Concordant developmental expression profiles of orthologues in highly divergent Bilateria

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Abstract

Bilateria are the predominant clade of animals on earth. Despite having evolved a large variety of body-plans and developmental modes, they are characterized by common morphological traits. However, it is not clear if clade-specific genes can be linked to these traits, distinguishing bilaterians from non-bilaterians, with their less complex body morphology. Comparing proteomes of bilaterian and non-bilaterian species in an elaborate computational pipeline we aimed to find and define a set of bilaterian-specific genes. Finding no high-confidence set of such genes, we nevertheless detected an evolutionary signal possibly uniting the highly diverse bilaterian taxa. Using a novel multi-species GO-enrichment method, we determined the functional repertoire of genes that are widely conserved among Bilateria. We found that these genes contribute to morphogenesis, neuronal-system and muscle development, processes that have been described as different between bilaterians and non-bilaterians. Analyzing gene expression profiles in three very distantly related bilaterian species, we find characteristic peaks at comparable stages of development and a delayed onset of expression in embryos. In particular, the expression of the conserved genes appears to peak at the phylotypic stage of different bilaterian phyla. In summary, our data underpin

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the orthologue conjecture and illustrate how development connects distantly related Bilateria after millions of years of divergence, pointing to processes potentially separating them from non-bilaterians.

Keywords: Evolution, Bilateria, Gene Ontology, Expression profile, Development

1. Introduction

Bilateria represent by far the largest monophyletic group in the animal kingdom. They comprise about 99% of the extant eumetazoans [1] and are classified into 32 phyla [2]. The taxon ‘Bilateria’ has been defined on the basis of morphological key innovations, namely bilateral symmetry, triploblasty, an enhanced nervous system and a complex set of cell types [3]. Probably the most striking observation is the existence of a large variety of body plans, accompanied by high morphological diversity in many bilaterian phyla [4].

It is thought that the major bilaterian phyla emerged in a fast radiation during the early Cambrian, about 540 million years ago [1], but the appearance of the common bilaterian ancestor, and its exact timing, is controversial [5, 6, 7, 8]. It is also difficult to define unifying morphological properties for larval or adult Bilateria, as their descendants underwent extensive re-modellings (including secondary reductions and simplifications) of body forms in the lineages leading to extant crown clades. Nevertheless, the general process of development from single-cell to adult is conserved among all animals. Recently, evidence for the hypothesis that the developmental transcriptome might be a conserved trait across diverse groups of animals has been found [9, 10]. In particular, several studies described a conserved phylotypic period mid development based on transcriptomic analyses [11, 12, 13], akin to the morphological hourglass model of developmental progression [14, 15], itself an extension of von Baers reverse funnel model of development in animals [16].

Despite these similarities in the global transcriptome, comparisons at the molecular level reveal that many developmentally important genes are older than the ancestor of Bilateria. Among them are determinants of dorso-ventral and anterior-posterior patterning, eye formation, segmentation, and heart development [5, 17, 18, 19, 20]. In a recent study, it was estimated that up to 85% of the genes present in any Bilaterian examined were already present in the ur-bilaterian [21], and only about 15% are of more recent

origin. Deep comparative genomics including non-bilaterian Metazoans, also revealed that the major developmental signaling pathways are already present in Cnidarians, Ctenophores, Placozoa and sponges [22, 23, 24], and must predate the origin of Bilateria. At the same time, it was shown that genes specific to large clades with divergent sub-taxa, like the deuterostomes, might exist [25]. We hypothesised that similar to the conserved developmental transcriptome and the gene novelties described to define the deuterostomes [25] there might be genes defining the Bilateria as a clade, setting them apart from their non-bilaterian ancestors.

Using reciprocal blast searches, orthology pipelines, and an array of filtering steps we identified a set of orthologues proteins that satisfy our initial criteria for Bilateria specificity. However, these proteins could not be determined to be actually clade-specific for Bilateria with highest-confidence after secondary validation. In fact, re-analysing an initial set of 85 orthogroups with new data from the ever growing database of available proteins we found only 35 orthogroups to remain “Bilateria-specific”. It must be assumed that this set would shrink even further with more data becoming available, in particular from more non-bilaterians.

Despite our limited confidence in the ability of these approaches to detect clade-specific genes in Bilateria, we observed an interesting signal in our data. Analysing the set of 85 orthogroups in more detail we found that these genes are not only retained in species separated by a billion years of independent lineage evolution, but that most of them act in key developmental processes. These genes show very similar and peculiar expression profiles across analogous developmental stages in highly derived model species in the fishes, the arthropods, and the nematodes. This finding supports the idea that evolutionary changes in the genetic machinery of development were coupled to the emergence of Bilateria and are still conserved in very divergent taxa today. Additionally, by finding similar developmental signals across the expression profiles for the most conserved orthologues in our orthogroups, our work also lends new support to the ortholog conjecture.

2. Results

2.1. Defining potential clade-specific proteins in Bilateria

The aim of our study was to identify genes that newly emerged in the ‘ur-bilaterian’ (Fig 1a) and have been retained in its descendant species.

To this end, our initial study was grounded in a comparison of ten bilaterian and seven non-bilaterian species with fully sequenced and annotated genomes as of 2011. We downloaded 383,586 protein sequences in total. About 70% (268,252) are from Bilateria, covering the three major clades Lophotrochozoa, Ecdysozoa and Deuterostomia, and about 30% (115,334) are non-bilaterian (Table 1). We discarded all bilaterian sequences that had a best BLAST hit below the threshold E -value of 10^{-5} with non-bilaterian sequences. We retained 13,582 bilaterian specific candidate proteins which were grouped into 1,867 clusters of orthologous proteins (COPs) using the OrthoMCL pipeline [26]. We condensed the raw set of 1,867 clusters of orthologous proteins by applying filters with different stringency for the taxa included (Fig 1) and obtained the following four sets

C: Each cluster must contain one or more representatives from each of the three major clades, Lophotrochozoa, Ecdysozoa and Deuterostomia. This set contains 506 clusters.

M: Each cluster must contain representatives from all model organisms, *D. rerio*, *D. melanogaster* and *C. elegans*. This set contains 160 clusters.

L: Each cluster contains representatives from all major clades, as in set *C*. However, only those clusters are permitted for which the representation of species is explained by at most one loss event along the species tree (set *C* does not have this restriction). This resulted in 125 clusters (see supplementary Table S1)

A: Each cluster contains representatives of all bilaterian species considered (no protein loss is admitted) except possibly *A. californica*. This set has 34 clusters.

On average, each of the nine bilaterian species is represented in 66.2% of the 506 ortholog clusters in set *C*. In set *M*, this percentage is 80.9%, and in set *L* it is 91.2% (see Table 2), reflecting the levels of stringency of the filtering criteria.

2.2. Orthologues conserved across divergent Bilateria

In our analysis, we included the highly divergent bilaterian model organisms *Caenorhabditis elegans*, *Drosophila melanogaster*, and *Danio rerio*.

98 These have very well curated and annotated genomes and are therefore help-
 99 ful to find genes which are also functionally conserved since more than
 100 500Myrs of independent evolution. To take advantage of this, we decided
 101 to use the intersection of sets M and L (termed $L' = L \cap M$) for further
 102 analysis. Compared to set L (125 clusters), this intersection lacks 31 clusters
 103 missing a *C. elegans* ortholog and 9 clusters missing an ortholog from *D.*
 104 *melanogaster*, resulting in a set of 85 orthogroups. Except for *A. californica*,
 105 the least represented species in these clusters is *S. purpuratus* (genes of this
 106 species occur in 63 of 85 clusters). By construction the model organisms (*C.*
 107 *elegans*, *D. melanogaster*, *D. rerio*) are represented in all clusters in L' . On
 108 average, a (bilaterian) species is represented in 93.1% of the clusters.

109 2.3. Clade-specificity declines with data availability

110 A general drawback of our experimental procedure is its reliance on cor-
 111 rectly identified and annotated genes. Since the majority of the organisms
 112 in our study are non-model organisms, they may suffer from incomplete or
 113 erroneous gene annotation. For example, we omitted the bilaterian species
 114 *A. californica* from downstream analyses because of its low quality protein
 115 annotation. It is also possible that truly existing genes are missing from
 116 the non-bilaterian dataset due to annotation or prediction errors or simple
 117 insufficient availability of sequenced species, leading to wrong inferences of
 118 bilaterian specificity. To test this possibility, we first verified by additional
 119 BLAST searches at NCBI that none of the 85 COPs in set L' had BLAST
 120 hits below an E -value of 10^{-5} in non-bilaterians or other eukaryotes avail-
 121 able in August 2014. To accomodate to the very fast growing amount of data
 122 from across the bilateria and non-bilateria becoming available in recent years,
 123 we decided to implement a second, more comprehensive test to validate the
 124 potential bilaterian specificity set L' . Conducting searches with Diamond
 125 and NCBI-BLAST against a wide array of protein data downloaded in Au-
 126 gust 2017 from NCBI we identified potential homologs for proteins in the 85
 127 COPs. Using OMA and phylogenetic inferences we could then identify non-
 128 bilaterian orthologs for all but 35 of the 85 set L' COPs. This showed that
 129 with additional genomes and transcriptomes becoming available, the number
 130 of bilaterian specific orthologous groups decreased drastically.

131 2.3.1. Most conserved orthologues

132 Although not being strictly bilaterian-specific, we regarded the set L' COPs
 133 as biologically interesting since they are conserved across huge evolutionary

distances with many speciation events separating them in the three model organisms (and additionally divergent enough from non-bilaterian sequences to not be detected in our first re-BLAST screen). We thus wondered what their biological function is and if this function could be correlated to a distinct process in the biology of Bilateria. As a result of our experimental design, but also due to different duplication histories, all COPs contain differing numbers of paralogs from different species. On average, we found most paralogs in Deuterostomia, followed by Ecdysozoa and Lophotrochozoa. The highest average paralog number is found for *D. rerio*, which might be a consequence of the additional genome duplication events in Teleosts [27]. Since the function of paralogs might differ from the function in the last common ancestor, we constructed an additional set of ortholog clusters free of paralogs: we extracted from each cluster in our original set L' the most conserved ortholog for each species, according to the PhastCons conservation score [28], and discarded the less conserved paralogs. This reduction does not affect the number of clusters, but the number of proteins within a cluster. In our downstream analyses we considered both versions, the ‘most conserved orthologs’ (MCO) and the ‘all orthologs’ (AO) catalog for each cluster.

2.4. Gene ontology and functional classification

GO terms reveal a link to development

To gain an overview over the biological functions of the proteins in set L' , we extracted and analyzed their associated GO terms. To quantify simultaneous enrichment in multiple species, we developed a novel generalized Fisher’s exact test for multiple species, which we term MSGEA (‘Multi Species GO Enrichment Analysis’; Fig 3 and Fig S1). In contrast to standard GO enrichment analysis, MSGEA does not solely focus on the over-representation of GO terms in single species, but is able to detect GO terms enriched across several species, even if not over-represented in any single species. Of special interest for our analysis were GO terms occurring across all three model organisms: such terms may indicate the conservation of biological function across large evolutionary time scales. To our knowledge, this novel method is the only test that is sensitive to coherent enrichment of GO terms across multiple species (see Methods). Applying MSGEA we extracted a list of GO terms which are likely associated to long-term retained functions of our candidate genes. Concentrating on the domain ‘biological process’ of the GO database, we find the terms ‘development’, ‘muscle’, ‘neuron’, ‘signaling’ and

170 ‘regulation’ to be strongly over-represented (Fig. 4 and supplementary Ta-
171 ble S4). The terms identified through in silico analysis suggest a prominent
172 role for these orthologues in development.

173 *Inference of biological function through literature mining*

174 Since orthology is a strictly phylogenetic criterion, and since GO classi-
175 fication draws heavily on sequence homology, without necessarily reflecting
176 conserved function, we decided to perform an in-depth human-curated lit-
177 erature search to collect additional evidence for the functional role of genes
178 in COP clusters. We mined the literature databases and compiled human
179 curated functional descriptions, based on experimental evidence, for the pro-
180 teins contained in set L' . We defined the six classes ‘Neuron related’, ‘Mor-
181 phology related’, ‘Muscle related’, ‘Signaling related’, ‘Regulation related’
182 and ‘Others’ (supplementary Table S5). We used functional descriptions ex-
183 tracted from the literature to assign each of the 85 set L' clusters to one
184 of these six classes (Fig. 4). The manual extraction of species- and protein-
185 specific functional information allowed us to compare and better interpret
186 functions across the three model organisms.

187 To compare this human-curated classification with the one based on the
188 GO database, we assigned all GO terms appearing in set L' to one of the
189 six classes above. Since a given protein may be associated with many GO
190 terms there is no one-to-one relationship of the human curated annotation
191 and the GO terms. For example, while the human curation assigns eight of
192 the 85 clusters (the left-most columns in Fig 4) to the category ‘Morphol-
193 ogy related’, morphology related GO terms occur in 33 clusters, scattered
194 throughout the six classes. Similarly, signaling related GO terms are found
195 in almost every cluster, while the class ‘Signaling related’ contains only 18
196 clusters. However, our manual curation is still broadly consistent with GO
197 annotations. For example, GO terms associated to ‘muscle’ do occur in the
198 class ‘Muscle related’ and GO terms related to ‘neuron’ are mostly found in
199 the ‘Neuron related’ clusters.

200 *2.5. Expression profiles*

201 To analyze expression of genes in set L' across developmental stages and
202 to compare it between model organisms, we used publicly available data for
203 *D. melanogaster*, *D. rerio* and *C. elegans*. We normalized expression values -
204 separately for each species and for each developmental stage - by the genome-
205 wide average, and then log-transformed them.

Pooled profiles

To obtain a global picture of the expression profiles of genes in set L' , we calculated mean and median profiles for each of the three model organisms, shown as orange lines in Fig 5 and Fig S3, top row. To see how expression of bilaterian-specific genes compares to background expression, we generated two different background sets for each species. The first is a random-background, obtained by randomly selecting genes from the model organism genomes and recording their expression (distribution shown as white boxplots in Fig 5). The second background, which we call the ‘matched-function’ background (orange boxplots), is a random collection of genes with GO terms matched to those found in set L' . The background distributions were constructed independently for each stage and for each set, and they may differ in size and content. We grouped the developmental stages from fertilized egg to the adult body into coarse grained, yet comparable, phases: ‘Blastoderm’, ‘Gastrulation and Organogenesis’, ‘Hatching to Larva’ and ‘Adult’ (separated by vertical lines in Fig 5).

For all three species, the set L' profiles roughly follow the shape of the background profiles across embryogenesis, however at a higher expression level (Fig 5). In *D. melanogaster*, expression of bilaterian-specific proteins is initially low, then rises until ‘dorsal closure’, after which it slightly decreases again. In *C. elegans*, expression is also initially low and then rises towards ‘ventral enclosure’. This pattern is mirrored in *D. rerio*, where expression peaks around the pharyngula stage. The profiles seen in *C. elegans* and *D. rerio* are congruent with the major cycle of tissue proliferation and differentiation during organogenesis in these species [29]. During ‘dorsal closure’ in *Drosophila*, ‘ventral closure’ in *C. elegans* and ‘pharyngula’ in *Danio*, the head, the nervous system and the bilaterally organized body develop [30]. In *Drosophila* we find a second expression peak during a second cycle of proliferation corresponding to metamorphosis. The matched-function profile largely resembles the profile of set L' . However, set L' genes show distinctly low expression in the very early embryonic stages, a pattern which is consistently seen in all species (Fig 5 and Fig S3). Another common feature is that expression of MCO genes is consistently higher than of AO genes (Fig 5).

Characteristic expression profiles

To further analyze the expression profiles, we clustered individual profiles based on their similarity and extracted a few typical ones for each species (Fig 5, bottom row). Methods exist to identify tissue-specific expression

243 profiles, or to differentiate between profiles, but no standard techniques are
 244 available to detect enrichment of profiles across a set of genes. To overcome
 245 this limitation, we employed an ad hoc 'profile enrichment' strategy. Briefly,
 246 we considered a COP expression profile relevant, if similar profiles were sig-
 247 nificantly more abundant among genes in our COPs than among other genes
 248 (for details, see Methods). We extracted all statistically significant profiles
 249 and then clustered them to distill representative ones. For each of the three
 250 model species qualitatively similar patterns are retrieved: an intersection of
 251 increasing expression profiles (yellow and green lines in Fig 5, 2nd row) with
 252 slightly decreasing profiles (dark red and dark blue lines). The intersection
 253 occurs right before the developmentally important events 'dorsal closure',
 254 'ventral enclosure' and 'pharyngula'. A second crossing of profiles can be
 255 seen in the fly, just before metamorphosis into the adult body. To interpret
 256 this observation, we considered the following functional classification.

257 *Expression profiles stratified by function and age*

258 We determined the mean expression profiles for the six functional classes
 259 - morphology- (8 set L' COPs), muscle- (11), neuron- (23), signaling- (18),
 260 regulation-related (20) and other genes (5) (Fig S2). We find upward and
 261 downward peaked expression patterns for the classes morphogenesis, neuron
 262 and muscle development (Fig S4), which are even emphasized compared to
 263 the pooled profiles of the global analysis. This is in particular true for ex-
 264 pression before the onset of gastrulation. On the contrary, the genes related
 265 to regulation and signaling display a rather constant profile, without the
 266 characteristic expression peaks of morphogenesis.

267 For species as different as animals, plants and fungi, it has been repeatedly
 268 observed that timing and level of expression during development depend on
 269 the evolutionary age of a protein [31, 32, 33, 30, 34]. For instance, one recur-
 270 rent finding was that older genes tend to show higher expression during early
 271 embryogenesis. To examine our proteins in this respect, we analysed whether
 272 the set L' expression profiles are more similar to the profiles of younger or
 273 of older genes, and we calculated background distributions considering the
 274 age of genes. Genes which arose before Bilateria (phylostratigraphic level
 275 less than 7, corresponding to Bilateria, according to the strata-numbers by
 276 Domazet-Lošo) were defined as 'older' and all others (level > 7) as 'younger'
 277 [32]. Expression profiles of genes (AO) in set L' match very well the profiles
 278 of the 'younger' genes with matched function (red lines and orange boxplots
 279 in Fig 5 and Fig S3). All three species show low expression of young and of

set L' genes during early development. Hence, this late onset of expression appear to be an evolutionary novelty of Bilateria. After ‘dorsal closure’ in *D. melanogaster*, ‘ventral closure’ in *C. elegans*, and ‘pharyngula’ in *D. rerio*, expression increases and older and younger profiles converge.

3. Discussion

The most significant innovations of bilaterian animals are a third germ layer (the mesoderm), a liquid filled body cavity surrounded by mesoderm (the coelom), a complex nervous system, and a large number of specialised cell types. The formation and presence of these features had a massive effect on the evolutionary possibilities and success of bilaterians. By analysing the genomes of bilaterian and non-bilaterian species we aimed at testing whether there is a genomic correlate to these morphological key innovations. Roughly a billion years of divergence between the most distant species, chromosome fusions, fissions and re-arrangements, genome duplications, expansions and losses of gene families, as well as myriads of nucleotide substitutions might have blurred the common genetic heritage of Bilateria. However, those genes and proteins which have evolved under steady purifying selection, i.e. play a key non-redundant role in extant Bilateria species, should still be identifiable as orthologs across Bilateria. We searched for genes that potentially evolved in the bilaterian ancestor and have been retained in extant bilaterians because such genes might have influenced the evolution of bilaterians. We were initially able to detect a set of 85 orthogroups (set L'), containing genes which are shared between widely divergent Bilateria and, at the same time, appeared to be without orthologues in non-Bilateria. We reason that this set had to be taken with caution, as for example there is no clear-cut threshold of sequence similarity which separates orthology from non-orthology. In such cases the decision, whether a gene with partial homology should be included into an orthogroup or not, is somewhat arbitrary. For the 85 set L' COPs we have considered partial homology to a non-bilaterian sequence as an insufficient criterion to exclude an orthogroup from our initial list. A similar BLAST strategy as implemented by us, however without subsequent orthology clustering, had been used before to determine the evolutionary origin of genes and to assign phylostratigraphic ages [35, 32, 36, 34]. One pitfall of this approach is that an overall alignment score, such as an Blast E -value, may lead to both false positive and false negative predictions. False positives arise from the (wrong) inclusion of a protein into the bilaterian-specific list, which

316 *does* have non-bilaterian orthologs, but which are missed due to low sequence
 317 similarity or due to absence from the database. False negative predictions
 318 arise from missing truly bilaterian-specific proteins, for instance when a con-
 319 served domain is identified in a protein for which a homologous domain is also
 320 present in non-bilaterians. One such example is the C2H2 zinc finger pro-
 321 tein CTCF which has been described as bilaterian-specific before [37]. Our
 322 search did not recover this protein, because multi-zinc finger proteins exist
 323 in cnidarians and their conserved Cys/His residues and linker regions evoke
 324 a similarity above threshold. As a result, our approach will wrongly treat
 325 such proteins as older and not bilaterian-specific and, consequently, eliminate
 326 them. Similar errors may affect other proteins with extended conserved do-
 327 mains. However, proteins that pass the filter should have a high probability
 328 to be true bilaterian novelties. In contrast, genes may be wrongly assigned
 329 to subclades which are younger than bilateria. For instance, this could be a
 330 consequence of non-orthologous gene displacement (NOGD) [38], a process
 331 in which functions are taken over by new or different proteins and the origi-
 332 nal orthologue is lost. Finally, we could have wrongly classified a protein as
 333 bilaterian-specific because faint homology to more anciently diverged species
 334 is missed.

335 Another limitation in our initial screen was the restriction to 16 high-
 336 quality genomes, from which we inferred bilaterian specificity. Firstly, it has
 337 now been shown that OrthoMLC, which we mainly relied on, is limited in
 338 finding distantly related orthologues if taxon sampling is coarse [39]. This
 339 problem could have been inflated by our restriction of candidates in our
 340 blast searches before running orthology prediction pipelines. Secondly, new
 341 entries to the databases may change the inferred phylostratigraphic age of an
 342 orthogroup. Being aware of this and in particular because sequencing efforts
 343 are increasingly directed towards non-standard model organisms in the last
 344 years, we have scrutinized and re-inspected our initial candidate set of set
 345 L' COPs with refined tools and new data. While the remaining sub-set of
 346 35 orthogroups might consist of bilaterian-specific proteins, it is likely that
 347 more data from so far not-yet-sequenced non-bilaterian species will also reveal
 348 orthologous for some of these proteins. These caveats raise doubts on the
 349 likelihood to detect high-confidence genes that are unequivocally bilaterian
 350 and at the same time widely retained in this taxon. Our results clearly raise
 351 the possibility that there are just a handful of Bilateria-specific genes, or
 352 even none at all. At the same time our approach of partially falsifying the
 353 results of the first orthology screen by implementing two additional checks

354 shows that if there is any such gene, careful and elaborate measures have
355 to be taken to validate potential candidates. At the very least all available
356 genomic and proteomic data from currently available databases have to be
357 screened.

358 Despite not being necessarily clade-specific, our set of genes is retained in
359 very distantly related bilaterian species, while at the same time not being re-
360 stricted to the group of classical, universally-conserved house keeping genes.
361 This is interesting to note since even highly conserved and important genes
362 can become lost, such as some Hox genes, e.g. from *C. elegans* [40] and the
363 nematodes in general. Our set is also significantly divergent from potential
364 non-bilaterian orthologs, suggesting either their origin or their rapid evolu-
365 tion in the ur-bilaterian. It thus appeared logically to ask which functions
366 these orthologues might have in bilaterian animals. In comparison to classical
367 enrichment analysis implemented in various software packages like blast2GO
368 [41], our MSGEA test allows for the first time to compare GO-enrichment
369 across several species. This is advantageous in the age of genomics where very
370 often more than one species or a number of samples is analysed. In our frame-
371 work of three model bilaterians, a fruit fly, a zebrafish, and a roundworm,
372 the MSGEA based GO term analysis and subsequent literature mining indi-
373 cated roles in development for the clustered genes. This link appears likely,
374 since the process of development, the construction of adult specimens from
375 single cells, is conserved in all animals and is also the phase when the com-
376 bined hallmarks of bilaterian life are brought together: bilateral symmetry,
377 triploblasty, the enhanced nervous system, and a set of complex cell types. In
378 particular, the ‘muscle’ and ‘neuron’ class of clusters is compatible with the
379 idea that key bilaterian innovations involved the central nervous system and
380 muscles [42]. In general the connection to development is in agreement with
381 other studies, such as the large scale modEncode study of model organisms
382 [29].

383 The conserved genes in set L' follow characteristic expression patterns
384 during development, which are shared between species separated by a bil-
385 lion years of independent evolution. In particular, we observe characteristic
386 low expression in early development and a peak towards the end of lar-
387 val development common to highly derived taxa such as nematodes, insects
388 and teleosts. The single (fish and nematode) and double peak (fly) pat-
389 terns which we observed are in agreement with results from the modEncode
390 project [43, 29]. Starting from co-expression analyses, the authors arrived
391 at a set of orthologous genes acting in comparable stages of *D. melanogaster*

392 and *C. elegans* development. The simpler, single peaked pattern in *C. ele-*
393 *gans* and *D. rerio* reflects up-regulation at ventral enclosure and pharyngula
394 stages, respectively. Thus, the expression profiles in these organisms mirror
395 their life-cycle, displaying shared peaks when the adult body-plan is assem-
396 bled. This phase, dominated by morphogenesis, has been interpreted as the
397 phylotypic stage in both organisms [44, 45]. In the holometabolous fly the
398 situation is more complex: a body morphology is built twice, for the larva
399 and then for the adult. These are two phases of increased cell proliferation
400 during gastrulation and the transition from pupa to adult. The two peaks
401 are also visible in the matched-function set, but they are less pronounced
402 in the random-background set. It thus appears the two peaks we observed
403 for our proteins reflect up-regulation during both phases of cell proliferation.
404 It will be interesting to analyse if similar patterns can be observed in other
405 species undergoing metamorphosis, particularly in vertebrates.

406 It has been hypothesised that differences in gene regulatory processes and
407 the deployment of GRNs may have been more important for the evolution
408 of Bilateria than the conservation of single key genes [46]. A conserved pro-
409 file of developmental gene expression was observed in several studies across
410 animal phyla, including in non-bilaterians, when developmental time course
411 data were analysed [12]. These studies suggest the existence of a phylotypic
412 stage late in development, but see [47, 12, 31]. We observed low expres-
413 sion of young genes that originated after the emergence of Bilateria and of
414 set L' genes in early development. We interpret this to indicate that early
415 embryonic processes, e.g. initial cell divisions, are governed by evolution-
416 ary old genes, which are common to all bilateria and non-bilatera. Thus
417 while, non-Bilateria have a similar complex distribution and abundance of
418 gene regulatory elements and systems [48, 49], and likely their own phylo-
419 typic stage, our data support the idea that a change in gene regulation in the
420 phase when adult morphology is shaped might be linked to the emergence of
421 Bilateria. What is more, the process appears to be conserved across immense
422 evolutionary distances.

423 In agreement with the general idea of the orthology-conjecture, paralogs
424 contained in set AO may be sub-functionalized, while the MCO genes are
425 more likely to retain their original function. This observation on the MCO
426 genes appears interesting in regard to the ortholog conjecture, which posits
427 that orthologues are more likely to retain function in divergent organisms
428 in comparison to paralogues, see e.g. [50]. While being challenged in the
429 past [51] more recent analyses have found support or at least some support

for the ortholog conjecture by comparing for example mammals or divergent model organisms [52, 53, 54]. Our data does give additional support to the idea of functional conservation of orthologues in very distant species and in particular potentially extends the conjecture to expression patterns. That paralogues are indeed more divergent in their function was for example observed in flies [55] and appear apparent in our data of the AO subset as well.

3.1. Conclusions

In this study we performed a systematic approach to identify bilaterian-specific genes. Previous investigations concluded that essentially all important developmental regulators precede the origin of bilaterians, and that rewiring of already existing factors was a hallmark of bilaterian evolution [24, 56, 23, 22, 48] and our results enhanced by a second BLAST and phylogeny based control step are in support of this. Future data from non-bilaterian species might in fact show that even the 35 groups of orthologues we now identified to be conserved across the three model species but without credible non-bilaterian orthologues were present before the split from Cnidaria. Conversely, it remains possible that genes independently lost in bilaterian crown groups have no non-bilaterian orthologues. Analysing the roles of genes we found conserved across crown groups in bilateria we found a strong link to development. Thus, our results indicate that Bilateria are unified by processes occurring late in embryonic development, which are shaped by a set of conserved genes. At the same time early developmental processes might be shared across Metazoa. Finally, our findings give new support to the ortholog conjuncture.

Materials and Methods

Using a comparative genomic approach, we aimed to identify genes shared among and specific to Bilateria. To represent the spectrum of the different bilaterian clades we selected ten species from the three major clades - Deuterostomia, Ecdysozoa and Lophotrochozoa - concentrating on species with a fully sequenced and annotated genome (Table 1). We included more species from Lophotrochozoa than from the other two clades, since well-annotated complete genome sequences from lophotrochozoan model-organisms were still under-represented in public databases at the beginning of our studies. To obtain a high contrast between Bilateria and Non-bilateria, we used seven

465 non-bilaterian species, as in a previous study [37]. Proteomes and data from
466 InterProScan [57] and the Gene Ontology consortium [58] were retrieved from
467 the sources cited in Table 1.

468 *Initial BLAST and Clustering*

469 To find orthologs we set up a stringent analysis pipeline. We first species
470 blasted all-versus-all among the 17 proteomes using the stand-alone BLAST
471 version 2.2.25+ with a cutoff of $E = 10^{-5}$. We then discarded all bilaterian
472 proteins which had a significant hit in any of the non-bilaterian sequences.
473 This filtering resulted in 13,582 candidate proteins. From these we con-
474 structed orthologous groups using two different ortholog finders, InParanoid
475 and OrthoMCL [26, 59], using default parameter settings. Both programs
476 were rated highly in benchmarking studies which analyzed the performance of
477 orthology-prediction methods [60, 61]. For the purpose of detecting orthologs
478 of very diverged species, we chose OrthoMCL version 2.0.2 and the associ-
479 ated MCL version 11-335, as they were shown to be more robust [60, 61] than
480 similar programs. Finally, we grouped the ortholog clusters into four sets (A ,
481 L , M , C ; Fig 1), satisfying different stringency conditions, see page 4. Due
482 to the poor sequence quality, we treated the Lophotrochozoon *A. californica*
483 differently: where available we included *A. californica* orthologs, but we did
484 not require them to be present in set A ('all species').

485 The above data sets are ordered by degree of initial confidence in the
486 bilaterian specificity of the respective proteins. At the same time they reflect
487 the level of conservation of the proteins across different species. Seeking to
488 be conservative regarding bilaterian specificity on the one hand (set A being
489 most conservative), but on the other hand to analyze a dataset which is as
490 comprehensive as possible, we focused our further analyses on the intersection
491 of sets L and M (called L' ; Fig 1) comprising 85 clusters.

492 *Validation with BLAST, OMA, and Phylogeny*

493 As our original dataset was bilaterian and non-bilaterian species was as-
494 sembled prior to 2012 and the steady flow of newly sequenced species has
495 added massive new numbers of protein predictions to online databases we
496 wanted to re-evaluate the potential bilaterian-specificity of our 85 final clus-
497 ters. To this end we downloaded 19,185,382 bilaterian and 497,273 non-
498 bilaterian proteins from the NCBI database in August 2017 and created
499 individual databases for taxa in bilateria and non-bilateria. Next we used
500 Diamond [62] to blast the *D. rerio*, *C. elegans*, and *D. melanogaster* proteins

for each of the 85 clusters against the individual taxa. Additionally, we used NCBI BLAST with the parameters described to be most sensitive in finding orthologs in [63] for the non-bilaterian taxa. For each of the 85 clusters we then collected the 10 best blastp hits per taxon. Along with the original model organism proteins we obtained 96,287 proteins in this way, which we used as input for the OMA orthology prediction pipeline [64]. From the OMA output we selected hierarchical cluster of orthologues (HOG) for those of the original 85 clusters where the HOG contained non-bilaterian proteins. Finally, we aligned all sequences per HOG with clustal-omega [65] and reconstructed a phylogeny using IQTrees with automated model selection and 1000 fast bootstrap replicates [66]. We then analysed these trees by eye.

Selecting most conserved orthologs

For the three model species *D. rerio*, *D. melanogaster*, and *C. elegans* we extracted the UCSC tracks of basewise PhastCons conservation scores [28] calculated across insects, teleosts and nematodes, respectively. We used these scores to rank all genes in a cluster according to their fraction of strongly conserved sites, i.e. sites with PhastCons score > 0.99 . We selected the highest-ranking one from each cluster as the 'Most Conserved Ortholog' (MCO). These genes likely retained the same function, even in highly diverged species – a conjecture based on the idea that strong conservation reflects long-term evolutionary (and functional) constraint and that neo- or sub-functionalized paralogs tend to be less conserved. We used the fraction of strongly conserved sites, instead of the average conservation score, since we are interested in the degree of conservation in function, not in sequence. We reasoned that functional conservation should be related to high conservation of alleles at functional sites, while the remaining bases can evolve fast. However, the two ways of measuring similarity are highly correlated on a genome-wide scale across all coding exons ($r = 0.91$ for *D. melanogaster*, 0.84 for *D. rerio* and 0.71 for *C. elegans* (Fig 2)). We performed all subsequent analyses on both sets: the set of 'All orthologs and paralogs' (AOs) and the set of MCOs.

Multi-Species Gene Enrichment Analysis

To examine function of the genes in set L' by *in silico* methods, we obtained their gene ontology (GO) terms for the three model species from the GO database [58]. To identify terms enriched in COP genes compared to the

genomic background, we employed two strategies: the conventional single-species enrichment analysis based on Fisher’s exact test and a novel method, termed multi-species gene enrichment analysis (MSGEA). Standard single-species Fisher’s exact tests for enrichment cannot capture a signal of moderate, but joint, enrichment across different species. Instead, with MSGEA we compute an exact p -value for a pooled set of species, employing the following steps. First, for each GO term we counted the number of descendants in the GO tree. Then, we computed the p -values for the terms occurring in any species and corrected for multiple testing [67]. More specifically, let $n_{go,s}$ be the count (i.e., number of occurrences) of the GO term go in species s contained in our set and let $N_{go,s}$ be the count for the whole genome. Furthermore, let $n_s = \sum_{go} n_{go,s}$ and $N_s = \sum_{go} N_{go,s}$. Assume that all species under consideration diverged at the same time and evolved independently after that (i.e. they have a starlike phylogeny). The null hypothesis of the MSGEA test is that a given GO term was not over-represented in the common ancestor. Therefore, a significant p -value means that the genome of the ancestor was already enriched in the GO term considered. Being conservative, assume that the null distributions of $n_{go,s}$ are independent hypergeometrics with parameters $N_{go,s}$, n_s and N_s in each species. The enrichment statistics X_{go} is then the sum of the normalized enrichments of $n_{go,s}$ across species, i.e. the sum of the z -scores:

$$X_{go} = \sum_s \frac{n_{go,s} - E(n_{go,s})}{SD(n_{go,s})} \sim \sum_s \frac{n_{go,s} - N_{go,s}n_s/N_s}{\sqrt{N_{go,s}n_s/N_s}} \quad (1)$$

where a Poisson approximation is used to define the score. Finally, the p -value is the probability

$$p_{go} = \text{Prob}(x \geq X_{go} \mid N_{go,s}, n_s, N_s),$$

where the distribution of x follows from the hypergeometric distributions of the $n_{go,s}$ with the above parameters.

For a single species, this test coincides with the standard one-tailed Fisher’s exact test for GO enrichment, and therefore is consistent. The exact estimation of p -values is computationally intensive; an optimized code, written in C, is available from the authors upon request. Since MSGEA is based on the hypothesis of independent evolution of each lineage, it can be applied only to starlike phylogenies. For the situation considered here, this assumption is met, since the “roundworm-fruit fly-zebra fish” phylogeny is

approximately starlike. We find that a considerable fraction (30-70%) of significant GO terms in our data set is detected by MSEA, but not by single species enrichment.

Detailed functional analysis of genes from model organisms

To mine literature databases for functional studies in model organisms with a focus on development we employed biomaRt: we used the Bioconductor module [68] biomaRt 2.19.3 to extract information for proteins present in cluster set L' . Wormbase release WS220 was queried for *C. elegans* proteins and ENSEMBL 75 for *D. melanogaster* and *D. rerio* proteins. We then searched the literature for experimental validations of protein function. We grouped the results in six major categories, described in Results and Discussion and summarized in supplementary Table S5. These categories represent prominent molecular functions during embryogenesis and development. Based on the retrieved annotations we assigned proteins in set L' to these categories.

Cross species expression profiles

We retrieved expression data for different developmental stages in *D. melanogaster* [69, 70], *D. rerio* [32] and *C. elegans* [71]. *D. rerio* data for adult stages were not used. The expression profiles were transformed by taking the logarithm to base 10 of the expression levels and then subtracting the $\log_{10}$ of the mean expression at each stage.

We devised the following test to find characteristic expression patterns: first, we computed Pearson’s correlation coefficient between expression profiles for different genes. For each profile in each set of the genes from 85 COPs, we performed two types of tests: (i) a Mann-Whitney test on the distribution of correlations, comparing the correlation of the profile with other bilaterian genes versus the correlation with genome-wide profiles, in order to detect profiles that are more correlated with the ones of other bilaterian genes than with the rest of the genome; (ii) for each profile, we classified the remaining genes as “highly correlated” or “not highly correlated” in expression, using correlation thresholds of $r = 0.5, 0.7$ and 0.9 ; then, we tested for enrichment of correlated profiles among Bilaterian genes by Fisher’s exact test.

A Benjamini-Hochberg correction for multiple testing was applied to the p -values resulting from these tests. All the significant profiles ($p < 0.05$) were

601 clustered based on their correlation coefficients r by complete-linkage hier-
 602 archical clustering implemented in the R statistics software package, using
 603 $1 - r$ as distance measure and selecting clusters at height $h = 0.75$. After
 604 clustering, profiles were normalized by their average expression across stages,
 605 then averaged across each cluster. The resulting profile shapes are shown in
 606 row 2 of Fig 5.

607 *Are expression profiles driven by gene function?*

608 We performed a randomisation test to explore if the mean (logarithmic)
 609 expression level of COPs genes could be explained by their function. For each
 610 developmental stage we checked if expression correlates with that of randomly
 611 selected genes possessing equal or similar GO terms, i.e. we checked if genes
 612 of different age, but similar ontology, would show similar expression profiles.
 613 We performed this analysis only on set L' .

614 For this purpose, we randomly sampled from the whole genome 100 sets
 615 with the same number of genes as are contained in set L' . We did this
 616 in two different ways. (i) The first (“random-background”) was a random
 617 sampling of genes from the genome. After sampling, we computed the mean
 618 normalized expression of the selected genes for each stage. We repeated this
 619 procedure 100 times, and thus obtained a distribution of normalized means,
 620 represented as boxplots (in white) in Fig 5, Fig S3 and Fig S4. (ii) The second
 621 was a “matched-function” sampling: first, for each GO term in the original
 622 set, we listed all genes with the same annotation and extracted from this list
 623 a number of random genes equal to the number of occurrences of the term;
 624 second, we pooled all the resulting lists; third, we extracted from this list a
 625 number of random genes equal to the number of genes in the original set.
 626 This way, we obtained sets of the same size and function (approximately,
 627 at least) as our original set. We applied this to the whole set L' and for
 628 all subsets corresponding to the six functional categories described above
 629 (Fig S4).

630 *Age index of proteins*

631 We used the phylostratigraphy from Domazet-Lošo et al. [32] for *D.*
 632 *melanogaster*, *D. rerio* and *C. elegans* to define two age-groups of proteins:
 633 (i) older proteins, with an origin in phylostrata 1 to 6; (ii) younger proteins,
 634 with an origin in phylostrata 8 and higher. Proteins that we identified as
 635 bilaterian-specific (i.e. the ones in our sets A , L , M and C) were excluded

636 from the two groups, irrespective of their original phylostratigraphic classifi-
637 cation. We repeated all the analyses described in the previous sections, and
638 compared separately our COPs genes with the genome-wide background of
639 older and of younger genes (Fig 5).

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Figures

Figure 1: **A**: Sketch of the genealogical relationship between the clades of Lophotrochozoa, Ecdysozoa and Deuterostomia and the species included in our analysis (Table 1). Legend: *M* – all model species are represented in a cluster; *C* – all clades are represented; *L* – at most one loss event along the tree (see supplementary Table S1); *A* – all of nine bilaterian species are represented. Sketch of the 'ur-bilaterian' after [4]. Blue dashed lines indicate examples of gene loss events. **B**: Absolute numbers of clusters of orthologous proteins (COPs) in the four sets. Gray region: detailed analyses are performed for 85 COPs in set $L' = L \cap M$.

Figure 2: Conservation scores for orthologous exons from PhastCons analysis. For each species, we show the correlation between scores (top), the distribution of the fraction of very conserved sites (middle), and of the average conservation score (bottom) among exons.

Figure 3: Number of significant GO terms identified with MSGEA in set L' ($p < 0.05$). Red histograms: considering all orthologs (AO) from a cluster; blue histograms: only the most conserved orthologs (MCO) are considered in the MSGEA analysis. Left: number of significant terms found for each single- or multi-species enrichment analysis. Right: number of significant terms found only in single/multiple-species enrichment analyses and in both kind of analyses. The large amount of GO terms found only in multiple species analysis illustrates the power of MSGEA (see also Fig S1).

Figure 4: Significant GO terms identified in set L' by applying MSGEA (see text). The upper part of the figure shows the GO terms which occur in each COP (x -axis). Those terms which occur at least in three COPs are spelled out with their GO-ID, otherwise they are collected in the lines 'additional ... GOs'. Only non-generic GO terms of level 4 and higher are recorded. The bottom six rows show the assignment of COPs to functional classes based on human-curated literature mining. As can be seen from the color code, automatic GO and human-curated functional assignments do not always coincide.

Figure 5: **Expression profiles** of different species. Top row: average (global) expression profiles for genes collected in set L' . Red line: pooled profiles for 'all orthologs and paralogs' (set 'AO'). Blue line: profiles for the 'most conserved orthologs' (set 'MCO'). The white and orange boxplots show the 'random' and 'matched-function' expression backgrounds, respectively (see text). Second row: Characteristic expression profiles. Different colors represent different clusters. Darker and lighter colors represent profiles which are similar among species. Bottom rows: distributions shown in boxplots are calculated from genes which are younger (phylostrata ≥ 7 according to the terminology of [32]) or older (phylostrata < 7) than bilateria. Background shading (from white to dark grey) represents developmental stages embryo, larva, pupa (fly only) and adult. For a detailed explanation of the developmental stages see supplementary Tables S2 and S3. Meaning of labels in row 2: G - gastrulation; DC - dorsal closure; MT - first muscle twitching; AM - transition to adult morphology; VC - ventral closure; E - 100% epiboly

Tables

Table 1: **Species considered** to contrast bilaterian and non-bilaterian protein repertoires

species	data source	no. proteins in database	no. proteins in all COPs	no. COPs w/ species ¹	no. proteins per COP ²
Bilateria					
Deuterostomia					
<i>Danio rerio</i>	Ensembl	41693	1190	428	2.78
<i>Strongylocentrotus purpuratus</i>	NCBI	42420	652	260	2.51
<i>Mus musculus</i>	Ensembl	40732	867	357	2.43
Ecdysozoa					
<i>Anopheles gambiae</i>	Ensembl	13133	380	307	1.24
<i>Drosophila melanogaster</i>	FlyBase	23849	989	392	2.52
<i>Caenorhabditis elegans</i>	WormBase	25634	602	291	2.07
Lophotrochozoa					
<i>Aplysia californica</i>	NCBI	1093	15	7	2.14
<i>Capitella teleta</i>	JGI	32415	642	376	1.71
<i>Helobdella robusta</i>	JGI	23432	420	325	1.29
<i>Lottia gigantea</i>	JGI	23851	492	337	1.46
Non-Bilateria					
<i>Amphimedon queenslandica</i>	NCBI	9908			
<i>Arabidopsis thaliana</i>	TAIR	28952			
<i>Dictyostelium discoideum</i>	Dictybase	13426			
<i>Hydra magnipapillata</i>	NCBI	17563			
<i>Nematostella vectensis</i>	JGI	27273			
<i>Saccharomyces cerevisiae</i>	SGD	6692			
<i>Trichoplax adhaerens</i>	JGI	11520			

1: number of COPs (out of $|C \cup M| = 519$), in which a given species is represented

2: average number of paralogs within a COP (number of proteins / number of COPs)

Table 2: COP distribution in different data sets

species	set C (506)	set M (160)	set L (125)	$L \cap M$ (85)	set A (34)	
Deuterostomia						
<i>D. rerio</i>	1169415	82.0	690 160 100	555 125 100	436 85 100	205 34 100
<i>S. purpuratus</i>	636 254	50.2	233 89 55.6	288 103 82.4	175 63 74.1	90 34 100
<i>M. musculus</i>	855 349	69.0	398 134 83.8	428 121 96.8	301 81 95.3	163 34 100
Ecdysozoa						
<i>A. gambiae</i>	373 300	59.3	156 120 75.0	143 112 89.6	107 78 91.8	51 34 100
<i>D. melanogaster</i>	964 379	74.9	494 160 100	387 116 92.8	317 85 100	143 34 100
<i>C. elegans</i>	589 278	54.9	359 160 100	231 94 75.2	207 85 100	101 34 100
Lophotrochozoa						
<i>C. teleta</i>	642 376	74.3	247 116 72.5	198 119 95.2	153 79 92.9	65 34 100
<i>H. robusta</i>	420 325	64.2	165 109 68.1	160 115 92.0	110 75 88.2	48 34 100
<i>L. gigantea</i>	492 337	66.6	224 117 73.1	217 121 96.8	169 81 95.3	84 34 100
[<i>A. californica</i>]	15 7	01.4	7 4 2.5	3 2 1.6	3 2 2.4	1 1 2.9

#P: number of proteins from given species in given set. #C: number of COPs (out of a maximum number x given in parentheses in the header line) which contain at least one protein from a given species. Ratio: percentage $\#C/x \cdot 100$

Supplementary Figures and Tables

Figure S1. Supplementary Figure related to Figure 3. MSGEA vs. single species enrichment analysis for clusters of orthologous proteins (COPs). Number of significant terms shown for each of the four sets A, L, M, and C (cf. Fig 3).

Figure S2. Supplementary Figure related to Figure 4. Absence/presence map of GO-slim terms. *x*-axis: GO-slim terms (including all GO levels); right *y*-axis: internal COP IDs.

Figure S3. Supplementary Figure related to Figure 5. Expression profiles with background distribution based on medians, instead of means. Color code and other labels as in Fig 2.

Figure S4. Supplementary Figure related to Figure 5. Expression profiles of set L' genes by functional classes. Background distributions based on mean expression values. Color code and other labels as in Fig 2. *D.m.*, 'muscle': there are no paralogs; therefore, only the blue line (for MCOs) is displayed.

Table S1. Supplementary Table related to Figure 1. Distribution of losses in set *L*.

number of clusters	species affected	lineage affected
[33]	[APL]	[APL only]
31	APL*, CEL	CEL only
22	APL*, STR	STR only
8	APL*, HEL	HEL only
6	APL*, ANO, DRO	insects
6	APL*, ANO	ANO only
4	APL*, MUS	MUS only
4	APL*, LOT	LOT only
4	APL*, CAP	CAP only
3	APL*, DRO	DRO only
2	APL*, HEL, CAP	annelids
1	ANO	ANO only
1	-	-

*due to the incomplete assembly and annotation status of the *A. californica* genome, APL was ignored when determining the number of losses. When *A. californica* is included only one cluster contains orthologs from all species (last line). Omitting *A. californica*, we find 33 clusters which contain orthologs from nine species (first line). In the remaining 91 clusters in set *L* an ortholog from at least one species is missing. Most losses (31 of 91) are *C. elegans*-specific (second line).

Table S2. Supplementary Table with annotation of x -axis tick marks in Fig 2, Fig S3 and Fig S4 according to [75] (*D. melanogaster*), [124] (*C. elegans*) and [48] (*D. rerio*).

bckgr-color	tickmark	devel. stage	bckgr-color	tickmark	devel. stage
		<i>D. rerio</i>			<i>D. melanogaster</i>
white	1	egg 0min	white	1	embryos 0-2hr
	2	zygote 15min		2	embryos 2-4hr
	3	cleavage 45min		3	embryos 4-6hr
	4	cleavage 1h15min		4	embryos 6-8hr
	5	cleavage 1h45min		5	embryos 8-10hr
	6	blastula 2h15min		6	embryos 10-12hr
	7	blastula 2h45min		7	embryos 12-14hr
	8	blastula 3h20min		8	embryos 14-16hr
	9	blastula 4h		9	embryos 16-18hr
	10	blastula 4h40min		10	embryos 18-20hr
	11	gastrula 5h20min		11	embryos 20-22hr
	12	gastrula 6h		12	embryos 22-24hr
	13	gastrula 7h	light grey	13	L1 larvae
	14	gastrula 8h		14	L2 larvae
	15	gastrula 9h		15	L3 larvae 12hr post molt
	16	gastrula 10h	grey	16	L3 larvaePS 1-2
	17	segmentation 10h20min		17	L3 larvaePS 3-6
	18	segmentation 11h		18	L3 larvaePS 7-9
	19	segmentation 11h40min		19	white prepupae
	20	segmentation 12h		20	WPP 12hr
	21	segmentation 13h		21	WPP 24hr
	22	segmentation 14h		22	pupae WPP 2d
	23	segmentation 15h		23	pupae WPP 3d
	24	segmentation 16h		24	pupae WPP 4d
	25	segmentation 17h	dark grey	25	adult male 1d
	26	segmentation 18h		26	adult male 30d
	27	segmentation 19h		27	adult male 5d
	28	segmentation 20h		28	adult female 1d
	29	segmentation 21h		29	adult female 5d
	30	segmentation 22h		30	adult female 30d
	31	segmentation 23h			
	32	pharyngula 1d1h			
	33	pharyngula 1d3h			
	34	pharyngula 1d6h			
	35	pharyngula 1d10h			
	36	pharyngula 1d14h			
	37	pharyngula 1d18h			
	38	hatching 2d			
	39	hatching 2d12h			
	40	hatching 3d			
light grey	41	larva 4d	white	1	stage1
	42	larva 6d		2	stage2
	43	larva 8d		3	stage3
	44	larva 10d		4	stage4
	45	larva 14d		5	stage5
	46	larva 18d		6	stage6
	47	larva 24d		7	stage7
	48	larva 30d		8	stage8
	49	larva 40d		9	stage9
dark grey	50	juvenile 45d	light grey	10	stage10
	51	juvenile 55d			
	52	juvenile 65d			
	53	juvenile 80d			

C. elegans

S-7

Table S4. Supplementary Table related to Figures 3 and 4. GO terms (of level ≥ 4) identified in 85 COPs of set L' . COPs ake-68, ake-73, ake-185, ake-217 and ake-224 did not contain GO terms of level ≥ 4 and do therefore not occur in this table.

GO-ID	classification	GO description	internal COP-ID (ake-###)
GO:0000122	regulation	negative regulation of transcription from RNA polymerase II promoter	114, 37, 82
GO:0000188	signalling	inactivation of MAPK activity	3
GO:0000226	muscle	microtubule cytoskeleton organization	120, 51
GO:0000278	morphology	mitotic cell cycle	135, 267
GO:0000280	signalling	nuclear division	16
GO:0000302	signalling	response to reactive oxygen species	118
GO:0000381	regulation	regulation of alternative nuclear mRNA splicing, via spliceosome	276
GO:0000387	signalling	spliceosomal snRNP assembly	203
GO:0000422	regulation	mitochondrion degradation	193
GO:0001525	morphology	angiogenesis	6
GO:0001653	signalling	peptide receptor activity	6
GO:0001666	signalling	response to hypoxia	135, 193
GO:0001708	morphology	cell fate specification	82
GO:0001709	morphology	cell fate determination	32
GO:0001751	neuron	compound eye photoreceptor cell differentiation	180, 32
GO:0001754	neuron	eye photoreceptor cell differentiation	64, 82
GO:0001756	morphology	somitogenesis	2, 37, 6
GO:0001946	morphology	lymphangiogenesis	82
GO:0001947	muscle	heart looping	3
GO:0001964	signalling	startle response	37, 55
GO:0002009	morphology	morphogenesis of an epithelium	120, 16, 171, 92
GO:0002074	muscle	extraocular skeletal muscle development	71
GO:0002119	morphology	nematode larval development	114, 131, 150, 155, 16, 171, 174, 180, 18, 203, 216, 232, 242, 255, 259, 279, 46, 71, 82, 92, 97
GO:0002121	signalling	inter-male aggressive behavior	52, 55
GO:0002385	signalling	mucosal immune response	155
GO:0003140	morphology	determination of left/right asymmetry in lateral mesoderm	3
GO:0003146	muscle	heart jogging	3
GO:0003707	signalling	steroid hormone receptor activity	114
GO:0003810	signalling	protein-glutamine gamma-glutamyltransferase activity	3
GO:0003917	regulation	DNA topoisomerase type I activity	3
GO:0003918	regulation	DNA topoisomerase (ATP-hydrolyzing) activity	3
GO:0003924	signalling	GTPase activity	3
GO:0003951	regulation	NAD ⁺ kinase activity	170
GO:0003954	regulation	NADH dehydrogenase activity	118
GO:0004091	signalling	carboxylesterase activity	51
GO:0004129	signalling	cytochrome-c oxidase activity	135
GO:0004190	signalling	aspartic-type endopeptidase activity	113
GO:0004221	signalling	ubiquitin thiolesterase activity	3
GO:0004252	signalling	serine-type endopeptidase activity	11
GO:0004308	signalling	exo-alpha-sialidase activity	3
GO:0004386	signalling	helicase activity	162
GO:0004518	signalling	nuclease activity	131
GO:0004519	signalling	endonuclease activity	131
GO:0004521	signalling	endoribonuclease activity	131
GO:0004553	signalling	hydrolase activity, hydrolyzing O-glycosyl compounds	51
GO:0004556	signalling	alpha-amylase activity	27
GO:0004672	signalling	protein kinase activity	144
GO:0004674	signalling	protein serine/threonine kinase activity	144
GO:0004725	signalling	protein tyrosine phosphatase activity	3
GO:0004812	regulation	aminoacyl-tRNA ligase activity	160, 32

... Table S4 continued

GO-ID	classification	GO description	internal COP-ID (ake- ###)
GO:0004842	signalling	ubiquitin-protein ligase activity	2
GO:0004887	signalling	thyroid hormone receptor activity	114
GO:0004888	signalling	transmembrane signaling receptor activity	6
GO:0004930	signalling	G-protein coupled receptor activity	3, 6
GO:0004948	signalling	calcitonin receptor activity	6
GO:0004991	signalling	parathyroid hormone receptor activity	6
GO:0005085	signalling	guanyl-nucleotide exchange factor activity	63
GO:0005086	signalling	ARF guanyl-nucleotide exchange factor activity	3
GO:0005089	signalling	Rho guanyl-nucleotide exchange factor activity	63
GO:0005184	neuron	neuropeptide hormone activity	6
GO:0005216	signalling	ion channel activity	144, 3, 52
GO:0005253	signalling	anion channel activity	19
GO:0005254	signalling	chloride channel activity	19, 52
GO:0005261	signalling	cation channel activity	144
GO:0005262	signalling	calcium channel activity	134
GO:0005267	signalling	potassium channel activity	144
GO:0005277	signalling	acetylcholine transmembrane transporter activity	251
GO:0005278	signalling	acetylcholine:hydrogen antiporter activity	251
GO:0005326	neuron	neurotransmitter transporter activity	31
GO:0005430	neuron	synaptic vesicle amine transmembrane transporter activity	64
GO:0005786	signalling	signal recognition particle, endoplasmic reticulum targeting	285
GO:0005802	signalling	trans-Golgi network	18
GO:0005913	signalling	cell-cell adherens junction	180
GO:0005975	signalling	carbohydrate metabolic process	27, 51
GO:0006030	morphology	chitin metabolic process	2
GO:0006120	regulation	mitochondrial electron transport, NADH to ubiquinone	118
GO:0006123	regulation	mitochondrial electron transport, cytochrome c to oxygen	135
GO:0006171	signalling	cAMP biosynthetic process	6
GO:0006184	signalling	GTP catabolic process	3
GO:0006200	regulation	ATP catabolic process	162, 3
GO:0006260	regulation	DNA replication	3
GO:0006265	regulation	DNA topological change	3
GO:0006281	regulation	DNA repair	33
GO:0006306	regulation	DNA methylation	6
GO:0006351	regulation	transcription, DNA-dependent	101, 2, 3, 37, 71
GO:0006355	regulation	regulation of transcription, DNA-dependent	101, 114, 2, 32, 37, 3, 71, 82
GO:0006357	regulation	regulation of transcription from RNA polymerase II promoter	101, 37
GO:0006364	regulation	rRNA processing	401
GO:0006396	regulation	RNA processing	225
GO:0006397	signalling	mRNA processing	203, 276
GO:0006412	regulation	translation	150, 216, 232, 242, 255, 259, 327, 97
GO:0006418	regulation	tRNA aminoacylation for protein translation	160, 32
GO:0006450	regulation	regulation of translational fidelity	327
GO:0006457	signalling	protein folding	112
GO:0006468	signalling	protein phosphorylation	144
GO:0006470	signalling	protein dephosphorylation	3
GO:0006486	signalling	protein glycosylation	2, 3
GO:0006508	signalling	proteolysis	113, 3
GO:0006511	signalling	ubiquitin-dependent protein catabolic process	3
GO:0006614	regulation	SRP-dependent cotranslational protein targeting to membrane	285
GO:0006629	signalling	lipid metabolic process	142
GO:0006694	signalling	steroid biosynthetic process	74
GO:0006754	regulation	ATP biosynthetic process	3

... Table S4 continued

GO-ID	classification	GO description	internal COP-ID (ake- ###)
GO:0006811	signalling	ion transport	144, 3, 52
GO:0006812	signalling	cation transport	3
GO:0006813	signalling	potassium ion transport	144, 26
GO:0006814	signalling	sodium ion transport	24, 3
GO:0006820	signalling	anion transport	19
GO:0006821	signalling	chloride transport	19, 52
GO:0006826	signalling	iron ion transport	3
GO:0006836	neuron	neurotransmitter transport	101, 251, 31, 64
GO:0006855	signalling	drug transmembrane transport	251, 64
GO:0006869	signalling	lipid transport	74
GO:0006874	signalling	cellular calcium ion homeostasis	5
GO:0006879	signalling	cellular iron ion homeostasis	3
GO:0006884	signalling	cell volume homeostasis	19
GO:0006885	signalling	regulation of pH	3
GO:0006886	signalling	intracellular protein transport	14
GO:0006898	signalling	receptor-mediated endocytosis	102, 120, 131, 150, 232, 242, 259, 279, 46, 92
GO:0006911	signalling	phagocytosis, engulfment	52, 62, 82
GO:0006914	signalling	autophagy	193
GO:0006915	signalling	apoptotic process	3
GO:0006952	signalling	defense response	152, 2
GO:0007005	regulation	mitochondrion organization	5
GO:0007015	neuron	actin filament organization	120
GO:0007021	muscle	tubulin complex assembly	188
GO:0007026	muscle	negative regulation of microtubule depolymerization	25
GO:0007030	signalling	Golgi organization	135
GO:0007165	signalling	signal transduction	101, 112, 174, 180, 2, 3, 6
GO:0007166	signalling	cell surface receptor signaling pathway	6
GO:0007173	signalling	epidermal growth factor receptor signaling pathway	37
GO:0007186	signalling	G-protein coupled receptor signaling pathway	3, 6
GO:0007218	neuron	neuropeptide signaling pathway	6
GO:0007219	signalling	Notch signaling pathway	37, 46
GO:0007265	signalling	Ras protein signal transduction	180
GO:0007276	signalling	gamete generation	171
GO:0007281	morphology	germ cell development	276
GO:0007287	signalling	Nebenkern assembly	54
GO:0007297	signalling	ovarian follicle cell migration	32
GO:0007304	morphology	chorion-containing eggshell formation	32
GO:0007349	signalling	cellularization	46
GO:0007368	morphology	determination of left/right symmetry	3
GO:0007391	morphology	dorsal closure	46
GO:0007398	morphology	ectoderm development	37
GO:0007399	neuron	nervous system development	16, 37, 46, 51, 82
GO:0007400	neuron	neuroblast fate determination	82
GO:0007402	neuron	ganglion mother cell fate determination	82
GO:0007405	neuron	neuroblast proliferation	82
GO:0007406	neuron	negative regulation of neuroblast proliferation	82
GO:0007409	neuron	axonogenesis	16, 203, 51, 82
GO:0007411	neuron	axon guidance	82
GO:0007416	neuron	synapse assembly	82
GO:0007417	neuron	central nervous system development	82
GO:0007419	neuron	ventral cord development	174, 82
GO:0007420	neuron	brain development	3, 82
GO:0007422	neuron	peripheral nervous system development	32, 37, 82
GO:0007423	morphology	sensory organ development	82
GO:0007424	morphology	open tracheal system development	101
GO:0007426	morphology	tracheal outgrowth, open tracheal system	180
GO:0007427	signalling	epithelial cell migration, open tracheal system	101
GO:0007443	morphology	Malpighian tubule morphogenesis	174

... Table S4 continued

GO-ID	classification	GO description	internal COP-ID (ake- ###)
GO:0007465	neuron	R7 cell fate commitment	82
GO:0007476	morphology	imaginal disc-derived wing morphogenesis	180
GO:0007498	morphology	mesoderm development	174, 37, 5
GO:0007507	muscle	heart development	16, 2, 60
GO:0007517	muscle	muscle organ development	16, 71
GO:0007519	muscle	skeletal muscle tissue development	16, 71
GO:0007527	muscle	adult somatic muscle development	169
GO:0007528	neuron	neuromuscular junction development	203, 51
GO:0007552	morphology	metamorphosis	114
GO:0007553	morphology	regulation of ecdysteroid metabolic process	114
GO:0007591	morphology	molting cycle, chitin-based cuticle	114
GO:0007606	signalling	sensory perception of chemical stimulus	3
GO:0007616	neuron	long-term memory	22
GO:0007619	signalling	courtship behavior	82
GO:0008026	regulation	ATP-dependent helicase activity	162
GO:0008036	signalling	diuretic hormone receptor activity	6
GO:0008045	neuron	motor axon guidance	46
GO:0008049	signalling	male courtship behavior	6
GO:0008081	signalling	phosphoric diester hydrolase activity	33
GO:0008083	signalling	growth factor activity	3
GO:0008088	neuron	axon cargo transport	51, 62
GO:0008101	signalling	decapentaplegic signaling pathway	32
GO:0008104	signalling	protein localization	82
GO:0008137	regulation	NADH dehydrogenase (ubiquinone) activity	118
GO:0008138	signalling	protein tyrosine/serine/threonine phosphatase activity	3
GO:0008168	signalling	methyltransferase activity	6
GO:0008188	neuron	neuropeptide receptor activity	6
GO:0008233	signalling	peptidase activity	3
GO:0008234	signalling	cysteine-type peptidase activity	3
GO:0008284	signalling	positive regulation of cell proliferation	32
GO:0008285	signalling	negative regulation of cell proliferation	82
GO:0008293	signalling	torso signaling pathway	180
GO:0008355	neuron	olfactory learning	51
GO:0008356	morphology	asymmetric cell division	82
GO:0008373	signalling	sialyltransferase activity	3
GO:0008380	regulation	RNA splicing	203, 276
GO:0008417	signalling	fucosyltransferase activity	2
GO:0008504	signalling	monoamine transmembrane transporter activity	64
GO:0008508	signalling	bile acid:sodium symporter activity	24
GO:0008582	neuron	regulation of synaptic growth at neuromuscular junction	51
GO:0008587	morphology	imaginal disc-derived wing margin morphogenesis	37
GO:0008595	morphology	anterior/posterior axis specification, embryo	180
GO:0008898	signalling	homocysteine S-methyltransferase activity	2
GO:0009303	regulation	rRNA transcription	131
GO:0009306	signalling	protein secretion	46
GO:0009651	signalling	response to salt stress	155
GO:0009792	morphology	embryo development ending in birth or egg hatching	118, 131, 150, 155, 16, 169, 174, 193, 203, 21, 216, 232, 242, 255, 259, 276, 323, 334, 46, 51, 71, 92, 97
GO:0009881	signalling	photoreceptor activity	26
GO:0009966	signalling	regulation of signal transduction	180
GO:0009996	morphology	negative regulation of cell fate specification	32
GO:0010001	neuron	glial cell differentiation	82
GO:0010171	morphology	body morphogenesis	114, 120, 16, 18, 71
GO:0010466	signalling	negative regulation of peptidase activity	162
GO:0010468	signalling	regulation of gene expression	401
GO:0010629	signalling	negative regulation of gene expression	112, 37

... Table S4 continued

GO-ID	classification	GO description	internal COP-ID (ake- ###)
GO:0010866	signalling	regulation of triglyceride biosynthetic process	155
GO:0010867	signalling	positive regulation of triglyceride biosynthetic process	142
GO:0014036	neuron	neural crest cell fate specification	3
GO:0014812	muscle	muscle cell migration	60
GO:0015077	signalling	monovalent inorganic cation transmembrane transporter activity	3
GO:0015143	signalling	urate transmembrane transporter activity	21
GO:0015238	signalling	drug transmembrane transporter activity	251, 64
GO:0015269	signalling	calcium-activated potassium channel activity	26
GO:0015297	signalling	antiporter activity	3
GO:0015299	signalling	solute:hydrogen antiporter activity	3
GO:0015385	signalling	sodium:hydrogen antiporter activity	3
GO:0015662	regulation	ATPase activity, coupled to transmembrane movement of ions, phosphorylative mechanism	3
GO:0015672	signalling	monovalent inorganic cation transport	144, 3
GO:0015721	signalling	bile acid and bile salt transport	24
GO:0015842	neuron	synaptic vesicle amine transport	64
GO:0015844	signalling	monoamine transport	64
GO:0015872	signalling	dopamine transport	64
GO:0015893	signalling	drug transport	251, 64
GO:0015934	signalling	large ribosomal subunit	232
GO:0015992	signalling	proton transport	135, 3
GO:0016057	neuron	regulation of membrane potential in photoreceptor cell	26
GO:0016070	regulation	RNA metabolic process	55
GO:0016079	neuron	synaptic vesicle exocytosis	31
GO:0016202	muscle	regulation of striated muscle tissue development	71
GO:0016286	signalling	small conductance calcium-activated potassium channel activity	26
GO:0016301	signalling	kinase activity	144, 26
GO:0016307	signalling	phosphatidylinositol phosphate kinase activity	14, 2
GO:0016310	signalling	phosphorylation	144, 170, 26
GO:0016311	signalling	dephosphorylation	3
GO:0016319	neuron	mushroom body development	114, 52
GO:0016337	signalling	cell-cell adhesion	3
GO:0016360	morphology	sensory organ precursor cell fate determination	37
GO:0016477	morphology	cell migration	46
GO:0016567	signalling	protein ubiquitination	2
GO:0016568	morphology	chromatin modification	3
GO:0016746	signalling	transferase activity, transferring acyl groups	251
GO:0016757	signalling	transferase activity, transferring glycosyl groups	2, 3
GO:0016772	signalling	transferase activity, transferring phosphorus-containing groups	144
GO:0016791	signalling	phosphatase activity	3
GO:0016798	signalling	hydrolase activity, acting on glycosyl bonds	27
GO:0016820	signalling	hydrolase activity, acting on acid anhydrides, catalyzing transmembrane movement of substances	3
GO:0016887	regulation	ATPase activity	3
GO:0017017	signalling	MAP kinase tyrosine/serine/threonine phosphatase activity	3
GO:0017053	signalling	transcriptional repressor complex	37
GO:0017127	signalling	cholesterol transporter activity	74
GO:0018149	signalling	peptide cross-linking	3
GO:0018990	morphology	ecdysis, chitin-based cuticle	114
GO:0018996	morphology	molting cycle, collagen and cuticulin-based cuticle	114, 131, 46, 82, 92
GO:0019730	signalling	antimicrobial humoral response	114
GO:0019888	signalling	protein phosphatase regulator activity	267
GO:0019895	regulation	kinesin-associated mitochondrial adaptor activity	54
GO:0019896	neuron	axon transport of mitochondrion	54
GO:0019915	signalling	lipid storage	179, 19, 20, 22, 51, 5, 74

... Table S4 continued

GO-ID	classification	GO description	internal COP-ID (ake-###)
GO:0021884	neuron	forebrain neuron development	82
GO:0021905	neuron	forebrain-midbrain boundary formation	3
GO:0022008	neuron	neurogenesis	171, 267, 279, 46, 62
GO:0030001	signalling	metal ion transport	18
GO:0030097	signalling	hemopoiesis	18
GO:0030111	signalling	regulation of Wnt receptor signaling pathway	400, 60
GO:0030154	morphology	cell differentiation	71
GO:0030163	signalling	protein catabolic process	92
GO:0030239	muscle	myofibril assembly	16, 5, 82
GO:0030240	muscle	skeletal muscle thin filament assembly	203, 5
GO:0030289	signalling	protein phosphatase 4 complex	267
GO:0030301	signalling	cholesterol transport	74
GO:0030307	signalling	positive regulation of cell growth	32
GO:0030382	regulation	sperm mitochondrion organization	54
GO:0030414	signalling	peptidase inhibitor activity	162
GO:0030421	signalling	defecation	16
GO:0030707	signalling	ovarian follicle cell development	32
GO:0030916	signalling	otic vesicle formation	174
GO:0031016	morphology	pancreas development	60
GO:0031453	signalling	positive regulation of heterochromatin assembly	155
GO:0031618	signalling	nuclear centromeric heterochromatin	155
GO:0032012	signalling	regulation of ARF protein signal transduction	3
GO:0032474	morphology	otolith morphogenesis	174
GO:0034220	signalling	ion transmembrane transport	135, 3, 52
GO:0034605	signalling	cellular response to heat	155
GO:0034707	signalling	chloride channel complex	52
GO:0035023	signalling	regulation of Rho protein signal transduction	63
GO:0035050	morphology	embryonic heart tube development	3
GO:0035071	signalling	salivary gland cell autophagic cell death	323, 32
GO:0035072	signalling	ecdysone-mediated induction of salivary gland cell autophagic cell death	114
GO:0035098	signalling	ESC/E(Z) complex	3
GO:0035147	signalling	branch fusion, open tracheal system	101
GO:0035188	morphology	hatching	267
GO:0035199	signalling	salt aversion	3
GO:0035238	signalling	vitamin A biosynthetic process	64
GO:0035282	morphology	segmentation	32
GO:0035304	signalling	regulation of protein dephosphorylation	267
GO:0035307	signalling	positive regulation of protein dephosphorylation	142
GO:0035335	signalling	peptidyl-tyrosine dephosphorylation	3
GO:0035462	morphology	determination of left/right asymmetry in dien-cephalon	3
GO:0035469	morphology	determination of pancreatic left/right asymmetry	3
GO:0035556	signalling	intracellular signal transduction	184, 63
GO:0036065	signalling	fucosylation	2
GO:0040002	morphology	collagen and cuticulin-based cuticle development	18
GO:0040010	signalling	positive regulation of growth rate	102, 131, 150, 16, 171, 174, 203, 216, 232, 242, 255, 259, 2, 279, 46, 71, 82, 84, 92, 97
GO:0040017	signalling	positive regulation of locomotion	46
GO:0040018	morphology	positive regulation of multicellular organism growth	114, 174, 19
GO:0040020	morphology	regulation of meiosis	276
GO:0040026	morphology	positive regulation of vulval development	180
GO:0040034	morphology	regulation of development, heterochronic	114
GO:0040035	morphology	hermaphrodite genitalia development	120, 16, 171, 92
GO:0042048	neuron	olfactory behavior	55
GO:0042051	neuron	compound eye photoreceptor development	46
GO:0042063	neuron	gliogenesis	37
GO:0042074	morphology	cell migration involved in gastrulation	60

... Table S4 continued

GO-ID	classification	GO description	internal COP-ID (ake- ###)
GO:0042127	morphology	regulation of cell proliferation	276
GO:0042332	signalling	gravitaxis	6
GO:0042673	neuron	regulation of retinal cone cell fate specification	82
GO:0042676	neuron	compound eye cone cell fate commitment	82
GO:0042745	morphology	circadian sleep/wake cycle	6
GO:0042749	morphology	regulation of circadian sleep/wake cycle	6
GO:0042803	signalling	protein homodimerization activity	155, 32, 37
GO:0042981	signalling	regulation of apoptotic process	134
GO:0043005	neuron	neuron projection	6
GO:0043053	signalling	dauer entry	113
GO:0043066	signalling	negative regulation of apoptotic process	32
GO:0043086	signalling	negative regulation of catalytic activity	153
GO:0043152	signalling	induction of bacterial agglutination	2
GO:0043282	muscle	pharyngeal muscle development	71
GO:0043401	signalling	steroid hormone mediated signaling pathway	114
GO:0043524	neuron	negative regulation of neuron apoptotic process	51
GO:0043620	regulation	regulation of DNA-dependent transcription in response to stress	60
GO:0045087	signalling	innate immune response	233
GO:0045165	morphology	cell fate commitment	37
GO:0045179	signalling	apical cortex	82
GO:0045214	signalling	sarcomere organization	16, 5
GO:0045468	neuron	regulation of R8 cell spacing in compound eye	37
GO:0045475	neuron	locomotor rhythm	6
GO:0045500	neuron	sevenless signaling pathway	46
GO:0045572	signalling	positive regulation of imaginal disc growth	102
GO:0045664	neuron	regulation of neuron differentiation	82
GO:0045676	neuron	regulation of R7 cell differentiation	82
GO:0045746	signalling	negative regulation of Notch signaling pathway	32
GO:0045793	signalling	positive regulation of cell size	120
GO:0045892	regulation	negative regulation of transcription, DNA-dependent	37
GO:0045893	regulation	positive regulation of transcription, DNA-dependent	102
GO:0045900	regulation	negative regulation of translational elongation	285
GO:0045944	signalling	positive regulation of transcription from RNA polymerase II promoter	101, 114, 155
GO:0045980	signalling	negative regulation of nucleotide metabolic process	153
GO:0046008	signalling	regulation of female receptivity, post-mating	2
GO:0046331	signalling	lateral inhibition	18
GO:0046488	signalling	phosphatidylinositol metabolic process	14, 2
GO:0046692	signalling	sperm competition	2
GO:0046716	muscle	muscle cell homeostasis	16, 5
GO:0046843	morphology	dorsal appendage formation	32
GO:0046854	signalling	phosphatidylinositol phosphorylation	14, 2
GO:0046928	neuron	regulation of neurotransmitter secretion	31
GO:0046982	signalling	protein heterodimerization activity	101, 37
GO:0046983	signalling	protein dimerization activity	2
GO:0047291	signalling	lactosylceramide alpha-2, 3-sialyltransferase activity	3
GO:0048102	signalling	autophagic cell death	323, 32
GO:0048190	morphology	wing disc dorsal/ventral pattern formation	323, 37
GO:0048311	regulation	mitochondrion distribution	54
GO:0048368	morphology	lateral mesoderm development	3
GO:0048477	morphology	oogenesis	114, 32
GO:0048488	neuron	synaptic vesicle endocytosis	134, 46
GO:0048500	signalling	signal recognition particle	285
GO:0048512	morphology	circadian behavior	6
GO:0048514	morphology	blood vessel morphogenesis	63
GO:0048644	muscle	muscle organ morphogenesis	5

... Table S4 continued

GO-ID	classification	GO description	internal COP-ID (ake- ###)
GO:0048666	neuron	neuron development	37
GO:0048676	neuron	axon extension involved in development	203
GO:0048738	muscle	cardiac muscle tissue development	16
GO:0048741	muscle	skeletal muscle fiber development	71
GO:0048749	neuron	compound eye development	32, 37, 51
GO:0048752	morphology	semicircular canal morphogenesis	174
GO:0048755	neuron	branching morphogenesis of a nerve	3
GO:0048812	neuron	neuron projection morphogenesis	3
GO:0048813	neuron	dendrite morphogenesis	37, 51, 82
GO:0048844	morphology	artery morphogenesis	6
GO:0048846	neuron	axon extension involved in axon guidance	14
GO:0048854	neuron	brain morphogenesis	37, 55
GO:0048885	neuron	neuromast deposition	82
GO:0048886	neuron	neuromast hair cell differentiation	82
GO:0048920	neuron	posterior lateral line neuromast primordium mi- gration	162
GO:0050768	neuron	negative regulation of neurogenesis	37
GO:0050771	signalling	negative regulation of axonogenesis	82
GO:0050790	signalling	regulation of catalytic activity	3, 63
GO:0050830	signalling	defense response to Gram-positive bacterium	153
GO:0050909	signalling	sensory perception of taste	82
GO:0050914	signalling	sensory perception of salty taste	3
GO:0051124	neuron	synaptic growth at neuromuscular junction	31
GO:0051216	morphology	cartilage development	174, 3
GO:0051260	signalling	protein homooligomerization	3
GO:0051403	signalling	stress-activated MAPK cascade	155
GO:0051608	signalling	histamine transport	64
GO:0055002	muscle	striated muscle cell development	71
GO:0055059	neuron	asymmetric neuroblast division	82
GO:0055060	neuron	asymmetric neuroblast division resulting in gan- glion mother cell formation	82
GO:0060028	neuron	convergent extension involved in axis elongation	400, 60
GO:0060029	morphology	convergent extension involved in organogenesis	60
GO:0060037	morphology	pharyngeal system development	174
GO:0060041	neuron	retina development in camera-type eye	64
GO:0060052	neuron	neurofilament cytoskeleton organization	51
GO:0060385	neuron	axonogenesis involved in innervation	82
GO:0060438	morphology	trachea development	174
GO:0060857	neuron	establishment of glial blood-brain barrier	152
GO:0060971	morphology	embryonic heart tube left/right pattern formation	3
GO:0061327	morphology	anterior Malpighian tubule development	174
GO:0070278	signalling	extracellular matrix constituent secretion	174
GO:0070527	signalling	platelet aggregation	3
GO:0070829	signalling	heterochromatin maintenance	155
GO:0070983	neuron	dendrite guidance	82
GO:0071436	signalling	sodium ion export	24, 3
GO:0071470	signalling	cellular response to osmotic stress	155
GO:0071688	muscle	striated muscle myosin thick filament assembly	203, 5
GO:0071805	signalling	potassium ion transmembrane transport	144, 26
GO:0072320	signalling	volume-sensitive chloride channel activity	19
GO:0072583	signalling	clathrin-mediated endocytosis	46
GO:0090305	signalling	nucleic acid phosphodiester bond hydrolysis	131
GO:2000223	signalling	regulation of BMP signaling pathway involved in heart jogging	3

Table S5. Supplementary Table related to Figures 1 and 4. Description of the 85 clusters in set L' .

internal ID	Flybase ID	Symbol	Name	Description	Ensembl ID	Symbol	Name	Description	Wormbase ID	Symbol	Name	Description	Supplementary References
			Fly				Fish				Worm		
Morphology related													
ake-114	FBgn0000448, FBgn0000568	Hr46, Eip75B	Hormone receptor-like in 46, Ecdysone-induced protein 75B	DHR3, ecdysone-inducible gene product involved in metamorphosis	ENSDARG00000043059	VDR	vitamin D receptor		WBGene00003622	nhr-23	nuclear hormone receptor homolog	required in all larval molts for hypodermal expression and restricted to epidermal cells	[177, 113, 60, 112, 42]
ake-46	FBgn0000414	Dab	Disabled		ENSDARG00000053091, ENSDARG00000031761	Dab2, BDP1	disabled 2, subunit of RNA polymerase III transcription initiation factor IIIB		WBGene00000894	dab-1	disabled ortholog	required for normal molting and meiotic arrest	[189, 65, 33, 63, 108, 156, 164]
ake-179	FBgn0264490	Eip93F	Ecdysone-induced protein 93F		ENSDARG00000070907	lcor	ligand dependent nuclear receptor corepressor		WBGene00011315	mbr-1			[13, 184, 56, 150]
ake-174	FBgn0026562	sparc	BM-40-SPARC		ENSDARG00000019353, ENSDARG00000074989	sparc, sparc1	secreted acidic cysteine rich glycoprotein, SPARC-like 1		WBGene00003893	ost-1	ortholog of the conserved basement membrane glycoprotein component osteonectin/-SPARC/BM-40 collagen	required for embryonic and larval development	[97, 18, 180, 58, 27, 200, 135]
ake-14	FBgn0259244				ENSDARG00000037845, ENSDARG00000039462, ENSDARG00000040118, ENSDARG00000054753, ENSDARG00000058960, ENSDARG00000060893, ENSDARG00000068218, ENSDARG00000076163	Col9a3, col10a1, col8a2, otolin 1	collagen		WBGene00000708	col-135			[111, 45, 3, 109]
ake-101	FBgn0039411	dys	dysfusion		ENSDARG00000055752, ENSDARG00000061920, ENSDARG00000087753	npas4	neuronal PAS domain protein 4 (a, b)		WBGene00000521	cky-1	encodes a member of the basic helix-loop-helix/Per-Arnt-Sim (bHLH-PAS) protein family		[101, 102]
ake-63	FBgn0035308				ENSDARG00000040614, ENSDARG00000076480, ENSDARG00000040614	Sestd1, mcf2l	SEC14 and spectrin domains, mcf.2 cell line derived transforming sequence-like b		WBGene00010111, WBGene00018887, WBGene00194671				[144, 214]
ake-32	FBgn0259176	bun	bunched		ENSDARG00000038306, ENSDARG00000075666	Tsc22d 3)	(1, TSC22 domain family, member (1, 3)		WBGene00016272, WBGene00011824				[64, 69, 7, 107]
Muscle related													
ake-16	FBgn0004028	wupA	wings up A	troponin I (TnI)	ENSDARG00000005841, ENSDARG00000013752, ENSDARG00000029069, ENSDARG00000029995, ENSDARG00000035958, ENSDARG00000042559, ENSDARG00000045592, ENSDARG00000052708, ENSDARG00000073766	tnni 2a.2, troponin I, skeletal, 2a.3, 2a.4, tal, fast, tandem 2b.2, 2b.1, duplicate 1c, 2a.1, 1b, 1d)			WBGene00006584, WBGene00006585, WBGene00006586, WBGene00006764	Tni- (1,3,4), unc-76	troponin I	encodes a member of the troponin family that affects body morphology, locomotion, egg laying, and epithelial morphogenesis	[37, 190, 73, 80, 157, 137, 132, 154, 88, 61, 194, 152]

... Table S5 continued

internal ID	Flybase ID	Symbol	Name	Description	Ensembl ID	Symbol	Name	Description	Wormbase ID	Symbol	Name	Description	References in Supplementary file 6
			Fly				Fish				Worm		
ake-5	FBgn0004169	up	upheld	troponin (TnT)	T ENSDARG00000002988, ENSDARG00000030270, ENSDARG00000037954, ENSDARG00000045822, ENSDARG00000068457	tnnt (2d, 3a, 2e, 3b)	Troponin T (2d, 2e -cardiac; 3a, 3b -skeletal; typ 1 -skeletal)		WBGene00006588	Tnt-3	troponin T	orthologous to the human gene TROPONIN T (TNNT2; OMIM:191045), which when mutated leads to disease	[37, 190, 73, 80, 157, 137, 132, 154, 88, 61, 194, 152]
ake-71	FBgn0002922	nau	nautilus		ENSDARG00000007277, ENSDARG00000029830, ENSDARG00000030110	Myf (5, 6, d1)	myogenic factor (5, 6, d1)		WBGene00001948	hlh-1	ortholog of the myogenic regulatory factor (MRF) subgroup of basic helix-loop-helix (bHLH) transcription factor	required during embryogenesis for proper bodywall muscle development and function, but not for bodywall muscle cell fate specification	[54, 8, 30, 12, 4, 143, 208, 125, 195, 170]
ake-33	FBgn0003016	osp	outspread		ENSDARG00000006385, ENSDARG00000073998, ENSDARG00000074705	Triobp, X	TRIO and F-actin binding protein, myosin phosphatase Rho interacting protein		WBGene00008666				[141]
ake-120	FBgn0035411	Girdin	Girdin		ENSDARG00000076189, ENSDARG00000078440	Ccdc88 (b, Aa)	coiled-coil domain containing (88B, 88Aa)		WBGene00013082				[162, 205, 159]
ake-188	FBgn0039869				ENSDARG00000016754	tbca	tubulin cofactor a		WBGene00021475				[103, 222]
ake-225	FBgn0037737	Pnn	Pinin		ENSDARG00000015851	pnn	pinin	desmosome associated protein	WBGene00011308				[204, 104, 210, 89, 90]
ake-169	FBgn0030699				ENSDARG00000010400	lrrfip2	leucine rich repeat (in FLII) interacting protein 2		WBGene00018998	flap-1			[43]
ake-113	FBgn0036196				ENSDARG00000040277, ENSDARG00000075172	Fbxo (32, 25)	F-box protein (32, 25)		WBGene00008439	mfb-1	F-box protein		[41, 99, 72]
ake-144	FBgn0030745				ENSDARG00000024047, ENSDARG00000041562	Tmem38a, X	transmembrane protein 38A, Uncharacterized protein		WBGene00013255, WBGene00013268				[212]
ake-68	FBgn0035772	Sh3beta	SH3-binding, glutamic acid-rich protein		ENSDARG00000021633, ENSDARG00000036878, ENSDARG00000058302	sh3bgr, sh3bgrl2	SH3 domain binding glutamic acid-rich protein, like		WBGene00013666				[140]
Neuron related													
ake-267	FBgn0030208	PPP4R2r	Protein phosphatase 4 regulatory subunit 2-related protein		ENSDARG00000026540	ppp4r2a	protein phosphatase 4 regulatory subunit 2a		WBGene00017064	Ppfr-2			[17]
ake-251	FBgn0015323	VACHT			ENSDARG00000006356, ENSDARG000000090189	Slc18a3a, X	solute carrier family 18 (vesicular acetylcholine) member 3a, Uncharacterized protein		WBGene00000481, WBGene00006756	Cha-1, unc-17	Cha-1 encodes a choline acetyltransferase that synthesizes acetylcholine, unc-17 encodes a synaptic vesicle acetylcholine transporter (VACHT), share a common promoter	expressed in neurons, and is required for viability, normal growth, locomotion, and sensitivity to acetylcholinesterase inhibitors	[44, 83, 51]

... Table S5 continued

internal ID	Flybase ID	Symbol	Name	Description	Ensembl ID	Symbol	Name	Description	Wormbase ID	Symbol	Name	Description	References in Supplementary file 6
			Fly				Fish					Worm	
ake-25	FBgn0051057	tau			ENSDARG00000055052, ENSDARG00000087616, ENSDARG00000089314	Mapt (a, b), tau a	microtubule-associated protein (a, b), microtubule-associated protein tau		WBGene00004212	ptl-1	microtubule-binding protein homologous to members of the tau/MAP2/MAF4 subfamily of microtubule-associated proteins	associated with several neurodegenerative disorders	[31, 55, 98, 16, 62, 114]
ake-82	FBgn0004595	pros	prospero		ENSDARG000000041952, ENSDARG00000055158	Prox 1a	(2, prospero homeobox (2, 1a)		WBGene00000448	Ceh-26	a protein that contains a prospero-related homeodomain		[46, 161, 6, 219]
ake-276	FBgn0039977				ENSDARG00000056235	smndc1	survival motor neuron domain containing 1		WBGene00004891	smr-1			[191]
ake-31	FBgn0041605	cpx	complexin		ENSDARG000000018997, ENSDARG00000061918	cplx2	Complexin 2, like		WBGene00022271	cpx-1			[142, 70, 133, 81, 34, 95, 218]
ake-64	FBgn0260964	Vmat	Vesicular monoamine transporter		ENSDARG000000015110, ENSDARG00000078157	Slc18a2, X	solute carrier family 18 (vesicular monoamine) member 2, solute carrier family 18 (vesicular monoamine transporter) member 1		WBGene00000295	cat-1	encodes a synaptic vesicular monoamine transporter	required for the presence of dopamine and serotonin in nerve terminals	[50]
ake-62	FBgn0040395	Unc-76			ENSDARG000000022260, ENSDARG00000023174, ENSDARG00000073784		fasciculation and elongation protein zeta (1, 2)		WBGene00006808	unc-76	coiled-coil protein that belongs to the FEZ (fasciculation and elongation protein; zygin/zeta-1) family of proteins	required for normal axonal outgrowth and hence, normal locomotion and is present in all axons throughout development	[119, 199, 10, 67, 38, 15, 139]
ake-37	FBgn0000591, FBgn0002631, FBgn0002633, FBgn0002735, FBgn0032741	E(spl), HLHm5, HLHm7, HLHm-beta, HLH-mgamma, Side	Enhancer of split, E(spl) region transcript m (5, 7, mbeta, mgamma), similar to Deadpan	Enhancer of split, E(spl) region transcript	ENSDARG00000007097, ENSDARG000000016363, ENSDARG000000019335, ENSDARG000000068168, ENSDARG00000069675	her (13, 8a, 2.2, 8.2), hes (6, 2.2)	Hairy-related (13, 8a, 8.2), hairy-related 8a, hairy and enhancer of split 2		WBGene00003008				[207, 9, 66, 115, 220, 57, 181, 39, 85]
ake-73	FBgn0032949, FBgn0052225	Lamp1, X			ENSDARG000000077944, ENSDARG000000092610		lysosomal-associated membrane protein 1		WBGene00015471	Imp-2	lysosomal associated membrane glycoprotein transmembrane protein		[68, 59]
ake-21	FBgn0031213, FBgn0034365	Galectin, X			ENSDARG000000092923, ENSDARG000000093059, ENSDARG000000037613, ENSDARG000000044001, ENSDARG000000077850	Galectin, lgals3l	Galactoside-binding		WBGene00002266, WBGene00002272, WBGene00008284, WBGene00017080, WBGene00002271, WBGene00002270, WBGene00002273, WBGene00002268	Lec-9,8,7,10,5,3			[87]
ake-155	FBgn0050420	Atf-2	Activating transcription factor-2		ENSDARG000000056156, ENSDARG000000074697	Npdc1, X	neural proliferation, differentiation and control 1		WBGene00000277	cab-1		involved in synaptic regulation	[1]

... Table S5 continued

internal ID	Flybase ID	Symbol	Name	Description	Ensembl ID	Symbol	Name	Description	Wormbase ID	Symbol	Name	Description	References in Supplementary file 6
	Fly				Fish				Worm				
ake-6	FBgn0030437, FBgn0033932, FBgn0052843, FBgn0260753	Hector, Dh44-R1, Dh31-R1, Pdfr	Hector, Diuretic hormone (44, 31) receptor / Pigment-dispersing factor receptor	Diuretic hormone 31 receptor / Pigment-dispersing factor receptor	ENSDARG00000003989, ENSDARG00000006678, ENSDARG00000011473, ENSDARG00000011571, ENSDARG00000018418, ENSDARG00000020957, ENSDARG00000021186, ENSDARG00000028845, ENSDARG00000062377, ENSDARG00000018494	Crhr1, pth2r, calcr1a, calcr1b, pth1rb, pth1ra, calcr	corticotropin, parathyroid, calcitonin hormone receptor		WBGene00007664, WBGene00014035		G-protein-coupled receptor (GPCR) that is a member of the secretin family		[44, 197, 117, 25, 100, 145]
ake-203	FBgn0036641	Smn	survival motor neuron		ENSDARG00000022045, ENSDARG00000059601, ENSDARG00000060326, ENSDARG00000060434, ENSDARG00000060805, ENSDARG00000014939, ENSDARG00000019753, ENSDARG00000023546, ENSDARG00000091306	map	microtubule-associated protein (1Ab, 1Aa, 1S, 1b)		WBGene00004887	smn-1	encodes a homolog of human cle SMN		[53]
ake-51	FBgn0259108	futsch							WBGene00007966, WBGene00009113, WBGene00009306				[79, 94, 175]
ake-26	FBgn0029761, SK		small conductance calcium-activated potassium channel			Kenn (1a, 1b, 3)	(1a, potassium intermediate/small conductance calcium-activated channel subfamily N member (2, 3, 1b, 1a)		WBGene00008570	Kcnl-2			[47]

... Table S5 continued

Internal ID	Flybase ID	Symbol	Name	Description	Ensembl ID	Symbol	Name	Description	Wormbase ID	Symbol	Name	Description	References in Supplementary file 6
	Fly				Fish				Worm				
ake-3	FBgn0034286, FBgn0034723, FBgn0037993, FBgn0040726, FBgn0040823, FBgn0051361, FBgn0052600, FBgn0053202, FBgn0053481, FBgn0085400	Dpr (13, 15, 6, 17, 8, 11, 7)		defective proboscis extension response	ENSDARG00000015607, ENSDARG000000017984, ENSDARG00000028912, ENSDARG00000029838, ENSDARG00000034092, ENSDARG00000034466, ENSDARG00000040669, ENSDARG00000041828, ENSDARG00000043445, ENSDARG00000045875, ENSDARG00000053027, ENSDARG00000054655, ENSDARG00000055245, ENSDARG00000057013, ENSDARG00000057886, ENSDARG00000058564, ENSDARG00000070065, ENSDARG00000070223, ENSDARG00000071050, ENSDARG00000076144, ENSDARG00000076981, ENSDARG00000078180, ENSDARG00000078824, ENSDARG00000079487, ENSDARG00000086166, ENSDARG00000086301, ENSDARG00000086919, ENSDARG00000087148, ENSDARG00000088811, ENSDARG00000089316, ENSDARG00000089404, ENSDARG00000089710, ENSDARG00000089993, ENSDARG00000090329, ENSDARG00000090874, ENSDARG00000091074, ENSDARG00000091388, ENSDARG00000091579, ENSDARG00000091661, ENSDARG00000091712, ENSDARG00000092031, ENSDARG00000092322, ENSDARG00000092520, ENSDARG00000093279, ENSDARG00000093880, ENSDARG00000093987, ENSDARG00000094374, ENSDARG00000094391, ENSDARG00000094524, ENSDARG00000095133, ENSDARG00000095134, ENSDARG00000095772	cadm3, unknown	cell adhesion molecule 3, Uncharacterized proteins	WBGene00007851, WBGene00021117, WBGene00021290, WBGene00021291, WBGene00006985	Clec- (245, 126, 122, zig-8	predicted secreted proteins	member of the immunoglobulin superfamily	[155, 71]	
ake-162	FBgn0039155	kal-1	Kallmann syndrome ortholog	1	ENSDARG00000004932, ENSDARG00000012896, ENSDARG00000087064	Kal1 (b, a)	Kallmann syndrome 1 (b, a) sequence	WBGene00002181	kal-1	cell surface protein that contains a WAP-type protease inhibitor domain	ortholog of human KAL-1, required for epithelial morphogenesis and axon branching	[178, 202]	
ake-84	FBgn0037443				ENSDARG00000046007, ENSDARG00000060954	Tmcc2, X	transmembrane and coiled-coil domain family (2, 1b)	WBGene00015800				[221, 84]	

... Table S5 continued

internal ID	Flybase ID	Symbol	Name	Description	Ensembl ID	Symbol	Name	Description	Wormbase ID	Symbol	Name	Description	References in Supplementary file 6
			Fly				Fish					Worm	
ake-24	FBgn0030638, FBgn0030756				ENSDARG00000009131, ENSDARG00000030588, ENSDARG00000095660	Slc10a3	(4, solute carrier family 10 (sodium/bile acid cotransporter family) member (4, 3, 1)		WBGene00022135				[120, 167]
ake-112	FBgn0035103, FBgn0042101	Vdup1, X	Vitamin D up-regulated protein 1	[[3]]	ENSDARG00000032444, ENSDARG00000067664		Uncharacterized proteins		WBGene00013043	arrd-16			[123, 28]
ake-224	FBgn0032221				ENSDARG00000035175	schip1	schwannomin interacting protein 1		WBGene00018074				[74, 134]
ake-334	FBgn0035676				ENSDARG00000000588	grasp	GRP1 (general receptor for phosphoinositides 1)-associated scaffold protein		WBGene00009272				[110]
Signalling related													
ake-74	FBgn0035028	start1			ENSDARG00000017809, ENSDARG00000074280	Startd3, X	START domain containing 3, StAR-related lipid transfer (START) domain containing 4		WBGene00017826, WBGene0001050				[11, 176]
ake-134	FBgn0261722	fwe	flower		ENSDARG00000090818		calcium channel flower domain containing 1		WBGene00008973				[173, 168, 122, 129]
ake-152	FBgn0039014				ENSDARG00000057322	tmem47	transmembrane protein 47		WBGene00006875	vab-9	claudin homolog	Claudins are in-orthologous tegral membrane to human proteins with brain cell mem- four transmembrane protein 1 brane sequences (BCMP1)	[185, 91, 186]
ake-52	FBgn0015558, FBgn0261444	tty	tweety		ENSDARG00000007678, ENSDARG00000010727, ENSDARG00000016934, ENSDARG00000034473	Ttyh21, 23a)	(3b, tweety homolog (3b, 2, 2 like, 3a)		WBGene00009632	ttyh-1			[23, 138]
ake-18	FBgn0029506, FBgn0033124, FBgn0033127, FBgn0033128, FBgn0033129, FBgn0033130, FBgn0033133, FBgn0033134, FBgn0033135, FBgn0043550	Tsp42E	Tetraspanin (e, c, f, 42E (e, c, f, g, g, h, i, h, i, k, l, n), k, l, n), Tetraspanin 68C		ENSDARG00000015015, ENSDARG00000025757, ENSDARG00000068233	Tspan18b, X, X	tetraspanin 18b, X, CD53 molecule		WBGene00006637, WBGene00006643, WBGene00006635, WBGene00006642, WBGene00006641, WBGene00006639	Tsp-(11, 17, 9, 16, 15, 13)			[93, 149, 29, 148]

... Table S5 continued

Internal ID	Flybase ID	Symbol	Name	Description	Ensembl ID	Symbol	Name	Description	Wormbase ID	Symbol	Name	Description	References in Supplementary file 6
	Fly				Fish				Worm				
ake-19	FBgn0035696, Best (2, Bestrophin 1)			(2, Bestrophin 2/1)	ENSDARG00000078331, Best (1, 4, Bestrophin (1, 4, ENSDARG00000078455, 2) ENSDARG00000079163				WBGene00015288, orthologous to the human gene WBGene00007404, VITELLIFORM WBGene00015628, MACULAR WBGene00007808, DYSTROPHY WBGene00007988, PROTEIN WBGene00008185, (VMD2) WBGene00008186, WBGene00008821, WBGene00011258, WBGene00020559, WBGene00011868, WBGene00021368, WBGene00013520, WBGene00013921, WBGene00022797, WBGene00014102, WBGene00014103			[35, 36, 193]	
ake-22	FBgn0037913	fabp	fatty acid bindin protein		ENSDARG00000007697, Fbap(7a, 3, fatty acid bind- ENSDARG00000023290, 7b) crabp1 ing protein (7 ENSDARG00000034650, (b, a) - brain, 3 - ENSDARG00000035904, muscle), cellular ENSDARG00000045926 retinoic acid binding protein 1 (a, b)			WBGene00002259, Lbp-(7, intracellular predicted to WBGene00002260, 8, 6, 5, fatty acid bind- function as an WBGene00002258, 9) ing protein intracellular WBGene00002257, (iFABP) transporter for WBGene00021486 small hydrophobic molecules such as lipids and steroid hormones			[2, 151, 147]		
ake-102	FBgn0036318		Wbp2 ortholog		ENSDARG00000056605	Wbp2	WW domain binding protein 2		WBGene00015955, WBGene00008404				[223]
ake-180	FBgn0021818	cnk	connector en- hancer of ksr		ENSDARG00000074236				WBGene00000564	cnk-1	protein that con- tains a SAM do- main, a PDZ do- main, and a PH domain		[40, 20, 198, 174]
ake-285	FBgn0038808	Srp14	Signal recog- nition particle protein 14		ENSDARG00000088272	srp14	signal recogni- tion particle 14		WBGene00017799				[49, 14]
ake-401	FBgn0030711				ENSDARG00000021112	c1d	C1D nuclear receptor co- repressor		WBGene00021785				[215]
ake-55	FBgn0032156, FBgn0034181				ENSDARG00000042892	paip1	poly(A) binding protein interact- ing protein 1		WBGene00018405				[130]
ake-60	FBgn0033166	Eaf	ELL-associated factor		ENSDARG00000010432, ENSDARG00000070795	Eaf (2, 1)	ELL associated factor (2, 1)		WBGene00017011	eaf-1			[187, 126, 21, 22, 19, 118, 169, 131, 127] [171]
ake-400	FBgn0035148				ENSDARG00000059177	tax1bp3	Tax1 (human T-cell leukemia virus type I) binding protein 3		WBGene00016678				
ake-20	FBgn0034345				ENSDARG00000013655, ENSDARG00000027154, ENSDARG00000042548	Tpd52l (1, tumor protein D52-like (2b, 2a, 1)			WBGene00008745				[24]
ake-92	FBgn0037299				ENSDARG00000028391, ENSDARG00000031836, ENSDARG00000039319	vps37	vacuolar protein sorting 37 ho- molog (B, C)		WBGene00016990	vps-37	endosomal sort- ing ESCRT-I complex that functions in endosomal sorting of mono- ubiquitinated membrane proteins		[160, 76]
ake-233	FBgn0036847				ENSDARG00000070512	tnrc5	trinucleotide re- peat containing 5		WBGene00007531				[203]

... Table S5 continued

internal ID	Flybase ID	Symbol	Name	Description	Ensembl ID	Symbol	Name	Description	Wormbase ID	Symbol	Name	Description	References in Supplementary file 6
			Fly				Fish				Worm		
ake-142	FBgn0036090				ENSDARG00000036064	Cnep1r1	CTD nuclear envelope phosphatase 1 regulatory subunit 1		WBGene00011828				[77]
ake-54	FBgn0262872	milt	milton		ENSDARG00000041304, ENSDARG00000078872	trak2	Regulation related trafficking protein kinesin binding (2, 1)		WBGene00020838				[98, 121, 201]
ake-11	FBgn0034067, FBgn0039648, FBgn0261534		l(2)34Fc lethal (2) 34Fc		ENSDARG00000060622, ENSDARG00000069103, ENSDARG00000093957				WBGene00007339, WBGene00007545, WBGene00013292				[166]
ake-118	FBgn0011455		l(3)neo18 lethal (3) neo18		ENSDARG00000070824	ndufb5	NADH dehydrogenase (ubiquinone) 1 beta subcomplex 5		WBGene00016118	ortholog of the NDUF5/SGDH subunit of the mitochondrial NADH dehydrogenase (ubiquinone) complex (complex I)			[192, 163]
ake-131	FBgn0262116				ENSDARG00000069336, ENSDARG00000069336, ENSDARG00000069461	Rnasek (b, a)	(b, ribonuclease, RNase K (b, a)		WBGene00016721, WBGene00009881				[106]
ake-135	FBgn0032833, FBgn0033020		Cytochrome c oxidase subunit IV		ENSDARG00000022509, ENSDARG00000032970	Cox4i (2, 1)	Cytochrome c oxidase subunit IV isoform (2, 1)		WBGene00012354				[146, 92, 183, 5, 165]
ake-150	FBgn0260407	mRpS23	mitochondrial Ribosomal protein S23		ENSDARG00000033973	Mrps23	mitochondrial ribosomal protein S23		WBGene00014224				[179, 158, 26, 136, 188]
ake-153	FBgn0040660				ENSDARG00000044092, ENSDARG00000067975	Atpif1 (b, a)	(b, ATPase inhibitory factor 1 (b, a)		WBGene00015248	mai-2	orthologous to mitochondrial intrinsic ATPase inhibitor protein IF(1))	blocks reverse action (ATP hydrolysis) by F(0)F(1)-ATPase when its (normally required) proton gradient is lost	[32, 96, 182]
ake-170	FBgn0033373				ENSDARG00000007181	nadk2	NAD kinase 2 mitochondrial polymerase (RNA) III (DNA directed) polypeptide G, like a		WBGene00012463				[86]
ake-171	FBgn0053051				ENSDARG00000012044, ENSDARG00000024687	polr3gla, polr3g			WBGene00021638				[206, 172, 209]
ake-216	FBgn0260460	mRpS34	mitochondrial ribosomal protein S34		ENSDARG00000057910	mrps34	mitochondrial ribosomal protein S34		WBGene00010905				[179, 158, 26, 136, 188]
ake-232	FBgn0037566	mRpL1	mitochondrial ribosomal protein L1		ENSDARG00000054606	mrpl1	mitochondrial ribosomal protein L1		WBGene00017997				[179, 158, 26, 136, 188]
ake-242	FBgn0031357	mRpL48	mitochondrial ribosomal protein L48		ENSDARG00000035167	mrpl48	mitochondrial ribosomal protein L48		WBGene00016989				[179, 158, 26, 136, 188]
ake-255	FBgn0038426	mRpS33	mitochondrial ribosomal protein S33		ENSDARG00000020015	mrps33	mitochondrial ribosomal protein S33		WBGene00009013				[179, 158, 26, 136, 188]

... Table S5 continued

internal ID	Flybase ID	Symbol	Name	Description	Ensembl ID	Symbol	Name	Description	Wormbase ID	Symbol	Name	Description	References in Supplementary file 6
	Fly					Fish					Worm		
ake-259	FBgn0032053	mRpL51	mitochondrial ribosomal protein L51		ENSDARG00000041340		mitochondrial ribosomal protein L51		WBGene00011740				[179, 158, 26, 136, 188]
ake-97	FBgn0034986	mRpS17	mitochondrial ribosomal protein S17		ENSDARG00000054164		mitochondrial ribosomal protein S17		WBGene00015487				[179, 158, 26, 136, 188]
ake-327	FBgn006411				ENSDARG00000056855		glutamyl-tRNA(Gln) amidotransferase subunit C		WBGene00013433				[52, 153]
ake-160	FBgn0038400				ENSDARG00000039374		apolipoprotein O-like		WBGene00019333	moma-1			[78]
ake-217	FBgn0025820	JTBR			ENSDARG00000070150		jumping translocation breakpoint		WBGene00002180	jtr-1	predicted trans-membrane protein that is related to the conserved jumping translocation breakpoint (JTB) family of proteins		[105]
ake-323	FBgn0037700				ENSDARG00000059357	sarnp	SAP domain containing ribonucleoprotein		WBGene00021813				[116, 213]
ake-193	FBgn0032200				ENSDARG00000040822, ENSDARG00000086461	Fundc 2)	(1, FUN14 domain containing (1, 2)		WBGene00011528				[211, 128]
Others													
ake-184	FBgn0031413				ENSDARG00000086655, ENSDARG00000089374		chromosome 3 open reading frame 83		WBGene00021131				
ake-185	FBgn0032488				ENSDARG00000020611		chromosome 19 open reading frame 47		WBGene00010002				

... Table S5 continued

internal ID	Flybase ID	Symbol	Name	Description	Ensembl ID	Symbol	Name	Description	Wormbase ID	Symbol	Name	Description	References in Supplementary file 6
	Fly				Fish				Worm				
ake-2	FBgn0016675, FBgn0031273, FBgn0031910, FBgn0040093, FBgn0040097, FBgn0040099, FBgn0040102, FBgn0040104, FBgn0040106, FBgn0040107, FBgn0053532, FBgn0053533, FBgn0259230, FBgn0259958, FBgn0262357		Lectin-(galC1, 46Ca, 30A, 28C, 24Db, 24A, 21Cb, 21Ca, 22C), Seminal fluid protein 24F	Galactose-specific C-type lectin, lectin-(46Ca, 30A, 28C, 24Db, 24A, 21Cb, 21Ca, 22C), Seminal fluid protein 24F	ENSDARG00000014624, ENSDARG00000021678, ENSDARG00000027399, ENSDARG00000034330, ENSDARG00000036745, ENSDARG00000038551, ENSDARG00000041159, ENSDARG00000041389, ENSDARG00000043736, ENSDARG00000043768, ENSDARG00000043769, ENSDARG00000045867, ENSDARG00000053113, ENSDARG00000058023, ENSDARG00000067495, ENSDARG00000070414, ENSDARG00000070850, ENSDARG00000070851, ENSDARG00000075003, ENSDARG0000007503, ENSDARG00000075634, ENSDARG00000076541, ENSDARG00000077136, ENSDARG00000077692, ENSDARG00000078750, ENSDARG00000079039, ENSDARG00000079107, ENSDARG00000086100, ENSDARG00000087115, ENSDARG00000088252, ENSDARG00000088614, ENSDARG00000088919, ENSDARG00000089758, ENSDARG00000090092, ENSDARG00000091095, ENSDARG00000091460, ENSDARG00000092749, ENSDARG00000092837, ENSDARG00000093391, ENSDARG00000093673, ENSDARG00000093909, ENSDARG00000094184, ENSDARG00000094225, ENSDARG00000094453, ENSDARG00000094750, ENSDARG00000095091, ENSDARG00000095335, ENSDARG00000095481, ENSDARG00000095757, ENSDARG00000013489	ly75, Un- lymphocyte antigen 75, Un- characterized proteins			WBGene00015631, Clec-(89, WBGene00009517, 167, 166, WBGene00009518, 172, 209, WBGene00018910, 174, 83, WBGene00044719, 84, 210, WBGene00021224, 187, 186) WBGene00021580, WBGene00021879, WBGene00021895, WBGene00023484, WBGene00022261, WBGene00014137, WBGene00014138		[196]		
ake-279	FBgn0030061					gltscr2	glioma tumor suppressor candidate region gene 2		WBGene00012692				[216, 217]
ake-27	FBgn0000078	Amy-d	Amylase distal		ENSDARG00000009443, ENSDARG00000030357				WBGene00008220				[82]

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