Profiling G protein-coupled receptors of *Fasciola hepatica* identifies orphan rhodopsins unique to phylum Platyhelminthes

Short title: Profiling G protein-coupled receptors (GPCRs) in *Fasciola hepatica*

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Abstract

G protein-coupled receptors (GPCRs) are established drug targets. Despite their considerable appeal as targets for next-generation anthelmintics, poor understanding of their diversity and function in parasitic helminths has thwarted progress towards GPCR-targeted anti-parasite drugs. This study facilitates GPCR research in the liver fluke, *Fasciola hepatica*, by generating the first profile of GPCRs from the *F. hepatica* genome. Our dataset describes 146 high confidence GPCRs, representing the largest cohort of GPCRs, and the most complete set of *in silico* ligand-receptor predictions, yet reported in any parasitic helminth. All GPCRs fall within the established GRAFS nomenclature; comprising three glutamate, 135 rhodopsin, two adhesion, five frizzled and one smoothened GPCR. Stringent annotation pipelines identified 18 highly diverged rhodopsins in *F. hepatica* that maintained core rhodopsin signatures, but lacked significant similarity with non-flatworm sequences, providing a new sub-group of potential flukicide targets. These facilitated identification of a larger cohort of 76 related sequences from available flatworm genomes, representing new members of existing groups of flatworm-specific rhodopsins. These receptors imply flatworm specific GPCR functions, and/or co-evolution with unique flatworm ligands, and could facilitate development of exquisitely selective anthelminthics. Ligand binding domain sequence conservation relative to deorphanised rhodopsins enabled high confidence ligand-receptor matching of seventeen receptors activated by acetylcholine, neuropeptide F/Y, octopamine or serotonin. RNA-Seq analyses showed expression of 101 GPCRs across various developmental stages, with the majority expressed most highly in the pathogenic intra-mammalian juvenile parasites. These data identify a broad complement of GPCRs in *F. hepatica*, including rhodopsins likely to have key functions in neuromuscular control and sensory perception, as well as frizzled and adhesion families implicated, in other species, in growth, development and

reproduction. This catalogue of liver fluke GPCRs provides a platform for new avenues into our understanding of flatworm biology and anthelmintic discovery.

Author Summary

Fasciola spp. liver fluke are important veterinary pathogens with impacts on human and animal health, and food security, around the world. Liver fluke have developed resistance to most of the drugs used to treat them (flukicides). Since no vaccines exist, we need to develop new flukicides as a matter of urgency. Most anthelmintic drugs used to treat parasitic worm infections operate by impeding the functioning of their nerve and muscle. In flatworms, most nervous signals are received by a type of receptor called a G protein-coupled receptor (GPCR). Since GPCRs control important parasite functions (e.g. movement, egg-laying, feeding), they represent appealing targets for new flukicides, but have not yet been targeted as such. This work exploited the *F. hepatica* genome to determine the quantity and diversity of GPCRs in liver fluke. We found more GPCRs in the *Fasciola* genome than have been reported in any other parasitic worm. These findings provide a foundation that for researchers to determine the functions of these receptors, and which molecules/ligands they are activated by. These data will pave the way to exploring the potential of *F. hepatica* GPCRs as targets for new flukicides.

68 ## Introduction

69 *Fasciola spp.* liver fluke are pathogens of veterinary ruminants that threaten the
70 sustainability of global meat and dairy production. Infection with *Fasciola*
71 (fasciolosis/fascioliasis) inhibits animal productivity through liver condemnation,
72 reduced meat and milk yields, and reduced fertility (for recent impact surveys see [1-
73 4]. *Fasciola* spp. also infect humans, with fascioliasis considered a neglected
74 tropical disease [5]. Anthelmintic chemotherapy currently carries the burden of fluke
75 control, since there are no liver fluke vaccines [6]. Six flukicidal active compounds
76 are available for general use, with on-farm resistance reported for all except
77 oxyclozanide [7]. Resistance to the frontline flukicide, triclabendazole, also exists in
78 human *F. hepatica* infections [8,9]. Given the absence of alternative control
79 methods, new flukicides are essential for secure future treatment of veterinary and
80 medical liver fluke infections.
81
82 The helminth neuromuscular system is a prime source of molecular targets for new
83 anthelmintics [10-12], not least because many existing anthelmintics (dichlorvos,
84 levamisole, morantel, piperazine, pyrantel, macrocyclic lactones, paraherquamide,
85 amino acetonitrile derivatives) act upon receptors or enzymes associated with
86 classical neurotransmission in nematodes [11] The G protein-coupled receptors
87 (GPCRs) that transduce signals from both peptidergic and classical
88 neurotransmitters are of broad importance to helminth neuromuscular function.
89 Despite industry efforts to exploit helminth GPCRs in the context of anthelmintic
90 discovery [13], only a single current anthelmintic (emodepside) has been attributed
91 GPCR-directed activity as part of its mode of action [14-16]. GPCRs are druggable
92 targets, since 33% of human prescription medicines are attributed a GPCR-based
93 mode of action [17].
94

95 Despite two *F. hepatica* genomes [18,19], no GPCR sequences have been reported
96 from *F. hepatica*. In contrast, GPCRs have been profiled in the genomes of
97 trematodes (*Schistosoma mansoni* and *Schistosoma haematobium* [20,21]),
98 cestodes (*Echinococcus multilocularis*, *E. granulosus, Taenia solium* and
99 *Hymenolepis microstoma* [22]), and planaria (*Schmidtea mediterranea, Girardia*
100 *tigrina* [21-24]). These datasets illustrated clear differences in the GPCR
101 complements of individual flatworm classes and species, with reduced complements
102 in parasitic flatworms compared to planarians.
103
104 This study profiles the GPCR complement of the temperate liver fluke *F. hepatica* for
105 the first time, permitting comparisons with previously characterised species that
106 inform evolutionarily and functionally conserved elements of flatworm GPCR
107 signalling. We have identified and classified 146 GPCRs by GRAFS family
108 (glutamate, rhodopsin, adhesion, frizzled, secretin) assignment [25], the majority of
109 which are expressed in *Fasciola* RNA-Seq datasets. These include clear
110 orthologues of GPCRs activated by known neurotransmitters, within which we
111 performed the deepest *in silico* ligand-receptor matching analyses to date for any
112 parasitic helminth. The latter predicted ligands for 17 *F. hepatica* GPCRs,
113 designating these as primary targets for deorphanisation. Intriguingly, the dataset
114 included a set of flatworm-expanded GPCRs lacking orthologues outside of phylum
115 Platyhelminthes. Evolution of such GPCRs across the parasitic flatworm classes
116 may have been driven by flatworm-specific functional requirements or co-evolution
117 with flatworm ligands, either of which could help support novel anthelmintic
118 discovery. This dataset provides the first description of GPCRs in liver fluke, laying a
119 foundation for future advances in GPCR-directed functional genomics and flukicide
120 discovery.
121

122 ## Results and Discussion

123 **A first look at GPCRs in the *F. hepatica* genome**

124 This study represents the first description of the GPCR complement of the temperate
125 liver fluke, *F. hepatica*. Using HMM-led methods to examine available *F. hepatica*
126 genome datasets, we identified 166 GPCR-like sequences in *F. hepatica* (Figure 1,
127 S1 Table). Figure 1B shows that 49.7% contained 7 TM domains, with 88% of
128 sequences containing at least four TMs. The remainder of this manuscript focuses
129 on 146 sequences containing ≥4TM domains (S1 Table; S2 Text). Twenty
130 sequences containing ≤3 TMs were analysed no further (Figure 1).

131

132 Our ≥4TM dataset (146 sequences) was comprised of three glutamate, 135
133 rhodopsin, two adhesion, five frizzled, and one smoothened GPCR. Sequence
134 coverage was generally good in terms of TM and extracellular domain
135 representation, so we did not attempt to extend truncated sequences into full-length
136 receptors. The overall dataset contained excellent representation of seven TM
137 domains, while N-terminal extracellular LBDs and cysteine-rich domains (CRD) were
138 also detected (in glutamate, frizzled/smoothened, adhesion families). However, we
139 could not identify N-terminal secretory signal peptides in any sequence, suggesting
140 incomplete sequence coverage at extreme N-termini. Rhodopsins are designated by
141 ubiquitously conserved motifs on TMs 2, 3, 6 and 7. All rhodopsin sequences
142 contained at least one of these motifs (Figure 2, S3 Table), including in the highly
143 diverged flatworm-specific rhodopsins described below.

144

145 Table 1 compares the *F. hepatica* GPCR complement with other flatworms,
146 illustrating that *F. hepatica* has the largest GPCR complement reported from any
147 parasitic flatworm to date. The bulk of the expansion involves rhodopsins, while the
148 other GRAFS families are comparable between *F. hepatica* and other flatworm

parasites. Secretin is the notable exception, at least one of which has been identified in every other species studied, but which was absent from the datasets scrutinized here.

Table 1. Comparison of the *Fasciola hepatica* G-protein coupled receptor (GPCR) complement with those reported from other flatworms. Species complements are shown in the context of GRAFS nomenclature [25]. * Saberi et al [24] described 566 GPCRs in *Schmidtea mediterrannea*, of which 516 fall within GRAFS nomenclature.

	F. hepatica	*S. mansoni*[21]	*S. mansoni*[20]	*S. haematobium*[20]	*E. multilocularis*[22]	*S. mediterrannea*[21]	*S. mediterrannea*[24]
Glutamate	3	2	2	2	5	9	11
Rhodopsin	135	105	59	53	48	418	461
Adhesion	2	3	-	-	4	9	14
Frizzled/Smoothened	6	5	4	4	5	11	10
Secretin	0	2	5	5	1	1	20
Total	**146**	**117**	**64**	**64**	**83**	**448**	**516***

Stringent annotation of flatworm-specific orphan rhodopsin GPCRs in *F. hepatica*

Encompassing 135 sequences, the rhodopsin family is the largest of the GRAFS classifications in *F. hepatica*. Rhodopsins comprise four subfamilies (α, β, γ and δ) [26]; we identified members of both α and β groups, with nucleotide-activated (P2Y) receptors (γ group), and olfactory (δ group) receptors absent from our dataset (Figures 1, 2; S1 Table). The *F. hepatica* α subfamily contained 38 amine receptors and three opsins, with the β subfamily comprised of at least 47 peptide receptors. Homology-based annotations were supported by an ML phylogeny (Figure 2A), which clearly delineated between amine and opsin α clades, and the peptide-activated β-rhodopsin clades. Amine and peptide receptors were further delineated

179 by additional phylogenetic and structural analyses, permitting high-confidence
180 assignment of putative ligands to 16 GPCRs (see below).
181
182 Six clades contain an additional 44 rhodopsin sequences with low scoring (median E
183 = $5.6e^{-5}$) similarity matches to a range of disparate α and β rhodopsins. Due to the
184 subsequent difficulty in designating these clades as amine, peptide or opsin, we
185 labelled them orphan rhodopsins ("R" clades in Figure 2A). Eighteen GPCRs within
186 the orphan clades displayed exceptionally low similarity scores relative to non-
187 flatworm sequences (Figure 2A,B). Seven returned no-significant hits in BLASTp
188 searches against non-flatworm members of the ncbi nr dataset (the most diverse
189 sequence dataset available to the research community), and the remaining eleven
190 scored E>0.01. Domain analysis (InterPro) identified rhodopsin domains
191 (IPR000276 or IPR019430) in thirteen of these (S1 Table, S3 Table), confirming their
192 identity as rhodopsin-like GPCRs. More troublesome to classify were five that, in
193 addition to lacking significant BLASTp identity to non-flatworm sequences, also
194 lacked any identifiable protein domains/motifs (with the exception of TM domains).
195 We annotated these as rhodopsins because: (i) They did not contain motifs/domains
196 representative of any other protein family; (ii) They displayed topological similarity to
197 GPCRs (ten had seven TM domains, seven had six TMs, one had five TM domains);
198 (iii) They contained at least two of the conserved rhodopsin motifs in TM domains 2,
199 3, 6 and 7 similar to those seen in the rest of the *F. hepatica* rhodopsins (Figure 2C;
200 S4 Table). As highly diverged rhodopsins with little or no sequence similarity versus
201 host species, these 18 *F. hepatica* receptors have obvious appeal as potential
202 targets for flukicidal compounds with exquisite selectivity for parasite receptors over
203 those of the host. This potential is contingent on future work demonstrating essential
204 functionality for these receptors; showing their wider expression across flatworm
205 parasites would enable consideration of anthelmintics with multi-species activity. To

206 investigate the latter question, we used BLASTp to search the 18 *F. hepatica*
207 rhodopsins against other available genomes representing phylum Platyhelminthes.
208
209 **An orphan family of lineage-expanded rhodopsins in flatworm genomes**
210 Although lacking similarity against non-flatworm datasets, each of the 18 lineage-
211 expanded *F. hepatica* rhodopsins returned high-scoring hits in BLASTp searches
212 against the genomes of other flatworms (WormBase Parasite release WBPS9). All
213 returns were subsequently filtered through a stringent five-step pipeline (Figure 3A)
214 consisting of: (i) Removal of duplicate sequences; (ii) Exclusion of sequences
215 containing fewer than four TM domains; (iii) A requirement for reciprocal BLASTp
216 against the *F. hepatica* genome to return a top hit scoring E<0.001 to one of the
217 original 18 *F. hepatica* queries; (iv) A requirement for BLASTp against ncbi nr non
218 flatworm sequences to return a top hit scoring E>0.01; (v) Removal of sequences
219 lacking conservation of the ubiquitous rhodopsin motifs seen in the divergent *F.*
220 *hepatica* rhodopsins (Figures 2C, 3C). The latter motifs were largely absent from
221 cestode rhodopsins (with the exception of a single sequence from *Diphyllobothrium*
222 *latum*, and three sequences from *Schistocephalus solidus*), and present in only two
223 sequences from a single monogenean (*Protopolystoma xenopodis*). This left our
224 final dataset consisting of 76 “flatworm-specific” rhodopsins (fwRhods; Figure 3B,
225 Table S4) in phylum Platyhelminthes, heavily biased towards trematodes (70
226 sequences). Nineteen sequences from nine species of cestode were omitted from
227 the final dataset despite meeting the inclusion criteria in most respects, because they
228 lacked conservation of ubiquitous rhodopsin motifs (filtering step (v)). Although their
229 further characterisation was beyond the scope of this study, they warrant more
230 detailed examination in future studies as potential cestode-specific rhodopsins. Note
231 that our filtering pipeline also excluded initial hits from *Gyrodactylus salaris*
232 (Monogenea), and the Turbellarians *Macrostomum lignano* and *S. mediterranea*.
233 Individual species complements of fwRhods showed some consistency (Figure 3B);

234 the trematodes *F. hepatica* and *Echinostoma caproni* (both phylum Platyhelminthes
235 Order Echinostomida) bore 18 and 19 sequences, respectively, most species of
236 family Schistosomatidae contained 3-4 sequences each. The inclusion of two
237 cestode species and a single monogenean may be an indication of the existence of
238 distantly related rhodopsins in those lineages, rather than a true measure of the
239 extent of cestode and monogenean fwRhod diversity. Again, proper classification of
240 these groups will require further more focused study that was beyond the scope of
241 the current work.
242
243 Our method for identification of fwRhods is supported by a similar BLAST-driven
244 approach used to identify highly diverged "hidden orthologues" in flatworms [27]. It
245 should be noted that the existence of sequences lacking sequence similarity to
246 genes of other species is not a new finding. "Taxonomically-restricted genes"
247 comprise 10-20% of every sequenced eukaryote genome, and may be essential for
248 phylum-specific morphological and molecular diversity [28]. How do our fwRhods
249 compare to previously reported groups of flatworm restricted GPCRs in *S. mansoni,*
250 *S. mediterrannea and E. granulosus* [21,22,24]? Phylogenetic comparisons of these
251 groups (Figure 3D) demonstrated that the previously described *Schmidtea* Srfb
252 cluster [24] and the PROF1 clade (*E. multilocularis, Schmidtea, S. mansoni* [21]) are
253 equivalent, and likely represent a single group. Our phylogeny added 23 fwRhods to
254 this clade, including three from *F. hepatica* (BN1106_s6156B000040, D915_03083,
255 D915_13002). Figure 3D designated the remaining fwRhods within additional pre-
256 existing groups [24], placing 34 within Rho-L (including eight from *F. hepatica*), nine
257 in Srfc (one from *F. hepatica*), four in Rho-R (one from *F hepatica*) and two in Srfa
258 (one from *F. hepatica*). Four fwRhod sequences were omitted from this tree due to
259 poor alignment.
260

261 Our approach to classifying flatworm-restricted rhodopsins was to err towards
262 stringency, and this may have resulted in erroneous exclusion of some sequences
263 from the dataset. There is no set definition for lineage specificity in the literature, but
264 ours is the most stringent yet applied to flatworm GPCRs. Applying our BLASTp
265 E≥0.01 cutoff (modified from Pearson [29]) to the previously described groups of
266 flatworm-specific rhodopsins [21,22,24], excludes 57 of the 62 PROF1s described
267 from *S. mansoni* and *S. mediterranea* and 287 of the 318 RhoL/R and Srfa/b/c
268 flatworm-specific clusters in *S. mediterranea*. Further pursuit of the extent of lineage
269 restricted GPCRs in the wider phylum was beyond the scope of this study, but we
270 are currently trawling for taxonomically restricted flatworm GPCRs on a phylum wide
271 scale.
272
273 We have established the existence of a group of rhodopsin GPCRs that appear
274 restricted to, and expanded in, phylum Platyhelminthes. By definition these
275 receptors are orphan (i.e. their native ligands are unknown), so key experiments
276 must focus on identifying their ligands and functions. Such experiments can exploit
277 the expanding molecular toolbox for flatworm parasites, which in *F. hepatica* includes
278 RNA interference (RNAi) [30-33], interfaced with enhanced in vitro maintenance
279 methods, and motility, growth/development and survival assays [31,34,35]. Our
280 phylogeny (Figure 2A) suggests that fwRhods are more similar to peptide than amine
281 receptors. If their heterologous expression can be achieved, one approach to
282 characterisation would be to screen them with the growing canon of peptide ligands
283 from flatworms [36-38], as well as from other genera, in a receptor activation assay.
284 Subsequent localisation of their spatial expression patterns would provide additional
285 data that would inform function.
286
287 **Predicting ligands for *F. hepatica* rhodopsin GPCRs**

288 In addition to the flatworm-specific fwRhod sequences described above, for which
289 the ligands and functions remain cryptic, we also identified many rhodopsins with
290 clear similarity to previously annotated GPCRs. Figure 2A shows the phylogenetic
291 delineation of these sequences into amine-, opsin- and peptide-like receptors,
292 distinctions that are supported by BLASTp comparisons with general (ncbi nr) and
293 lineage-specific (superphylum level) datasets, as well as by gross domain structure
294 (InterProScan) (S1 Table). These data provided a foundation for deeper
295 classification of putative ligand-receptor matches.
296
297 The structure and function of GPCR LBDs can be studied using molecular modelling
298 to predict interactions with receptor-bound ligands. These predictions can then be
299 validated by targeted mutagenesis of residues within the LBD, measuring impacts
300 with downstream signalling assays. Such experiments have been performed in
301 model vertebrates and invertebrates, enabling identification of evolutionarily
302 conserved binding residues/motifs. These data inform assignment of putative
303 ligands to newly discovered receptors. Since mutagenesis experiments have not yet
304 been performed in flatworm GPCRs, we employed a comparative approach to
305 identify 17 *F. hepatica* rhodopsins with LBD motifs diagnostic of receptors for NPF/Y,
306 5-HT, octopamine (Oct) or acetylcholine (ACh) (Figure 4; S4 Table), thus enabling *in*
307 *silico* ligand-receptor matching of these GPCRs.
308
309 Comparison of *F. hepatica* rhodopsins by structural alignment with LBD residues
310 conserved across vertebrate NPY and dipteran NPF receptors [39-44] identified
311 three peptide receptors with more than 75% identity across 9 ligand-interacting
312 positions (Figure 4A). The two highest scoring GPCRs (BN1106_s3169B000088
313 and D915_05685) are also found, in our phylogenetic analysis (S5 Figure) in the
314 same clade as the deorphanized NPF/Y receptors of human (HsNPYR2), *Glossina*
315 *mortisans* (Glomo-NPFR) and *S. mediterranea* (SmedNPYR1). These data

316 designate these three *F. hepatica* GPCRs as prime candidates for further work to
317 deorphanize and confirm these receptors as NPF/Y-activated, and to probe the
318 biology of NPF/Y receptors in parasitic flatworms. A single NPF/Y receptor has been
319 functionally characterised in *S. mediterranea*, displaying a role in the maintenance of
320 sexual maturity [24]. If related functions are conserved in liver fluke NPF/Y receptors
321 they could have appeal as therapeutic targets in adult fluke that could interrupt
322 parasite transmission, although their utility for the control of acute fasciolosis, caused
323 by migrating juveniles, would be open to question.
324
325 Broad phylogenetic comparison of our peptide receptor set with a comprehensive
326 collection of deorphanized bilaterian rhodopsin GPCRs (S5 Figure), identified *F.*
327 *hepatica* receptors similar to those for myomodulin, FLP, luqin and Neuropeptide KY
328 (NKY). These ligands have all been predicted or demonstrated in previous
329 biochemical or in silico studies of flatworm neuropeptides [36-38]. We also
330 uncovered *F. hepatica* GPCRs with phylogenetic similarity to allatotropin, allatostatin,
331 thyrotropin-releasing hormone and sex peptide receptors. These ligands have not
332 yet been reported in flatworms, although the existence of allatostatin-like receptors in
333 flatworms is supported by the inter-phyla activity of arthoropod allatostatins in
334 helminth (including flatworm) neuromuscular assays [45].
335
336 No *F. hepatica* neuropeptide sequences have been published yet, but our
337 unpublished data suggest the presence of at least 36 neuropeptide genes in the *F.*
338 *hepatica* genome (Duncan Wells, Queen's University Belfast, personal
339 communication). These ligands would facilitate deorphanisation of heterologously-
340 expressed peptide GPCRs (S1 Table). This is essential work, since although two
341 planarian peptide receptors have been deorphanised [23,24], no flatworm parasite
342 peptide GPCRs have been ligand matched yet. Receptor deorphanisation provides
343 a starting point for drug discovery, by enabling development of agonists or

344 antagonists that modulate the interaction of a GPCR with its cognate ligand. Such
345 compounds could form the basis of ligand series for screening pipelines that would
346 lead to new flukicides [46,47].
347
348 Serotonin (5-hydroxytryptamine, 5-HT) is abundant throughout flatworm nervous
349 systems, and is considered the primary flatworm excitatory neurotransmitter [48].
350 Deorphanized GPCRs activated by 5-HT have been described in turbellarians and
351 trematodes, with an *S. mansoni* 5-HT receptor (Sm5HTR) involved in neuromuscular
352 control [49-51]. Five *F. hepatica* rhodopsins (Figure 4B) bore appreciable (≥80%)
353 positional identity in amino acids shown to be key ligand-interacting residues in the
354 human 5HT1A LBD [52,53]. Notably, these residues were also conserved in the
355 deorphanized *S. mansoni* 5-HT receptor (Sm5HTR, Smp_126730) [51]. Three of the
356 sequences (BN1106_s362B000177, BN1106_s81B000700 and
357 BN1106_s10B000515) also resembled Sm5HTR in our phylogenetic analysis,
358 identifying them as likely 5-HT receptors. The remaining two (D915_00277 and
359 BN1106_s1436B000114) appeared phylogenetically more similar to an *S. mansoni*
360 dopamine receptor (Smp_127310) [54]. These annotations provide rational starting
361 points for receptor deorphanization using functional genomic and/or heterologous
362 expression tools. We found that *F. hepatica* dopamine-like receptors, identified by
363 phylogeny (S5 Figure), displayed poor conservation (max 56% overall identity) to the
364 human D2 LBD [55]. Due to this lack of selectivity, we did not annotate any *F.*
365 *hepatica* GPCRs as dopamine receptors.
366
367 Although common in other invertebrates, octopamine has not yet been directly
368 demonstrated as a neurotransmitter in flatworms. Evidence for its presence is
369 indirect, based on tyramine β-hydroxylase (octopamine's biosynthetic enzyme)
370 activity in cestodes and planaria [56,57]. Three rhodopsins (Figure 4C) showed
371 100% conservation of the arthropod octopamine LBD, as determined from

372 *Periplaneta americana* and *Bombyx mori* [58,59], with an additional four showing
373 88% conservation. Of these seven rhodopsins, four resolved in close phylogenetic
374 proximity to *Drosophila* mushroom body octopamine receptors (D915_02972),
375 *Drosophila* octopamine beta-receptors (D915_08505 and BN1106_s1016B000108)
376 (S5 Figure) or a *Drosophila* tyramine receptor (D915_05578), denoting these as
377 high-confidence octopamine receptors. These data provide further evidence in
378 support of a functional role for this enigmatic classical neurotransmitter in flatworms.
379
380 Acetylcholine has species-specific impacts on flatworm neuromuscular preparations
381 *in vitro*, with myoinhibitory effects in *Fasciola* [60]. Two putative muscarinic
382 acetylcholine receptors (mAChRs), shared highest LBD identity with a Rat M3 ACh
383 receptor (Figure 4D) [61]. Although these were only 67% identical to the rat
384 sequence, the five ligand-interacting residues within their LBDs were 100% identical
385 to those of a deorphanised *S. mansoni* mAChR, known to be involved in
386 neuromuscular coordination (SmGAR) [62]. These receptors (D915_00814 and
387 BN1106_s1913B000092) were also the most similar to SmGAR in our phylogeny (S5
388 Figure) so we consider them amongst our high confidence candidates for
389 deorphanization.
390
391 ***F. hepatica* glutamate receptors bear divergent glutamate binding domains**
392 At least three glutamate-like GPCRs exist in *F. hepatica* (Figure 5A, S1 Table). All
393 three are defined by significant BLASTp similarity (median $E=2.3e^{-34}$) to metabotropic
394 glutamate receptors (mGluRs), and/or by the presence of InterPro GPCR family 3
395 (Class C) domains IPR017978, IPR000162 or IPR000337. Phylogenetic analysis of
396 these GPCRs was performed alongside receptors representative of the various Class
397 C subgroups (Figure 5) [63], including Ca^{2+}-sensing receptors, γ-aminobutyric acid
398 type B ($GABA_B$) receptors, metabotropic glutamate (mGluR) receptors, and
399 vertebrate taste receptors; for reference we also included the two previously reported

400 mGluRs from *S. mansoni* [21]. One *F. hepatica* GPCR (BN1106_s2924B000081)
401 resolved alongside Smp_052680, which has previously been described as an *S.*
402 *mansoni* mGluR; these receptors form a close outgroup from the mGluR clade,
403 supporting their designation as mGluRs. A second *F. hepatica* glutamate receptor
404 (BN1106_s1717B000113) also has a close *S. mansoni* ortholog (Smp_128940), both
405 of which reside in an orphan outgroup that is of uncertain provenance. The third *F.*
406 *hepatica* glutamate receptor resides within another orphan group, close to human
407 GPR158 and GPR179, two closely related class C GPCRs expressed respectively in
408 the human brain and retina [64]. Although these receptors have been linked with
409 specific disease states [65,66], their ligands remain unknown.
410
411 Divergence within the LBD can inform the ligand selectivity of Class C receptors
412 [21,67]. To further classify the two orphan glutamate GPCRs described above, we
413 generated multiple sequence alignments to analyse the conservation of established
414 agonist-interacting residues between mammalian mGluR and $GABA_B$ receptors and
415 our *F. hepatica* GPCRs. These analyses identified no significant conservation of
416 either mGluR or $GABA_B$ LBD residues (Figure 6B). Figure 6B also includes the
417 previously reported *S. mansoni* glutamate receptors [21], where Smp_052660
418 contained a relatively well-conserved LBD with Smp_062660 appearing more
419 atypical. Since all three *F. hepatica* glutamate GPCRs bear atypical LBDs with
420 respect to both $GABA_B$ and mGluR, it remains difficult to unequivocally define their
421 ligand selectivity on the basis of conserved motifs. Nevertheless, the lack of *in silico*
422 evidence for *F. hepatica* $GABA_B$ GPCRs reflects the dominance of $GABA_A$-like
423 pharmacology, which suggests that flatworm GABA signal transduction is probably
424 entirely mediated by ionotropic receptors [48,68].
425
426 **The Wnt binding domain is conserved in *F. hepatica* frizzled/smoothened**
427 **receptors**

428 Ten frizzled (fzd) GPCRs and a single smoothened (smo) GPCR are recognised in
429 the human genome. In *F. hepatica* we identified five fzd-like sequences and one
430 smo-like sequence (Figure 6; Table S1; Table S7). All of these show high scoring
431 similarity to annotated sequences in the ncbi nr dataset (median E=3.8e^{-83}), and all
432 five fzd contain InterPro domain IPR000539, with the single smo containing domain
433 IPR026544 (Table S1). Phylogenetic analysis of these alongside vertebrate and
434 invertebrate receptors placed all in close proximity to existing fzd/smo groups (Figure
435 6A). Four *F. hepatica* fzd had individual direct orthologs with the four known *S.*
436 *mansoni* fzd [21].
437
438 *Frizzled* receptors are activated by cysteine-rich glycoprotein ligands known as Wnts
439 (Wingless and Int-1), and are involved in developmental signalling through at least
440 three different signalling pathways [69]. Crystallography of mouse fz8, docked with
441 *Xenopus* wnt8, identified 14 amino acids within the fz8 CRD that make contact with
442 the Wnt8 ligand [69]. Positional conservation of these residues is apparent when fz8
443 is aligned with the five *F. hepatica* fzd sequences (Figure 6B; S6 Table), suggesting
444 conservation of the wnt-frizzled interaction between liver fluke and vertebrates.
445
446 Two Wnt ligands have been described in *S. mansoni* [70,71] ; our BLAST searches
447 identified at least three Wnt-like sequences in the *F. hepatica* genome
448 (BN1106_s198B000330.mRNA-1, BN1106_s1256B000163.mRNA-1,
449 BN1106_s737B000430.mRNA-1; Figure 6C). These showed conservation of the 23
450 conserved cysteine residues that are diagnostic of Wnt glycoproteins [72]. Norrin, a
451 non-Wnt protein ligand, can also activate Fz4, and the canonical β-catenin pathway.
452 The amino acids involved in norrin binding to the fz4 CRD have also been
453 determined [73], but we did not observe conservation of these in any of the *F.*
454 *hepatica* fzd. Similarly, BLASTp searches of human norrin (Uniprot Q00604) against
455 the *F. hepatica* genome did not return significant hits, suggesting that the norrin-fz

signaling axis may not function in liver fluke. Smoothened receptors are structurally similar to frizzleds, but operate in a ligand-independent fashion within hedgehog signaling pathways that control several developmental processes [74]. Model organism genomes typically contain only one smoothened gene (SMO); this was the case in *S. mansoni* and *S. mediterranea* [21], and here we have identified a single *F. hepatica* smoothened (BN1106_s1509B000194 ; Figure 6; Table S1).

Fzd/smo GPCRs are involved broadly in the control of cellular development. Our discovery of fzd/smo GPCRs, and their Wnt ligands, in *F. hepatica* opens avenues towards probing molecular aspects of development and differentiation in the putative stem cells/neoblasts of liver fluke [35]. Neoblasts are the cells that impart the regenerative capacity of free-living turbellarian flatworms [75], and neoblast-like cells also represent the only proliferating cells in several parasitic species [76-78]. Therefore, these cells are important in understanding fundamental fluke biology and represent potential repositories of unique anthelmintic targets, capable of inhibiting worm growth or development. The presence of both receptor and ligand sequences will permit functional genomic dissection of Wnt-Frizzled ligand-receptor signalling networks, aimed at elucidating their roles in the development and differentiation of liver fluke neoblast- like cells. These FhGPCRs will enable comparisons between the biology of parasitic and free-living flatworms, where Wnt signaling is known to be essential for anterior-posterior polarity in regenerating planaria [79,80].

Class B (Adhesion and Secretin) receptors

Class B receptors incorporate both adhesions and secretins. Adhesions are characterised by a long N-terminal extracellular domain (ECD) that includes several functional motifs. These ECDs are auto-proteolytically cleaved into two subunits that subsequently reassemble into a functional dimer [26]. We identified two Class B sequences in the *F. hepatica* genome (S7 Figure, S1 Table), both of which

484 (scaffold181_78723-79604, and BN1106_s537B000355) contained GPCR class B
485 InterPro domain IPR000832 and displayed closest BLASTp similarity (E=5.6e^{-7}) to
486 latrophilin-like receptors. These data suggest that both are adhesions, rather than
487 secretins. Phylogenetic analysis of these GPCRs alongside human Class B
488 receptors supports the definition of scaffold181_78723-79604 as an adhesion,
489 alongside two previously reported *S. mansoni* adhesions (Smp_176830,
490 Smp_099670) [21], while the other receptor appears more divergent.
491 BN1106_s536B000355 pairs with another known *S. mansoni* adhesion
492 (Smp_058380) [21], although both sit in closer proximity to human secretins than
493 adhesions.
494
495 Deorphanization of a handful of adhesions matches them with a complex assortment
496 of ligands including collagen, transmembrane glycoproteins, complement proteins
497 and FMRFamide-like neuropeptides [81]. This assortment of potential ligands, and
498 their expression in almost every organ system has led to the proposal of a diverse
499 range of functions for vertebrate adhesions. The *F. hepatica* adhesion complement
500 of two GPCRs is greatly reduced compared to the 33 receptors known in humans; in
501 other flatworms 14, 4 and 1 adhesions have been described in *S. mediterranea, E.*
502 *multilocularis* and *S. mansoni*, respectively [21,22,24]. Functional characterisation
503 will be a challenging task given the wide range of possible functions to be assayed;
504 an appealing starting point would be to investigate roles in neoblast motility prior to
505 differentiation, given that mammalian adhesion GPCRs are involved in the control of
506 cellular migration [81].
507
508 **Developmental expression**
509 Using RNA-Seq methods, we were able to confirm expression of 101 GPCRs across
510 libraries representing several *F. hepatica* life-stages. These datasets included
511 publically available reads from individual developmental stages [18], and a

512 transcriptome that we generated in-house for 21-day liver stage ex-vivo juveniles
513 (juv2). Since these datasets were generated independently and clearly display
514 distinct sequence diversities, we avoided any further direct comparisons between
515 Cwiklinski juv1 and our juv2 datasets. Each dataset is analysed separately, below.
516
517 Figure 7A illustrates detection of 83 GPCRs across Cwiklinski's developmentally
518 staged RNA-Seq datasets. These comprised four FZD, thirteen aminergic
519 rhodopsins, two opsins, 41 peptidergic rhodopsins, and 23 orphan rhodopsins. The
520 latter included nine fwRhods. Clustering within Figure 7A's expression heatmap
521 shows clear developmental regulation of GPCR expression, outlining nine GPCRs
522 with relatively higher expression in adults, two with higher expression in 21d
523 juveniles, 64 GPCRs preferentially expressed in either 1h, 3h or 24h NEJs, and six
524 receptors expressed most highly in eggs. GPCR classes appear to be randomly
525 distributed across these expression clusters, giving little opportunity to infer function
526 from expression. Adult-expressed GPCRs include five orphan fwRhods, three
527 peptide receptors including a putative NPF/Y receptor, and a predicted octopamine-
528 gated aminergic rhodopsin. The majority of expressed GPCRs occurred in the NEJ-
529 focused expression cluster. Given data implicating GPCRs in motility,
530 growth/development and sensory perception [11], it is no surprise to find high levels
531 of GPCR expression in the NEJs, which must navigate and burrow their way from the
532 gut lumen into the liver parenchyma, while also sustaining rapid growth from the start
533 of the infection process. The high expression in these stages, of receptors that we
534 predict to be activated by myomodulators such as ACh, FMRFamide, GYIRFamide,
535 myomodulin, myosuppressin and 5-HT, provide tentative support for these
536 predictions. The focused expression of six GPCRs in eggs suggests potential roles
537 in the control of cellular proliferation and fate determination processes that occur
538 during embryonation of liver fluke eggs. This complement did not include frizzled or
539 adhesion GPCRs that are traditionally implicated in the control of development,

540 instead consisting of rhodopsins (including an angiotensin-like peptide receptor, two
541 octopamine-like amine receptors, one opsin receptor and one fwRhod receptor).
542
543 Focusing on the pathogenic 21-day juvenile stage, we detected 76 GPCRs in our
544 juv2 datasets, and 29 in the corresponding juv1 samples from Cwiklinski's dataset
545 (Figure 7B). Our juv2 dataset included three glutamate, one adhesion, four frizzled,
546 one smoothened, and 67 rhodopsins. The identity of the receptors expressed here
547 again attest to the key role of neuromuscular co-ordination in this highly motile life
548 stage, which must penetrate and migrate through the liver parenchyma *en route* to
549 the bile ducts. Amongst the receptors expressed in this stage and thought to have a
550 role in neuromuscular function are several activated by classical neurotransmitters
551 including ACh, dopamine and 5-HT. The peptide receptors include some with
552 phylogenetic similarity to receptors for myoactive flatworm peptides (FMRFamide,
553 GYIRFamide, NPF) [11], as well as receptors from other invertebrates activated by
554 peptide ligands known to have excitatory effects on flatworms (allatostatin A,
555 myomodulin, proctolin) [82]. The presence of highly expressed GPCRs with
556 probable neuromuscular functions in liver stage juveniles, points to the importance of
557 studying these receptors with a view to flukicide discovery. The damage caused by
558 migrating juvenile fluke requires that new flukicides are effective against this stage.
559 The neuromuscular GPCRs expressed in migrating juveniles provide compelling
560 targets for new drugs.
561
562 **Conclusions**
563 GPCRs are targets for 33% of human pharmaceuticals [17], illustrating the appeal of
564 GPCRs as putative anthelmintic targets. This study provides the first description of
565 the *F. hepatica* GPCR complement permitting consideration of a GPCR target-based
566 screening approach to flukicide discovery. To facilitate the deorphanization
567 experiments that will precede compound screening efforts, we have described a set

568 of high confidence rhodopsin ligand-receptor pairs. We identified these GPCRs,
569 including receptors for ACh, octopamine, 5HT and NPF/Y, through phylogenetic
570 comparison with existing deorphanised receptors and positional conservation of
571 ligand-interacting residues within ligand binding domains. Our additional descriptions
572 of flatworm-specific rhodopsins support the potential for synthetic ligands to be
573 parasite-selective anthelmintics.
574

575 ## Materials and Methods

576 **Liver fluke sequence databases**

577 We exploited two *F. hepatica* genome assemblies available from WormBase
578 ParaSite [83], generated by Liverpool University
579 (http://parasite.wormbase.org/Fasciola_hepatica_prjeb6687/Info/Index/; [18], and
580 Washington University, St Louis
581 (http://parasite.wormbase.org/Fasciola_hepatica_prjna179522/Info/Index/; [19].
582

583 **Identification of GPCR-like sequences from *F. hepatica***

584 Figure 1 summarises our GPCR discovery methodology, which employed Hidden
585 Markov Models (HMMs) constructed from protein multiple sequence alignments
586 (MSAs) of previously described *S. mansoni* and *S. mediterranea* GPCR sequences
587 [21]. Individual HMMs were constructed for each GRAFS family [25]. Alignments
588 were generated in Mega v7 (www.megasoftware.net) [84] using the Muscle algorithm
589 with default parameters. HMMER v3 (http://.hmmer.org) was employed to construct
590 family-specific HMMs (*hmmbuild*) from alignments and these were searched
591 (*hmmsearch*) against a predicted protein dataset from *F. hepatica* genome
592 PRJEB6687 consisting of 33,454 sequences [18]. Returned sequences were filtered
593 for duplicates and ordered relative to the *hmmsearch* scoring system, enabling the
594 classification of hits according to the GRAFS family to which they showed most
595 similarity (i.e. highest score, lowest E value). All remaining returns were then used
596 as BLAST queries (BLASTp and tBLASTn) to identify matching, or additional,
597 sequences originating from the PRJEB6687 and PRJNA179522 genomes (Figure 1).
598 Where sequences appeared in both genomes, we kept the longest annotated
599 sequence (S1 Table).
600

601 **GPCR annotation**

602 Sequences resulting from HMM searches were filtered by transmembrane (TM)
603 domain composition, using hmmtop (http://www.sacs.ucsf.edu/cgi-bin/hmmtop.py)
604 [85,86]. Sequences containing ≥4 TMs were analysed as described below.
605
606 **Homology analyses**
607 All GPCRs were used as BLASTp [87] queries, to identify their closest (highest
608 scoring) match in the ncbi non-redundant (nr) protein sequence dataset
609 (https://blast.ncbi.nlm.nih.gov/Blast.cgi), with default settings and the "Organism" field
610 set to exclude Platyhelminthes (taxid: 6157). All GPCRs were additionally searched
611 against more phylogenetically limited datasets, by using the "Organism" field to limit
612 the BLASTp searches to: (i) Basal phyla, Ctenophora (taxid:10197), Porifera
613 (taxid:6040), Placozoa (taxid:10226), Cnidaria (taxid:6073); (ii) Superphylum
614 Lophotrochozoa (taxid: 1206795), excluding phylum Platyhelminthes (taxid: 6157);
615 (iii) Superphylum Ecdysozoa (taxid: 1206794); (iv) Superphylum Deuterostomia
616 (taxid: 33511). For BLASTp searches against other flatworms, we performed local
617 BLAST+ [88] on the WBPS9 release of WormBase Parasite, which included
618 predicted protein datasets from 30 flatworm species. In all cases, we recorded the
619 single highest scoring hit, or recorded "no significant similarity found" in cases where
620 no hits were returned (Table S1); sequences generating both GPCR hits and "no
621 hits" were retained. Where the top hit was not to a GPCR, that sequence was
622 removed from the dataset.
623
624 **Domain composition**
625 GPCR identities were confirmed using InterProScan Sequence Search
626 (www.ebi.ac.uk/interpro/search/sequence-search) [89] and/or HMMER HMMScan
627 (www.ebi.ac.uk/Tools/hmmer/search/hmmscan) [90]. Again, sequences returning
628 non-GPCR domains were omitted from the dataset, with all others retained.
629

630 **Motif identification**

631 As an additional measure of confidence in our identifications, we analysed the
632 presence/absence of key motifs diagnostic of receptor families and subfamilies.
633 These analyses were performed for rhodopsins generally, the ligand binding domains
634 (LBDs) of rhodopsin receptors for acetylcholine (ACh), neuropeptide F/Y (NPF/Y),
635 octopamine and serotonin (5-hydroxytryptamine, 5HT), and for the LBDs of
636 glutamate and frizzled/smoothened families. Motifs were identified via protein
637 multiple sequence alignment (MSA) of GPCRs, performed in MAFFT
638 (www.mafft.cbrc.jp/alignment/server) [91]. Only identical amino acids were accepted
639 at each site, with conservation expressed as % identity across all sites. Motif
640 illustrations (Figures 3 & 7) were generated using WebLogo 3
641 (http://weblogo.threeplusone.com) [92].
642

643 **Phylogenetic reconstruction**

644 Maximum likelihood (ML) phylogenetic trees were constructed using PhyML
645 (http://www.phylogeny.fr) [93], from protein MSA generated in MAFFT
646 (www.mafft.cbrc.jp/alignment/server/) [91]. Alignments were manually edited (in
647 Mega v7) to include only TM domains, by removing extramembrane blocks aligned
648 with human glutamate, rhodopsin, adhesion or frizzled proteins. Trees were
649 constructed from these TM-focused alignments in PhyML using default parameters,
650 with branch support assessment using the approximate likelihood ratio test (aLRT),
651 under "SH-like" parameters. Trees, exported from PhyML in newick format were
652 drawn and annotated in FigTree v1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/).
653

654 **RNA-Seq analyses**

655 Expression of *F. hepatica* GPCRs was investigated in publically available and in-
656 house generated RNA-Seq datasets. These included developmentally staged
657 Illumina transcriptome reads associated with one of the *F. hepatica* genome projects

658 [18] (reads accessed from the European Nucleotide Archive at
659 http://www.ebi.ac.uk/ena/data/search?query=PRJEB6904). These samples
660 originated from distinct developmental stages of US Pacific Northwest Wild Strain *F.*
661 *hepatica* (Baldwin Aquatics), including egg (*n*=2), metacercariae (met; *n*=4), *in vitro*
662 NEJs 1h post-excystment (NEJ1h; *n*=1), *in vitro* NEJs 3h post-excystment (NEJ3h;
663 *n*=2), *in vitro* NEJs 24h post-excystment (NEJ24h; *n*=2), *ex-vivo* liver-stage juveniles
664 (juv1; *n*=1) and *ex-vivo* adult parasites (Ad; *n*=3). Our in-house datasets were
665 generated from *ex vivo* liver stage *F. hepatica* juveniles (Italian strain, Ridgeway
666 Research Ltd, UK), recovered from rat (Sprague Dawley) hosts at 21 days following
667 oral administration of metacercariae (juv2; *n*=3). Total RNA, extracted with Trizol
668 (ThermoFisher Scientific) from each of the 3 independent biological replicates, was
669 quantified and quality checked on an Agilent Bioanalyzer, converted into paired-end
670 sequencing libraries and sequenced on an Illumina HiSeq2000 by the Centre for
671 Genomic Research at the University of Liverpool, UK. RNA samples were spiked
672 prior to library construction with the ERCC RNA Spike-In Mix (ThermoFisher
673 Scientific) [94]. All read samples were analysed using the TopHat and Cufflinks
674 pipeline [95-100], with mapping against PRJEB6687 genome sequence and
675 annotation files (accessed from WormBase Parasite;
676 http://parasite.wormbase.org/ftp.html). Data were expressed as number of fragments
677 mapped per million mapped reads per kilobase of exon model (FPKM). In juv2
678 datasets we discarded GPCRs represented by fewer than 0.5 FPKM (the minimum
679 linear sensitivity that we detected with our ERCC spike in); for the staged datasets,
680 we included only receptors represented by ≥0.5 FPKM in at least one life stage.
681 Heatmaps were generated with heatmapper (http://www.heatmapper.ca/) [101] set
682 for Average Linkage, and Pearson Distance Measurement.
683

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1083

1084 **Figure Captions**

1085 **Fig 1: Methods for discovery and annotation of *Fasciola hepatica* G protein**
1086 **coupled receptors (FhGPCRs).** (A) Hidden Markov Models (HMMs) representing
1087 glutamate, rhodopsin, adhesion, frizzled/smoothened and secretin families, and two
1088 rhodopsin subfamilies, were built from protein multiple sequence alignments of
1089 *Schistosoma mansoni* and *Schmidtea mediterranea* GPCRs [21]. HMMs were built
1090 and searched respectively using the *hmmbuild* and *hmmsearch* modules of HMMER
1091 v3.0. Searches were performed against two publically available *F. hepatica*
1092 genomes using *hmmsearch* and basic local alignment search tool (BLAST) tools.
1093 Each putative FhGPCR sequence was assessed for transmembrane (TM) domain
1094 composition with *hmmtop* before classification using tools including BLASTp,
1095 Interproscan and CLANS. (B) The largest proportion (49%) of FhGPCRs carried the
1096 full complement of 7 TMs, with 88% of sequences bearing at least 4 TMs. (C)
1097 GRAFS composition of 146 FhGPCRs carrying ≥4 TMs. (D) Rhodopsins were
1098 subject to further classification, including BLASTp vs datasets representing major
1099 non-flatworm animal phyla and superphyla. These rhodopsin homology
1100 classifications fed back into phylogenetic analyses versus deorphanised bilaterian
1101 GPCRs to confirm their putative ligand selectivity, with a final analysis of ligand
1102 binding domain composition comparing conservation of ligand interacting residues
1103 for characterised GPCRs reported in the literature with our *F. hepatica* assignments.
1104

1105 **Fig 2: Phylogenetic classification of *Fasciola hepatica* rhodopsin G protein-**
1106 **coupled receptors. (**A) Maximum-likelihood cladogram of *F. hepatica* rhodopsins.
1107 Phylogeny delineated clades containing rhodopsins with distinct homologies (RA,
1108 amine; RP, peptide; RO, opsin: R, orphan rhodopsin). The orphan clades contained
1109 sequences with generally low BLASTp similarity to their closest non-flatworm
1110 BLASTp hit, but concentrated within them were 18 sequences with exceptionally low
1111 (E>0.01) BLASTp similarity to non-flatworm sequences (fwRhods). The tree was

1112 midpoint rooted and was generated from a multiple protein sequence alignment
1113 trimmed to TM domains I-VII. Numbers at nodes indicate statistical support from
1114 approximate likelihood ratio test (aLRT). Tip colours are coded according to the E-
1115 value scale (as indicated) of that GPCR's closest BLASTp match in the ncbi nr
1116 database, excluding phylum Platyhelminthes. (B) Summary of sequence similarity
1117 comparisons between GPCRs within each rhodopsin clade, and their closest
1118 BLASTp hits in major phylogenetic groups (Basal: Cnidaria, Ctenophora, Porifera,
1119 Placozoa; superphylum Lophotrochozoa, omitting Platyhelminthes; Superphylum
1120 Ecdysozoa; superphylum Deuterostomia; phylum Platyhelminthes). BLASTp E-value
1121 (median) is summarised in each case, colour coded as a heat map on the same
1122 colour scale as (A). The number of GPCRs comprising each *F. hepatica* clade (*n*) is
1123 also indicated. (C) Sequence diversity within ubiquitous rhodopsin motifs of the
1124 majority (117) of the *F. hepatica* rhodopsins (upper panel), compared to those motifs
1125 in 18 *F. hepatica* fwRhods (lower panel). The mammalian consensus motifs are
1126 illustrated above the top panel, along with an illustration of the location of each motif
1127 within the rhodopsin 7TM domain structure. Some variability is visible within the TM2
1128 and TM6 motifs, but TM3 and TM7 motifs are well conserved.
1129
1130 **Fig 3: Identification of flatworm-specific rhodopsins (fwRhods) in genomes**
1131 **from phylum Platyhelminthes.** (A) The 18 *Fasciola hepatica* GPCRs in our dataset
1132 that had poor BLASTp similarity (E>0.01) to non-flatworm sequences in the ncbi nr
1133 dataset (lsGPCRs), were used as queries in BLASTp searches of flatworm genomes
1134 in WormBase Parasite (release WBPS9). All hits scoring E<0.01 were back-
1135 searched by BLASTp against our *F. hepatica* GPCR dataset. Sequences scoring
1136 E<0.01 against one of the original *F. hepatica* GPCRs were retained as matches.
1137 These sequences were then filtered to identify those lacking matches in ncbi nr,
1138 lacking non-GPCR protein domains, possessing at least 4 transmembrane (TM)
1139 domains, and containing rhodopsin motifs consistent with those seen in the majority

1140 of *F. hepatica* rhodopsins (see C). (B) This process identified 76 fwRhods in phylum
1141 Platyhelminthes, the majority (70) of which were from class Trematoda. Small
1142 numbers were returned from classes Cestoda and Monogenea. Note that no
1143 fwRhods fitting these criteria were identified in class Turbellaria. (C) Sequence
1144 diversity within ubiquitous rhodopsin motifs of 18 *F. hepatica* fwRhods (upper panel),
1145 compared to those motifs in the 58 fwRhods identified in the wider phylum (lower
1146 panel); motifs are broadly similar between *F. hepatica* and the rest of the phylum.
1147 (D) Maximum likelihood phylogeny of 76 fwRhods, alongside flatworm-specific
1148 rhodopsins described previously (70 platyhelminth rhodopsin orphan family 1
1149 (PROF1) [21,22], and 245 *S. mediterranea* G protein coupled receptor [GCRs,
1150 comprising RhoL, RhoR, Srfa, Srfb and Srfc families, reported as lacking non-
1151 flatworm homologues [24]) with branches coloured to indicate Family (dark blue,
1152 PROF1; mid blue, Srfa; cyan, Rho-L; green, Rho-R; orange, Srfb; purple, Srfc; red,
1153 fwRhod). Tree was rooted to a human rhodopsin (P08100) and was generated from
1154 an alignment trimmed to transmembrane domains I-VII. Numbers at nodes indicate
1155 statistical support from approximate likelihood ratio test (aLRT).
1156
1157 **Fig 4: Conservation of ligand-interacting residues between 17 *Fasciola***
1158 ***hepatica* G protein-coupled receptors (GPCRs) and structurally characterized**
1159 **homologues from other species.** (A) Neuropeptide F/Y receptor ligand binding
1160 residues as characterised by mutagenesis in human neuropeptide Y receptor NPY1R
1161 [39-43], and conserved in *Anopheles gambiae* (Ag) and *Drosophila melanogaster*
1162 (Dm) neuropeptide F receptors (NPFR) [44]. Numbering relative to HsNPY1R. (B)
1163 Serotonin (5-hydroxytryptamine; 5HT) receptor ligand binding residues as
1164 characterised by mutagenesis in human 5HT receptor (Hs5HT1A) [102], and
1165 conserved in *Schistosoma mansoni* 5HTR [51]. Numbering relative to Hs5HT1A.
1166 (C) Octopamine receptor (OaR) ligand binding residues as characterised by
1167 homology modelling of the *Periplaneta americana* (Pa) [58], and mutational analysis

1168 of the *Bombyx mori* (Bm) [59] octopamine receptor ligand binding domain.
1169 Numbering relative to PaOAR, except for Y412 which is shown relative to BmOAR.
1170 (D) Acetylcholine receptor ligand binding residues as characterised by homology
1171 modelling of the *S. mansoni* G protein-coupled acetylcholine receptor (SmGAR) [62];
1172 numbering relative to SmGAR. In each case, only *F. hepatica* sequences displaying
1173 at least 75% identity across the stated ligand binding residues are shown. Relative
1174 positions of residues across seven transmembrane domains (TM1-7) are shown. TM
1175 diagrams are not to scale.
1176
1177 **Fig 5: *Fasciola hepatica* glutamate G-protein coupled receptors (GPCRs)**
1178 **display divergent phylogeny and ligand binding domain (LBD) composition.**
1179 (A) Maximum likelihood phylogeny containing three *F. hepatica* glutamate receptors,
1180 alongside representative receptors from the various recognised GPCR Class C
1181 subgroups (subclasses indicated by blue boxes: Ca^{2+}, Ca^{2+}-sensing receptor;
1182 $GABA_B$, γ-aminobutyric acid type B receptors; mGluR, metabotropic glutamate
1183 receptors; Orphan, receptors with no known ligand; Taste, vertebrate taste
1184 receptors). Two previously reported *Schistosoma mansoni* glutamate receptors are
1185 also included; *F. hepatica* sequences are coloured red, *S. mansoni* are coloured
1186 blue, all others are black. Node numbers indicate statistical support as determined
1187 by approximate likelihood ratio test (aLRT). Tree was midpoint rooted. (B)
1188 Conservation of ligand-interacting residues between vertebrate $GABA_B$ and
1189 metabotropic glutamate receptors (mGluR), and *F. hepatica* class C GPCRs.
1190 Agonist-interacting residues were identified by multiple protein sequence alignment
1191 of *F. hepatica* glutamate receptors against mutationally-identified ligand interacting
1192 residues (those causing a significant reduction in receptor signalling activity), from
1193 mouse $GABA_B$ receptor (top panel), or selected human mGluR subtypes (lower
1194 panel). Identical amino acids in *F. hepatica*/*S. mansoni* GPCRs are represented by
1195 white text on black background, functionally conserved amino acids by black text on

1196 grey background. In lower panel, mutations causing a significant reduction in mGluR
1197 receptor activity are bold and numbered, with the region of the glutamate molecule
1198 bound by each residue indicated (COOH, C-terminus; NH_2, N-terminus). For
1199 references see [63,103,104].
1200
1201 **Fig 6: Frizzled/smoothened seven transmembrane receptors and wnt ligands in**
1202 ***Fasciola hepatica.*** (A) Maximum likelihood phylogeny containing six *F. hepatica*
1203 frizzled/smoothened receptors, alongside those from *Schistosoma mansoni,*
1204 *Drosophila melanogaster, Caenorhabditis elegans* and *Homo sapiens* (identified by
1205 FSMP, d, c and h, respectively). *F. hepatica* sequences are coloured red, *S.*
1206 *mansoni* are coloured blue, all other species are coloured black. Radial labels
1207 indicate human frizzled clusters (hClust) I-IV, and the smoothened clade. Node
1208 numbers indicate statistical support as determined by approximate likelihood ratio
1209 test (aLRT). Tree was rooted against a *Dictyostelium* frizzled sequence (dicty-fslJ-1).
1210 Tree composition adapted from [21]. (B) WebLogo comparison of ligand interacting
1211 residues between mouse fz1-10 (top panel) and *F. hepatica* frizzled receptors.
1212 Numbering in top panel x-axis is relative to mouse fz8 [69]. (C) Three wnt-like
1213 sequences exist in *F. hepatica.* Shading indicates positions of 22 characteristic Cys
1214 residues, positions numbered relative to *D. melanogaster* wnt-1 (Dro-wnt-1).
1215
1216 **Fig 7: Expression profiling of 101 G protein-coupled receptors (GPCRs) in**
1217 ***Fasciola hepatica* life stages.** (A) Expression heatmap generated from log_2 FPKM
1218 values of 83 GPCRs identified from developmentally staged RNA-seq libraries. Life
1219 stages are represented in columns (Egg; Met, metacercariae; NEJ_1h, newly-
1220 excysted juvenile collected 1h post excystment; NEJ_3h, NEJ collected 3h post-
1221 excystment; NEJ_24h, NEJ collected 24h post-excystment; Juv_21d, liver stage
1222 juvenile parasites collected from murine livers 21 days following oral administration of
1223 metacercariae; Adult, adult parasites collected from the bile ducts of bovine livers).

1224 Rows indicate individual GPCRs, as denoted by the ID and phylogeny columns. The
1225 latter indicates receptor classification and predicted ligand where available (see S1
1226 Table). Expression cluster column indicates clusters of GPCRs with highest
1227 expression focused in particular life stages. (B) Detection of 76 GPCRs in Illumina
1228 RNA-Seq libraries generated from *F. hepatica* 21 day liver-stage juveniles, recovered
1229 *ex vivo* from rat infections. Data show expression of three glutamate (G), one
1230 adhesion (A), four frizzled (F), one smoothened (S) and 67 rhodopsin (R) GPCRs.
1231 The rhodopsins include representatives of amine (RA1, RA3), opsin (RO), peptide
1232 (RP1-7), and orphan (R2,3,4,6). Data points (each at *n*=3) represent mean $\log_2$
1233 FPKM ± 95% confidence intervals, as calculated by cuffdiff. In both panels, flatworm
1234 rhodopsins (fwRhods) are marked in red text. ACh, acetylcholine; AstA. Allatostatin
1235 A; Dop, dopamine; FMRFa, FMRFamide; GHS, growth hormone secretagogue;
1236 GYIRFa, GYIRFamide; Myom, myomodulin; Myos, myosuppressin; NPF/Y,
1237 neuropeptide F/Y; Oct, octopamine; Pkt, prokineticin; Tyr, tyramine; 5HT, 5-
1238 hydroxytryptamine.
1239

1240 **Supporting Information Captions**

1241 **S1 Table. *Fasciola hepatica* G protein coupled receptor (GPCR) dataset**
1242 **summary.** Table describes sequences containing 4-9 transmembrane (TM)
1243 domains. Each sequence is defined in terms of GRAFS family, and annotated for
1244 TM composition, sequence length, phylogeny, domain composition and homology
1245 relative to various datasets.

1246

1247 **S2 Text. *Fasciola hepatica* G protein-coupled receptor (GPCR) protein**
1248 **sequence dataset.**

1249

1250 **S3 Table. Flatworm-specific rhodopsins (fwRhods) in *Fasciola hepatica* and**
1251 **other flatworms.** Species and genome IDs of sequences that have ≥4
1252 transmembrane (TM) domains, and lack high-scoring orthologues in non-flatworms
1253 (BLASTp score E≥0.01 vs ncbi nr excluding Platyhelminthes), and show
1254 conservation of at least two of the four ubiquitous rhodopsin motifs. Each sequence
1255 is annotated for protein domains where present (Pfam HMMScan). Accessions refer
1256 to WormBase ParaSite.

1257

1258 **S4 Table. Rhodopsin ubiquitous motifs and ligand binding domains for ACh,**
1259 **NPF/Y 5-HT, octopamine.** Note data are in individual tabs. Rhodopsin: Sequence
1260 motifs extracted from alignment of *F. hepatica* rhodopsins, corresponding to
1261 ubiquitous rhodopsin motifs of TMs 2, 3, 6 and 7; Acetylcholine, NPF, 5-HT,
1262 Octopamine: Amino acids extracted from alignment of *F. hepatica* rhodopsins with
1263 mutationally or structurally-characterised GPCRs (comparators). Summary: Percent
1264 identity of ACh, NPF, 5-HT and Oct receptor LBDs, indicating most conserved LBD
1265 sequences showing at least 75% identity.

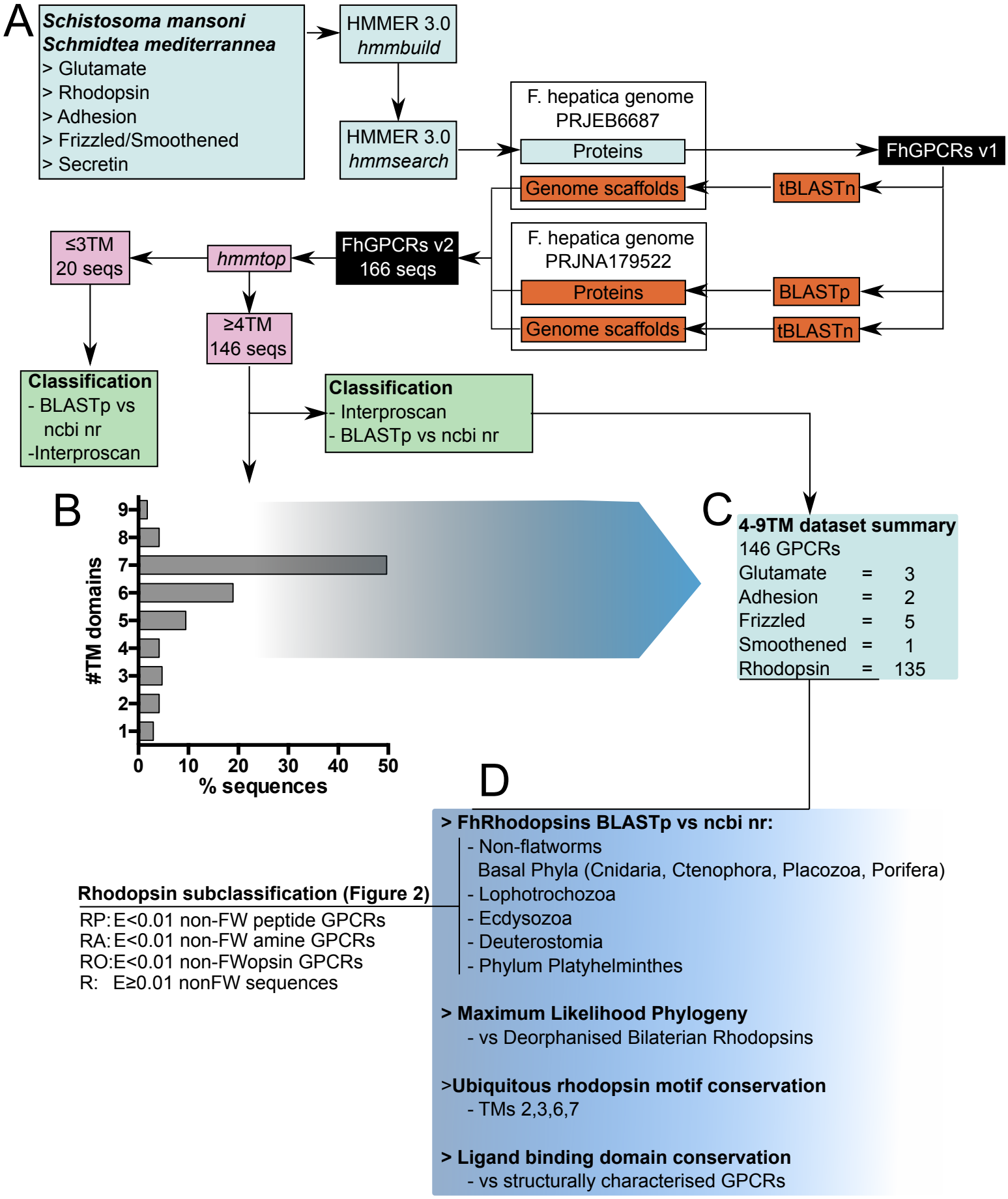
1266

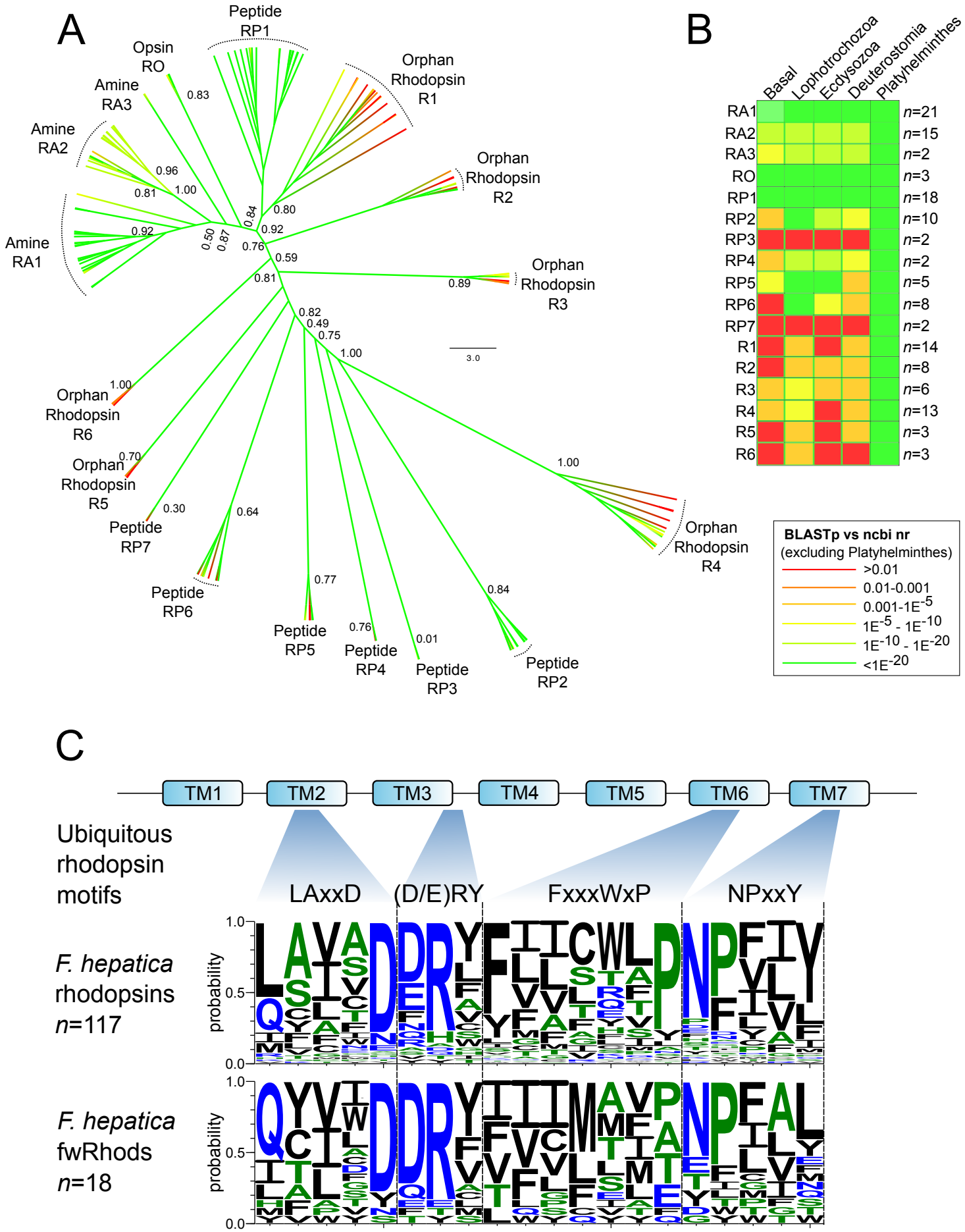
1267 **S5 Fig. Phylogenetic comparison of *Fasciola hepatica* GPCRs with**
1268 **deorphanised bilaterian GPCRs.** (A) Peptide receptors (*F. hepatica* black or
1269 magenta as described in Fig x); (B) Amine receptors (*F. hepatica* dark blue); In all
1270 cases, non-flatworm receptors are coloured light blue. In (A) outer labels indicate
1271 positions of receptors for neuropeptide families previously reported in flatworms
1272 (McVeigh et al., 2009; Collins et al., 2010; Koziol et al., 2016); in (B), outer labels
1273 represent major groups containing phylogenetically similar *F. hepatica* sequences.
1274 Trees were midpoint rooted, maximum likelihood phylogenies of transmembrane
1275 domains I-VII. Numbers at nodes indicate statistical support from approximate
1276 likelihood ratio test (aLRT). Scale bars at the centre of each tree indicate number of
1277 substitutions per site. Abbreviations: ACh, acetylcholine; CCAP, crustacean
1278 cardioactive peptide; Dop, dopamine; FLP, FMRFamide-like peptide; GrH,
1279 gonadotropin-releasing hormone; Luq, luqin; Mmd, myomodulin; NKY, neuropeptide
1280 KY; NPF/Y, neuropeptide F/Y; Oct, octopamine; PK, pyrokinin; SIFa, SIFamide; Tyr,
1281 tyramine; SmGPR, schistosome GPCRs; 5HT, 5-hydroxytryptamine.
1282
1283 **S6 Table. Frizzled receptor ligand binding domain motifs.** Amino acids
1284 extracted from alignment of *F. hepatica* frizzled and smoothened GPCRs with
1285 mutationally characterised mouse fz8, also showing positionally-conserved residues
1286 in mouse fz1-10 (top panel) and *F. hepatica* frizzled receptors. Numbering in top row
1287 relative to mouse fz8 (Janda et al., 2012). Green boxes indicate identical residues in
1288 *F. hepatica* vs mammalian Fzd.
1289
1290 **S7 Figure: Adhesion receptor phylogeny.** Maximum likelihood phylogeny of
1291 *Fasciola hepatica* adhesion/secretin-like GPCRs alongside class B GPCRs from
1292 human and *Schistosoma mansoni*. Tree was a midpoint rooted, maximum likelihood
1293 phylogeny of transmembrane domains I-VII. Numbers at nodes indicate statistical

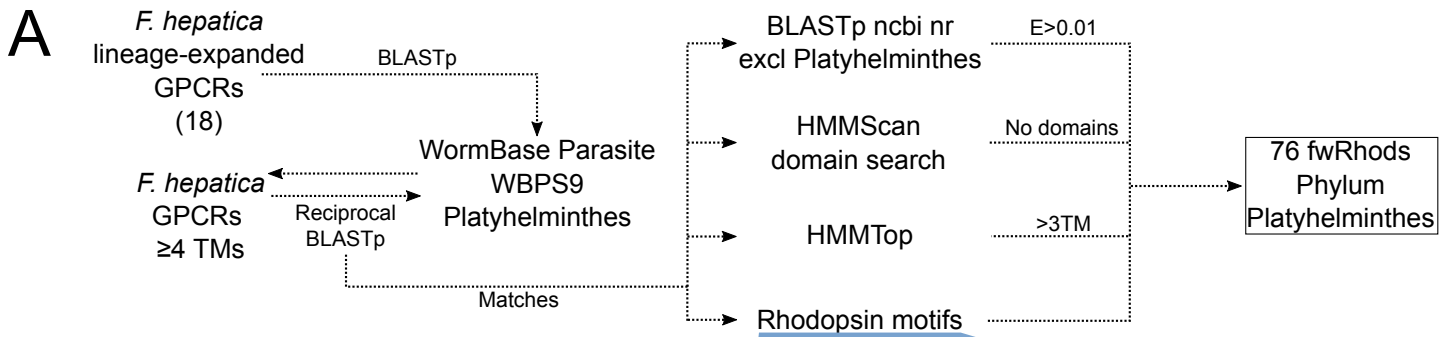
1294 support from approximate likelihood ratio test (aLRT). Scale bars at the centre of

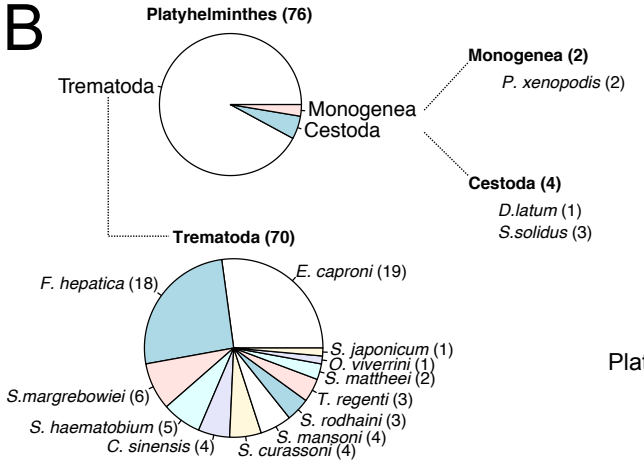
1295 each tree indicate number of substitutions per site.

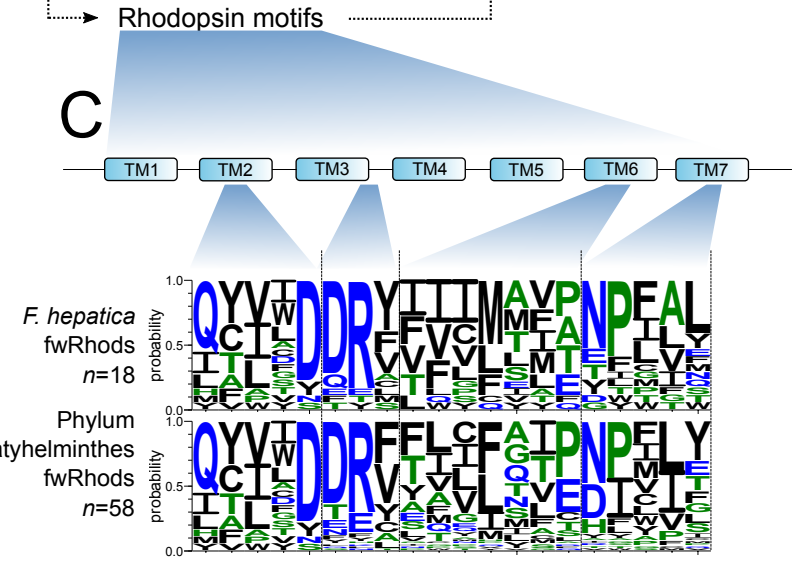
1296

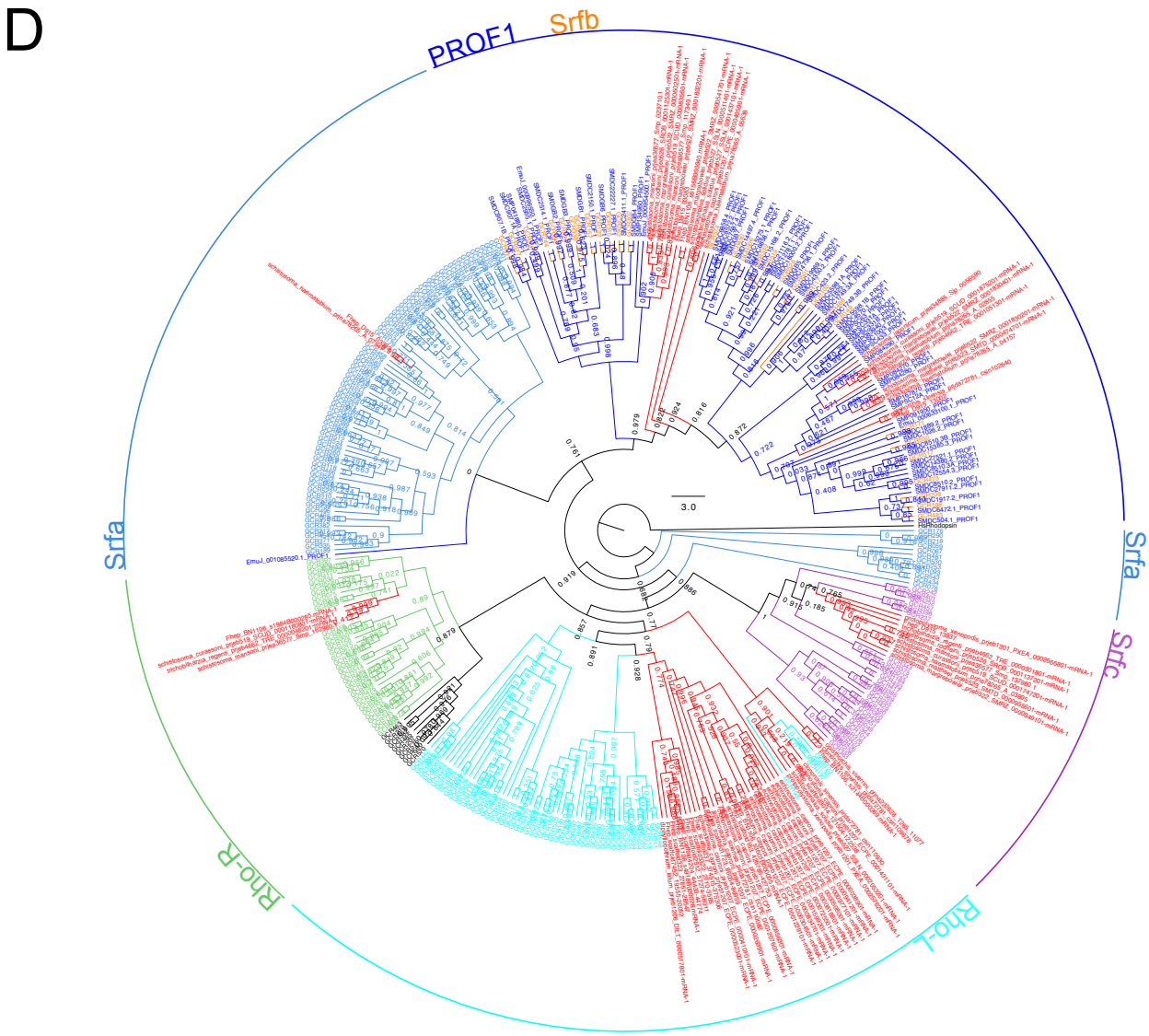

A
Schistosoma mansoni
Schmidtea mediterrannea
> Glutamate
> Rhodopsin
> Adhesion
> Frizzled/Smoothened
> Secretin
HMMER 3.0
hmmbuild
HMMER 3.0
hmmsearch
F. hepatica genome
PRJEB6687
Proteins
Genome scaffolds
FhGPCRs v1
tBLASTn
F. hepatica genome
PRJNA179522
Proteins
Genome scaffolds
BLASTp
tBLASTn
FhGPCRs v2
166 seqs
hmmtop
≤3TM
20 seqs
≥4TM
146 seqs
Classification
- BLASTp vs
ncbi nr
-Interproscan
Classification
- Interproscan
- BLASTp vs ncbi nr
B
#TM domains
% sequences
C
4-9TM dataset summary
146 GPCRs
Glutamate = 3
Adhesion = 2
Frizzled = 5
Smoothened = 1
Rhodopsin = 135
D
> FhRhodopsins BLASTp vs ncbi nr:
- Non-flatworms
Basal Phyla (Cnidaria, Ctenophora, Placozoa, Porifera)
- Lophotrochozoa
- Ecdysozoa
- Deuterostomia
- Phylum Platyhelminthes
> Maximum Likelihood Phylogeny
- vs Deorphanised Bilaterian Rhodopsins
>Ubiquitous rhodopsin motif conservation
- TMs 2,3,6,7
> Ligand binding domain conservation
- vs structurally characterised GPCRs
Rhodopsin subclassification (Figure 2)
RP: E<0.01 non-FW peptide GPCRs
RA: E<0.01 non-FW amine GPCRs
RO: E<0.01 non-FWopsin GPCRs
R: E≥0.01 nonFW sequences

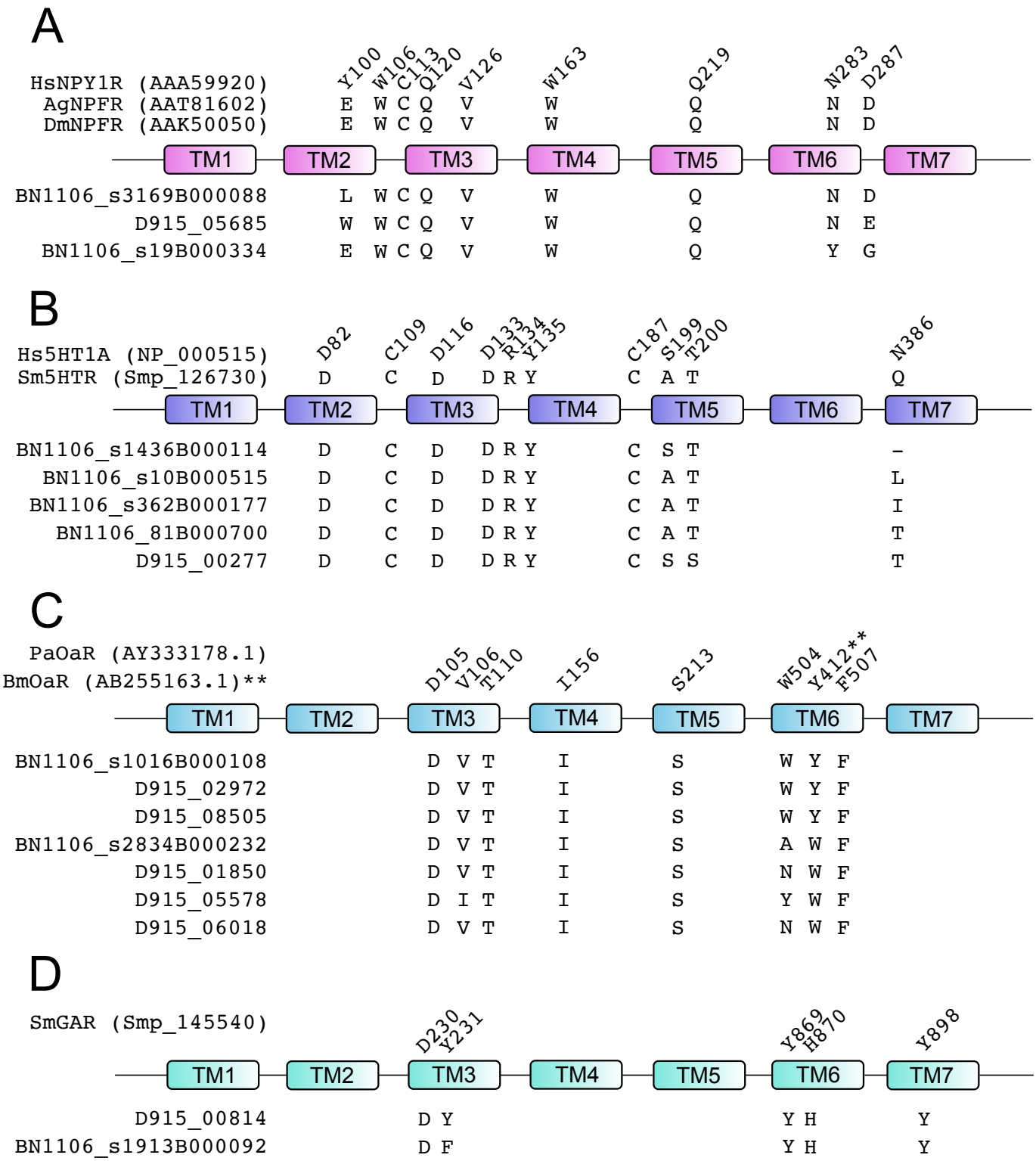

A
Peptide RP1
Opsin RO
Amine RA3
Amine RA2
Amine RA1
Orphan Rhodopsin R1
Orphan Rhodopsin R2
Orphan Rhodopsin R3
Orphan Rhodopsin R4
Orphan Rhodopsin R5
Orphan Rhodopsin R6
Peptide RP2
Peptide RP3
Peptide RP4
Peptide RP5
Peptide RP6
Peptide RP7
3.0
B
Basal
Lophotrochozoa
Ecdysozoa
Deuterostomia
Platyhelminthes
RA1 n=21
RA2 n=15
RA3 n=2
RO n=3
RP1 n=18
RP2 n=10
RP3 n=2
RP4 n=2
RP5 n=5
RP6 n=8
RP7 n=2
R1 n=14
R2 n=8
R3 n=6
R4 n=13
R5 n=3
R6 n=3
BLASTp vs ncbi nr
(excluding Platyhelminthes)
>0.01
0.01-0.001
0.001-1E-5
1E-5 - 1E-10
1E-10 - 1E-20
<1E-20
C
TM1
TM2
TM3
TM4
TM5
TM6
TM7
Ubiquitous rhodopsin motifs
LAxxD
(D/E)RY
FxxxWxP
NPxxY
F. hepatica rhodopsins n=117
F. hepatica fwRhods n=18
probability
1.0
0.5
0.0


A
F. hepatica
lineage-expanded
GPCRs
(18)
BLASTp
WormBase Parasite
WBPS9
Platyhelminthes
F. hepatica
GPCRs
≥4 TMs
Reciprocal
BLASTp
Matches
BLASTp ncbi nr
excl Platyhelminthes
E>0.01
HMMScan
domain search
No domains
HMMTop
>3TM
Rhodopsin motifs
76 fwRhods
Phylum
Platyhelminthes


B
Platyhelminthes (76)
Trematoda
Monogenea
Cestoda
Monogenea (2)
P. xenopodis (2)
Cestoda (4)
D.latum (1)
S.solidus (3)
Trematoda (70)
F. hepatica (18)
E. caproni (19)
S. japonicum (1)
O. viverrini (1)
S. mattheei (2)
T. regenti (3)
S. rodhaini (3)
S. mansoni (4)
S. curassoni (4)
C. sinensis (4)
S. haematobium (5)
S.margrebowiei (6)


C
TM1
TM2
TM3
TM4
TM5
TM6
TM7
F. hepatica
fwRhods
n=18
Phylum
Platyhelminthes
fwRhods
n=58
probability
1.0
0.5
0.0


D
PROF1
Srfb
Srfa
Srfc
Rho-L
Rho-R
3.0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Y100 W106 C113 Q120 V126 W163 Q219 N283 D287
HsNPY1R (AAA59920) E W C Q V W Q N D
AgNPFR (AAT81602) E W C Q V W Q N D
DmNPFR (AAK50050) E W C Q V W Q N D
TM1 TM2 TM3 TM4 TM5 TM6 TM7
BN1106_s3169B000088 L W C Q V W Q N D
D915_05685 W W C Q V W Q N E
BN1106_s19B000334 E W C Q V W Q Y G
B
D82 C109 D116 D133 R134 Y135 C187 S199 T200 N386
Hs5HT1A (NP_000515) D C D D R Y C A T Q
Sm5HTR (Smp_126730)
TM1 TM2 TM3 TM4 TM5 TM6 TM7
BN1106_s1436B000114 D C D D R Y C S T -
BN1106_s10B000515 D C D D R Y C A T L
BN1106_s362B000177 D C D D R Y C A T I
BN1106_81B000700 D C D D R Y C A T T
D915_00277 D C D D R Y C S S T
C
PaOaR (AY333178.1)
BmOaR (AB255163.1)**
D105 V106 T110 I156 S213 W504 Y412** F507
TM1 TM2 TM3 TM4 TM5 TM6 TM7
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D915_08505 D V T I S W Y F
BN1106_s2834B000232 D V T I S A W F
D915_01850 D V T I S N W F
D915_05578 D I T I S Y W F
D915_06018 D V T I S N W F
D
SmGAR (Smp_145540)
D230 Y231 Y869 H870 Y898
TM1 TM2 TM3 TM4 TM5 TM6 TM7
D915_00814 D Y Y H Y
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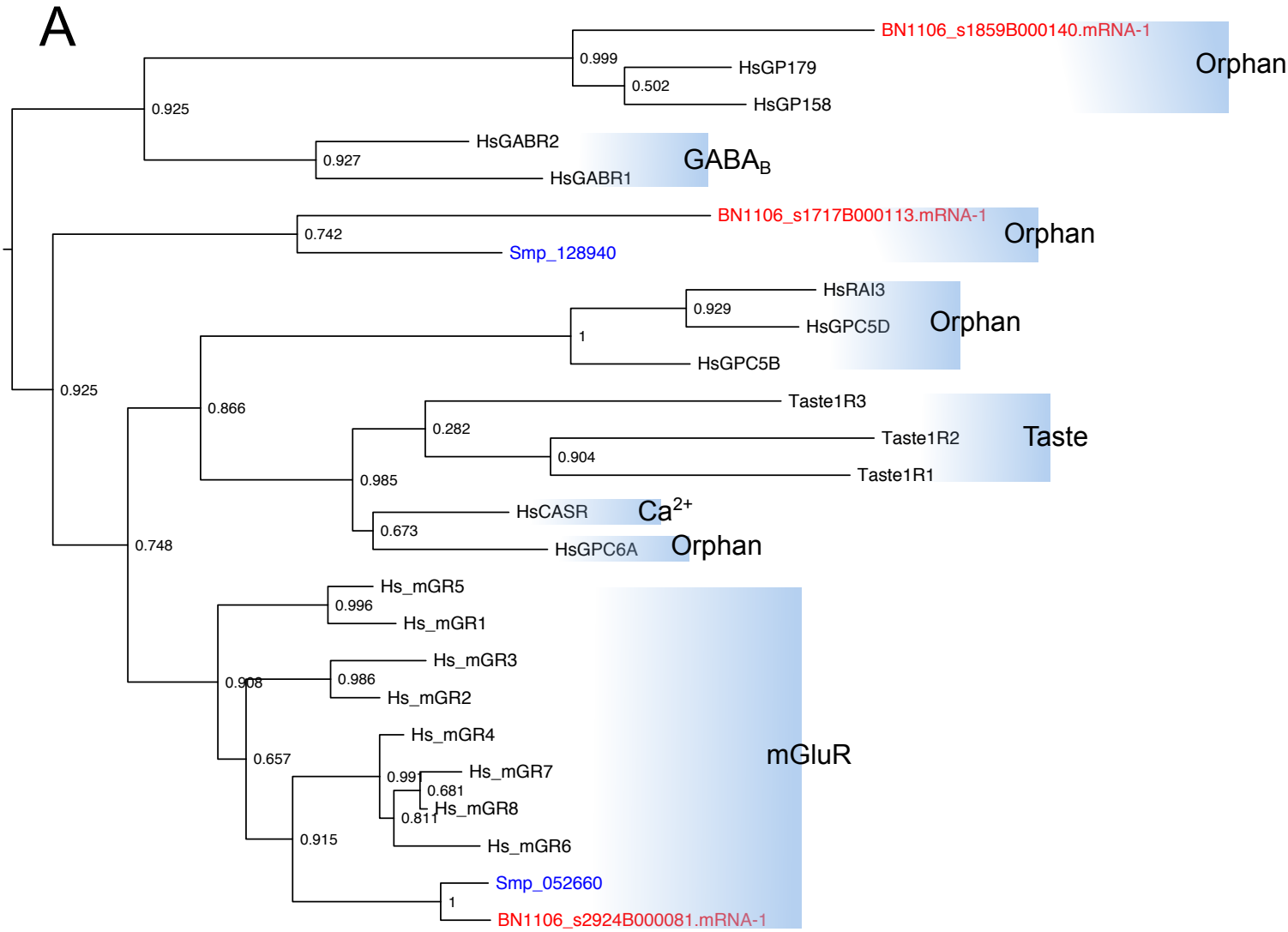
A

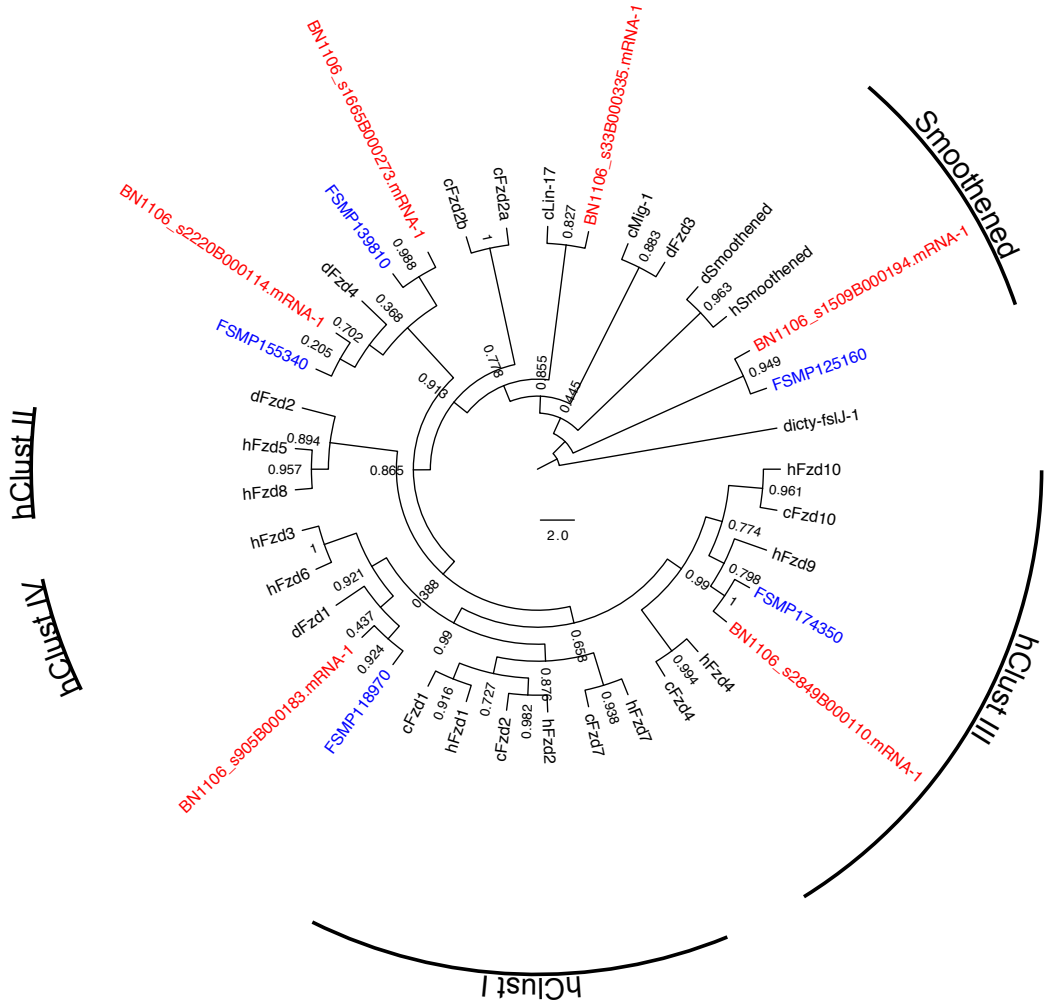


B

MmGABA$_{B1}$	S246	S269	S270	E465	D471
BN1106_s2924B000081	–	–	–	–	–
BN1106_s1717B000113	S	D	A	D	N
BN1106_s1859B000140	F	L	N	C	Y
Smp_128940	S	A	S	D	N
Smp_052660	S	T	T	E	D

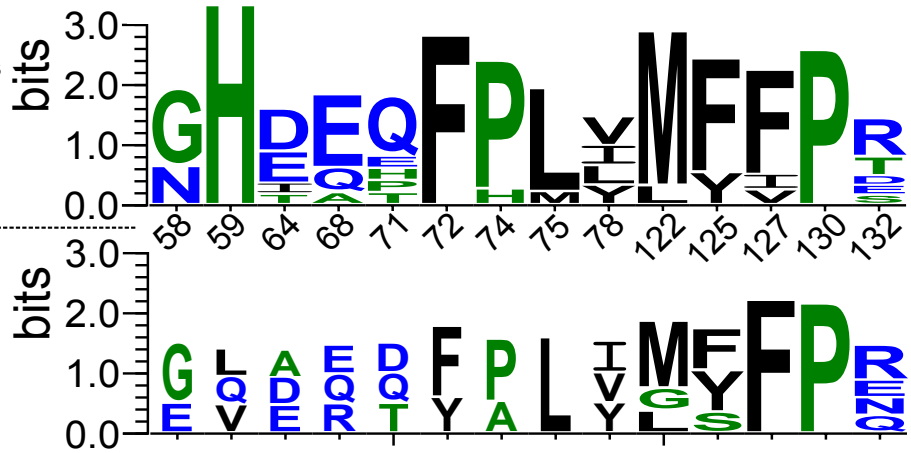
Glu binding region:		COOH		αNH$_2$	αNH$_2$	αNH$_2$	αNH$_2$			αNH$_2$		COOH
mGluR1	Y74	R78	S	S165	T188	D208	Y236	E292	G293	D318	D	K409
mGluR2	R57	R	Y	S145	T168	D188	Y216	R	S	D295	R	K377
mGluR3	R64	R68	Y150	S151	T174	D194	Y222	R277	S	D301	Q306	K
mGluR4	K74	R78	G	S159	T182	D	Y	N	E	D	K	K
mGluR7	N	R	G	S	T	D	Y	N	D	D	K	K
mGluR8	K71	R75	A	S	T	D	Y	N	E	D309	K	K
BN1106_s2924B000081	–	–	–	–	–	–	–	–	–	–	–	–
BN1106_s1717B000113	I	F	I	S	D	A	Y	S	S	N	D	A
BN1106_s1859B000140	–	L	–	–	–	–	A	D	V	N	–	P
Smp_128940	N	L	Q	S	A	I	H	T	S	N	T	S
Smp_052660	R	R	Y	S	T	D	Y	G	M	D	E	K

A



B

Ligand-contacting amino acids
M. musculus fz1-10,
numbering relative to fz8



Aligned positions
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BN1106_s1665B000273
BN1106_s2894B000110
BN1106s33B000335
BN1106_s905B000183

C

Amino acid position relative to Dro-wnt-1

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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                BN1106_s737B000430 --- --- ACS CDC GCS CKC ACS TCW YCR LCN CNRLCC CDC CCEVRCKVC RCK
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                BN1106_s198B000330 --- --- ACS CGC GCS CKC SCE TCW FCH RCV CEYLCC CNC CCKVICQTC TCN
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                BN1106_s1256B000163 ECQ NCT SCK CGC GCG CRP L-- --- YCQ ECL CVHLCC CRC CCRVECQTC VCN
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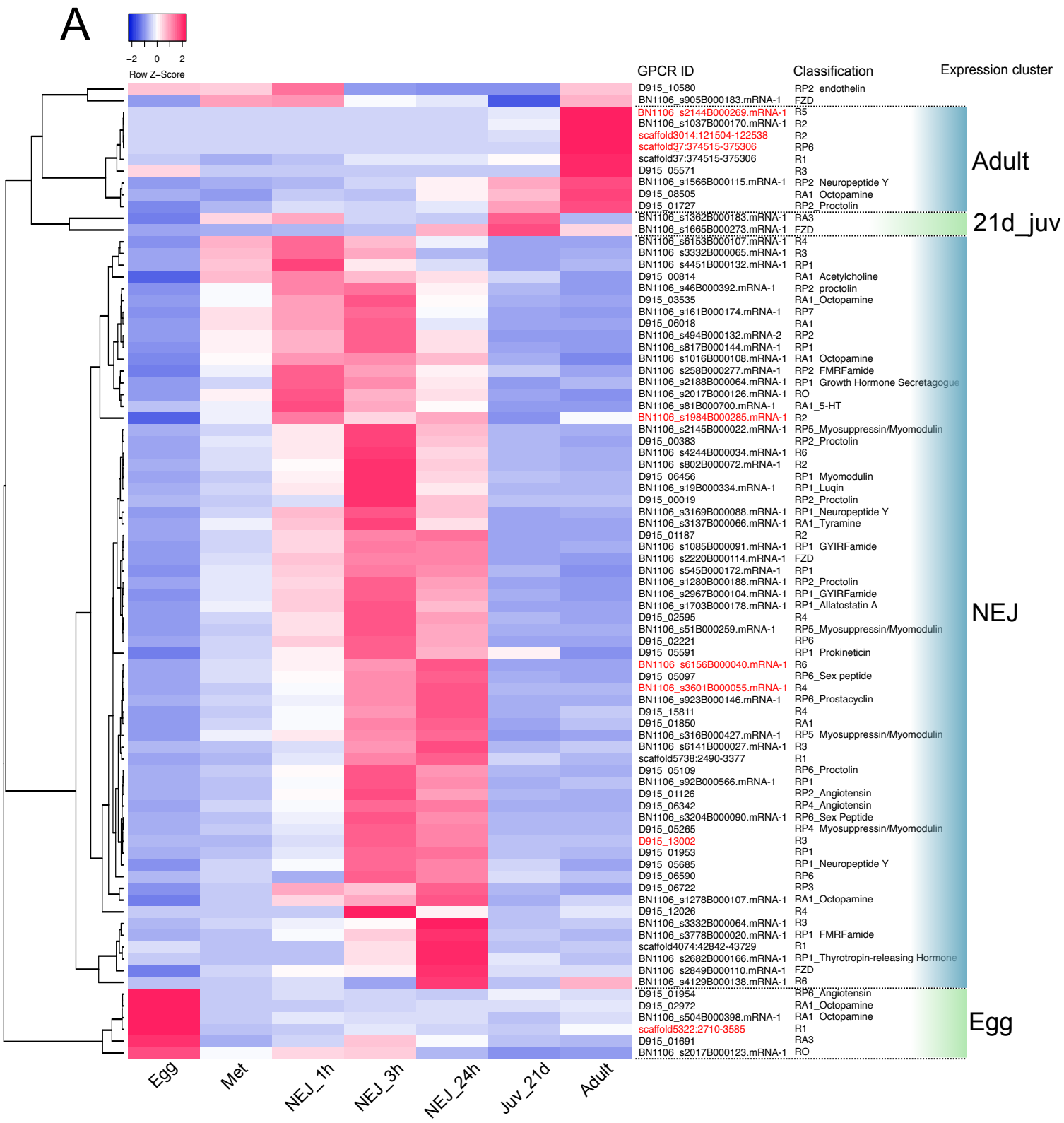
A
−2 0 2
Row Z−Score
GPCR ID
Classification
Expression cluster
D915_10580 RP2_endothelin
BN1106_s905B000183.mRNA-1 FZD
BN1106_s2144B000269.mRNA-1 R5
BN1106_s1037B000170.mRNA-1 R2
scaffold3014:121504-122538 R2
scaffold37:374515-375306 RP6
scaffold37:374515-375306 R1
D915_05571 R3
BN1106_s1566B000115.mRNA-1 RP2_Neuropeptide Y
D915_08505 RA1_Octopamine
D915_01727 RP2_Proctolin
BN1106_s1362B000183.mRNA-1 RA3
BN1106_s1665B000273.mRNA-1 FZD
BN1106_s6153B000107.mRNA-1 R4
BN1106_s3332B000065.mRNA-1 R3
BN1106_s4451B000132.mRNA-1 RP1
D915_00814 RA1_Acetylcholine
BN1106_s46B000392.mRNA-1 RP2_proctolin
D915_03535 RA1_Octopamine
BN1106_s161B000174.mRNA-1 RP7
D915_06018 RA1
BN1106_s494B000132.mRNA-2 RP2
BN1106_s817B000144.mRNA-1 RP1
BN1106_s1016B000108.mRNA-1 RA1_Octopamine
BN1106_s258B000277.mRNA-1 RP2_FMRFamide
BN1106_s2188B000064.mRNA-1 RP1_Growth Hormone Secretagogue
BN1106_s2017B000126.mRNA-1 RO
BN1106_s81B000700.mRNA-1 RA1_5-HT
BN1106_s1984B000285.mRNA-1 R2
BN1106_s2145B000022.mRNA-1 RP5_Myosuppressin/Myomodulin
D915_00383 RP2_Proctolin
BN1106_s4244B000034.mRNA-1 R6
BN1106_s802B000072.mRNA-1 R2
D915_06456 RP1_Myomodulin
BN1106_s19B000334.mRNA-1 RP1_Luqin
D915_00019 RP2_Proctolin
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BN1106_s3137B000066.mRNA-1 RA1_Tyramine
D915_01187 R2
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BN1106_s1280B000188.mRNA-1 RP2_Proctolin
BN1106_s2967B000104.mRNA-1 RP1_GYIRFamide
BN1106_s1703B000178.mRNA-1 RP1_Allatostatin A
D915_02595 R4
BN1106_s51B000259.mRNA-1 RP5_Myosuppressin/Myomodulin
D915_02221 RP6
D915_05591 RP1_Prokineticin
BN1106_s6156B000040.mRNA-1 R6
D915_05097 RP6_Sex peptide
BN1106_s3601B000055.mRNA-1 R4
BN1106_s923B000146.mRNA-1 RP6_Prostacyclin
D915_15811 R4
D915_01850 RA1
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BN1106_s6141B000027.mRNA-1 R3
scaffold5738:2490-3377 R1
D915_05109 RP6_Proctolin
BN1106_s92B000566.mRNA-1 RP1
D915_01126 RP2_Angiotensin
D915_06342 RP4_Angiotensin
BN1106_s3204B000090.mRNA-1 RP6_Sex Peptide
D915_05265 RP4_Myosuppressin/Myomodulin
D915_13002 R3
D915_01953 RP1
D915_05685 RP1_Neuropeptide Y
D915_06590 RP6
D915_06722 RP3
BN1106_s1278B000107.mRNA-1 RA1_Octopamine
D915_12026 R4
BN1106_s3332B000064.mRNA-1 R3
BN1106_s3778B000020.mRNA-1 RP1_FMRFamide
scaffold4074:42842-43729 R1
BN1106_s2682B000166.mRNA-1 RP1_Thyrotropin-releasing Hormone
BN1106_s2849B000110.mRNA-1 FZD
BN1106_s4129B000138.mRNA-1 R6
D915_01954 RP6_Angiotensin
D915_02972 RA1_Octopamine
BN1106_s504B000398.mRNA-1 RA1_Octopamine
scaffold5322:2710-3585 R1
D915_01691 RA3
BN1106_s2017B000123.mRNA-1 RO
Adult
21d_juv
NEJ
Egg
Egg
Met
NEJ_1h
NEJ_3h
NEJ_24h
Juv_21d
Adult
B
Log2(FPKM)
2
1
0
-1
ACh
Dop
Oct
5HT
Tyr
FMRFa
Luqin
GYIRFa
NPF/Y
Myomodulin
Ast A
GHS
Pkt
Angiotensin
Endothelin
FMRFa
NPF/Y
Proctolin
FMRFa
Myom/Myos
Proctolin
Sex peptide
Prostacyclin
BN1106_s1984B000285_fwRhod
BN1106_s6156B000040_fwRhod
G A F S
RA1
RA3
RO
RP1
RP2
RP3
RP4
RP5
RP6
RP7
R2
R3
R4
R6
R
GPCR families