteins
140 produced, 99 of which could be identified [25]. Among these were proteases, protease
141 inhibitors, heat shock proteins, redox/antioxidant enzymes, ion-binding proteins, and venom
142 allergen-like (SmVAL) proteins. Wang et al. [26] also provided an analysis of proteins released
143 by *S. mansoni* miracidia and noted several of the same features, and also provided a foundation
144 for further study of neurohormones produced by *S. mansoni* larvae. Cultured mother sporocysts
145 were a component of SAGE tag generation by Williams et al. [27]. Highlights of 6d and 20d
146 cultured mother sporocysts transcript expression were an up-regulation of HSP 70, HSP 40, egg
147 protein, and trypsinogen 1-like all exclusive to miracidia and sporocyst stages. LongSAGE was
148 utilized by Taft et al. [29] to identify transcripts from miracidia, or 6d or 20d cultured mother
149 sporocysts grown in medium conditioned by sporocysts or by products derived from the Bge (*B.*
150 *glabrata* embryo) cell line. Amongst the groups studied, 432 transcripts were differentially
151 expressed (DE), which was also dependent on whether or not the sporocysts had been
152 conditioned in medium with Bge cell products. Wang et al. [12] in a functional study of germinal
153 cells in intramolluscan stages of *S. mansoni*, noted similarities in molecular signatures with the
154 neoblast stem cells produced by planarians.

155 Here, our primary focus is on presentation of RNA-Seq results for *S. mansoni* from the same
156 field-derived Kenyan snails that comprised the *B. pfeifferi* transcriptomic study of Buddenborg et
157 al. [22]. Briefly, field-derived snails found negative upon isolation and shedding were exposed
158 experimentally to *S. mansoni* miracidia hatched from eggs from fecal samples from local
159 schoolchildren. These exposed snails were harvested 1 or 3 days later. Additionally, field snails
160 found to be naturally shedding *S. mansoni* cercariae were chosen for study. Our goal was to
161 provide *in vivo* views of establishment of early mother sporocyst development and shedding
162 stages for snails and parasites taken directly from natural transmission sites. We did not
163 investigate longer exposure intervals following experimental exposures because we did not
164 want these snails to lose their field characteristics. Additionally, we supplemented these
165 observations with results from a set of independent microarray experiments of *S. mansoni* in *B.*
166 *glabrata* acquired at 2, 4, 8, 16, and 32d. These time points cover some additional stages in
167 development including production, release and migration of daughter sporocysts. Our approach
168 is distinctive in its focus on *in vivo* life cycle stages, the inclusion of both snails naturally infected
169 from an endemic area in western Kenya and of laboratory-maintained snails, and the use of two

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170 transcriptome technologies for co-validation of expression data. We examined specific groups of
171 transcripts to gain distinctive insights on intramolluscan development. The database we provide
172 should also provide helpful information in eventually achieving a deeper understanding of the
173 interactome that is the essence of this dynamic host-parasite interaction.
174

175 **METHODS**

176 **Ethics statement and sample collection**

177 Details of recruitment and participation of human subjects for procurement of *S. mansoni*
178 eggs from fecal samples collection are described in Mutuku et al. [30] and Buddenborg et al.
179 [22]. The Kenya Medical Research Institute (KEMRI) Ethics Review Committee (SSC No. 2373)
180 and the University of New Mexico (UNM) Institution Review Board (IRB 821021-1) approved all
181 aspects of this project involving human subjects. All children found positive for *S. mansoni* were
182 treated with praziquantel following standard protocols. This project was undertaken with
183 approval of Kenya's National Commission for Science, Technology, and Innovation (permit
184 number NACOSTI/P/15/9609/4270), National Environment Management Authority
185 (NEMA/AGR/46/2014) and an export permit has been granted by the Kenya Wildlife Service
186 (0004754).

187 Snail exposures for RNA-Seq experiments are described in detail in Buddenborg et al. [22].
188 Briefly, field-collected *B. pfeifferi* were simultaneously exposed to 20 miracidia each from pooled
189 fecal samples (5 individuals) for 1d and 3d. Field-collected, cercariae-producing snails were
190 used for the shedding sample group. Biological triplicates were sequenced for each sample
191 group using Illumina HiSeq 2000 (Illumina, Carlsbad CA) at the National Center for Genome
192 Resources (NCGR) in Santa Fe, NM. In addition, one naturally shedding *B. pfeifferi* snail was
193 sequenced on a 454 sequencer (Roche, Basel Switzerland) to improve *S. mansoni* transcript
194 assembly but these sequences were not used for quantification. See Buddenborg et al. [22] for
195 RNA extraction, library preparation, sequencing procedures, and sequencing summaries.
196

197 ***S. mansoni* microarray experiments**

198 The M-line strain of *B. glabrata* infected with *S. mansoni* PR1 strain was used in the
199 microarray experiments to monitor parasite transcriptional changes that occur during infection.
200 Both snail and trematode were maintained at UNM as previously described [31]. Snails were
201 exposed to 10 miracidia each of *S. mansoni* for 2d, 4d, 8d, 16d, or 32d (shedding snails), with
202 biological triplicate replicates for each time point. An uninfected *B. glabrata* group was also used
203 to account for cross-hybridization from mixed snail-trematode samples. Total RNA was

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204 extracted as previously described [32] and treated with DNAse I (Ambion UK) to remove gDNA
205 contamination. RNA was quantified on a NanoDrop ND-1000 spectrophotometer and quality-
206 assessed using an Agilent 2100 bioanalyzer. cDNA synthesis, amplification, labeling, and
207 hybridization were performed as previously described [32].

208 A publicly available *S. mansoni* microarray (NCBI GEO accession GPL6936) representing
209 19,244 unique *S. mansoni* contigs (38,460 total experimental probes) was used with the
210 following modification: all array probes were duplicated to allow for an added level of
211 replicability. The transcript probes contained on the array were designed to profile 15 different
212 developmental stages. Thus, many of the molecules likely important to larval development are
213 present as well. Microarray images were recovered from a GenePix 4100A (Axon Instrument
214 Inc.) dual channel laser scanner.

215 Raw data was averaged from replicates in each experimental group (2d, 4d, 8d, 16d, 32d),
216 and for replicates in the uninfected snail group (Bg-only). For each experimental group, the
217 mean and standard deviation were calculated, and values falling below one standard deviation
218 from the mean were removed from further analysis. Features that were non-reactive for any of
219 the groups used in this study, amounting to 26,581 probes, were removed as well as those that
220 were cross-reactive with the Bg-only group (787 probes). The average Bg-only value was
221 subtracted from experimental groups for each probe. Calculated expression values less than 1
222 were removed from the analysis and the remaining values were transformed by log base 2.

223 An updated annotation of array features was performed using BLASTn with the NCBI
224 nucleotide database (sequence identity >70%, E-value <10^{-06}), BLASTp with the NCBI non-
225 redundant protein database (sequence identity >40%, E-value <10^{-06}). Array features were
226 matched to their homologous *S. mansoni* transcript by BLASTn against the assembled *S.*
227 *mansoni* transcripts. These homologous transcripts were used for analyses comparing across
228 Illumina and array samples.

229

230 ### ***S. mansoni*** **transcriptome assembly and annotation**

231 An overview of our analysis pipeline is shown in S1 Fig. After pre-processing all Illumina
232 reads, those from Illumina and 454 sequencing that did not map back to the *B. pfeifferi*
233 transcriptome or identified symbionts were assembled into contigs (assembled, overlapping
234 reads). The separation of host, parasite, and symbiont reads is described in detail in
235 Buddenborg et al. [22]. We employed Trinity v2.2 RNA-Seq *de novo* assembler [33,34] for *de*
236 *novo* and genome-guided transcriptome assembling using paired-end reads only. The *S.*
237 *mansoni de novo* assembly consisted of reads that did not map to the *B. pfeifferi* transcriptome

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238 or symbionts after alignment with Bowtie2 v2.2.9 [35]. The genome-guided transcriptome
239 assembly was performed using STAR v.2.5 2-pass alignment [36] to the *S. mansoni* genome
240 (GeneDB: *S. mansoni* v5.2).
241

242 *Schistosoma mansoni* genome-guided and *de novo* Trinity assemblies were concatenated
243 and redundancy reduced using CD-Hit-EST at 95% similarity [37]. The resulting sequences
244 were screened against *B. glabrata* (VectorBase: BglaB1) and *S. mansoni* genomes, peptides,
245 and mRNAs using BLASTx and BLASTn (sequence identity >70%, E-value < 10^{-12}). Sequences
246 with blast results to *B. glabrata* were removed and remaining *S. mansoni*-specific sequences
247 are henceforth referred to as transcripts.

248 All assembled transcripts were annotated based on their closest homologs and predicted
249 functional domains in the following databases and tools: BLASTp with NCBI non-redundant
250 protein database (sequence identity >40%, E-value <10^{-06}), BLASTn with NCBI nucleotide
251 database (sequence identity >70%, E-value < 10^{-06}), BLASTn consensus of top 50 hits
252 (sequence identity >70%, E-value < 10^{-06}), Gene Ontology [38], KEGG [39], and InterProScan5
253 [40].

254 *Schistosoma mansoni* transcript-level quantification was calculated with RSEM (RNA-Seq
255 by expectation maximization) [41] and TPM (Transcripts Per kilobase Million) values were used
256 for downstream analyses. TPM is calculated by normalizing for transcript length and then by
257 sequencing depth ultimately allowing us to compare the proportion of reads that mapped to a
258 specific transcript [42,43]. Full Blast2Go [44] annotations were performed on all assembled *S.*
259 *mansoni* transcripts.
260

261 RESULTS AND DISCUSSION

262 Illumina-derived *S. mansoni* transcriptomic characteristics

263 Throughout this discussion, a "transcript" is defined as assembled *S. mansoni* contigs
264 formed from overlapping reads with the understanding that this includes both full-length
265 transcripts, partial transcripts, and isoforms. For our Illumina-based study, a total of 23,602
266 transcripts made up our combined genome-guided and *de novo* assembled *S. mansoni*
267 intramolluscan transcriptome. Microarray and Illumina expression data can be found in S1 File.
268 *Schistosoma mansoni* assembly metrics are provided in S1 Table. When all raw reads from
269 each infected snail were mapped to the *S mansoni* transcripts, 1d, 3d, and shedding replicates'
270 mapping percentages ranged from 4.01-5.46, 1.48-4.05, and 4.36-9.72, respectively (S2 Fig).
271 The principal component analysis (PCA) plot (S3 Fig) shows that the percentage of *S. mansoni*

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272 reads varies, and that 1d and 3d groups show more variation between replicates than do
273 shedding replicates. It is not surprising that the transcriptional responses among early replicates
274 at 1 an 3d are more variable in this natural system involving both genetically variable snails and
275 schistosomes, especially as compared to shedding snails which have reached a steady state of
276 continued cercarial production. Also, because the parasite stages at 1 and 3d are small relative
277 to their hosts, uniform sampling of their contributions may be harder to achieve.
278

279 S1 Table. RNA-Seq statistics and *S. mansoni de novo* assembly metrics

Filtered reads used in *S. mansoni de novo* assemblies	**222,593,797**
***De novo* assembled contigs**	
Trinity *de novo* Illumina	18,860
Genome-guided Trinity *de novo* Illumina	26,993
Genome-guided Trinity *de novo* 454	5,767
***S. mansoni* transcripts**	**23,602**
% GC	35.40%
N_{50}	1,412
Median transcript length	479
Average transcript length	841.92
Transcripts ≥500nt	11,419 (48.4%)

280

281 **Overall Illumina and microarray *S. mansoni* transcript expression**

282 Transcripts with ≥1 Log_2 TPM in at least one replicate per group in Illumina samples and
283 features with fluorescence ≥ 1 in microarrays were considered for expression analyses. Based
284 on these cutoffs, over fifteen thousand different transcripts representing 7,252 *S. mansoni*
285 genes were detected in 1d *S. mansoni* infections. Following a dip in 3d samples, even more *S.*
286 *mansoni* transcripts were expressed in shedding snails (Fig 1A). The decline noted in the 3d
287 Illumina samples may reflect that at least one replicate returned fewer *S. mansoni* reads in
288 general or may simply reflect a sampling consideration due to the small size of the parasites
289 relative to the snail at this time point. Sustained expression of a large number of transcripts with
290 a general trend towards higher expression in shedding snails was also noted in the microarray
291 data (Fig 1B). Particularly for the Illumina results, some of the transcripts enumerated represent
292 different portions of the same original full-length mRNAs as well as different isoforms, so the
293 actual number of expressed genes is approximately half as many as the number of recorded
294 transcripts. Nonetheless, the variety produced is impressive and generally supported by our
295 microarray results as well (at least 6,000 features expressed at all time points).

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296
297 Fig 1. (A) *Schistosoma mansoni* transcripts with ≥1 Log_2 TPM in at least one replicate per group
298 in Illumina samples. (B) Array features with fluorescence ≥1 in microarray analyses.
299
300 Our datasets generated by Illumina and microarray analyses might be expected to return
301 different results for at least four different reasons: 1) the two methods are totally different in
302 approach; 2) the host snail species and *S. mansoni* strains differed; 3) the time points sampled
303 differed; and 4) the transcripts represented on the array are more limited than whole
304 transcriptomic sequencing provided by Illumina. However, they also provide independent views
305 of the same basic process, so some comparisons are warranted, especially so for shedding
306 snails when the same developmental stage could be compared between techniques. S4 Fig
307 shows for shedding snails the positive correlation of microarray fluorescence in averaged
308 replicates with ≥1 fluorescence (then Log_2 transformed) and Illumina RNA-Seq taking the
309 average of replicates with ≥1 Log_2 TPM. The positive correlation between the two platforms at
310 32d is a likely indication of the steady state of transcription achieved by *S. mansoni* at the stage
311 of ongoing cercarial production. The venn diagram (S4 Fig) serves as a reminder of the greater
312 overall coverage that is achieved in the Illumina samples.
313
314 Particularly noteworthy in both Illumina and array samples was that a large number of
315 transcripts was shared across all time points (Fig 2). Among Illumina groups, >15% of all
316 transcipts are expressed constitutively and among all microarray groups, >34% of all probes
317 were expressed constitutively. This is suggestive of a core transcriptome required of
318 schistosomes living in snails (see below for more details as to what comprises this core
319 transcriptome). When comparing both early time points (1-4d) and shedding time points from
320 both Illumina and array methods, venn diagrams not surprisingly indicate that Illumina RNA-Seq
321 detects more *S. mansoni* transcripts (S5 Fig). In addition, S5 Fig shows less overlap in
322 expression profiles between Illumina and array methods at early time points than for shedding
323 snails, again suggestive of more variation amongst the sampled early time points for reasons
324 already stated above.
325
326 Fig 2. (A) Venn diagrams of *S. mansoni* replicates from Illumina sample groups. (B) Venn
327 diagram of *S. mansoni* transcripts with ≥1 Log_2(TPM) in at least one replicate per group. (C)
328 Venn diagram of expressed unique *S. mansoni* probes with >1 log_2 fluorescence in the
329 microarray.

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330

331 **The intra-molluscan metabolic landscape**

332 After successful penetration of the snail host, digeneans alter their metabolism to depend
333 completely on the resources available in the molluscan host and shift their energy budget
334 towards sporocyst and/or rediae development. One of the unique evolutionary innovations of the
335 Neodermata is the syncytial tegument, a vital aspect of digenean biology providing both
336 protection and a highly efficient route to acquire nutrients from the host species [45]. Through
337 the tegument, schistosomes acquire most nutrients and other key molecules via facilitated or
338 active transport using transmembrane transporters [46]. Glucose transporters are expressed in
339 both adult and larval stages of *S. mansoni* [47,48]. While miracidia in water employ aerobic
340 energy metabolism, after 24 hours of *in vitro* cultivation, sporocysts shift their metabolism
341 towards lactate production [49]. Expression of glucose transporters is particularly important in
342 initial establishment (1d) and in shedding snails (Fig 3). By the 3d day post infection, the
343 parasite up-regulates metabolic processes that are part of the purine salvage pathway and
344 nucleotide biosynthesis, highlighting its transition to reproduction processes and mitosis. This is
345 concurrent with a down-regulation of phosphorylation and general mitochondrial metabolic
346 activities. This highlights the transition to the less aerobic regime within the host, and the shift to
347 a tightly regulated reproductive program rather than active migration within the host or the
348 environment. It has also been observed that daughter sporocysts have fewer mitochondria
349 [50,51]. This shift to anaerobic mode of energy production is reversed by the presence of fully-
350 formed cercariae developing in the sporocysts of actively shedding snails and is corroborated by
351 the fact that aerobic respiration is especially active in the tails of cercariae [52]. This is expected
352 due to the fact that cercariae, once released from the snail, have an active lifestyle and must
353 generate enough energy from a limited amount of stored glycogen. Oxidative phosphorylation,
354 aided by the greater availability of oxygen in the aquatic environment, helps cercariae fulfill their
355 demanding energy requirements.

356

357 Fig 3. Glucose, amino acid, and nucleoside transmembrane transporters present in
358 intramolluscan *S. mansoni* stages.

359

360 While obtaining organic carbon from the host fulfills the energetic requirements of the
361 parasite, any actual growth is nitrogen dependent. Acquiring amino acids and other important
362 building-block molecules is thus paramount to the parasite's fitness. Tegumental amino acid
363 transporters have not been previously been reported in *S. mansoni* sporocysts [46] but here we

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364 provide evidence of the expression of several amino acid transporters across all intramolluscan
365 time points, some of which may be tegumental. A glutamate transporter was the highest
366 expressed amino acid transporter across all replicates in the Illumina samples. This is
367 concurrent with an increase of amino acid biosynthesis by 3d which continues, but to a lesser
368 degree in shedding snails (e.g. Alanine transaminase EC: 2.6.1.2, Glutamate Synthase EC
369 1.4.1.13). Nucleoside transporters were also abundantly expressed, especially in shedding
370 snails, as noted both by Illumina and microarray results.

371 Components of receptor-mediated endocytosis are present in the transcriptome of free-living
372 and adult stages of *S. mansoni* [23]. Transcripts necessary for clathrin-mediated endocytosis,
373 including clathrin assembly proteins, low-density lipoproteins, and adapter complex Ap2 were
374 present in our intramolluscan transcriptome. The regulator of endocytosis, dynamin, was also
375 present. These transcripts may be used in endocytosis to bring in lipids needed to make
376 membranes. Expression of transcripts involved in receptor-mediated endocytosis, and possibly
377 also in exocytosis, was high immediately upon miracidial transformation in 1d *S. mansoni*
378 mother sporocysts.

379 We identified additional putative transmembrane transporters using the Transporter
380 Classification Database (www.tcdb.org) [53]. For microarray samples at 16d and 32d, two
381 transmembrane NADH oxidoreductases and two annexins were most highly expressed (Fig 4).
382 For Illumina samples, the most abundantly expressed transporters at 1d were AAA-ATPase and
383 protein kinase superfamilies whereas the nuclear pore complex, H+ or translocating NADH
384 dehydrogenase, and endoplasmic reticular retrotranslocon families were dominant in 3d and
385 shedding groups. ABC transporters were present in all Illumina samples, with 27 ABC
386 transporter transcripts expressed in shedding snails. Three transcripts, identified as an ATP-
387 binding cassette sub-family F member 2-like isoform X1, isoform X2, and ATP-binding cassette
388 sub-family E member 1-like were highly expressed in every Illumina replicate. It has been
389 suggested that ABC transporters serve an excretory function for adult schistosomes, playing a
390 role in the removal of xenobiotics and/or influencing interactions with the definitive host [54]. The
391 high expression of ABC transporters in intramolluscan stages, particularly in shedding snails,
392 suggests they have an important and as yet not fully appreciated role in development. Perhaps
393 they play a role in elimination of wastes associated with production of cercariae or facilitate
394 release of factors that modify the immediate environment of the daughter sporocysts to favor
395 their continued productivity of cercariae.

396

397 Fig 4. Transmembrane families/superfamilies represented in microarray samples.

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398

399 Protein kinases phosphorylate intracellular proteins in order to alter gene expression and
400 are responsible for many basic cellular functions. In schistosomes, kinases are predicted to play
401 a role in host invasion, sensory behavior, growth, and development [55]. Because of their
402 importance, kinases have been used as potential pharmaceutical targets against *S. mansoni*
403 [56]. A BLASTx homology search of kinases from Kinase SARfari
404 (https://www.ebi.ac.uk/chembl/sarfari/kinasesarfari) confirmed the representation of 19 kinases
405 from 4 different superfamilies on the microarray, and 154 kinases belonging to 7 different
406 superfamilies expressed in Illumina 1d, 3d, and shedding samples (S6 Fig). The highest
407 expressed protein kinases are members of the group CMGC which includes MAPK growth and
408 stress response kinases, cell cycle cyclin dependent kinases, and kinases for splicing and
409 metabolic control.

410

411 **Protease and protease inhibitor transcripts expressed at different stages of parasite**
412 **development**

413 The protease-encoding genes of parasitic helminths have undergone gene duplication and
414 divergence, and by enabling helminths to process diverse proteinaceous substrates are
415 believed to be critical to establishment and perpetuation of infection [57,58]. Helminth proteases
416 and protease inhibitors have proven useful as markers for diagnostics purposes, or as targets
417 for drugs or vaccines [58-60]. In the snail host, larval schistosomes use proteases for nutrient
418 acquisition, to create the space needed for their expansive growth, and for defense functions,
419 potentially destroying or inhibiting lytic host proteases [61]. Miracidia release proteases to
420 facilitate entry into the snail host, often into dense tissue of the head-foot [62]. *In vitro* studies of
421 cultured mother sporocysts have revealed secretion of proteases facilitating degradation of snail
422 hemolymph proteins such as hemoglobin [61].

423 We observed that intramolluscan *S. mansoni* devotes considerable effort to making
424 proteases and protease inhibitors with 397 protease transcripts and 77 protease inhibitor
425 transcripts represented in at least one time point (Fig 5). Replicates of each Illumina time point
426 with the lowest percentage of *S. mansoni* reads (1d-R1, 3d-R2, shedding-R3) also had the least
427 abundant number of transcripts identified as proteases and protease inhibitors. One-day
428 infections (see 1d-R2 and 1d-R3) with higher read counts indicative of robust development
429 show expression of a gamut of *S. mansoni* proteases that somewhat surprisingly resemble
430 those produced by *S. mansoni* in shedding snails. Coincidentally, we noted the snail host up-

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431 regulated expression of protease inhibitors especially during larval establishment at 1d and 3d
432 [22].
433
434 Fig 5. Intramolluscan expression of *S. mansoni* proteases and protease inhibitors organized by
435 catalytic binding site for the proteases or MEROPS database clan for the protease inhibitors.
436 Individual protease inhibitor clans contain inhibitors that have arisen from a single evolutionary
437 origin. See https://www.ebi.ac.uk/merops/inhibitors/ for details.
438
439 At all time points more *S. mansoni* proteases were present than protease inhibitors and, in
440 general, protease inhibitors and proteases increased in abundance and expression as infection
441 progressed. For both Illumina and microarray samples, shedding snails had both the greatest
442 number of proteases and protease inhibitors expressed relative to other time points, and the
443 highest expression levels of proteases and protease inhibitors.
444 As expected, elastases, an expanded family of serine proteases in *S. mansoni* [63], were
445 the most highly expressed proteases in *S. mansoni* from both *B. glabrata* and *B. pfeifferi*
446 shedding snails (Fig 6). We identified 9 elastase transcripts including those previously
447 designated as cercarial elastases 1a and 2b and found in daughter sporocysts and cercariae
448 [63]. Although elastases are known to be used in definitive host skin penetration, active
449 translation of SmCE2b into protein sequences is seen prior to exiting the snail and was
450 postulated to be involved in facilitating egress from the snail [63].
451
452 Fig 6. Prominent proteases of interest include elastases (A), leishmanolysins (B), and
453 cathepsins (C).
454
455 Our data not only corroborate the presence of SmCE2b in shedding snails, but also reveal
456 this and other *S. mansoni* elastases to be present at all time points we examined, in contrast to
457 microarray results previously reported with early stage sporocysts from *in vitro* cultures [29]. For
458 example, even at 1d (see 1d-R2) we found high expression of six *S. mansoni* elastases, some
459 of which are those noted prominently in cercariae [63]. Our microarray samples also show
460 expression of several elastases at all time points. It is not unusual to think that early-stage larval
461 *S. mansoni* would express protease activity as they too must implement host penetration. Wu et
462 al. [25] noted a conspicuous absence of elastase proteins in *in vitro* larval transformation
463 products but other proteases present suggested an obvious degree of overlap between cercarial
464 versus larval protease repertoires.

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465 Leishmanolysin (also called invadolysin), a metalloprotease, is the second most abundant
466 type of secreted protease of cercariae after elastases [64]. Functional studies of leishmanolyin
467 in larval *S. mansoni* suggested this protease is capable of interfering with the migration of *B.*
468 *glabrata* hemocytes and may influence the establishment of infection [65]. Leishmanolysin has
469 also been detected among the proteins accompanying transformation of miracidia to mother
470 sporocysts [25]. We detected leishmanolysin transcripts at all time points, and they were most
471 abundant in shedding snails, likely indicative of their representation in developing cercariae (Fig
472 6B).

473 Cathepsins are papain-like cysteine proteases and have been identified in the *S. mansoni*
474 miracidia proteome, transforming miracidia, and mother sporocysts [61,66] and are implicated in
475 tissue penetration, digestion and immune evasion in the definitive host [58,67-70]. Cathepsins
476 take the place of tissue-invasive elastases in the cercariae of avian schistosomes [71]. Of two
477 cathepsin B transcripts we noted, we found one expressed in all replicates except from 1d-R1
478 and 3d-R2, the early-stages replicates noted to have lower *S. mansoni* read counts (Fig 6C).
479 *Schistosoma mansoni* expresses cathepsin B in the flame cells of cercariae where they are
480 believed to play a role in osmoregulation and/or secretion [72]. Cathepsin C, involved in
481 acquisition of oligopeptides and free amino acids by larval schistosomes [73], was also
482 identified by Illumina at 1d, 3d, and in shedding snails, with the exception of replicate 3d-R2
483 which had a pre-patent amphistome infection. Cathepsins L1 and L3 were highly expressed by
484 mother sporocyst stages (2d, 3d, 4d, 8d samples) in the microarray samples. At 16d, when
485 daughter sporocysts are migrating through host tissue and hemolymph to the digestive gland,
486 the proteases produced most closely resemble those from 32d shedding infections, including
487 cathepsin C.

488 In contrast to proteases, there is relatively little information about protease inhibitors and
489 their roles in parasite development and survival (see [59] for a thorough review of schistosome
490 protease inhibitors). One of the better-characterized groups is the serine protease inhibitors
491 (serpins; MEROPS clan ID, family I4) that may play a role in both post-translational regulation of
492 schistosome proteases and defense against host proteases [74]. Serpins were expressed in all
493 the time points sampled but we observed the highest expression of serpins at 1d and in
494 shedding snails. The most abundant protease inhibitors in the Illumina samples (1d, 3d,
495 shedding) were those that belong to the JF clan which is interesting because it is by no means
496 the most abundantly represented clan, comprised of only one family called cytotoxic T-
497 lymphocyte antigen-2-alpha (CTLA-2α), known to induce apoptosis of T-lymphoma cells in
498 schistosome-infected mice [75]. This gene homolog is not represented on the *S. mansoni*

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499 microarray which accounts for its absence in those samples. The homologous CTLA-2α
500 transcripts expressed in the intramolluscan stages of *S. mansoni* may play a similar role in
501 apoptosis or immunomodulation in snails to facilitate maintenance of long-term infections.
502 Transcripts identified as the protease inhibitor aprotinin (IB clan), a trypsin inhibitor, were
503 moderately expressed in Illumina 1d-R2 and R3 replicates and in all replicates of shedding
504 snails. In the plasma of *Biomphalaria*, the phenoloxidase enzyme laccase, whose activity is
505 enhanced by trypsin, induces a negative impact on late (7-9 week) *S. mansoni* infections [76].
506 We noted an up-regulation of snail-produced trypsins in *B. pfeifferi* shedding *S. mansoni*
507 cercariae [22] as compared to uninfected controls. By inhibiting snail-produced trypsins, *S.*
508 *mansoni* daughter sporocysts and/or developing cercariae within may disable an important snail
509 defense strategy.
510

511 **The *S. mansoni* venom allergen-like proteins (SmVALs)**

512 The provocatively named venom allergen-like proteins (SmVAL2, 3/23, 9, 15, 26/28, and 27)
513 have been identified as secreted larval transformation proteins [25]. SmVAL proteins can be
514 found throughout miracidia and sporocyst parenchymal cell vesicles and in germinal cells with
515 evidence for involvement in larval tissue remodeling and development by regulating snail matrix
516 metalloproteinases [77]. One and 3d Illumina samples showed variable expression of SmVALs
517 1, 11, 14, and 22 (Fig 7). Replicates from shedding snails had more consistent SmVAL profiles,
518 with 14 different SmVAL homologs found among Illumina replicates and 9 SmVAL homologs in
519 the 32d microarray samples. SmVAL1 was ubiquitously expressed across 1d, 3d, and shedding
520 Illumina samples. Chalmers et al. [78] also noted abundant SmVAL transcripts in the infective
521 stages of *S. mansoni*, namely miracidia and cercariae. SmVALs 4 and 24 transcripts, localized
522 to the preacetabular glands of developing cercariae [79] were also the highest expressed
523 SmVAL transcripts we found in shedding *S. mansoni*. SmVAL16 was localized close to the
524 neural ganglia of adult male worms [79]; we detected its expression at 1d, 3d, and shedding
525 time points. The repertoire of SmVAL proteins secreted during transformation may differ from
526 the SmVAL transcripts being produced and this may account for the differences in the SmVAL
527 transcripts we report here versus previously published proteomic findings.
528

529 Fig 7. The venom allergen-like proteins of intramolluscan *S. mansoni*
530

531 ***S. mansoni* intramolluscan G-protein coupled receptors (GPCRs)**

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532 G-protein coupled receptors or GPCRs are the largest superfamily of transmembrane
533 proteins in eukaryotes responsible for facilitating signaling affecting various downstream
534 functions like development, reproduction, neuronal control of musculature and more [80,81]. As
535 receptors, GPCRs are involved in mediating a variety of processes critical to schistosome
536 survival including mediating host-parasite interactions, reproduction, and mating [82] (Liang et
537 al. 2016). Praziquantel has been identified as a GPCR ligand acting to modulate serotoninergic
538 signaling [83]. Several *in silico* studies identifying and characterizing the *S. mansoni*
539 "GPCRome" [84,85] culminated in the classification of a broad range of phylogenetically distinct
540 clades/classes of GPCRs [86]. *S. mansoni* microarray studies have reported diverse expression
541 patterns of individual GPCRs, with the overall highest expression occurring in 3-7 week worms,
542 indicating that they are associated with complex stage-specific roles [29,86].
543 In intramolluscan stages, we identified 78 GPCR transcripts from our Illumina samples, and
544 26 probes from microarray samples (Fig 8). Many (38%) of the Illumina GPCR transcripts were
545 A FLPR-like, a GPCR class containing receptors similar to FMRFamide GPCRs that invoke
546 muscle fiber contractions in schistosomes by increasing calcium transport across voltage-gated
547 calcium channels [87]. Shedding snails had the most diverse representation of GPCRs. One
548 transcript, homologous to an identified *S. mansoni* GPCR (Smp_193810) with unknown
549 function, was expressed at all time points with markedly high expression in both Illumina and
550 microarray samples from shedding snails. A GPCR sensing the biogenic amine 5HT
551 (Smp_126730) and known to be distributed throughout the adult worm's nervous system [80],
552 was expressed at 1d and shedding Illumina samples (no homologous probe was found on the
553 microarray). Its presence in intramolluscan stages suggests that serotonin-stimulated movement
554 is essential throughout the life cycle of schistosomes. At 1d and shedding, a type 1 serotonin
555 receptor is down-regulated in *B. pfeifferi* and at 3d, kynurenine 3-monooxygenase (important for
556 its ability to degrade tryptophan and limit concentrations of serotonin) is up-regulated [22].
557 Serotonin is a molecule of relevance to both the snail and parasite, and interference with its
558 levels may be relevant to castration of snails (see concluding comments).
559
560 Fig 8. G-protein coupled receptors expressed in Illumina and microarray *S. mansoni*
561 intramolluscan time points.
562
563 **Neuropeptides and neural development**
564 Studies on neuropeptides (peptide hormones) in planarian flatworms and their homologs
565 in *S. mansoni* have identified their influence in locomotion, feeding, host location,

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566 regeneration, and development [88,89]. Lu et al. [90] reported the expression of putative
567 neuropeptides and transcripts suspected to be involved in neural development from paired
568 and unpaired female and male adult worms. Seventeen transcripts were identified as
569 neuropeptide receptors from the Illumina transcriptome, all of which were GPCRs (Fig 9).
570 Neuropeptides and their receptors were mostly absent at 1d and 3d but abundant in
571 shedding-R1 and R2. In shedding-R3, the replicate with a muted *S. mansoni* response, only
572 one neuropeptide receptor (neuropeptide Y receptor) was expressed. Shedding-R3 was
573 curious in that protein 7b2 and NPP-1 (GFVRIamide) prepropeptide were highly expressed.
574 Only one putative neuropeptide precursor (NPP-1 prepropeptide) was identified on the
575 microarray with only ~1 Log_2 fluorescence at 32d and was not present in any other sample.
576 In adult worms, GFVRIamide is localized to neurons that run along the cerebral commissure
577 towards the oral sucker [89]. Allatostatin receptor, a GPCR with ovary-specific transcription in
578 adult *S. mansoni* [86], is important for reproductive development in *Schistosoma japonicum*
579 adult females [91]. Four transcripts homologous to allatostatin receptor were expressed
580 primarily in shedding *S. mansoni* replicates. Our results indicate expanded roles for
581 neuropeptides and neural development transcripts previously uncharacterized in
582 intramolluscan stages of *S. mansoni*.
583 We identified 33 of the 39 genes found to be involved in neural development by Lu et al.
584 [90] in our Illumina *S. mansoni* transcriptome (Fig 9). Cell polarity proteins were the highest
585 expressed transcripts involved in neural development at 1d, 3d, 16d, and shedding snails. 2d
586 array *S. mansoni* showed little activity of transcripts related to neural development. In 4d and
587 8d samples, notch and septate junction transcripts were the most highly expressed neural
588 development transcripts. Notch transcripts are highly expressed in eggs but not in cercariae
589 and are thought to be mainly involved in *S. mansoni* oogenesis and embryogenesis within the
590 vertebrate host [92] but have been implicated in neurogenesis [23]. Lu et al. [90] found SOX to
591 be transcribed in the ovary of paired and unpaired females and its expression in germ balls
592 has also been established [93]. Three transcripts homologous to the *S. mansoni* SOX
593 transcription factor were present predominantly in 1d and shedding time points reinforcing
594 the role of SOX transcription in embryonic and germinal cell development.
595
596 Fig 9. Transcripts involved in neural development
597
598 **Transcripts associated with germinal cells and asexual reproduction of schistosomes in**
599 **snails**

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600 A prominent feature of the complex developmental program of sporocysts in snails is the
601 presence of germinal cells that give rise to embryos that come to contain both the somatic cells
602 that eventually divide to comprise the bodies of either sporocysts or cercariae and more
603 germinal cells. These germinal cells are then poised to give rise to the next generation. None of
604 this asexual polyembryonic process involves the formation of gametes or evidence of
605 fertilization. Germinal cells in *S. mansoni* sporocysts have been shown to share common
606 molecular features with planarian neoblasts or stem cells, prompting the suggestion that the
607 digenetic nature of the life cycle of schistosomes and other digenetic trematodes may have
608 evolved because of the adaptation of a system of preservation of these stem cell-like germinal
609 cells [12].
610 Consistent with Wang et al. [12] we observed expression of fibroblast growth factor
611 receptors (*fgfr*), *vasa*, *argonaute2* (*ago2*), and *nanos* transcripts shown to be associated with
612 long-term maintenance of neoblast stem cells (Fig 10A). Expression of *fgfr2, argonaut-2* and
613 especially *vasa* are expressed in all samples, suggestive of their importance in intramolluscan
614 development. The microarray had an additional *fgfr* feature (*fgfr4*) that was not detected in the
615 Illumina transcriptome. Our results are in agreement with Wang et al. [12] that *nanos-1* is not
616 expressed in sporocysts, consistent with their suggestion that *nanos-1* expression is exclusive
617 in adult *S. mansoni* [66]. *Nanos-2* expression was observed in every replicate of every time
618 point with the exception of the 2d microarray sample. It has been proposed that there are two
619 populations of germinal cells, *nanos*$^+$ and *nanos*$^-$, with the latter population proliferating much
620 more rapidly [12]. *Vasa* is needed for proliferation of both *nanos*$^+$ and *nanos*$^-$ stem cell
621 populations and *ago2* is required for proliferation of only *nanos*$^-$ cells. It is hypothesized that the
622 two populations exist for different purposes: one a more undifferentiated stem cell-like
623 population and the other a more differentiated one ready to enter embryogenesis [94].
624
625 Fig 10. Transcripts associated with maintenance of neoblast stem cells in platyhelminthes (A)
626 and transcripts potentially involved in meiosis and/or homologous recombination in asexually
627 reproducing *S. mansoni* (B).
628
629 The proliferation of sporocysts and then cercariae by digenetic trematodes in snails is now
630 generally considered to be an asexual process, one that does not involve gamete formation or
631 fertilization [95], and it is frequently assumed that the progeny produced from a single
632 miracidium are genetically the same. However, there are also persistent claims that the process
633 is best considered as apomictic parthenogenesis [96]. Some observations indicate that the *S.*

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634 *mansoni* cercariae arising from a single miracidium are not genetically identical but exhibit some
635 variation with respect to representation of repetitive elements that has been attributed to mitotic
636 recombination [97,98]. Khalil and Cable [99] examined germinal development in rediae of
637 *Philopthalmus megalurus* and concluded the process was diploid parthenogenesis. They
638 observed the presence of cells interpreted to be oögonia entering meiotic prophase I up to the
639 stage of diakinesis that was then followed by the cell returning to interphase rather than
640 proceeding through meiosis. Such a process might also allow for some recombination among
641 the progeny produced during intramolluscan development.

642 Although the preponderance of evidence is surely against the occurrence of meiosis,
643 gamete formation or fertilization during intramolluscan development [95], there may be peculiar
644 remnants of these processes represented, especially considering that most accounts of the
645 evolution of digenetic trematodes favor the interpretation that the ancestral state was likely the
646 sexually reproducing adult worm which was followed at a later time by the addition of asexual
647 proliferative larval development in molluscs [100]. Might there then be peculiar remnant
648 signatures of meiosis in intramolluscan larvae? We identified homologs to two known meiosis
649 prophase-specific transcripts in our Illumina samples (Fig 10B), which were originally
650 characterized in mice: meiosis express protein 1 (MEIG1) known to be involved in
651 chromosome/chromatin binding in meiosis [101] and highly expressed during meiosis prophase
652 1 [102], and meiosis-specific nuclear structural protein 1 (MNS1). Anderson et al. [103] identified
653 a MEIG transcript expressed in adult male and female *S. mansoni* with a potential role in
654 gamete production but no possible functional role was suggested to explain its high expression
655 in eggs. MNS1 is specifically expressed in mice during the pachytene stage of prophase 1 of
656 meiosis. Retinoic acid (RA) initiates meiosis and although retinoic acid is not implicated in
657 development of *S. mansoni*, we did see expression of retinoic acid receptor RXR. Of the
658 putative meiosis stage-specific homologs, only RXR was present as a feature on the *S. mansoni*
659 microarray, and it showed increasing expression as intramolluscan development progressed.
660 Further study is warranted to learn if the transcripts we observed from intramolluscan stages are
661 perhaps indicative of some tendency for occasional formation of bivalents without associated
662 gamete formation or fertilization, or of a general repurposing of these molecules for use in many
663 kinds of cellular reproduction, including asexual reproduction.

664 Six recombinase transcripts were expressed in our Illumina samples: three RAD51
665 homologs, two cassette chromosome recombinase b homologs, and one trad-d4 homolog.
666 Recombinases like RAD51 are up-regulated in the testis and ovary of adult *S. mansoni* as
667 compared to whole worm controls [104] and in female adult *S. japonicum* when compared to

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668 males [105]. Recombinases can repair breaks in DNA as a result of DNA damage or that occur
669 during homologous recombination during meiosis. At least one transcript of another
670 recombinase, topoisomerase II, was expressed in every time point. Among other functions,
671 topoisomerase II interacts with the meiosis-specific RecA-like protein Dmc1 or RAD51 to
672 facilitate pairing of homologous chromosomes during chromosome strand exchange [106].
673

674 **Glycosyltransferase expression in intramolluscan stages**

675 Molecular mimicry has been an area of interest with respect to schistosome-snail
676 interactions since the early 1960s with the hypothesis that parasites express host-like molecules
677 to evade host immune responses [107]. Several studies have highlighted antigenic similarities
678 between miracidia and mother sporocysts and *B. glabrata* hemolymph proteins [108,109], and
679 the glycans on *S. mansoni* glycoproteins and glycolipids have been extensively studied,
680 including for their potential role in mediating host mimicry [25,110-114]. Yoshino et al. [115]
681 showed that antibodies to *S. mansoni* glycotopes bound more extensively to cell-free
682 hemolymph (plasma) from snails susceptible to infection than plasma from resistant strains, and
683 suggested host-mimicking glycotopes could be a determining factor in compatibility during early
684 larval stages. Consequently, we were interested in examining *S. mansoni* glycosyltransferases
685 because of the role they play in generation of glycan moieties on lipids and proteins. Among
686 others, one group of glycosyltranferases we found to be prominent in *S. mansoni* intramolluscan
687 stages were fucosyltransferases (FTRs). Several of the surface membrane glycoconjugates of
688 *S. mansoni* that interact with *B. glabrata* are fucosylated [116] and are suspected to be involved
689 in host mimicry [110]. We found 22 unique Illumina-assembled FTRs transcripts and 8 fucosyl-
690 transferase-specific probes represented on the microarray (Fig 11).
691

692 Fig 11. Fucosyltransferases of intramolluscan *S. mansoni*
693

694 Fitzpatrick et al. [29] observed two major clades of FTRs expressed by *S. mansoni,* those
695 expressed in miracidia and mother sporocyst stages (alpha 1,6 fucosyltransferases D and E)
696 and those expressed primarily in sexually mature adults (alpha 1,3 fucosyltransferases B, L, F).
697 We did not observe an obvious stage-specific demarcation in FTRs expression but rather
698 observed a broad range of FTR transcripts, including those Fitzpatrick et al. [29] observed
699 primarily in sexually mature adults. They were expressed at all time points with highest diversity
700 being at 1d and in shedding snails. Alpha 1,6 fucosyltransferase H was expressed ubiquitously
701 across all Illumina and microarray samples. We also saw expression of five O-

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702 fucosyltransferase transcripts exclusively at 1d and in shedding snails. O-fucosyltransferases
703 add a fucose residue to the oxygen on a side chain of either serine or threonine residues in a
704 glycoprotein. Heavy expression in shedding snails was not surprising because cercariae
705 possess a prominent fucose-rich glycocalyx [117].
706 A transcriptional regulatory protein, KRAB-A domain-containing protein and dolichyl-
707 diphosphooligosaccharide--protein glycosyltransferase subunit DAD1 homolog that performs
708 post-translational protein glycosylation, were among the most abundantly expressed
709 transcripts across all Illumina samples.
710

711 **Sporocyst defense and stress responses**

712 It is reasonable to expect that *S. mansoni* intramolluscan stages are under some duress
713 from host immune responses, and we noted that snail Cu,Zn superoxide dismutases (SOD)
714 were upregulated at both 1d and 3di in the highly compatible snail *B. pfeifferi* from which the *S.*
715 *mansoni* Illumina transcripts discussed here were also obtained [22]. The H_2O_2 resulting from
716 SOD activity is known to be toxic to *S. mansoni* sporocysts [46,118] and is a main factor
717 responsible for killing early larval *S. mansoni* in some *B. glabrata* resistant strains [46,118].
718 Organisms can remove harmful intracellular hydrogen peroxide with catalases, glutathione
719 peroxidases, and peroxiredoxins. Schistosomes lack catalases [119,120] and have low levels of
720 glutathione peroxidases with limited antioxidant abilities [121]. It is suggested that
721 peroxiredoxins are the schistosome's main defense against damage from hydrogen peroxide
722 [122]. In our data, thioredoxin peroxidases, peroxiredoxins that scavenge H_2O_2 using thioredoxin
723 [123], are consistently (and highly) expressed throughout all time points in array and Illumina
724 samples (Fig 12). Thioredoxin peroxidases reduce hydroperoxides with thioredoxin as a
725 hydrogen donor. *S. mansoni* also expresses SOD activity and *S. mansoni*-encoded Mn- and
726 Cu/Zn-type SODs are expressed in every replicate of both Illumina and array-sequenced
727 samples (Fig 12). Because the abundance of *S. mansoni* SOD transcripts was consistently
728 modest, we suggest that their function is not to mount an anti-snail counter-offensive but rather
729 to take care of the intracellular anti-oxidative needs of the parasite.
730

731 Fig 12. Sporocyst defense and stress factors

732

733 Cytochrome P450 proteins have been associated with stress responses and in detoxification
734 reactions. *Schistosoma mansoni* has but a single cytochrome P450-encoding gene and the
735 associated protein activity has been shown to be essential for survival in both adult worms and

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736 eggs, although its underlying function in schistosomes remains unclear [124]. Cytochrome P450
737 transcripts showed minimal expression at 1d, were absent at 3d, and had modest (~3 Log_2
738 TPM) in all shedding replicates.

739 Heat shock proteins (HSPs) are often produced under conditions of stress, but they are also
740 constitutively expressed in actively synthetic cells to serve as chaperones and to facilitate
741 protein folding [125]. *Schistosoma mansoni* sHSPs 16 and 20 and HSPs 70 and 90 were all
742 found among proteins released during *in vitro* miracidium to mother sporocyst transformation
743 [25]. We found sHSPs 16, 20, and 40 and HSPs 60 and 70 but not 90, to be expressed
744 throughout intramolluscan stages with the highest expression seen in HSPs 20, 40, and 70 (Fig
745 12C). sHSP 20 contributes up to 15% of the soluble proteins of miracidia [126] and is a
746 prominent protein identified in miracidia by LC-MS/MS [26]. sHSP 40 has been identified as a
747 soluble egg antigen responsible for eliciting immunopathological reactions in the definitive host
748 that result in granuloma formations [127]. Ishida and Jolly [128] showed that in the absence of
749 HSP 70, cercariae do not orient or penetrate normally, providing additional functional roles for
750 HSP 70 beyond stress responses. We noted that *S. mansoni* HSP 70 was expressed at high
751 levels in cercariae-producing shedding samples.

752 In addition to defending themselves from attack by host immune components, in long-lived
753 host-parasite associations as represented by *S. mansoni* in *B. pfeifferi*, it might also be
754 reasonable to expect digenean sporocysts to contribute to the stability and maintenance of the
755 host-parasite unit as a whole. Rediae of some digenean species do this in the form of actively
756 attacking newly-colonizing trematode infections [129]. In complex, natural transmission foci such
757 as the one from which our samples originated, *S. mansoni*-infected *B. pfeifferi* snails are
758 constantly exposed to a variety of viruses, bacteria, and infectious eukaryotes [22]. Therefore, it
759 seems reasonable that while imposing considerable stresses on its hosts, possibly including
760 immunosuppression, that larval schistosomes might also be expected to contribute to the well-
761 being of the host-parasite unit by expressing transcripts that contribute to repression or
762 elimination of additional parasites.

763 One way to gain insight into *S. mansoni* sporocyst capabilities in this regard was to review
764 what is known for free-living flatworms, such as the planarian *Dugesia japonica,* that are
765 regularly challenged by pathogenic and non-pathogenic bacteria in their habitats. Planarians are
766 capable of phagocytosing and destroying pathogens like *Staphylococcus aureus* and
767 *Mycobacterium tuberculosis* [130,131]. Conserved homologs to human genes, such as MORN2
768 (membrane occupation and recognition nexus-2 protein) are present and known to play a role in
769 LC3-associated phagocytosis (LAP) elimination of bacterial pathogens in human macrophages

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770 and in flatworms. Homologs of 34 transcripts of putative flatworm anti-bacterial factors [131]
771 were expressed in *S. mansoni* intramolluscan stages (Fig 12D). Homologs to dual specificity
772 phosphatases were the most prominent group of anti-bacterial factors. They were expressed at
773 all time points, with a general increase in expression with development time. Homologs of
774 MORN2 (membrane occupation and recognition nexus-2 protein) were also present throughout
775 intramolluscan development. MORN2 plays a role elimination of bacterial pathogens in human
776 macrophages as well as in flatworms. MORN2 is present in all replicates in 1d, 3d, and
777 shedding *S. mansoni* samples. Shedding *S. mansoni* samples in both Illumina and microarray
778 contain the most flatworm bacterial defense homologs. How these putative schistosome
779 defense factors may be deployed in sporocysts that lack a gut and that do not engage in
780 phagocytosis as far as we know, remains to be seen.

781 Among possible *S. mansoni* anti-immune factors we noted to be highly expressed in our
782 samples was calreticulin, previously shown to be present in the excretory/secretory products of
783 *S. mansoni* sporocysts (Fig 13). Because of its calcium-binding capability, Guillou et al. [132]
784 suggested calreticulin may interfere with hemocyte spreading and interfere with their ability to
785 initiate encapsulation responses.

786

787 Fig 13. Expression of calreticulin in *S. mansoni*

788

789 **Evidence of amphistome-mediated suppression of *S. mansoni* sporocyst development**

790 As noted in Buddenborg et al. [22], Illumina replicate 3d-R2 harbored a pre-patent infection
791 of an amphistome species, presumptive *Paramphistomum sukari*, known to be common in *B.*
792 *pfeifferi* in Kenya, including from the habitat from which these snails were obtained [133]. The
793 presence of an amphistome infection is of interest because previous studies indicate that
794 amphistomes and schistosomes interact in distinctive ways in the intramolluscan environment,
795 with amphistomes having a permissive effect in enabling development of a schistosome that
796 might not have otherwise developed in a particular snail species (e.g. [134]). In our context, the
797 effect of the amphistome appears to be the opposite, based both on field results which suggest
798 that amphistome infections supplant *S. mansoni* infections (Laidemitt, personal communication),
799 and on our transcriptional results. In general, overall *S. mansoni* transcription in the 3d replicate
800 with the amphistome was dampened relative to 3d replicates lacking the amphistome. This
801 dampening took the form of both fewer numbers of *S. mansoni* transcripts expressed, and for
802 those that were expressed, representation at lower copy numbers. It did not appear that specific
803 highly expressed *S. mansoni* transcripts were targeted in any selected way by the presence of

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804 the amphistome. In fact, the *S. mansoni* transcriptome in the amphistome-containing replicate
805 most closely resembles that seen for *S. mansoni* in 1d infections (1d-R1), suggestive of an
806 amphistome-imposed delay in development.
807

808 **Known unknowns and unknown unknowns**

809 Although many of the highly expressed transcripts found in our Illumina samples at 1d,
810 3d, and shedding time points fell into one (or more) known functional categories, there
811 remained several that either had an annotation and were not in one of our functional
812 categories of interest or had no annotation and remain unknown. One of the advantages of a
813 systematic sequencing approach is the discovery of transcripts for which we have no *a priori*
814 knowledge yet that may play a key role in *S. mansoni* intramolluscan development.
815 Transcripts with unknown characteristics are important to point out and briefly discuss
816 because they may provide brand new insights into molecular functions not yet characterized
817 but that may prove to be important for development and maintenance of infection in the snail
818 host.

819 The transcripts we highlight in this section function in cytoskeletal maintenance, iron
820 sequestration, oxidation-reduction, and transcription/protein regulation and modification.
821 Cytoskeletal proteins tektin and tubulin beta chain were abundantly expressed in all Illumina
822 *S. mansoni* samples. The tegument of the schistosome changes in shape and size and has
823 to closely interact with its hosts, requiring cytoskeletal molecules to be continually recycled
824 and renewed [40]. A gene, *nifu*, with homology to nitrogen fixation genes in bacteria, was
825 highly expressed in all Illumina samples and it likely functions in the *de novo* synthesis of
826 iron-sulfur clusters in mitochondria regulating cellular iron homeostasis [135]. Iron is
827 sequestered by *S. mansoni* from its hosts and is known to be essential to several metabolic
828 processes for adult development and reproduction [136]. Disrupting iron homeostasis has
829 been an area of interest for therapeutics against schistosomes [137]. Two enzymes involved
830 in the oxidation-reduction process were noted to be highly up-regulated in all samples:
831 NADH-plastoquinone oxidoreductase and NADH ubiquinone oxidoreductase chain 3. A
832 transcript identified as a putative stress associated endoplasmic reticulum protein was also
833 highly expressed in all samples and is linked to the stabilization of membrane proteins during
834 stress and facilitates subsequent glycosylation [138]. Lastly, an endothelial differentiation-
835 related factor 1 transcript was constitutively expressed. It is also expressed preferentially in
836 adult male *Schistosoma japonicum* worms [139].

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837 The SmSPO-1 gene, first identified in late stage sporocysts [140], is present in all replicates
838 and time points with increasing expression from mother sporocyst to cercariae production.
839 SmSPO-1 is secreted as a lipid bilayer-binding protein that binds to host cell surfaces and
840 induces apoptosis and has been well-characterized in cercariae during skin penetration [141]. In
841 addition to penetrating host tissue, intramolluscan stages of *S. mansoni* must also expand into
842 dense host tissue as they grow and must migrate from the head-foot to the digestive gland, all
843 activities for which SmSPO-1 activity may be critical.

844 We also see several egg CP391B-like and egg CP391S-like transcripts expressed at 1d, 3d,
845 and shedding *S. mansoni* samples. Egg proteins have been reported as differentially expressed
846 in mother sporocyst stages when compared to free-living miracidia [27].

847 With respect to “unknown unknowns,” a *de novo* assembled transcript
848 (TRINITY_DN6450_c0_g1_i1_len=386_path=[654) had no annotation in any database and a
849 targeted annotation revealed only that the transcript coded for a protein with a distinctive
850 cytoplasmic, transmembrane helix, non-cytoplasmic, and another transmembrane helix. This
851 transcript may be a novel and unique *B. pfeifferi* transmembrane protein.

852 Because the transcripts mentioned above are abundant in every replicate across all
853 Illumina samples, they represent potential novel targets for eliminating or moderating *S.*
854 *mansoni* parasite development within *Biomphalaria* snails.

855

856 CONCLUDING COMMENTS

857 As a miriacidium penetrates a snail, it rapidly enters a radically different milieu from what it
858 has previously experienced, and a number of pre-made proteins are released into its new
859 surroundings to effect transition to the mother sporocyst stage adapted for intramolluscan
860 existence [25,26,46]. Our 1d Illumina samples include many transcripts distinctive from the
861 proteins associated with transformation, indicative of the switch to the needs of existence as
862 sporocysts. As examples, we found representation of different SmVALs, heat shock proteins,
863 protease transcripts (elastases), neural development proteins or neurohormones among 1d
864 Illumina samples than seen as proteins in miracidial transformation products.

865 From our earliest Illumina samples, it is evident that *S. mansoni* orchestrates a complex
866 transcriptional program within its snail hosts, with a significant percentage of its genetic
867 repertoire (an estimated 66**%** of the *S. mansoni* genome) engaged (see also [23] who reports
868 that 50-60% genes are expressed in each *S. mansoni* stage). This is particularly evident at the
869 stage of production of cercariae, when large amounts of parasite tissue are present and
870 production and differentiation of the relatively complex cercarial bodies are underway. Also

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871 noteworthy is that a core transcriptome required of life in a snail host can be identified which
872 includes transcripts involved in a central glycolysis pathway and the TCA cycle, and for
873 transmembrane transporters for monosaccharides, amino acids, steroids, purines and
874 pyrimidines, indicative of the dependence of sporocysts on their hosts for key molecular building
875 blocks. Several of these like the amino acid transporters are noted for the first time in
876 schistosome sporocysts. Metabolically, early stage schistosomes focus primarily on acquisition
877 of molecular building blocks and nutrients with a distinct switch from storage to expending these
878 components towards cercariae production in patent stage shedding *S. mansoni*. A role for
879 receptor-mediated endocytosis in sporocyst nutrition should also not be excluded [142].
880 Transcripts for enzymes required for macromolecular synthesis and cell proliferation, the latter a
881 prominent and perpetual part of intramolluscan development and cercarial production, were also
882 part of the core transcriptome. Oxidoreductase activity and cell redox homeostasis are among
883 the most abundant functions across all larval stages of *S. mansoni*.

884 With respect to particular *S. mansoni* transcripts that may be key to successful
885 intramolluscan development and/or that comprise parts of the "interactome" with snail
886 transcripts, we highlight several findings below. First, by virtue of using both field-derived *B.*
887 *pfeifferi* and *S. mansoni* from infected children, our Illumina study allows for a broader range of
888 outcomes particularly as measured in the early stages of infection, and the variability that results
889 can provide distinctive insights. For instance, cases where early sporocysts seem to be thriving
890 with respect to read count are accompanied by production of larger quantities of factors
891 associated with infectivity like proteases, fucosyltransferases, SmVALs, and GPCRs. Also, poor
892 transcriptomic productivity for *S. mansoni* sporocysts has been associated with presence of
893 other digenean species, unknown to us to be present at the time of exposure to *S. mansoni*, but
894 that interfere with *S. mansoni* development [134] (Laidemitt, personal communication).

895 One of the surprising things about the genome of *S. mansoni* and of other parasitic
896 helminths is the dearth of genes such as cytochrome P450s involved in degradation of
897 xenobiotics (*S. mansoni* has but one such gene with unknown function), potentially including
898 harmful-snail produced factors as well. The ABC transporters we have noted to be highly
899 expressed in sporocysts may function to compensate [54].

900 Of particular interest was the expression of a diverse array of proteases and protease
901 inhibitors at all intramolluscan stages, including some proteases like elastases characteristic of
902 cercariae that were also produced by early sporocyst stages. The up-regulation by the snail host
903 of protease inhibitors during the larval establishment period at 1d and 3d [22] seems a likely
904 response to prevent parasite establishment. Also of note was expression of *S. mansoni*

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905 protease inhibitors that might inhibit the action of snail trypsin-like proteases up-regulated late in
906 infection [22]. These protease inhibitors may prevent the activation of the phenoloxidase
907 enzyme laccase, whose activity induces a negative impact on late (7-9 week) *S. mansoni*
908 infections [76].

909 G-coupled protein receptors (GPCRs) were also well represented in *S. mansoni*
910 intramolluscan stages and are likely to play several important roles in schistosome
911 development. One such GPCR is expressed at 1d and in shedding snails and is known to bind
912 serotonin [143]. We noted at the same time points that *B. pfeifferi* down-regulated production of
913 a type 1 serotonin receptor and additionally, at 3d kynurenine 3-monooxygenase which
914 degrades tryptophan and can limit concentrations of serotonin is up-regulated [22]. It seems
915 reasonable to continue to suspect serotonin of playing a role in parasitic castration. It stimulates
916 egg production when given to castrated snails [144]. By expressing the appropriate serotonin
917 GPCR, and possibly down-regulating the host receptor, *S. mansoni* sporocysts may limit
918 availability of serotonin to the snail.

919 Other factors also worthy of additional consideration with respect to parasitic castration are
920 *S. mansoni* neuropeptides Y and F and their receptors which were expressed particularly in
921 shedding snails. In snails, neuropeptide Y has been associated with decreased egg production
922 [145] and neuropeptide Y receptor was up-regulated at 1 day and in shedding *B. pfeifferi* snails
923 [22]. Whether these neurotransmitters produced by *S. mansoni* might directly affect snail
924 reproduction is not known. Ovipostatins which have a suppressive effect on egg laying in the
925 snail *Lymnaea stagnalis* were found to be up-regulated in shedding *B. pfeifferi* so it is possible
926 their expression may be targeted by *S. mansoni* in some manner as well. We did not see
927 obvious changes in some snail neuroendocrine factors associated with reproduction like
928 calfluxin or schistosomin [22]. Wang et al. [91] in a proteomic study of neuropeptides from *B.*
929 *glabrata*, including snails with 12 day infections with *S. mansoni*, found lower levels of many
930 snail reproductive neuropeptides. The extent to which *S. mansoni* and other digenetic
931 trematodes might effect snail reproduction through interference with their neuroendocrine
932 systems as proposed by de Jong-Brink [145] remains a topic worthy of more study. As noted by
933 Humphries [21], it is also possible that castration is more a consequence of depletion of
934 nutrients and alterations of metabolism imposed by metabolically demanding larval
935 schistosomes.

936 Insights provided by the study of planarians were important to our interpretation of our
937 results in two ways. The first was to confirm in intramolluscan *S. mansoni* samples the common
938 expression of genes associated with maintenance of stem cell-like germinal cells, including

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fibroblast growth factor receptors (*fgfr*), *vasa*, *argonaute2* (*ago2*), and *nanos-2* [12]. The second was to examine sporocysts for evidence of homologs of transcripts known to be involved in antibacterial responses in *Dugesia japonica* [131], for which 34 were found. Whether these factors are actually deployed in anti-bacterial or other defense responses remains to be seen. Their presence is somewhat peculiar because sporocysts lack phagocytic activity, unlike the gut cells of planarians. However, perhaps anti-bacterial proteins are deployed along sporocyst membranes, or sporocysts may engage in limited forms of endocytosis [142] that might result in engulfment of bacteria or their products. Possible anti-snail hemocyte factors like calreticulin [132] were also expressed by sporocysts. The possibility that sporocysts contribute to discouraging or preventing growth of third party symbionts that could compromise the snail-schistosome functional unit, especially in light of the need of schistosme sporocysts to compromise components of host immunity is also a topic worthy of additional study.

One advantage of next gen sequencing is its potential to provide unexpected insights. We were surprised to see two transcripts (MEIG1 and MNS1) associated specifically with prophase I of meiosis and discuss possible interpretations based on previous cytological studies of germinal cell development [99], observations that might help to explain differences among *S. mansoni* cercariae in genetic content [97]. The expression of recombinases in sporocysts might be consistent with a partial entry into meiosis up to diakinesis, or with mitotic recombination, the latter suggested by Grevelding [97] to account for genetic differences among cercariae derived from the same miracidium.

Finally, we note that many more highly represented transcripts were found, including those encoding both genes with suspected or unknown functions whose connection with intramolluscan development remain to be elucidated. With ever more complete transcriptional profiles becoming available for schistosomes in their snail hosts, the stage is set for further studies employing the best tools available for gene knockout to address the functional roles of these and the many other transcripts we and others have noted. Of particular interest will be to determine if ingenious use of this information can be made to specifically target and prevent the development of sporocysts and their production of human-infective cercariae, thereby opening a much-needed additional front in the effort to control and eliminate human schistosomiasis.

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973 S1 File. *Schistosoma mansoni* microarray and Illumina RNASeq expression data and annotation
974
975 S1 Fig. Overall assembly and differential expression pipeline for *S. mansoni* reads in dual RNA-
976 Seq.
977
978 S2 Fig. (A) Read mapping statistics for each replicate of our *S. mansoni* transcriptome.
979 Percentage of all reads mapped are graphed on the left y-axis in dark bars and overall number
980 of reads graphed on the right y-axis in light bars. (B) Number of transcripts expressed above ≥1
981 Log_2 (TPM) in each Illumina sample replicate.
982
983 S3 Fig. PCA plot of all transcripts expressed in replicates from 1d, 3d, and shedding Illumina
984 groups.
985
986 S4 Fig. (A) Linear regression of 32d array probes measured in log_2 fluoresence and shedding
987 Illumina expression results measured in log_2 TPM. Only homologous probes and transcripts
988 were included in the scatterplot. (B) Venn diagram of shared and unique features expressed
989 between microarray and Illumina time points (32d).
990
991 S5 Fig. Venn diagram comparing expression results of early *S. mansoni* development
992
993 S6 Fig. *Schistosoma mansoni* kinases identified at 1d, 3d, and shedding time points, organized
994 by kinase family.
995 TK: phosphorylate tyrosine residues; TKL: "tyrosine kinase-like" serine-threonine protein kinases; STE: mostly protein
996 kinases involved in MAP (mitogen-activated protein) kinase cascades; CK1: casein kinases; AGC: cyclic-nucleotide-
997 dependent family (PKA, PKG), PKC, and relatives; CAMK: calcium/calmodulin modulation activity; CMGC: cyclin-
998 dependent kinases, MAP kinases, glycogen synthase kinases, and CDK-like kinases. The figure was generated using
999 KinomeRender.
1000

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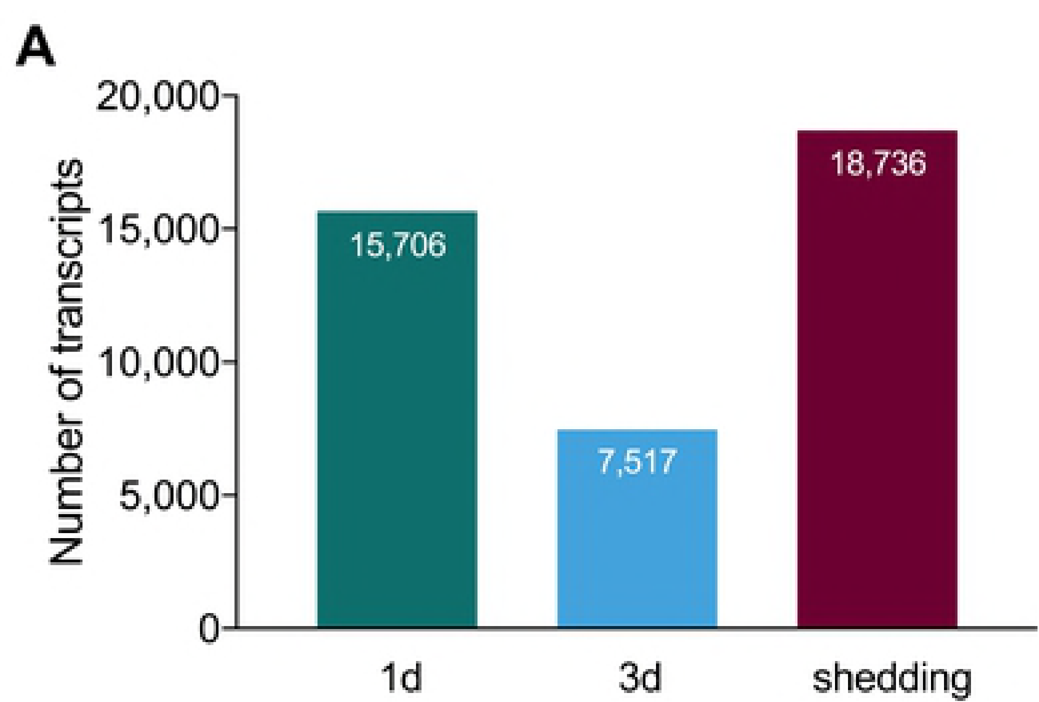

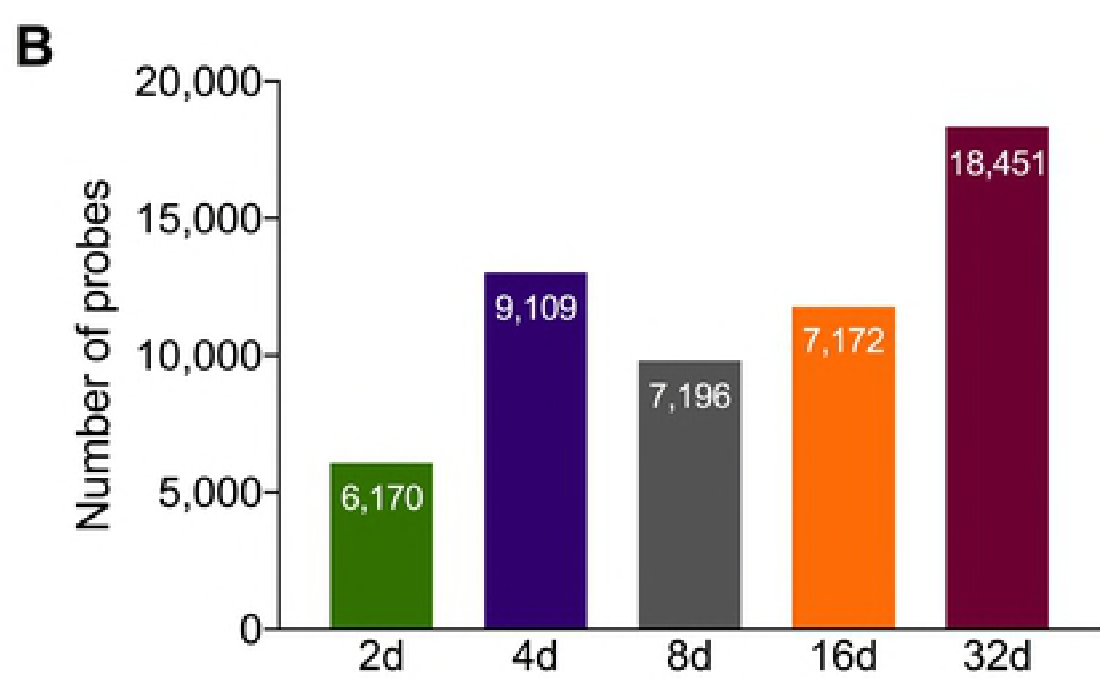

Fig 1

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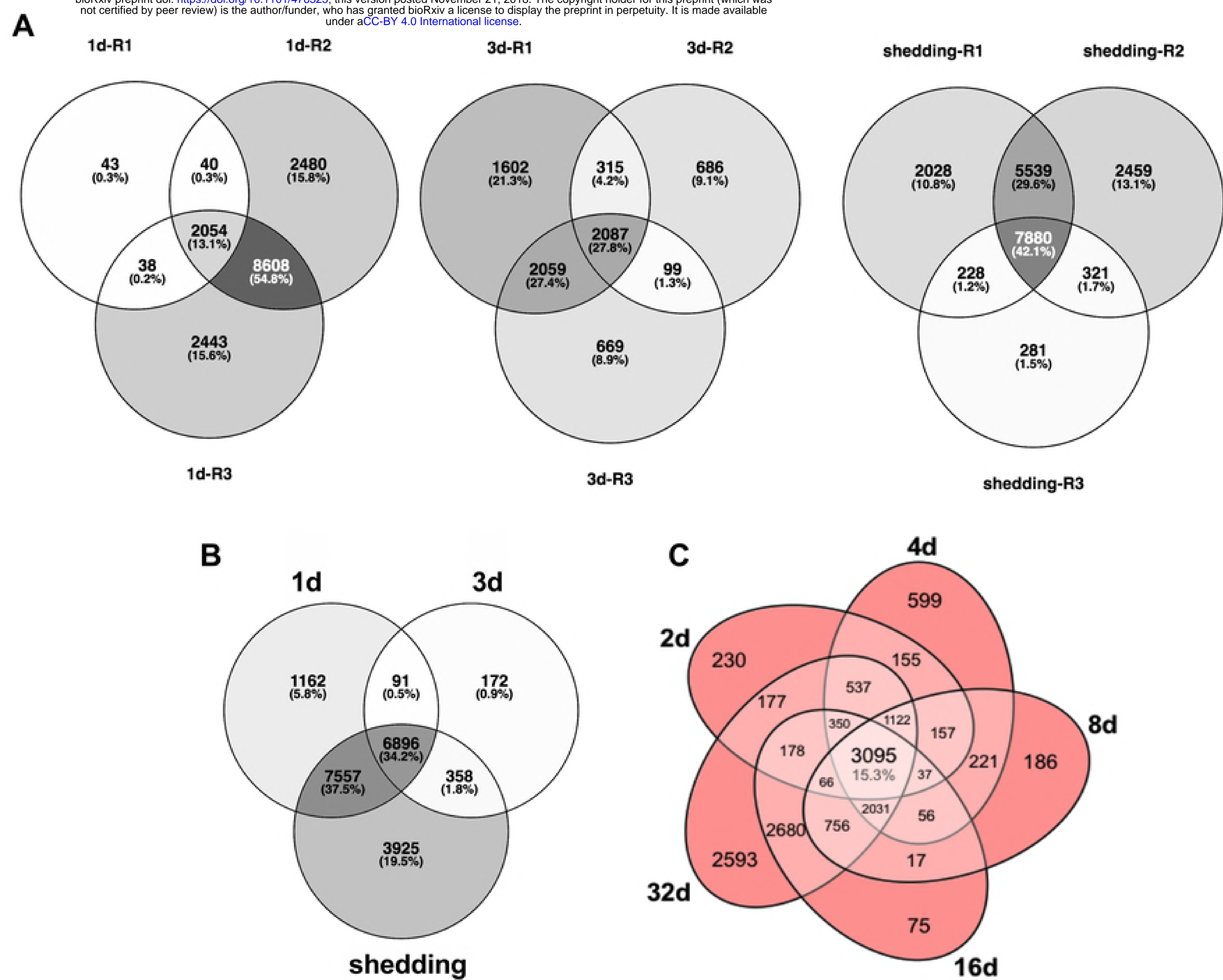

Fig 2

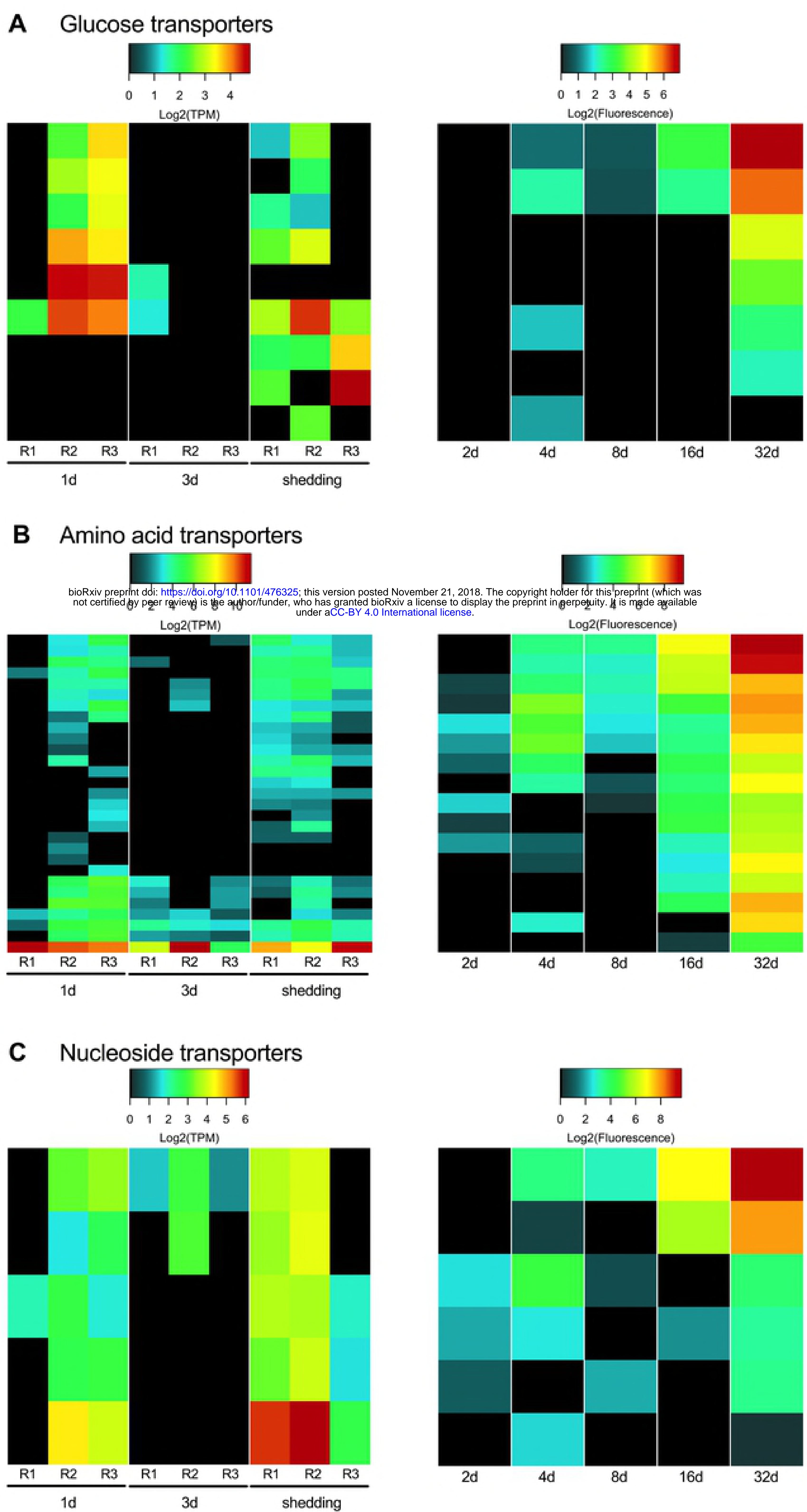

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Fig 3

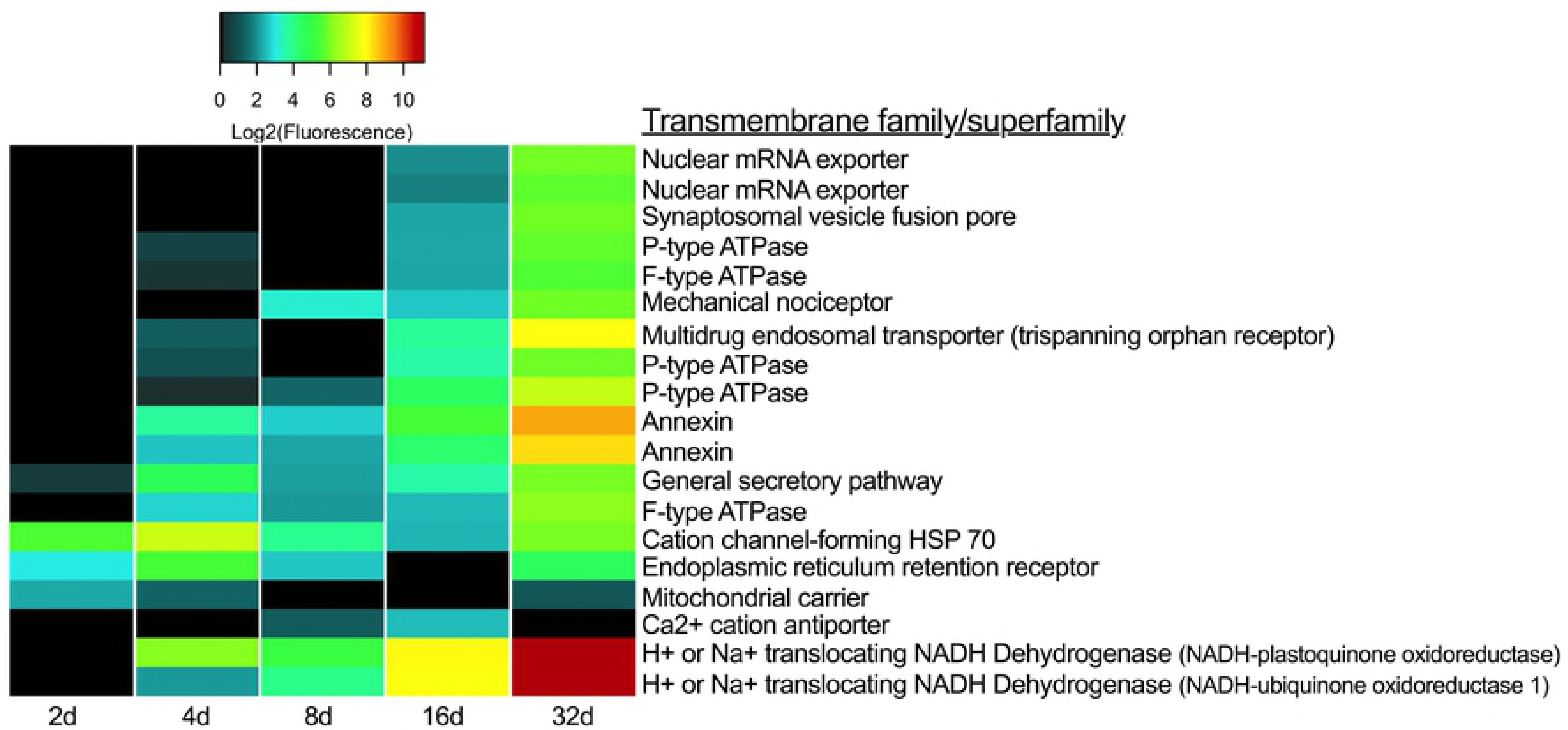

Fig 4

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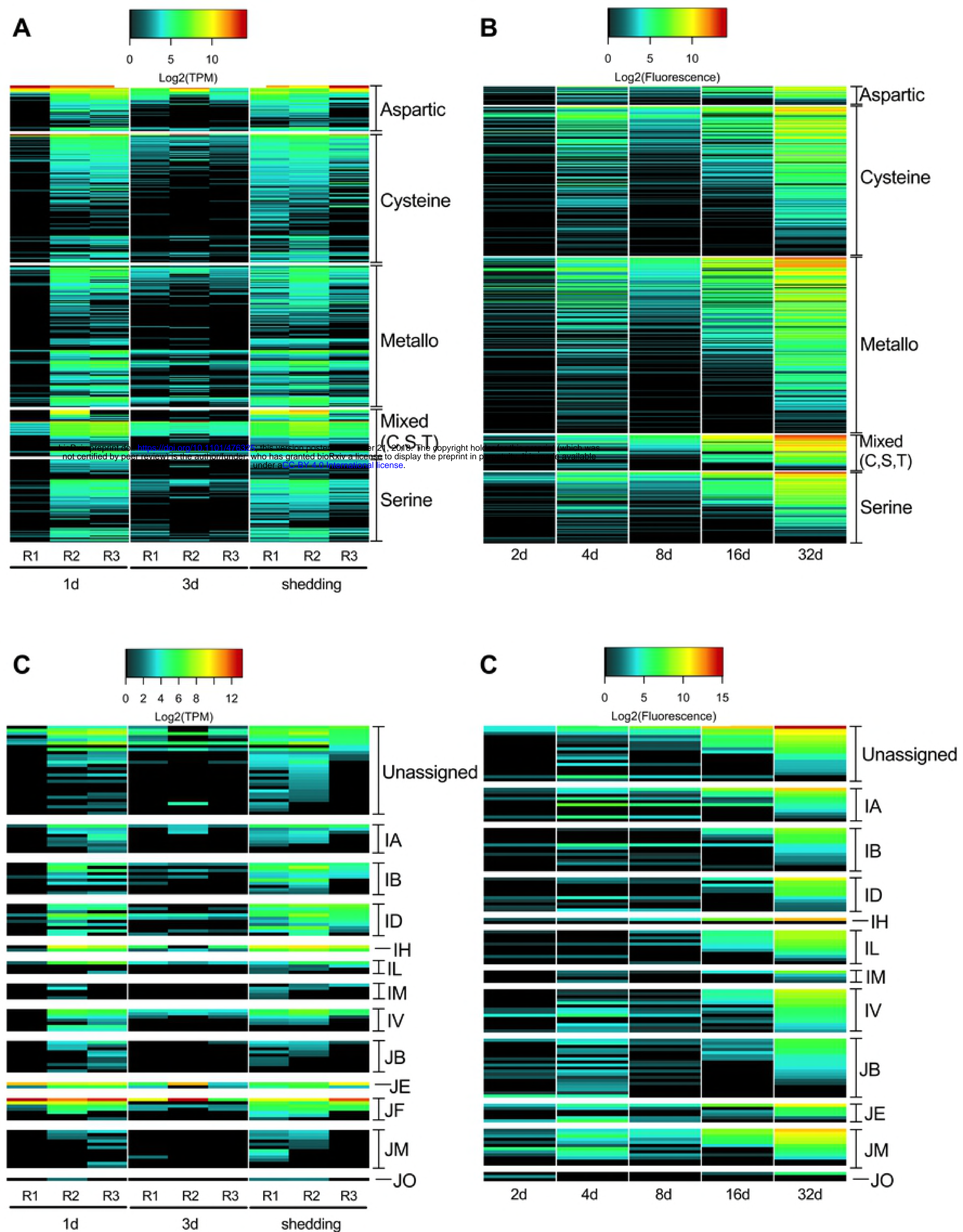

Fig 5

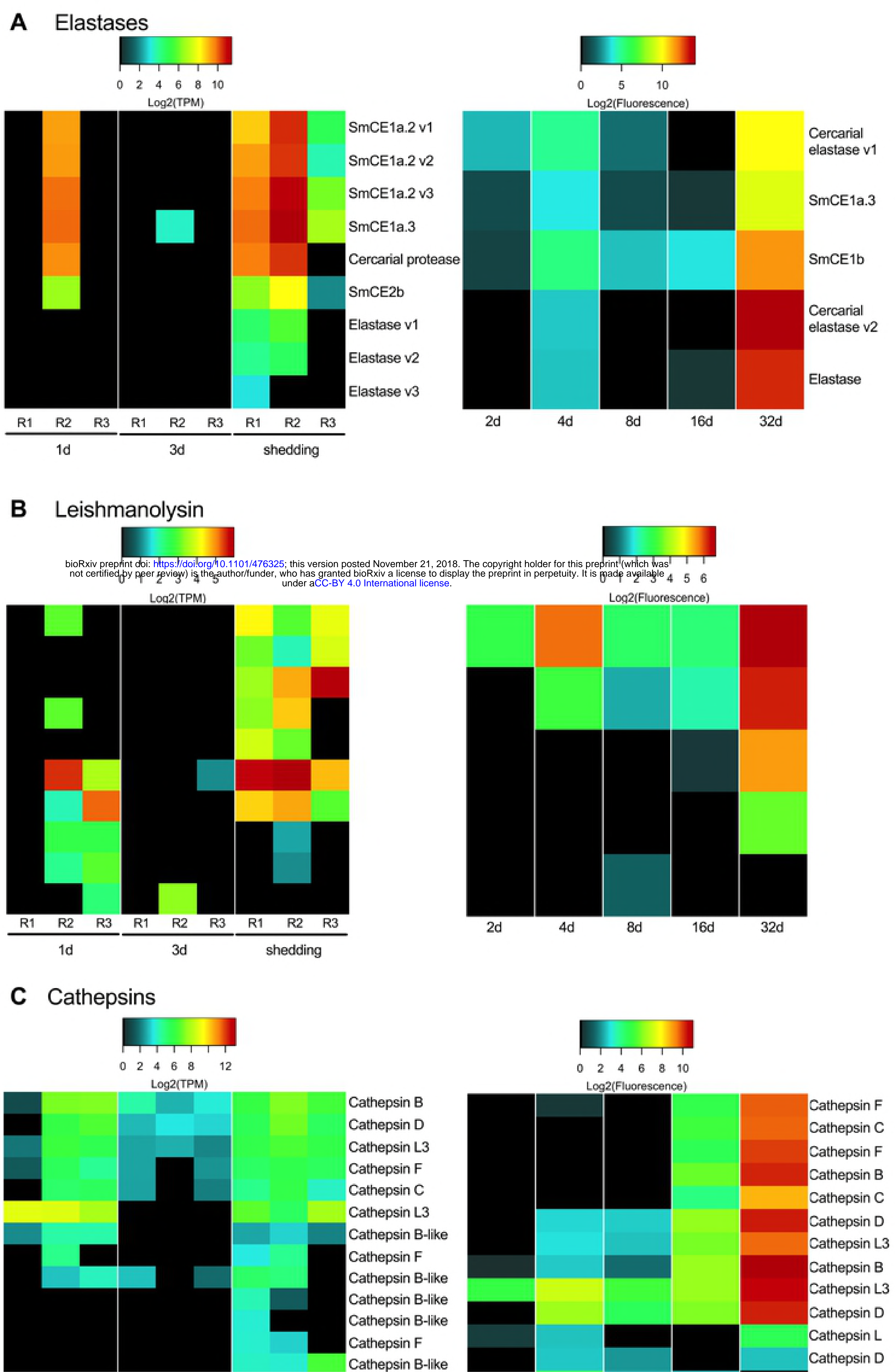

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Fig 6

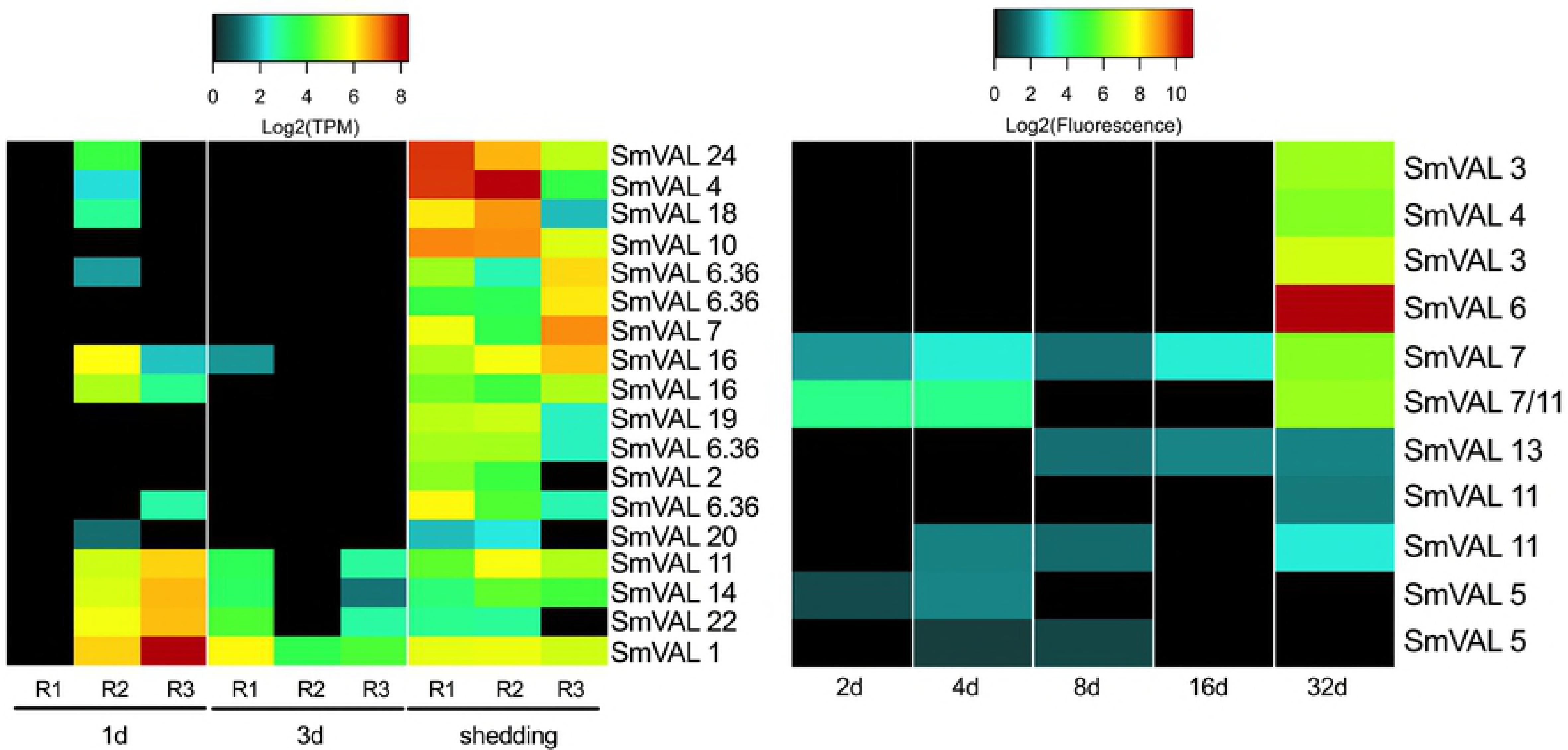

Fig 7

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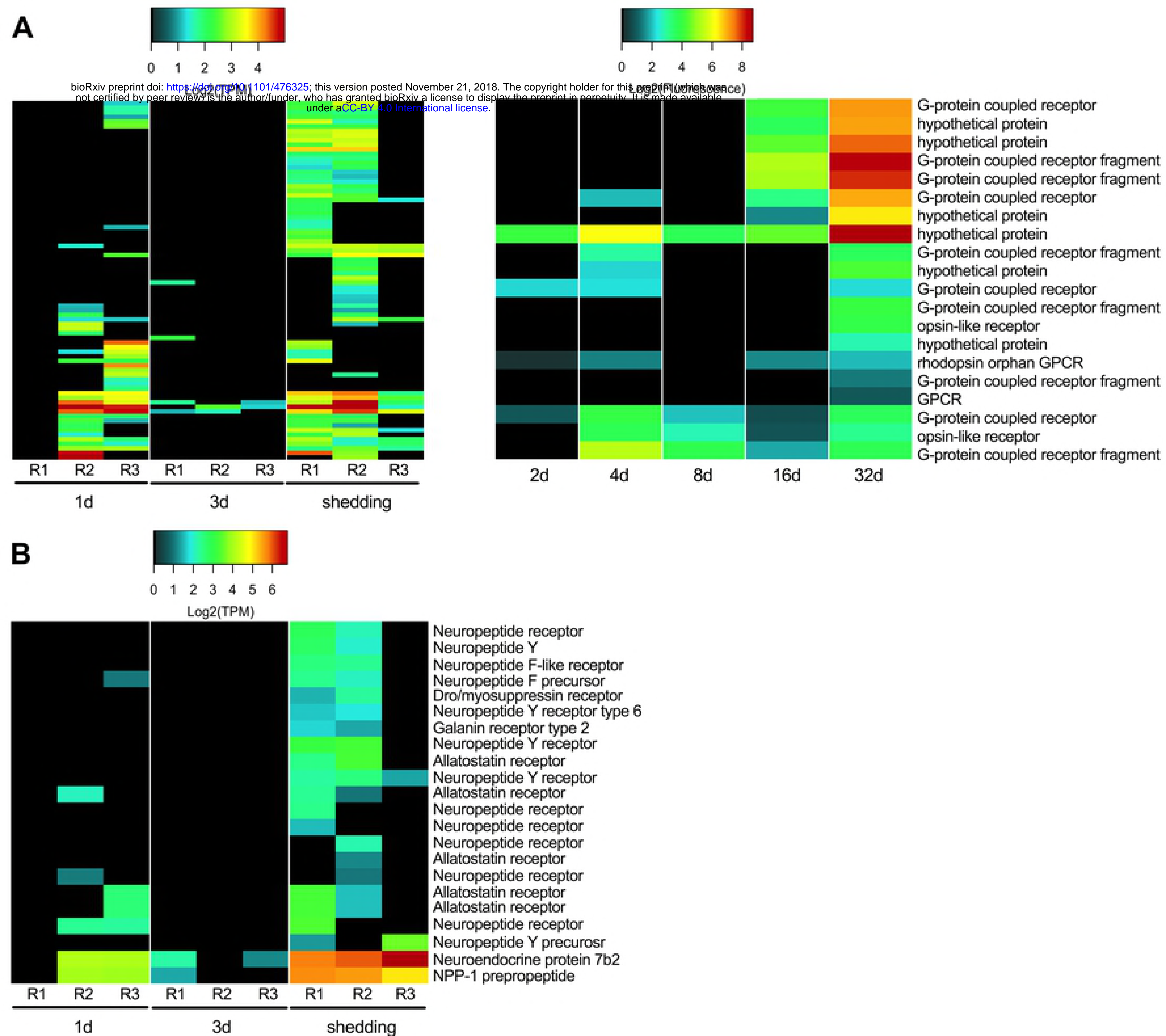

Fig 8

0 2 4 6

Log2(TPM)

Axon guidance proteins

Cell polarity proteins

Delta-like proteins

Dihydroceramide destaurase

53Gsx family homeobox protein

Jagged proteins

MAGUK homologs

Neuronal differentiation proteins

Notch

Septate junction proteins

Serine/threonin kinase

Sox transcription factor

Synapse formation protein

R1 R2 R3 R1 R2 R3 R1 R2 R3

1d 3d shedding

0 2 4 6 8

Log2(Fluorescence)

Cell polarity proteins

Notch

Septate junction proteins

Serine/threonin kinase

2d 4d 8d 16d 32d

Fig 9

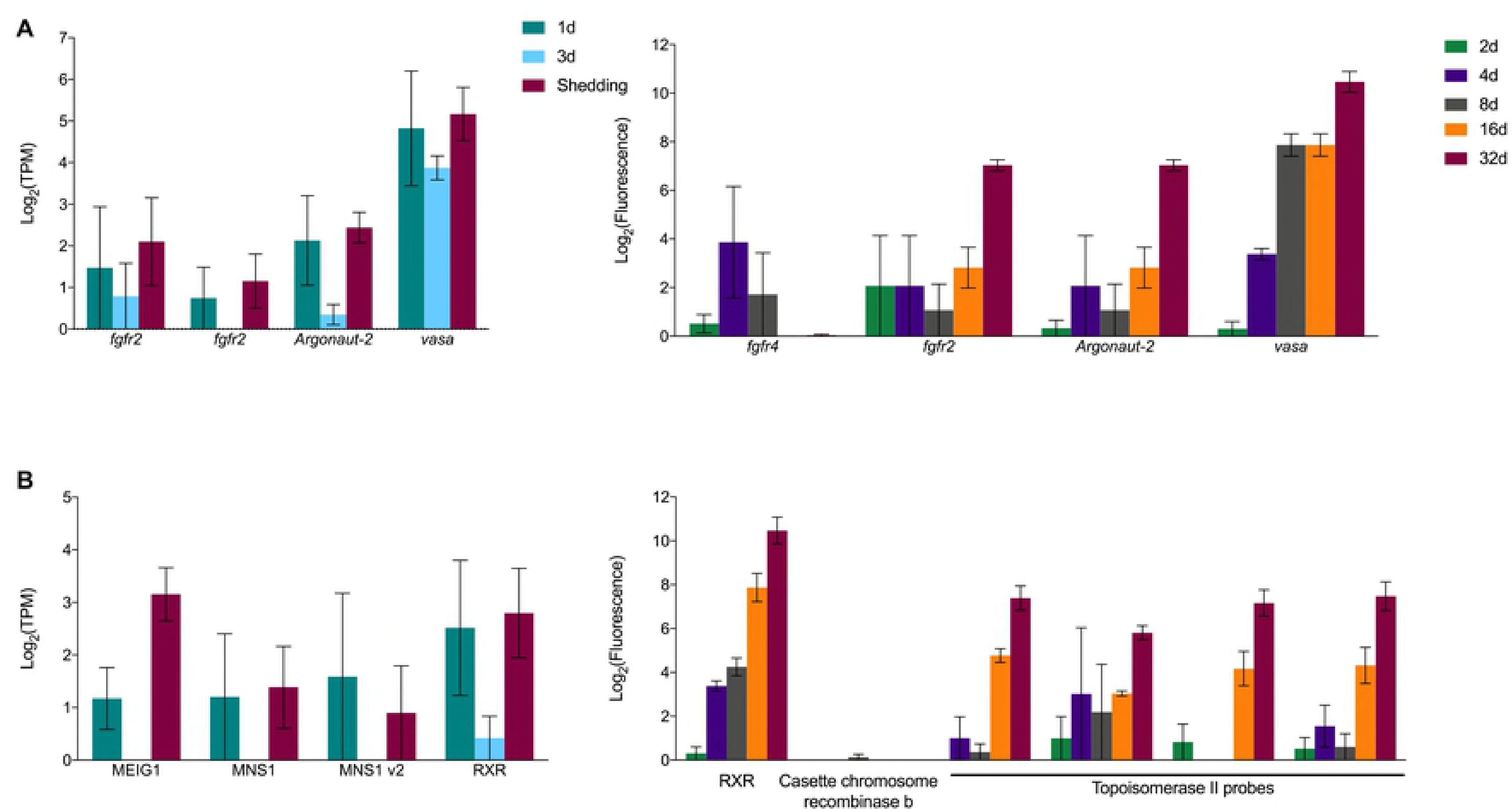

Fig 10

0 1 2 3 4 5 6
Log2(TPM)

R1 R2 R3 R1 R2 R3 R1 R2 R3
1d 3d shedding

0 2 4 6
Log2(Fluorescence)

2d 4d 8d 16d 32d

Fig 11

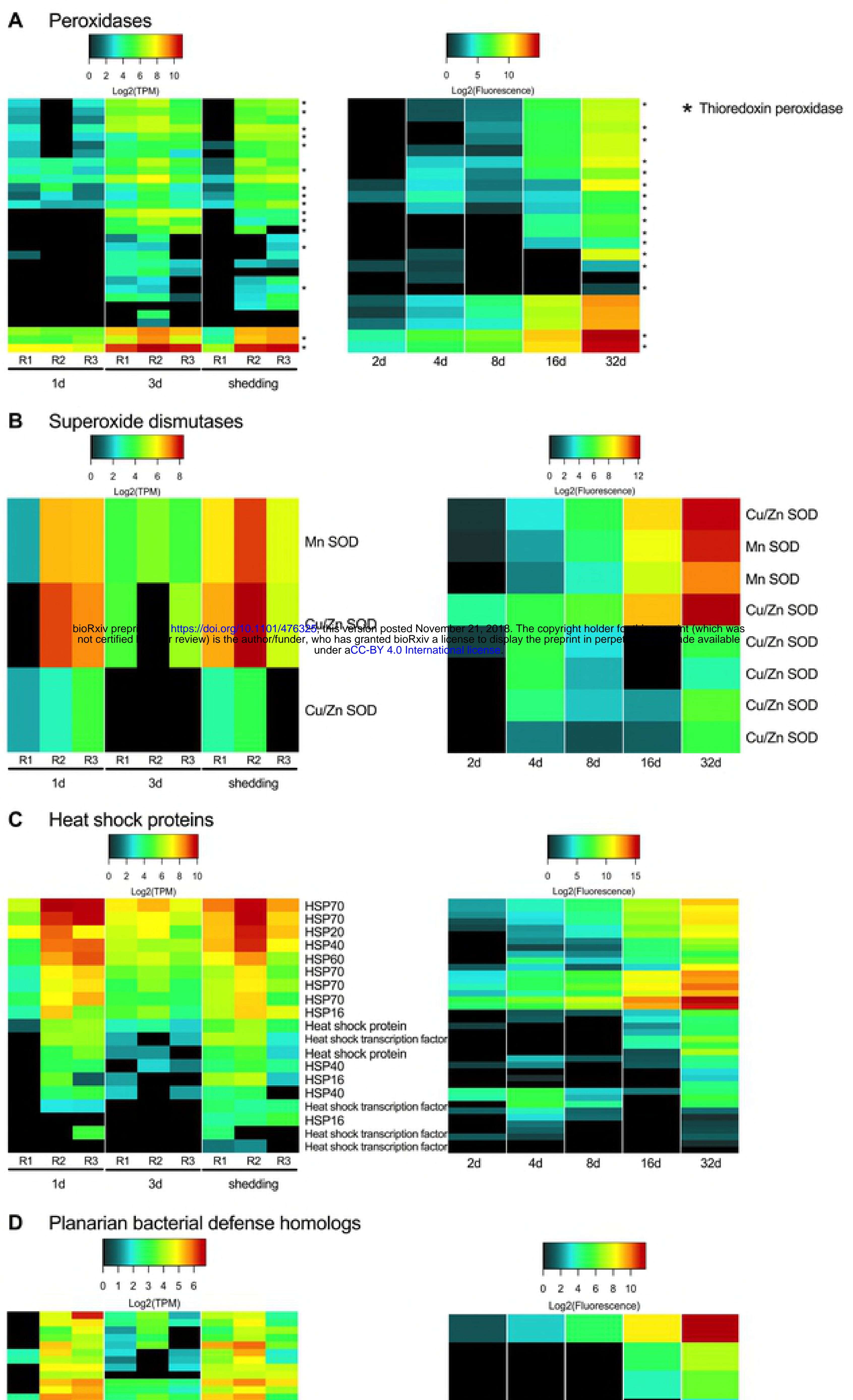

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Fig 12

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Log2(Fluorescence)
15
10
5
0
2d
4d
8d
16d
32d
Calreticulin isoform 2
Calreticulin

Log2(TPM)
10
8
6
4
2
0
1d
3d
Shedding
Calreticulin

Fig 13