Transposable element ‘roo’ attaches to nuclear matrix of the 
Drosophila melanogaster

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Abstract

The genome of eukaryotes is organized into structural units of chromatin loops. This higher order organization is supported by a nuclear skeleton called the nuclear matrix. The genomic DNA associated with the nuclear matrix is called the matrix associated region (MAR). Only a few genome-wide screens have been attempted, although many studies have characterized locus-specific MAR DNA sequences. In this study, a MAR DNA library was prepared from the Drosophila melanogaster Meigen (Diptera: Drosophilidae) genome. One of the sequences identified as a MAR was from a long terminal repeat region of ‘roo’ retrotransposon (roo MAR). Sequence analysis of roo MAR showed its distribution across the D. melanogaster genome. roo MAR also showed high sequence similarity with a previously identified MAR in Drosophila, namely the ‘gypsy’ retrotransposon. Analysis of the genes flanking roo MAR insertions in the Drosophila genome showed that genes were co-ordinately expressed. The results from the present study in D. melanogaster suggest this sequence plays an important role in genome organization and function. The findings point to an evolutionary role of retrotransposons in shaping the genomic architecture of eukaryotes.

Keywords: genome organization, MAR DNA, retrotransposon
Abbreviations: CTCF, CCCTC-binding factor; LTR, long terminal repeat; MAR, matrix associated region; NuMat, nuclear matrix
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Introduction

Chromatin in the eukaryotic nucleus is known to be organized into loop domains. Intranuclear space is compartmentalized into structural and functional domains (Spellman and Rubin 2002; Sexton et al. 2007; Kadauke et al. 2009; Cremer and Cremer 2010). The structural features of the nucleus are the nuclear membrane, nucleolus, and heterochromatic and euchromatic domains. The major functions involving chromatin, such as transcription, replication, repair, splicing, silencing, etc., are orchestrated in the non-chromatin space of the nucleus (Cook et al. 1999; Lanctot et al. 2007). The nuclear matrix (NuMat) has been proposed to play an important role in this structural and functional organization, as proteins related to the nuclear functions have been found to be physically associated with NuMat (Berezney and Wei 1998; Kallapagoudar et al. 2010).

Biochemically, NuMat is made of protein, RNA, and DNA. Protein and RNA constitute the bulk of NuMat, and only a small amount of DNA (~1%) is found to be associated with it (Berezney and Coffey 1977). The DNA sequences associated with NuMat are called matrix-associated or scaffold-attachment regions (MARs/SARs). The MARs bind to NuMat and provide an anchor for higher order chromatin organization. This association is dynamic and varies in a cell-specific manner (Fey and Penman 1988; Dworetzky et al. 1990; Cai et al. 2003; Varma and Mishra 2011).

Earlier studies indicated that the association of MARs with NuMat leads to the formation of 50–200 kb chromatin loops that can act as independent functional domains (Jackson et al. 1990; Cremer and Cremer 2001). MAR DNA sequences range between 300 and 1000 bp in length and are AT rich (Boulikas 1993). These sequences were shown to have special sequence motifs, such as A-box (AATAAAAA/CAA) and T-box (TTTTATTTT), and were also shown to bind to topoisomerase II, boundary element associated factor, and CCCTC-binding factor (CTCF) (Gasser and Laemmli 1986; Dunn et al. 2003; Pathak et al. 2007; Phillips et al. 2009). Many times they also coincided with replication origin (Amati and Gasser 1988). Though MARs contain specialized sequences, no consensus sequence motif had been identified before our study. It is presumed that the MAR property is determined by the structural similarities more than by the sequence similarity (Yamamura and Nomura 2001).

Computational programs that screen for genome wide occurrence of MAR sequences are far from perfect but they have useful predictive value (Evans et al. 2007). In the present study, a MAR DNA library from Drosophila melanogaster Meigen (Diptera: Drosophilidae) embryos was prepared. The long terminal repeat region (LTR) of transposable element ‘roo’ was found as one of the MARs. Earlier studies have shown that a 350-bp sequence at the 5’-UTR of the gypsy transposon also had a nuclear matrix binding property (Nabirochkin et al. 1998). The sequence alignment of roo MAR with the NuMat associated region of gypsy showed very high similarity. Interestingly, a significant proportion of genes present in the flanking region of roo transposon were found to be expressed in adult testes and ovaries. These findings point to the importance of transposable elements in genome organization and evolution.
**Materials and Methods**

**Isolation of MAR DNA of 0–16 hours old *Drosophila melanogaster* embryos**

Embryos (0–16 hrs old) were obtained from a laboratory population of *D. melanogaster* (Canton-S) maintained at 25° C. Embryos were collected and weighed. NuMat was prepared according to published protocol from 0.1 g of embryos (Mirkovitch et al. 1984) with modifications as mentioned in Pathak et al. (2007) (Figure 1). Briefly, nuclei were isolated in nuclear isolation buffer (15 mM Tris pH 7.4, 40 mM KCl, 1 mM EDTA, 0.1 mM EGTA, 0.1 mM PMSF, 0.25 mM spermidine, and 0.5% (v/v) Triton-X 100) with 0.25 M sucrose. The nuclear pellet was digested with digestion buffer (20 mM Tris pH 7.4, 20 mM KCl, 70 mM NaCl, 10 mM MgCl2, 0.125 mM spermidine, 1 mM PMSF, 0.5% Triton-X 100, 10 U/μL RNase In, and 40 U/μL DNase I) at 4° C for 1 hr to remove chromatin. Extraction was carried out sequentially with 0.4 M NaCl and then with 2.0 M NaCl, each for 5 min, in extraction buffer (10 mM Hepes pH7.5, 4 mM EDTA, 0.25 mM spermidine, 0.1 mM PMSF, 0.5% (v/v) Triton X-100). The final pellet after extraction was washed 2 times with wash buffer (5 mM Tris, 20 mM KCl, 1 mM EDTA, 0.25 mM spermidine, 0.1 mm PMSF), and DNA was isolated from the pellet using a DNeasy Blood and Tissue kit (Qiagen, www.qiagen.com).

**Preparation of MAR DNA library**

The isolated MAR DNA was made blunt end with DNA polymerase I, large (Klenow) fragment (New England Biolabs, www.neb.com) and ligated to pMOS blunt end vector (Amersham kit, GE Healthcare, www.gelifesciences.com) according to the manufacturer’s instructions. Transformed colonies were screened on blue-white selection and checked for inserts by restriction enzyme digestions. DNA inserts in the plasmids were sequenced by the cycle sequencing method using the Big Dye terminator version 1.1 cycle sequencing kit (Applied Biosystems, www.appliedbiosystems.com) and an ABI Prism 310 Automated DNA sequencer (Applied Biosystems) with M13F and T7 primers.

**Analysis of library sequences**

The library sequences were analyzed for MAR potential by MAR-WIZ program (Singh 2000) under the default parameters setting. The results are given in Table 1.

The MAR sequences were also analyzed for binding sites of DNA-binding proteins, such as boundary element associated factor, GAGA factor, zeste-white 5, suppressor of hairy wing, and dCTCF, using a bioinformatic tool known as “chromatin domain boundary element search tool – cdBEST” (Srinivasan and Mishra 2012). These proteins are known to interact with chromatin domain boundaries, and most of them have also been shown to bind with MARs. The results of the analysis are presented in Supplementary Table 1.
Analysis of MAR18 (roo MAR) sequence

The library sequences were aligned with the *Drosophila* genome using NCBI-BLAST program (http://www.ncbi.nlm.nih.gov/). Of these, the MAR18 sequence was found to correspond to the LTR of roo transposon. Before proceeding further with any analysis, we first wanted to validate that the LTR of roo was actually associated with NuMat. To do this, an *in vivo* MAR assay was performed. Primers were designed to PCR amplify a region that enclosed the MAR18 sequence in the LTR of roo element (forward primer: 5’CCGCTCTCAAATAGTCCC3’; reverse primer: 5’CCTACCTTTTGGTAGGGA3’; amplicon size: 299 bp). As controls, primers were designed that amplified sequences of the *D. melanogaster* genome from an exon (in arc gene: forward primer: 5’GGAGAGGATTCAGGTCACA3’; reverse primer: 5’GTAGGGGAGAGCAAC3’; amplicon size: 280 bp), an intron (in Wnt6 gene: forward primer: 5’GAGAGACGTTTTCTGTAAC3’; reverse primer: 5’CTTACCAATCGACCTGTT3’; amplicon size: 514 bp), or an intergenic region (5’ of Wnt4 gene: forward primer: 5’GATCTAGGGCCATGGTAAA3’; reverse primer: 5’CGAGAGCTGAACCGAATC3’; amplicon size: 497 bp). These control fragments were from regions close to roo insertions. The amplicons were resolved on a 1.2% TAE-agarose gel and transferred onto Nylon NY+ membrane in 20X SSC by capillary transfer. MAR DNA (obtained as mentioned above from *D. melanogaster* embryos) was labelled with 32P-dATP by the random primer labelling method. Hybridization was carried out at 60°C in 0.5 M sodium phosphate/7% SDS for 16 hr. The blot was washed stringently and exposed to a phosphor-imager screen for 4 hr. The results are presented in Figure 2.

After validating that the roo LTR sequence was indeed retained in NuMat, *in silico* analysis of the transposon insertion sites in the *Drosophila* genome was performed. The NCBI-BLAST results were observed in a whole genome view. The 190 bp sequence was analyzed by MAR-WIZ to find out the sequences with high MAR potential. The roo MAR sequence was aligned to the previously identified MAR in gypsy transposon using CLUSTAL-W program (www.clustal.org). The results are presented in Figure 3.

Analysis of genes that flank roo insertion sites in the *Drosophila* genome

The sequence locations of the roo transposon insertions in the whole genome of *D. melano-
**Results**

**Isolation of MAR DNA from *D. melanogaster* embryos**

NuMat was prepared from 0–16 hr old *D. melanogaster* embryos using standard protocol (Figure 1A). Standard nuclear isolation protocols use hypertonic salt extraction to remove digested DNA. Alternative protocols using low salt extraction have been developed with the argument that physiological levels of salt may better preserve the ultrastructure. However, a survey of literature shows that both methods reveal similar ultrastructural features (reviewed in Nickerson 2001). We used the high salt extraction method, modified so that the salt extraction was performed slowly in a step-wise manner (from low to high salt) in the presence of mild detergent. This ensured that the extraction process is gentle and avoids artifacts. From the NuMat pellet, MAR DNA was isolated. The size of MAR DNA ranged between 100 and 500 bp. Upon digestion of the isolated MAR DNA with DNase I, it was confirmed that the isolated...
fragments were DNA and not RNA (Figure 1B). The MAR DNA library was made according to the protocol described in the Methods. Despite repeated efforts, cloning did not give many colonies, probably because the MAR DNA were AT rich sequences with secondary structures. Such sequences are not tolerated well by the bacteria and hence are difficult to clone (Godiska et al. 2010; Leach and Lindsay 1986). The obtained MAR DNA clones were checked for inserts by restriction digestion. The size of the inserts ranged from 100 to 500 bp, correlating well with the size of the MAR DNA used for ligation. The clones were sequenced, and all the sequences obtained were found to be unique (Table 1).

Analysis of the MAR DNA clones with MAR-WIZ and cdBEST programs

All the MAR clones were analyzed for the NuMat binding properties by in silico analysis. As no single property is attributed to NuMat association, we checked for AT%, origin of replication sites, topoisomerase II cleavage sites, AT richness (regularly spaced AT repeats), ATC rule (a stretch of 20 or more nucleotides of A, T, or C) ,and MAR score (all the individual parameters were considered, and those that had a potential higher than the threshold were given) with MAR-WIZ program (Singh 2000). Sixteen of the 35 sequences showed AT% of more than 60% (Table 1). Origin of replication sites were found in all the MAR sequences except 3. Two-thirds of the sequences showed AT richness. Sixteen sequences showed topoisomerase II sites. ATC rule was also followed by many of the clones, and most importantly all the clones showed maximum threshold for matrix association. All the sequences satisfied more than one rule of NuMat association. This analysis clearly indicated that the obtained sequences have potential to associate with NuMat, and the library represents a subset of the whole genome of MAR DNA sequences from D. melanogaster embryos.

The binding motifs of a few DNA binding proteins, such as boundary element associated factor, GAGA factor, zeste-white 5, dCTCF, and suppressor of hairy wing, were also checked for in the cloned sequences, as these proteins are reported to bind to chromatin domain boundaries as well as MAR sequences. Several boundaries have been shown to associate with NuMat, so whether any of the sequences had a potential for boundary activity was also checked. To check this, the cdBEST program (Srinivasan and Mishra 2012) was used. The program can be used for identification of recognition sequences of boundary interacting proteins as well as for identifying potential boundaries. The results (Supplementary Table 1) show that none of the MAR sequences cloned were predicted to be a potential boundary. Of the boundary/MAR interacting proteins, the boundary element associated factor binding site was present in 10 sequences (~29%), the GAGA factor binding site was present in 10 sequences (~29%), and the zeste-white 5 binding site was present in 4 (~10%) of the sequences. Although this data set is small, it indicates that all MAR sequences may not necessarily act as boundaries and vice-versa. Further, MAR and boundary property, if present on the same sequence, may be separable and not overlapping.

LTR sequence from roo transposon is enriched in NuMat

One of the clones from the library, labeled as MAR18, corresponded to an 190 bp sequence in the LTR of roo retrotransposon (Figure 2A). The complete roo retrotransposon element is 8.7 Kb, with a terminal repeat of 429 bp (Kaminker et al. 2002). The association of
roo MAR with NuMat was validated by the in vivo MAR assay by Southern blotting. Primers were designed to amplify the LTR region of roo encompassing the MAR18 sequence. As controls, exonic, intronic, and intergenic regions close to roo insertion sites in the Drosophila genome were used. A signal in the roo MAR lane indicates the presence of complimentary sequences in the labelled MAR pool used as a probe. The absence of signals in the other lanes indicates that those sequences were not present in MAR in situ (Figure 2B). This experiment confirmed that the roo LTR element is associated with the NuMat in vivo.

**In silico analysis of roo MAR sequence**

Upon BLAST analysis, roo MAR was shown to be present 250 times in the genome (56, 47, 44, 59, and 46 times on X, 2L, 2R, 3L, and 3R chromosomes respectively) (Figure 3A). roo MAR sequences were found both at intergenic and intronic regions but never in an exon. Sometimes it was present more than once within the same intronic or intergenic region. The sequence of roo MAR when analyzed using MAR-WIZ showed a region of maximum matrix association that extended from 95 bp to 135 bp of the LTR (Figure 3B). This region had an origin of replication sequence (ATTTA), a curved DNA sequence (TTTAAA), an A-box (AAATAAAA), and a region that conformed with ATC rule (underlined in the sequence). The other 2 regions with lower MAR potential also harbored origin of replication sequences and were AT rich. The sequence was further checked for its similarity with an already known MAR DNA sequence in Drosophila gypsy retrotransposon. Alignment showed overall 40–50% sequence similarity. In the gypsy MAR sequence, topoisomerase II recognition sites are labelled as 1 to 7, and regions showing ATC rule are underlined (Figure 3C) (Nabirochkin et al. 1998). The topoisomerase II recognition sequence numbered “7,” and the regions following ATC rule, showed high sequence conservation among gypsy and roo MAR. Furthermore, an A-box was present in both sequences. Thus, the 2 sequences were similar in regions important for MAR association.

**Analysis of roo-flanking genes in the Drosophila genome**

FlyBase showed 193 insertions of roo in the whole genome of which 151 were in the sequenced region. Of the 151 places where roo transposon was inserted, 85 sites had a gene in the vicinity of those expressed in testes and ovaries (Supplementary Tables 2, 3), a significant 56% of the 151 sequenced roo insertions. Of the rest, expression data for genes around 11% of the roo insertions were either not available or the genes were not expressed in adult tissue. The remaining 33% insertions had associated genes expressed in other tissues (Figure 4). This analysis indicated a potential role for roo transposon in genome organization and regulated expression of distant genes via NuMat association.

**Discussion**

The genome in eukaryotes needs MAR regions to demarcate chromatin into domains and to regulate gene expression (Heng et al. 2004; Razin et al. 2007). Many MARs have been characterized and are found to lie in genomic as well as intergenic regions of the genome. MARs have been shown to topologically constrain DNA into loops. This plays an important role in compact packaging of the chromatin (Mirkovitch et al. 1984). As they are DNA sequences with special properties, several in silico programs attempt to predict these sequences on a genome-wide scale. MARs can target a DNA locus to a desired location for a specific function (Yusufzai and
Felsenfeld 2004). For example, in Drosophila, the scs’ boundary sequence that demarcates hsp70 heat shock locus behaves as a MAR. It binds to the boundary element associated factor and localizes to the NuMat (Pathak et al. 2007). A similar example is gypsy retrotransposon, which is known to behave as an insulator. Gypsy DNA, along with its binding proteins, is located in the NuMat, and the intervening DNA between 2 gypsy insertions was found to be arranged in a loop (Byrd and Corces 2003). Mutation in the gypsy binding protein leads to disruption of the loop. In the context of spatial organization, such MAR-associated localization could simply reflect changes in transcriptional status or changes in organization of chromatin structure.

In the present study, it was found that an abundant retrotransposon roo had a region that can bind to the NuMat. Transposon roo has been shown to be transcribed in a development and tissue-specific manner, and elements within the retrotransposon have been shown to act as cis-regulatory elements (Bronner et al. 1995). The transposon is distributed throughout the genome on all chromosomes. The genes flanking the transposon insertion site appeared to be coordinately regulated, as a sizable fraction of them were expressed in testes or ovaries. It would be ideal for the cell to have a few sequences and multiply them many times to organize the genome instead of having different sequences for different regions. These repeat sequences could provide the mechanism to identify coordinately regulated genes and cluster them in appropriate regions for regulated expression. Transposons like roo, by virtue of NuMat association, can act as a tool to direct the spatial organization of the genome and regulate expression. As they are mobile elements, they can lead to the creation of new domains by moving along the genome and helping in evolution. The findings of our study strengthen the idea of the role of mobile genetic elements in genome organization and gene regulation (Kazazian 2004; Tomilin 2008).

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Table 1. Characteristics of MAR DNA library sequences (Based on MAR-WIZ). Individual scores for origin of replication (ORI), Topoisomerase II (TopoII) sites, AT richness, and ATC rule are given for forward and reverse strands in F/R format.

| MAR Seq. No | Seq length | AT% | ORI pattern | TOPO II | AT richness | ATC rule | MAR Score 1 |
|-------------|------------|-----|-------------|---------|-------------|----------|-------------|
| MAR1        | 277        | 54.6| 3/2         | 0       | 2/2         | 0        | +           |
| MAR2        | 141        | 54.4| 0/2         | 0       | 0           | 0        | +           |
| MAR3        | 62         | 60.3| 0/0         | 0       | 2/3         | 0        | +           |
| MAR4        | 177        | 69.6| 4/4         | 0       | 15/16       | 0        | +           |
| MAR5        | 74         | 64.5| 1/2         | 0       | 5/3         | 0        | +           |
| MAR6        | 224        | 74.6| 6/6         | 1/0     | 14/14       | 20/7     | +           |
| MAR7        | 72         | 65.9| 1/2         | 0/1     | 6/4         | 0/7      | +           |
| MAR8        | 158        | 42.4| 0           | 1/0     | 0           | 0        | +           |
| MAR9        | 128        | 49  | 1/1         | 1/0     | 0           | 0        | +           |
| MAR10       | 306        | 50.3| 1/1         | 0       | 0           | 0        | +           |
| MAR11       | 82         | 64.2| 1/2         | 0       | 6/3         | 0        | +           |
| MAR12       | 278        | 57.7| 4/3         | 0       | 5/5         | 1/0      | +           |
| MAR13       | 73         | 62.6| 1/2         | 0       | 5/3         | 0        | +           |
| MAR14       | 165        | 69  | 3/3         | 0       | 16/18       | 0        | +           |
| MAR15       | 301        | 49.5| 1/0         | 0       | 0           | 0        | +           |
| MAR16       | 301        | 50.2| 1/0         | 0       | 0           | 0        | +           |
| MAR17       | 123        | 62.9| 1/0         | 0       | 1/3         | 0/3      | +           |
| MAR18       | 190        | 59.3| 4/1         | 0       | 2/1         | 0        | +           |
| MAR19       | 100        | 46.9| -           | -       | -           | -        | -           |
| MAR20       | 116        | 65.5| 1/2         | 0       | 0           | 0        | +           |
| MAR21       | 329        | 63.2| 5/6         | 2/0     | 9/0         | 5/4      | +           |
| MAR22       | 235        | 48.6| 6/6         | 0       | 14/14       | 7/20     | +           |
| MAR23       | 242        | 65.3| 5/4         | 3/0     | 3/1         | 2/16     | +           |
| MAR24       | 323        | 61.3| 4/3         | 1/0     | 5/5         | 2/0      | +           |
| MAR25       | 395        | 48.4| 3/4         | 1/0     | 0           | 2/0      | +           |
| MAR26       | 117        | 66.6| 3/2         | 0       | 1/3         | 0/6      | +           |
| MAR27       | 148        | 69.6| 2/3         | 0       | 4/2         | 6/0      | +           |
| MAR28       | 281        | 59.4| 2/4         | 0/1     | 0           | 10/0     | +           |
| MAR29       | 297        | 59.5| 4/3         | 0/1     | 6/5         | 8/0      | +           |
| MAR30       | 125        | 52  | 1/0         | 1/0     | 0           | 0        | +           |
| MAR31       | 77         | 49.6| 0/1         | 1/0     | 0           | 0        | +           |
| MAR32       | 580        | 51.4| 4/7         | 1/1     | 0           | 2/0      | +           |
| MAR33       | 179        | 58.1| 2/1         | 0       | 4/1         | 0        | +           |
| MAR34       | 359        | 53.8| 7/8         | 1/0     | 15/16       | 2/0      | +           |
| MAR35       | 364        | 63.3| 7/5         | 39/15   | 14/14       | 399/13   | +           |
**Supplementary Table 1.** Binding sites for various boundary/MAR interacting proteins in the MAR DNA library sequences (Based on cdBEST).

| MAR. Seq No | Sequence length | BEAF | GAGA factor | Zw5 | dCTCF | Su(Hw) |
|------------|-----------------|------|-------------|-----|-------|--------|
| MAR1       | 277             | 0    | 0           | 0   | 0     | 0      |
| MAR2       | 141             | 0    | 0           | 0   | 0     | 0      |
| MAR3       | 62              | 0    | 1           | 0   | 0     | 0      |
| MAR4       | 177             | 0    | 0           | 0   | 0     | 0      |
| MAR5       | 74              | 0    | 1           | 0   | 0     | 0      |
| MAR6       | 224             | 0    | 0           | 0   | 0     | 0      |
| MAR7       | 72              | 0    | 1           | 0   | 0     | 0      |
| MAR8       | 158             | 0    | 0           | 0   | 0     | 0      |
| MAR9       | 128             | 2    | 0           | 0   | 0     | 0      |
| MAR10      | 306             | 2    | 0           | 0   | 0     | 0      |
| MAR11      | 82              | 0    | 1           | 0   | 0     | 0      |
| MAR12      | 278             | 1    | 0           | 1   | 0     | 0      |
| MAR13      | 73              | 0    | 1           | 0   | 0     | 0      |
| MAR14      | 165             | 0    | 0           | 0   | 0     | 0      |
| MAR15      | 301             | 2    | 0           | 0   | 0     | 0      |
| MAR16      | 301             | 2    | 0           | 0   | 0     | 0      |
| MAR17      | 123             | 0    | 0           | 0   | 0     | 0      |
| MAR18      | 190             | 1    | 0           | 0   | 0     | 0      |
| MAR19      | 100             | 0    | 0           | 0   | 0     | 0      |
| MAR20      | 116             | 0    | 0           | 0   | 0     | 0      |
| MAR21      | 329             | 0    | 0           | 0   | 0     | 0      |
| MAR22      | 235             | 0    | 0           | 0   | 0     | 0      |
| MAR23      | 242             | 0    | 1           | 0   | 0     | 0      |
| MAR24      | 323             | 0    | 0           | 0   | 0     | 0      |
| MAR25      | 395             | 0    | 1           | 0   | 0     | 0      |
| MAR26      | 117             | 1    | 0           | 0   | 0     | 0      |
| MAR27      | 148             | 0    | 1           | 0   | 0     | 0      |
| MAR28      | 281             | 1    | 0           | 0   | 0     | 0      |
| MAR29      | 297             | 1    | 0           | 1   | 0     | 0      |
| MAR30      | 125             | 0    | 0           | 0   | 0     | 0      |
| MAR31      | 77              | 0    | 0           | 0   | 0     | 0      |
| MAR32      | 580             | 0    | 2           | 0   | 0     | 0      |
| MAR33      | 179             | 1    | 0           | 1   | 0     | 0      |
| MAR34      | 559             | 0    | 1           | 2   | 0     | 0      |
| MAR35      | 364             | 0    | 0           | 0   | 0     | 0      |
### Supplementary Table 2.

| S. No | Symbol | Chrm Arm | Sequence location | Associated gene orientation | Associated gene name | Summary of FlyAtlas Anatomical Expression Data |
|-------|--------|----------|-------------------|---------------------------|----------------------|-----------------------------------------------|
| 1     | rro[1L]2p5[2] | 2L      | 13563-15906       | upstream                  | CO11023              | Expression at high levels in the following post-embyronic organs or tissues: adult testis. |
|       |         |          |                   | downstream                | Ig11                 | No expression detected in any larval or adult organs/tissues. |
|       |         |          |                   |                           | Within                |                                     |
|       |         |          |                   |                           | L(3)gl               |                                     |
| 2     | rro[1L]2p81 | 2L      | 686074-605555     | upstream                  | CO2839               | No FlyAtlas data available because no Alph2 ProbeSet aligns to an exon of CO2839. |
|       |         |          |                   | downstream                | Hsp60B               |                                    |
|       |         |          |                   |                           | Within                |                                     |
|       |         |          |                   |                           | ds                   | Expression at moderate levels in the following post-embyronic organs or tissues: larval fat body. |
| 3     | rro[1L]2p83 | 2L      | 976935-984512     | upstream                  | CG4341               | Expression at high levels in the following post-embyronic organs or tissues: adult brain. |
|       |         |          |                   | downstream                | IA-2                 | Expression at moderate levels in the following post-embyronic organs or tissues: adult head, larval/adult central nervous system. |
|       |         |          |                   |                           |                       | Expression at moderate levels in the following post-embyronic organs or tissues: adult head, larval/adult central nervous system. |
| 4     | rro[1L]2p84 | 2L      | 995780-1005818    | upstream                  | CG4341               | Expression at moderate levels in the following post-embyronic organs or tissues: adult brain. |
|       |         |          |                   | downstream                | IA-2                 | Expression at moderate levels in the following post-embyronic organs or tissues: adult head, larval/adult central nervous system. |
|       |         |          |                   |                           |                       | Expression at moderate levels in the following post-embyronic organs or tissues: adult head, larval/adult central nervous system. |
| 5     | rro[2R]2p29 | 2L      | 2100430-2100522   | upstream                  | CG2674               | Expression at moderate levels in the following post-embyronic organs or tissues: adult male reproductive system. |
|       |         |          |                   | downstream                | CO4259               | Expression at moderate levels in the following post-embyronic organs or tissues: adult heart, fat body, adult spermatozoa. |
|       |         |          |                   |                           | Within                |                                     |
|       |         |          |                   |                           | dp3                  | Two or more Alph2 ProbeSets detect exons of this gene. This is a summary of the tissue expression peaks exhibited in at least one of these ProbeSets. |
| 6     | rro[3R]4m[1] | 3L      | 3545808-3545809   | upstream                  | Ch0-13               | Expression at moderate levels in the following post-embyronic organs or tissues: adult male reproductive system. |
|       |         |          |                   | downstream                | CR38322              |                                             |
|       |         |          |                   |                           | Within                |                                     |
|       |         |          |                   |                           | dm                   | Expression at moderate levels in the following post-embyronic organs or tissues: larval/adult hindgut, adult heart. |
| 7     | rro[3R]311 | 2L      | 5827763-5838712   | upstream                  | CG01644              | Expression at moderate levels in the following post-embyronic organs or tissues: larval fat body. |
|       |         |          |                   | downstream                | CO0895               | Expression at high levels in the following post-embyronic organs or tissues: adult testis. |
|       |         |          |                   |                           | Within                |                                     |
|       |         |          |                   |                           | trnsat1f              | Little or no expression detected in any larval or adult organs/tissues. |
| 8     | rro[5R]5m[1] | 5L      | 6426091-6431563   | upstream                  | CG0527               | Many larval and adult organs/tissues expressed at moderate levels. Expression at high levels in the following post-embyronic organs or tissues: adult head, larval/adult hindgut, larval/adult midgut. |
|       |         |          |                   | downstream                | CO81101              | Many larval and adult organs/tissues expressed at moderate levels. Expression at high levels in the following post-embyonic organs or tissues: adult head, larval/adult hindgut, larval/adult midgut. |
| 9     | rro[5R]315 | 2L      | 04315004-0430013  | upstream                  | CG0827               | Many larval and adult organs/tissues expressed at moderate levels. Expression at high levels in the following post-embyonic organs or tissues: adult head, larval/adult hindgut, larval/adult midgut. |
|       |         |          |                   | downstream                | E2k1103              |                                             |
| 10    | rro[1L]706 | 2L      | 6156014-6145512   | upstream                  | retm                 | Expression at high levels in the following post-embyonic organs or tissues: adult fat body, invariant spermatheca. |
|       |         |          |                   | downstream                |                      | Expression at moderate levels in the following post-embyonic organs or tissues: adult head, larval/adult hindgut, adult heart, larval fat body, larval salivary gland, adult female reproductive system, adult carasus. |
|       |         |          |                   |                           | partial overlap      |                                     |
|       |         |          |                   |                           | CO9527               | Many larval and adult organs/tissues expressed at moderate levels. Expression at high levels in the following post-embyonic organs or tissues: larval/adult midgut, larval/adult fat body. |
| 11    | rro[1L]319 | 2L      | 7343381-7350438   | upstream                  | wrt       | Expression at moderate levels in the following post-embyonic organs or tissues: larval hindgut, adult salivary gland. |
|       |         |          |                   | downstream                | Wnt10               | No expression detected in any larval or adult organs/tissues. |
|       |         |          |                   |                           | Within                |                                     |
|       |         |          |                   |                           | Wnt6                | Little or no expression detected in any larval or adult organs/tissues. |
| 12    | rro[1L]706 | 2L      | 8431781-8431781   | upstream                  | Aimp200              | High or moderate levels of expression observed in all larval and adult organs/tissues. Expression at high levels in the following post-embyonic organs or tissues: adult head, larval/adult hindgut, larval/adult midgut, larval/Majgut, larval/adult fat body, larval salivary gland, larval trachea, adult female reproductive system, adult male reproductive system. |
|       |         |          |                   | downstream                | D1                  | Expression at moderate levels in the following post-embyonic organs or tissues: larval central nervous system, adult ovary. |
|       |         |          |                   |                           | Within                |                                     |
|       |         |          |                   |                           | grk                  | Expression at moderate levels in the following post-embyonic organs or tissues: adult crop, adult hindgut, adult heart, adult ovary, larval/adult carasus. |
### Supplementary Table 2. Continued.

| 13 | roo | grk | 2L | 8431781-8433781 | Alco200 |
|----|-----|-----|----|-----------------|--------|
|    |     |     |    |     | High or moderate levels of expression observed in all larval and adult organs/tissues. Expression at high levels in the following post-embryonic organs or tissues: adult head, adult eye, larval/adult central nervous system, larval/adult midgut, larval hindgut, larval Malpighian tubules, adult heart, larval/adult fat body, larval salivary gland, larval trachea, adult female reproductive system, adult male reproductive system, larval/adult carcass. Expression at moderate levels in the following post-embryonic organs or tissues: adult crop, adult hindgut, adult Malpighian tubules, adult salivary gland. |
|    |     |     |    |     | Expression at moderate levels in the following post-embryonic organs or tissues: larval central nervous system, adult ovary. |
|    |     |     |    |     | Expression at moderate levels in the following post-embryonic organs or tissues: adult crop, adult hindgut, adult Malpighian tubules, adult salivary gland. |
|    |     |     |    |     | Expression at moderate levels in the following post-embryonic organs or tissues: larval central nervous system, adult ovary. |
| 14 | roo | grk | 2L | 8431781-8433781 | Alco200 |
|    |     |     |    |     | High or moderate levels of expression observed in all larval and adult organs/tissues. Expression at high levels in the following post-embryonic organs or tissues: adult head, adult eye, larval/adult central nervous system, larval/adult midgut, larval hindgut, larval Malpighian tubules, adult heart, larval/adult fat body, larval salivary gland, larval trachea, adult female reproductive system, adult male reproductive system, larval/adult carcass. Expression at moderate levels in the following post-embryonic organs or tissues: adult crop, adult hindgut, adult Malpighian tubules, adult salivary gland. |
|    |     |     |    |     | Expression at moderate levels in the following post-embryonic organs or tissues: larval central nervous system, adult ovary. |
|    |     |     |    |     | Expression at moderate levels in the following post-embryonic organs or tissues: adult crop, adult hindgut, adult Malpighian tubules, adult salivary gland. |
|    |     |     |    |     | Expression at moderate levels in the following post-embryonic organs or tissues: larval central nervous system, adult ovary. |
| 15 | roo | mnu201 | 5L | 8441909-8441909 | Chrnc-14 |
|    |     |     |    |     | Two or more Affy2 Probesets identify exons of this gene. This is a summary of the tissue expression peaks exhibited in at least one of these Probesets. Expression at high levels in the following post-embryonic organs or tissues: adult ovary. Expression at moderate levels in the following post-embryonic organs or tissues: larval central nervous system, larval trachea. |
|    |     |     |    |     | Expression at moderate levels in the following post-embryonic organs or tissues: adult ovary. |
|    |     |     |    |     | Expression at moderate levels in the following post-embryonic organs or tissues: larval central nervous system, larval trachea. |
| 16 | roo | fn2 | 2L | 8453476-8461588 | U26 |
|    |     |     |    |     | Little or no expression detected in any larval or adult organs/tissues. |
|    |     |     |    |     | Expression at moderate levels in the following post-embryonic organs or tissues: larval central nervous system, adult ovary. |
|    |     |     |    |     | Expression at moderate levels in the following post-embryonic organs or tissues: larval central nervous system, adult ovary. |
| 17 | roo | Try29F | 2L | 0000335-0007786 | CG9588 |
|    |     |     |    |     | Expression at high levels in the following post-embryonic organs or tissues: adult midgut, larval/adult hindgut. |
|    |     |     |    |     | Expression at moderate levels in the following post-embryonic organs or tissues: larval midgut, adult hindgut. |
|    |     |     |    |     | Expression at high levels in the following post-embryonic organs or tissues: larval/adult midgut, larval/adult hindgut, larval Malpighian tubules, adult fat body, adult female reproductive system, adult carcass. |
| 18 | roo | CO18072 | 2L | 10940995-10956155 | CG9588 |
|    |     |     |    |     | High or moderate levels of expression observed in all larval and adult organs/tissues. Expression at high levels in the following post-embryonic organs or tissues: larval central nervous system, adult crop, larval/adult fat body, larval salivary gland, larval ovary. |
|    |     |     |    |     | Expression at moderate levels in the following post-embryonic organs or tissues: adult head, adult eye, adult central nervous system, larval/adult midgut, larval/adult hindgut, larval/adult Malpighian tubules, adult heart, adult salivary gland, larval trachea, adult female reproductive system, adult male reproductive system, larval/adult carcass. |
|    |     |     |    |     | Expression at high levels in the following post-embryonic organs or tissues: larval/adult midgut, larval/adult hindgut, larval Malpighian tubules, adult heart, adult salivary gland, larval trachea, adult female reproductive system, adult male reproductive system, larval/adult carcass. |
|    |     |     |    |     | Expression at high levels in the following post-embryonic organs or tissues: larval/adult midgut, larval Malpighian tubules. |
|    |     |     |    |     | Expression at moderate levels in the following post-embryonic organs or tissues: adult/adult midgut, larval/adult hindgut, larval/adult Malpighian tubules. |
| 19 | roo | CO312P | 2L | 10940995-10956155 | CG07988 |
|    |     |     |    |     | Expression at high levels in the following post-embryonic organs or tissues: larval/adult midgut, larval Malpighian tubules. |
|    |     |     |    |     | Expression at moderate levels in the following post-embryonic organs or tissues: adult/adult midgut, larval Malpighian tubules. |
|    |     |     |    |     | Expression at high levels in the following post-embryonic organs or tissues: adult head, adult eye, adult central nervous system, adult crop, larval/adult midgut, larval/adult hindgut, larval/adult Malpighian tubules, adult heart, larval/adult fat body, larval/adult salivary gland, larval trachea, adult female reproductive system, adult male reproductive system, larval/adult carcass. |
|    |     |     |    |     | Expression at moderate levels in the following post-embryonic organs or tissues: larval central nervous system, adult ovary. |
|    |     |     |    |     | Expression at high levels in the following post-embryonic organs or tissues: adult head, adult eye, adult central nervous system, adult crop, larval/adult midgut, larval/adult hindgut, larval/adult Malpighian tubules, adult heart, larval/adult fat body, larval/adult salivary gland, larval trachea, adult female reproductive system, adult male reproductive system, larval/adult carcass. |
|    |     |     |    |     | Expression at moderate levels in the following post-embryonic organs or tissues: larval central nervous system, adult ovary. |
|    |     |     |    |     | Expression at high levels in the following post-embryonic organs or tissues: adult head, adult eye, adult central nervous system, adult crop, larval/adult midgut, larval/adult hindgut, larval/adult Malpighian tubules, adult heart, larval/adult fat body, larval/adult salivary gland, larval trachea, adult female reproductive system, adult male reproductive system, larval/adult carcass. |
| 20 | roo | Adh | 2L | 14662056-14663710 | C01528 |
|    |     |     |    |     | Two or more Affy2 Probesets identify exons of this gene. This is a summary of the tissue expression peaks exhibited in at least one of these Probesets. Expression at high levels in the following post-embryonic organs or tissues: larval carcass. Expression at moderate levels in the following post-embryonic organs or tissues: adult head, larval hindgut, larval trachea. |
|    |     |     |    |     | Expression at moderate levels in the following post-embryonic organs or tissues: adult head, larval hindgut, larval trachea. |
|    |     |     |    |     | Expression at moderate levels in the following post-embryonic organs or tissues: larval hindgut, larval Malpighian tubules, larval/adult fat body, larval trachea, adult female reproductive system, larval/adult carcass. |
|    |     |     |    |     | Expression at high levels in the following post-embryonic organs or tissues: larval carcass. |
### Supplementary Table 2. Continued.

|    |    |    |    |    |
|----|----|----|----|----|
| 21 | ros ([684] | 2L | 1675241-1677541 |    |
|    |    |    |    |    |
|    | upstream |    | CG13258 |    |
|    | downstream |    | Cyp35d1 |    |
|    |    |    | Expression at high levels in the following post-embryonic organs or tissues: adult head, adult eye, larval Malpighian tubules, adult heart, larval fat body, adult spermathecae, adult carcass. Expression at moderate levels in the following post-embryonic organs or tissues: adult central nervous system, larval midgut, larval/adult hindgut, adult Malpighian tubules. |
|    |    |    |    |    |
|    | Within |    | Mhc |    |
|    |    |    | Nearly all larval and adult organs/tissues expressed at moderate or high levels. Expression at high levels in the following post-embryonic organs or tissues: adult head, adult eye, adult crop, larval/adult midgut, larval/adult hindgut, adult heart, larval tracheae, adult spermathecae, adult male accessory gland, larval/adult carcass. Expression at moderate levels in the following post-embryonic organs or tissues: adult central nervous system, larval adult Malpighian tubules, adult fat body, adult ovaries. |
|    |    |    |    |    |
| 22 | ros ([462] | 2L | 18651931-18661019 |    |
|    |    |    |    |    |
|    | upstream |    | allEss |    |
|    | downstream |    | Art1 |    |
|    |    |    | Expression at high levels in the following post-embryonic organs or tissues: adult head, adult eye, adult brain. Expression at moderate levels in the following post-embryonic organs or tissues: adult tests. |
|    |    |    |    |    |
|    | Within |    | rdo |    |
|    |    |    | Two or more Affy2 Probesets identify genes of this gene. This is a summary of the tissue expression peaks exhibited in at least one of these ProbeSets. Expression at moderate levels in the following post-embryonic organs or tissues: adult head, adult eye, larval/adult central nervous system. |
|    |    |    |    |    |
| 23 | ros ([1676] | 2L | 19652628-19674003 |    |
|    |    |    |    |    |
|    | upstream |    | CG10366 |    |
|    | downstream |    | scw |    |
|    |    |    | No expression detected in any larval or adult organs/tissues. |
|    |    |    |    |    |
|    | Within |    | Lpr |    |
|    |    |    | Expression at moderate levels in the following post-embryonic organs or tissues: adult eye, larval/adult central nervous system, adult ovary. |
|    |    |    |    |    |
|    | upstream |    | scw |    |
|    |    |    | No expression detected in any larval or adult organs/tissues. |
|    |    |    |    |    |
| 24 | ros ([419] | 2L | 10703592-10712660 |    |
|    |    |    |    |    |
|    | downstream |    | CG10462 |    |
|    |    |    | Expression at moderate levels in the following post-embryonic organs or tissues: adult eye, larval central nervous system, adult ovary. |
|    |    |    |    |    |
|    | Within |    | Lpr |    |
|    |    |    | Expression at moderate levels in the following post-embryonic organs or tissues: adult eye, larval/adult central nervous system, adult ovary. |
|    |    |    |    |    |
|    | upstream |    | CR13605 |    |
|    |    |    |    |    |
| 25 | ros ([488] | 2L | 20340566-20345988 |    |
|    |    |    |    |    |
|    | downstream |    | Ls |    |
|    |    |    | Many larval and adult organs/tissues expressed at moderate levels. Expression at high levels in the following post-embryonic organs or tissues: larval central nervous system, adult ovary. Expression at moderate levels in the following post-embryonic organs or tissues: adult head, adult eye, adult central nervous system, adult crop, larval/adult midgut, larval/adult hindgut, adult heart, adult fat body, larval/adult salivary gland, larval tracheae, adult female reproductive system, adult male accessory gland, larval carcass. |
|    |    |    |    |    |
|    | Within |    | spir |    |
|    |    |    | Two or more Affy2 Probesets identify genes of this gene. This is a summary of the tissue expression peaks exhibited in at least one of these ProbeSets. Expression at high levels in the following post-embryonic organs or tissues: adult central nervous system. Expression at moderate levels in the following post-embryonic organs or tissues: adult head, adult eye, larval central nervous system, larval/adult midgut, larval hindgut, larval Malpighian tubules, adult ovary. |
|    |    |    |    |    |
| 26 | ros ([495] | 2L | 21401991-21403521 |    |
|    |    |    |    |    |
|    | upstream |    | mrv3 |    |
|    | downstream |    | His-Poi:CR131010 |    |
|    |    |    | No AffyAtlas data available because no Affy2 Probeset aligns to an exon of His-Poi:CR131010. |
|    |    |    |    |    |
| 27 | ros ([147] | 2L | 21410996-21450230 |    |
|    |    |    |    |    |
|    | upstream |    | His3:CG33812 |    |
|    | downstream |    | His1:CG33813 |    |
|    |    |    | No AffyAtlas data available because no Affy2 Probeset aligns to an exon of His1:CG33813. |
|    |    |    |    |    |
| 28 | ros ([501] | 2L | 21507831-21570223 |    |
|    |    |    |    |    |
|    | downstream |    | Lam1p |    |
|    |    |    | High levels of expression observed in all larval and adult organs/tissues. Expression at high levels in the following post-embryonic organs or tissues: adult head, adult eye, larval/adult central nervous system, adult crop, larval/adult midgut, larval/adult hindgut, larval/adult Malpighian tubules, adult heart, larval/adult fat body, larval/adult salivary gland, larval tracheae, adult female reproductive system, adult male reproductive system, larval/adult carcass. |
|    |    |    |    |    |
| 29 | ros ([508] | 2L | 21597085-21604770 |    |
|    |    |    |    |    |
|    | upstream |    | CR24546 |    |
|    | downstream |    | CG2201 |    |
|    |    |    | Many larval and adult organs/tissues expressed at moderate levels. Expression at high levels in the following post-embryonic organs or tissues: adult eye, larval Malpighian tubules, larval fat body, adult ovary. Expression at moderate levels in the following post-embryonic organs or tissues: larval/hindgut, larval Malpighian tubules, adult heart, adult fat body, larval/adult salivary gland, larval tracheae, adult female reproductive system, larval/adult carcass. |
|    |    |    |    |    |
| 30 | ros ([521] | 2L | 21981738-21990843 |    |
|    |    |    |    |    |
|    | upstream |    | CG11034 |    |
|    | downstream |    | CG16693 |    |
|    |    |    | Expression at moderate levels in the following post-embryonic organs or tissues: adult tests. |
|    |    |    |    |    |
|    | Within |    | CG2328 |    |
|    |    |    | Expression at high levels in the following post-embryonic organs or tissues: adult tests. |
| Supplementary Table 2. Continued. |
|----------------------------------|
| **upstream** | **Rpl38** | High level of expression observed in all larval and adult organs/tissues. Expression at high levels in the following post-embryonic organs or tissues: adult head, adult eye, larval/adult central nervous system, adult crop, larval/adult midgut, larval/adult Malpighian tubules, adult heart, larval/adult fat body, larval/adult salivary gland, larval trachea, adult male reproductive system, adult male reproductive system, larval/adult carcass. |
| **downstream** | **p320Gna** | High or moderate levels of expression observed in all larval and adult organs/tissues. Expression at high levels in the following post-embryonic organs or tissues: adult head, adult eye, larval/adult central nervous system, larval hindgut, larval/adult Malpighian tubules, adult heart, larval/adult fat body, larval/adult salivary gland, larval trachea, adult ovary, larval carcass. Expression at moderate levels in the following post-embryonic organs or tissues: adult crop, larval/adult midgut, larval hindgut, larval/adult Malpighian tubules, adult heart, larval/adult salivary gland, larval trachea, adult ovary, larval carcass. |
| 31 | roo1[1250] | 2R | 438509-438957 | 2.16 |
| 32 | roo1[1608] | 2R | 2181670-2208433 | 2.16 |
| 33 | roo1[764] | 2R | 3097401-3105090 | 2.16 |
| 34 | roo1[775] | 2R | 4213422-4218850 | 2.16 |
| 35 | roo1[784] | 2R | 5106254-5109083 | 2.16 |
| 36 | roo1[785] | 2R | 5297755-5248875 | 2.16 |
| 37 | roo1[1670] | 2R | 5367617-5378100 | 2.16 |
| 38 | roo1[1603] | 2R | 5755405-5757777 | 2.16 |
| 39 | roo1[1602] | 2R | 5746685-5746833 | 2.16 |
### Supplementary Table 2. Continued.

| Gene ID | Description | Genotype or Tissue | Expression Pattern |
|---------|-------------|-------------------|-------------------|
| **upstream** | CG1510 | CG12744 | CG18446 |
| **downstream** | dbx | No expression detected in any larval or adult organ/tissue. |
| **Within** | Spt7C | Spce11B | CG5412/4 |
| **upstream** | CG850 | CG2374 | CG4228/6 |
| **downstream** | CG7560 | CG6484 | CG4228 |
| **Within** | Spt7C | mir-869 | L |
| **upstream** | CG5363 | CG686F | slub |
| **downstream** | Choose the best option. | Get the best solution. | Choose the best option. |

Note: The table continues with additional entries, but they are not fully visible in the provided excerpt. For a complete view, please consult the full document.
### Supplementary Table 2. Continued.

| Gene | Chromosome | Description | Expression Profile | Notes |
|------|-------------|-------------|-------------------|-------|
| 50   | roo|1855 | RC2 | upsteam | CO|4|2697 | No FlyAtlas data available because no Affy Predicts aligns to an exon of CO|4|2697 | Little or no expression detected in any larval or adult organs/tissues. |
| 51   | roo|1852 | RC2 | downstream | CO|1|1102 | Expression at moderate levels in the following post-embryonic organs or tissues: larval fat body. |
| 52   | roo|1856 | RC2 | upsteam | LBR | Expression at high levels in the following post-embryonic organs or tissues: adult testis. |
| 53   | roo|1807 | RC2 | downstream | Cen|1 | Expression at moderate levels in the following post-embryonic organs or tissues: larval central nervous system, adult ovary. |
| 54   | roo|1808 | RC2 | upsteam | CRO|281 | Expression at moderate levels in the following post-embryonic organs or tissues: larval fat body, adult testis. |
| 55   | roo|1883 | RC2 | downstream | bino | Expression at moderate levels in the following post-embryonic organs or tissues: larval Malpighian tubules. |
| 56   | roo|1598 | J2L | upsteam | CO|4|3100 | Expression at moderate levels in the following post-embryonic organs or tissues: larval Malpighian tubules, larval adult central nervous system, larval adult midgut, larval Malpighian tubules, larval fat body. |
| 57   | roo|1665 | J2L | upsteam | CO|4|3106 | No expression detected in any larval or adult organs/tissues. |
| 58   | roo|1806 | J2L | upsteam | RNA|CR3|2481 | Many larval and adult organs/tissues expressed at moderate levels. Expression at high levels in the following post-embryonic organs or tissues: adult eye, adult crop, larval Malpighian tubules, larval adult midgut, larval adult salivary gland, larval testes, adult female reproductive system, adult male accessory gland, larval/adult caecum. |
| 59   | roo|1808 | J2L | downstream | CG|1|3890 | Expression at moderate levels in the following post-embryonic organs or tissues: larval fat body. |
| 60   | roo|1903 | J2L | downstream | Babylon | Expression at high levels in the following post-embryonic organs or tissues: adult heart. |
| 61   | roo|1911 | J2L | downstream | CG|1|5972 | Many larval and adult organs/tissues expressed at moderate levels. Expression at high levels in the following post-embryonic organs or tissues: larval fat body. |
| 62   | roo|1927 | J2L | upstream | CO|1|2027 | Expression at high levels in the following post-embryonic organs or tissues: adult testis. |
| 63   | roo|1050 | J2L | downstream | RNAi | Expression at high levels in the following post-embryonic organs or tissues: larval Malpighian tubules, larval adult central nervous system, larval adult midgut, larval Malpighian tubules, larval adult fat body. |
| 64   | roo|1936 | J2L | downstream | CO|1|2599 | Expression at high levels in the following post-embryonic organs or tissues: larval adult midgut. |
| 65   | roo|1708 | J2L | downstream | CG|1|1147 | Expression at high levels in the following post-embryonic organs or tissues: larval adult midgut. |
|      | J2L | downsteam | CO|4|747 | Expression at high levels in the following post-embryonic organs or tissues: larval adult midgut. |
**Supplementary Table 2. Continued.**

| Gene ID | Organism | Expression Description | Value |
|---------|----------|------------------------|-------|
| roo[1944] JL 0880673-0888106 | upstream | vd | Two or more Affy2 Probesets identify exons of this gene. This is a summary of the tissue expression peaks exhibited in at least one of these Probesets. Expression will be in adult and adult organs/tissues ranges from low to undetected. Expression at moderate levels in the following post-embryonic organs or tissues: adult head, adult body, larval trachea, adult ovaries. |
| roo[952] JL 7000981-7019096 | downstream | Pot2 | Expression at high levels in the following post-embryonic organs or tissues: adult head, adult body, larval trachea, adult ovaries. Expression at moderate levels in the following post-embryonic organs or tissues: adult eye. |
| CG1247 | upstream | Ck | Expression at moderate levels in the following post-embryonic organs or tissues: adult eye, adult body, larval hindgut, adult testis. |
| roo[1053] JL 8474028-8484028 | upstream | Gtgb | Many larval and adult organs/tissues expressed at moderate levels. Expression at high levels in the following post-embryonic organs or tissues: larval central nervous system. Expression at moderate levels in the following post-embryonic organs or tissues: adult head, adult eye, adult central nervous system, adult crop, adult midgut, larval/ adult hindgut, larval/ adult Malpighian tubules, adult heart, adult fat body, larval salivary gland, larval trachea, adult spermatheca, larval/ adult ovaries. |
| Within | Hn | | Expression at moderate levels in the following post-embryonic organs or tissues: adult hindgut, adult testes. |
| roo[952] JL 9009283-9015423 | downstream | CG4477 | Expression at high levels in the following post-embryonic organs or tissues: adult hindgut. Expression at moderate levels in the following post-embryonic organs or tissues: adult head, adult eye, adult central nervous system, adult crop, larval adult midgut, larval hindgut, adult heart, larval/ adult salivary gland, larval trachea, adult spermatheca, larval/ adult ovaries. |
| CG13163 | upstream | ZCH3 | Expression at moderate levels in the following post-embryonic organs or tissues: adult brain. |
| roo[868] JL 0263771-0271460 | downstream | GPR-LA | Expression at high levels in the following post-embryonic organs or tissues: adult hindgut, larval Malpighian tubules, larval fat body, larval salivary gland, larval trachea, adult ovary, larval ovaries. |
| Within | Olsen-RIB | | No expression detected in any larval or adult organs/tissues. |
| CG2027 | downstream | CG408 | Nearly all larval and adult organs/tissues expressed at moderate or high levels. Expression at high levels in the following post-embryonic organs or tissues: adult head, larval central nervous system, adult midgut, larval Malpighian tubules, adult heart, adult fat body, larval salivary gland, adult female reproductive system, adult ovaries. Expression at moderate levels in the following post-embryonic organs or tissues: adult eye, adult central nervous system, adult crop, larval midgut, larval/ adult hindgut, larval fat body, adult salivary gland, adult male accessory gland, larval ovaries. |
| roo[959] JL 9494556-9495238 | Within | math | Nearly all larval and adult organs/tissues expressed at moderate levels. Expression at high levels in the following post-embryonic organs or tissues: adult crop, larval hindgut. Expression at moderate levels in the following post-embryonic organs or tissues: adult head, adult eye, larval/ adult central nervous system, larval/ adult midgut, larval/ adult hindgut, larval/ adult Malpighian tubules, adult heart, larval/ adult fat body, larval/ adult salivary gland, larval tracheae, adult female reproductive system, adult male accessory gland, larval/ adult ovaries. |
| CG6085 | downstream | CG4356 | Two or more Affy2 Probesets identity exons of this gene. This is a summary of the tissue expression peaks exhibited in at least one of these Probesets. Expression at high levels in the following post-embryonic organs or tissues: adult head, adult eye, larval adult central nervous system, adult crop, larval adult hindgut, insensitized spermatheca. |
| roo[952] JL 9928283-9931543 | upstream | midE | Expression at moderate levels in the following post-embryonic organs or tissues: adult brain. |
| Within | CG3500 | | No FlyAtlas data available because no Affy2 Probesets align to any exons of CG3500. |
| CG03710 | upstream | CG8089 | 1 or no expression detected in any larval or adult organs/tissue. |
| roo[1653] JL 13587701-13687238 | downstream | mnt-299 | expression peak detected in at least one of these Probesets. Expression at moderate levels in the following post-embryonic organs or tissues: larval adult central nervous system, larval trachea, adult testis. |
| roo[982] JL 11402211-11503152 | upstream | dhh | Two or more Affy2 Probesets identity exons of this gene. This is a summary of the tissue expression peaks exhibited in at least one of these Probesets. Expression at high levels in the following post-embryonic organs or tissues: adult head, adult eye, larval adult central nervous system, larval adult midgut, larval/testis, larval salivary gland, larval/female reproductive system. |
| roo[982] JL 11402211-11503152 | downstream | CG1482 | Two or more Affy2 Probesets identity exons of this gene. This is a summary of the tissue expression peaks exhibited in at least one of these Probesets. Expression at moderate levels in the following post-embryonic organs or tissues: larval adult Malpighian tubules, adult heart. |
| Within | cz | | Expression at moderate levels in the following post-embryonic organs or tissues: larval adult central nervous system, larval trachea, adult ovary. |
| roo[982] JL 11402211-11503152 | upstream | CG7000 | Two or more Affy2 Probesets identity exons of this gene. This is a summary of the tissue expression peaks exhibited in at least one of these Probesets. Expression at moderate levels in the following post-embryonic organs or tissues: adult eye, larval/adult central nervous system. |
| CG1482 | downstream | CG1482 | Expression at high levels in the following post-embryonic organs or tissues: larval adult midgut, larval adult hindgut. Expression at moderate levels in the following post-embryonic organs or tissues: larval adult Malpighian tubules, adult heart. |
| roo[1653] JL 13587701-13687238 | upstream | bvu-3 | expression peak detected in at least one of these Probesets. Expression at moderate levels in the following post-embryonic organs or tissues: larval adult central nervous system, larval trachea, adult testis. |
| CG12814 | downstream | CG1482 | Expression at high levels in the following post-embryonic organs or tissues: larval adult midgut, larval adult hindgut. Expression at moderate levels in the following post-embryonic organs or tissues: larval adult Malpighian tubules, adult heart. |
| roo[982] JL 11402211-11503152 | upstream | CG1482 | Two or more Affy2 Probesets identity exons of this gene. This is a summary of the tissue expression peaks exhibited in at least one of these Probesets. Expression at high levels in the following post-embryonic organs or tissues: larval adult hindgut, larval adult ovaries. |
| CG1482 | downstream | CG1482 | Expression at high levels in the following post-embryonic organs or tissues: larval adult midgut, larval adult hindgut. Expression at moderate levels in the following post-embryonic organs or tissues: larval adult Malpighian tubules, adult heart. |
| roo[11010] JL 15022552-15023440 | downstream | ON11297 | Two or more Affy2 Probesets identity exons of this gene. This is a summary of the tissue expression peaks exhibited in at least one of these Probesets. Expression at moderate levels in the following post-embryonic organs or tissues: larval adult central nervous system. |
| Within | Phn-C3 | | Expression at high levels in the following post-embryonic organs or tissues: adult head, adult brain, larval hindgut, adult Malpighian tubules, larval heart, insensitized spermatheca, adult male accessory gland, larval testes. |
## Supplementary Table 2. Continued.

| Gene   | Gene Symbol | Description                                                                 |
|--------|-------------|-----------------------------------------------------------------------------|
| roo    | 1012       | Expression at moderate levels in the following post-embryonic organs or tissues: adult salivary gland, adult ovaries. |
| JL     | 16938883-1694797 | High or moderate levels of expression observed in all larval and adult organs/tissues. |
| N473E  |             | Expression at high levels in the following post-embryonic organs or tissues: adult head, adult eye, larval/adult central nervous system, adult crop, larval/adult midgut, larval/adult Malpighian tubules, adult heart, larval/adult fat body, larval/adult salivary gland, larval triches, adult spermatheca, larval/adult ovaries. |
|         |             | Expression at moderate levels in the following post-embryonic organs or tissues: adult ovaries. |
| CB4343 |             | Adult male reproductive system. |
| Obp73a |             | No expression detected in any larval or adult organs/tissues. |
| CG7724 |             | Expression at moderate levels in the following post-embryonic organs or tissues: adult head, adult eye, adult crop, adult Malpighian tubules, adult salivary gland, adult ovaries. |
| CG1413 |             | Two or more Afly2 probesets identify exons of this gene. This is a summary of the tissue expression peaks exhibited in at least one of these probesets. Expression at moderate levels in the following post-embryonic organs or tissues: adult head, adult eye, larval/adult central nervous system, adult crop, larval/adult midgut, larval/adult Malpighian tubules, adult heart, larval/adult fat body, larval/adult salivary gland, larval triches, adult spermatheca, larval/adult ovaries. |
| CG4393 |             | Two or more Afly2 probesets identify exons of this gene. This is a summary of the tissue expression peaks exhibited in at least one of these probesets. Expression at moderate levels in the following post-embryonic organs or tissues: larval/adult central nervous system, larval midgut, larval hindgut, larval Malpighian tubules, larval/adult fat body, larval/adult salivary gland, larval triches, adult ovaries, larval/adult ovaries. |
| COJ2198 |            | Expression at high levels in the following post-embryonic organs or tissues: larval/adult salivary gland. |
| snr1   |             | Little or no expression detected in any larval or adult organs/tissues. |
| Avr1   |             | Expression at high levels in the following post-embryonic organs or tissues: larval/adult midgut, larval Malpighian tubules, adult heart, larval fat body, virgin spermatheca. |
| CG1413 |             | Expression at moderate levels in the following post-embryonic organs or tissues: adult head, adult eye, larval/adult central nervous system, adult crop, larval/adult hindgut, adult Malpighian tubules, adult heart, larval/adult fat body, larval/adult salivary gland, larval triches, adult female reproductive system, adult male accessory gland, larval/adult ovaries. |
| CG1407 |             | Expression at moderate levels in the following post-embryonic organs or tissues: larval/adult central nervous system, adult crop, larval Malpighian tubules, larval body, larval salivary gland, larval triches, adult female reproductive system, adult testes, larval/adult ovaries. |
| Iiz-I1 |             | Expression at high levels in the following post-embryonic organs or tissues: adult ovaries. |
| CG1187 |             | Expression at moderate levels in the following post-embryonic organs or tissues: larval/adult ovaries. |
| CG7362 |             | Expression at moderate levels in the following post-embryonic organs or tissues: adult head, adult eye, adult central nervous system, adult crop, larval/adult hindgut, adult Malpighian tubules, adult heart, adult fat body, adult ovaries, adult ovaries. |
| Bake6  |             | Little or no expression detected in any larval or adult organs/tissues. |
| Pps6   |             | Expression at high levels in the following post-embryonic organs or tissues: larval fat body, larval salivary gland. |
| CG7115 |             | Expression at moderate levels in the following post-embryonic organs or tissues: adult head, adult eye, larval/adult central nervous system, adult crop, larval/adult midgut, adult Malpighian tubules, adult fat body, adult salivary gland, adult reproductive system, adult ovaries. |
| CG1413 |             | Expression at moderate levels in the following post-embryonic organs or tissues: adult ovaries. |
| CG8262 |             | No Afly2 data available because no Afly2 Probeset aligns to an exon of CG8262. |
| CG1413 |             | Expression at moderate levels in the following post-embryonic organs or tissues: adult midgut, larval/adult hindgut, larval adult Malpighian tubules. |
| CG1413 |             | No expression detected in any larval or adult organs/tissues. |
| CG1413 |             | High levels of expression observed in all larval and adult organs/tissues. |
| CG1413 |             | Expression at high levels in the following post-embryonic organs or tissues: adult head, adult eye, larval/adult central nervous system, adult crop, larval/adult midgut, larval/adult hindgut, larval Malpighian tubules, adult heart, larval/adult fat body, larval/adult salivary gland, larval triches, adult female reproductive system, adult male reproductive system, larval/adult ovaries. |
| CG1413 |             | Expression at high levels in the following post-embryonic organs or tissues: adult ovaries. |
| CG1413 |             | High levels of expression observed in all larval and adult organs/tissues. |
| CG1413 |             | Expression at high levels in the following post-embryonic organs or tissues: adult head, adult eye, larval/adult central nervous system, adult crop, larval/adult midgut, larval/adult hindgut, larval Malpighian tubules, adult heart, larval/adult fat body, larval/adult salivary gland, larval triches, adult spermatheca, adult male reproductive system, larval/adult ovaries. |
| CG1413 |             | Expression at high levels in the following post-embryonic organs or tissues: adult ovaries. |
### Supplementary Table 2. Continued.

| Gene | Strain | Expression | Description |
|------|--------|------------|-------------|
| upstream | hkb | Expression at moderate levels in the following post-embryonic organs or tissues: larval central nervous system. |
| downstream | CG1719 | Nearly all larval and adult organ/tissues expressed at moderate or high levels. Expression at high levels in the following post-embryonic organs or tissues: adult head, adult eye, adult heart, larval/adult fat body, adult spermathecae, adult testes, adult ovaries. |
| partial overlap | CG1190 | Expression at moderate levels in the following post-embryonic organs or tissues: adult head, adult eye, adult central nervous system. |
| upstream | lds | Expression at moderate levels in the following post-embryonic organs or tissues: larval central nervous system, adult ovary, adult testes. |
| downstream | CD208a | High or moderate levels of expression observed in all larval and adult organ/tissues. Expression at high levels in the following post-embryonic organs or tissues: adult head, adult eye, larval/adult central nervous system, adult ovary, larval/adult midgut, larval/adult hindgut, larval/Malpighian tubules, larval tracheae, adult ovary, adult salivary gland, adult testes. |
| Within | dxx | Two or more Affy2 Probesets identify genes of this probe. This is a summary of the tissue expression pattern exhibited at least once of these Probesets. Expression at moderate levels in the following post-embryonic organs or tissues: adult head, adult fat body, larval adult hindgut, larval/Malpighian tubules, larval tracheae, adult ovary. |
| upstream | CG1162 | No expression detected in any larval or adult organ/tissues. |
| downstream | Cg3402 | Expression at moderate levels in the following post-embryonic organs or tissues: larval central nervous system, adult ovary, adult testes. |
| upstream | Cg3402 | Expression at moderate levels in the following post-embryonic organs or tissues: larval central nervous system, adult ovary, adult testes. |
| downstream | Gr65c | No expression detected in any larval or adult organ/tissues. |
| upstream | Kp78b | Two or more Affy2 Probesets identify genes of this probe. This is a summary of the tissue expression pattern exhibited at least once of these Probesets. Expression at moderate levels in the following post-embryonic organs or tissues: larval central nervous system, adult testes. |
| downstream | nBp4L04 | Expression at moderate levels in the following post-embryonic organs or tissues: adult head, adult eye, adult heart. |
| upstream | Kp78a | Two or more Affy2 Probesets identify genes of this probe. This is a summary of the tissue expression pattern exhibited at least once of these Probesets. Expression at moderate levels in the following post-embryonic organs or tissues: larval central nervous system, adult testes. |
| downstream | CG1012 | Nearly all larval and adult organ/tissues expressed at moderate or high levels. Expression at high levels in the following post-embryonic organs or tissues: larval/adult central nervous system. Expression at moderate levels in the following post-embryonic organs or tissues: larval head, larval eye, larval heart, larval midgut, larval hindgut, larval salivary gland, larval testes. |
| upstream | d-erp | Expression at moderate levels in the following post-embryonic organs or tissues: adult testes. |
| downstream | CG3199 | Expression at moderate levels in the following post-embryonic organs or tissues: adult head, adult heart. Expression at high levels in the following post-embryonic organs or tissues: adult head. |
| upstream | CG3199 | Expression at moderate levels in the following post-embryonic organs or tissues: adult head. |
| downstream | CG1190 | Expression at moderate levels in the following post-embryonic organs or tissues: adult head. |
| upstream | Sir2 | Expression of moderate levels in the following post-embryonic organs or tissues: adult head, adult midgut, larval tracheae. |
| downstream | CG1221 | Expression at high levels in the following post-embryonic organs or tissues: larval/adult central nervous system. Expression at moderate levels in the following post-embryonic organs or tissues: larval head. |
| upstream | CR2483 | Expression of moderate levels in the following post-embryonic organs or tissues: larval fat body. |
| downstream | Dfs | Expression at moderate levels in the following post-embryonic organs or tissues: adult testes. |
| upstream | CG1517 | Expression at moderate levels in the following post-embryonic organs or tissues: larval fat body. |
| downstream | CG2022 | Two or more Affy2 Probesets identify genes of this probe. This is a summary of the tissue expression pattern exhibited at least once of these Probesets. Expression at high levels in the following post-embryonic organs or tissues: adult head, adult fat body, larval tracheae. |
| upstream | Suf2 | Expression at moderate levels in the following post-embryonic organs or tissues: adult head, adult midgut, larval tracheae. |
| downstream | CR4283 | Expression at high levels in the following post-embryonic organs or tissues: larval/adult central nervous system. Expression at moderate levels in the following post-embryonic organs or tissues: larval head. |
| upstream | CG1190 | No expression detected in any larval or adult organ/tissues. |
| downstream | Df2a | Expression at high levels in the following post-embryonic organs or tissues: adult testes. |
| upstream | G62 | Expression at moderate levels in the following post-embryonic organs or tissues: adult head, adult heart, adult midgut, larval tracheae. |
| downstream | min502H | No expression detected in any larval or adult organ/tissues. |
### Supplementary Table 2. Continued.

| Gene ID | Description | Expression Levels |
|---------|-------------|-------------------|
| 105     | No expression detected in any larval or adult organs/tissues. | Expression at high levels in the following post-embryonic organs or tissues: adult head, adult eye, adult cerci. Expression at moderate levels in the following post-embryonic organs or tissues: larval head, larval head, adult larval head, adult larval head, larval head, larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larval head, adult larva,
| ID   | Genotype | Genotype | Strain   | Accession | Description                                                                 |
|------|----------|----------|----------|-----------|-----------------------------------------------------------------------------|
| 118  | roo[1400] | 5SK      | 26221995-26255252 | expression observed in all larval and adult organs/tissues. Expression at high levels in the following post-embryonic organs or tissues: adult head, adult eye, larval/adult central nervous system, adult crop, larval/adult midgut, larval/adult hindgut, larval/adult Malpighian tubules, adult heart, larval/adult fat body, larval/adult salivary gland, larval trachea, adult female reproductive system, adult male reproductive system, larval/adult accessory glands. |
| 119  | roo[14733] | X        | 577096-64191 | expression observed at high levels in the following post-embryonic organs or tissues: larval hindgut. |
| 120  | roo[77] | X        | 721690-728423 | expression observed in all larval and adult organs/tissues. Expression at high levels in the following post-embryonic organs or tissues: adult head, adult eye, larval/adult midgut, larval Malpighian tubules, adult heart, larval/adult fat body, larval/adult salivary gland, larval trachea, adult female accessory glands. |
| 121  | roo[13] | X        | 957844-960579 | expression observed at high levels in the following post-embryonic organs or tissues: adult head, adult eye, larval/adult midgut, larval Malpighian tubules, adult heart, larval/adult fat body, larval/adult salivary gland, larval trachea, adult female accessory glands. |
| 122  | roo[20] | X        | 1651196-1640305 | expression observed at high levels in the following post-embryonic organs or tissues: adult head, adult eye, larval/adult midgut, larval Malpighian tubules, adult heart, larval/adult fat body, larval/adult salivary gland, larval trachea, adult female accessory glands. |
| 123  | roo[25] | X        | 2209326-2382220 | expression observed at high levels in the following post-embryonic organs or tissues: larval/adult midgut, larval Malpighian tubules, adult heart, larval/adult fat body, larval/adult salivary gland, larval trachea, adult female accessory glands. |
| 124  | roo[8] | X        | 2685706-2685712 | expression observed at high levels in the following post-embryonic organs or tissues: larval/adult Malpighian tubules. |
| 125  | roo[8] | X        | 2691741-2691745 | expression observed at high levels in the following post-embryonic organs or tissues: adult head, adult eye, larval/adult midgut, larval Malpighian tubules, adult heart, larval/adult fat body, larval/adult salivary gland, larval trachea, adult female accessory glands. |
| 126  | roo[8] | X        | 2717876-2726365 | expression observed at high levels in the following post-embryonic organs or tissues: larval/adult Malpighian tubules. |
| 127  | roo[34] | X        | 31122594-3123684 | expression observed at high levels in the following post-embryonic organs or tissues: larval/adult Malpighian tubules, larval/adult central nervous system, larval/adult salivary gland, larval trachea, adult male reproductive system. |
| 128  | roo[37] | X        | 3387180-3386280 | expression observed at high levels in the following post-embryonic organs or tissues: larval/adult central nervous system, larval/adult salivary gland, larval trachea, adult male reproductive system. |
| 129  | roo[39] | X        | 3491639-3496347 | expression observed at high levels in the following post-embryonic organs or tissues: larval/adult central nervous system, larval/adult salivary gland, larval trachea, adult female reproductive system. |
| 130 | roo/38 | X | 349597-2386220 | upstream | CG2275 | Expression at moderate levels in the following post-embyronic organs or tissues: larval central nervous system, larval/adult salivary gland, larval trachea, adult female reproductive system, adult male accessory gland, larval carcass. |
|-----|--------|---|----------------|---------|--------|---------------------------------------------------|
|     |        |   |                | downstream | Ifp7 | Expression at moderate levels in the following post-embyronic organs or tissues: adult thoraco-abdominal ganglion. |
|     |        |   |                | Within | AbhR | Expression at moderate levels in the following post-embyronic organs or tissues: adult eye, adult brain. |
| 131 | roo/41 | X | 350362-2350100 | upstream | CG2275 | Expression at moderate levels in the following post-embyronic organs or tissues: larval central nervous system, larval/adult salivary gland, larval trachea, adult female reproductive system, adult male accessory gland, larval carcass. |
|     |        |   |                | downstream | Ifp7 | Expression at moderate levels in the following post-embyronic organs or tissues: adult thoraco-abdominal ganglion. |
|     |        |   |                | Within | AbhR | Expression at moderate levels in the following post-embyronic organs or tissues: adult eye, adult brain. |
| 132 | roo/1631 | X | 3503177-3508877 | upstream | CG2275 | Expression at moderate levels in the following post-embyronic organs or tissues: larval central nervous system, larval/adult salivary gland, larval trachea, adult female reproductive system, adult male accessory gland, larval carcass. |
|     |        |   |                | downstream | Ifp7 | Expression at moderate levels in the following post-embyronic organs or tissues: adult thoraco-abdominal ganglion. |
|     |        |   |                | Within | AbhR | Expression at moderate levels in the following post-embyronic organs or tissues: adult eye, adult brain. |
| 133 | roo/33 | X | 682861-6487004 | upstream | CG2261 | Two or more Ayf2/Probehets identify clones of this gene. This is a summary of the tissue expression peaks established at least one of these Probehets. Expression at moderate levels in the following post-embyronic organs or tissues: adult eye. |
|     |        |   |                | downstream | SIF3 | Expression at high levels in the following post-embyronic organs or tissues: adult testis. |
|     |        |   |                | Within | CG12680 | Expression at moderate levels in the following post-embyronic organs or tissues: adult testis. |
| 134 | roo/1649 | X | 4884312-4891287 | upstream | CG12680 | Expression at high levels in the following post-embyronic organs or tissues: adult testis. |
|     |        |   |                | upstream | SIF3 | Expression at high levels in the following post-embyronic organs or tissues: adult testis. |
|     |        |   |                | downstream | CG12680 | Expression at moderate levels in the following post-embyronic organs or tissues: adult testis. |
| 135 | roo/157 | X | 4885900-4898013 | upstream | CG12680 | Expression at moderate levels in the following post-embyronic organs or tissues: adult testis. |
|     |        |   |                | downstream | Ifp7 | Expression at moderate levels in the following post-embyronic organs or tissues: larval/adult salivary gland, larval trachea, adult eye, adult male accessory gland, larval carcass. |
|     |        |   |                |Within | AbhR | Expression at moderate levels in the following post-embyronic organs or tissues: adult eye, adult brain. |
| 136 | roo/78 | X | 7013333-7024364 | upstream | CG2265 | Two or more Ayf2/Probehets identify clones of this gene. This is a summary of the tissue expression peaks established at least one of these Probehets. Expression at high levels in the following post-embyronic organs or tissues: adult eye, adult brain. |
|     |        |   |                | downstream | r4gA | Expression at moderate levels in the following post-embyronic organs or tissues: adult head, adult eye, larval/adult central nervous system, adult adip, adult/adult midgut, larval/adult hindgut, larval Malpighian tubules, larval/adult fat body, larval/adult salivary gland, larval trachea, adult eye, larval male accessory gland, larval carcass. |
|     |        |   |                | Within | c1.11 | Expression at moderate levels in the following post-embyronic organs or tissues: adult head, adult eye, larval/adult central nervous system, adult adip, adult/adult midgut, larval/adult hindgut, larval Malpighian tubules, adult head, adult/adult fat body, larval/adult salivary gland, larval trachea, adult eye, larval male accessory gland, larval carcass. |
| 137 | roo/189 | X | 8842847-8850298 | upstream | CG2265 | Two or more Ayf2/Probehets identify clones of this gene. This is a summary of the tissue expression peaks established at least one of these Probehets. Expression at high levels in the following post-embyronic organs or tissues: adult eye, adult brain. |
|     |        |   |                | downstream | r4gA | Expression at moderate levels in the following post-embyronic organs or tissues: adult head, adult eye, larval/adult central nervous system, adult adip, adult/adult midgut, larval/adult hindgut, larval Malpighian tubules, larval/adult fat body, larval/adult salivary gland, larval trachea, adult eye, larval male accessory gland, larval carcass. |
|     |        |   |                | Within | c1.11 | Expression at moderate levels in the following post-embyronic organs or tissues: adult head, adult eye, larval/adult central nervous system, adult adip, adult/adult midgut, larval/adult hindgut, larval Malpighian tubules, larval/adult fat body, larval/adult salivary gland, larval trachea, adult eye, larval male accessory gland, larval carcass. |
| 138 | roo/104 | X | 9181058-9181058 | upstream | CG1265 | Expression at high levels in the following post-embyronic organs or tissues: larval/adult midgut, larval Malpighian tubules, adult fat body. |
|     |        |   |                | downstream | CG1265 | Expression at moderate levels in the following post-embyronic organs or tissues: larval/adult midgut, larval Malpighian tubules, adult fat body. |
|     |        |   |                | partial overlap | CG1265 | Expression at moderate levels in the following post-embyronic organs or tissues: larval/adult midgut, larval Malpighian tubules, adult fat body. |
|     |        |   |                | Within | CG1265 | Expression at moderate levels in the following post-embyronic organs or tissues: larval/adult midgut, larval Malpighian tubules, adult fat body. |
| 139 | roo/136 | X | 10165191-10170482 | upstream | CG1265 | Expression at high levels in the following post-embyronic organs or tissues: adult head, larval Malpighian tubules, adult fat body. |
|     |        |   |                | downstream | CG1265 | Expression at moderate levels in the following post-embyronic organs or tissues: adult head, larval Malpighian tubules, adult fat body. |
|     |        |   |                | partial overlap | CG1265 | Expression at moderate levels in the following post-embyronic organs or tissues: adult head, larval Malpighian tubules, adult fat body. |
|     |        |   |                | Within | CG1265 | Expression at moderate levels in the following post-embyronic organs or tissues: adult head, larval Malpighian tubules, adult fat body. |
| 140 | roo/100 | X | 10580139-10585217 | upstream | CG1265 | Expression at moderate levels in the following post-embyronic organs or tissues: adult head, larval Malpighian tubules, adult fat body. |
|     |        |   |                | downstream | CG1265 | Expression at moderate levels in the following post-embyronic organs or tissues: adult head, larval Malpighian tubules, adult fat body. |
|     |        |   |                | Within | X11.ben | Expression at moderate levels in the following post-embyronic organs or tissues: adult head, larval Malpighian tubules, adult fat body. |
|     |        |   |                | upstream | CG1265 | Expression at moderate levels in the following post-embyronic organs or tissues: adult head, larval Malpighian tubules, adult fat body. |
|     |        |   |                | downstream | CG1265 | Expression at moderate levels in the following post-embyronic organs or tissues: adult head, larval Malpighian tubules, adult fat body. |
| 141 | roo/146i | X | 10819018-10819187 | upstream | CG1265 | Expression at high levels in the following post-embyronic organs or tissues: larval/adult midgut, larval Malpighian tubules, adult fat body. |
|     |        |   |                | downstream | CG1265 | Expression at moderate levels in the following post-embyronic organs or tissues: larval/adult midgut, larval Malpighian tubules, adult fat body. |
|     |        |   |                | Within | CG1265 | Expression at moderate levels in the following post-embyronic organs or tissues: larval/adult midgut, larval Malpighian tubules, adult fat body. |
| 142 | roo/111 | X | 11647655-11644743 | upstream | CG1265 | Expression at high levels in the following post-embyronic organs or tissues: larval/adult midgut, larval Malpighian tubules, adult fat body. |
|     |        |   |                | downstream | CG1265 | Expression at moderate levels in the following post-embyronic organs or tissues: larval/adult midgut, larval Malpighian tubules, adult fat body. |
|     |        |   |                | Within | CG1265 | Expression at moderate levels in the following post-embyronic organs or tissues: larval/adult midgut, larval Malpighian tubules, adult fat body. |
| 143 | roo/123 | X | 11599111-11500825 | upstream | CG1265 | Expression at high levels in the following post-embyronic organs or tissues: larval/adult midgut, larval Malpighian tubules, adult fat body. |
|     |        |   |                | downstream | CG1265 | Expression at moderate levels in the following post-embyronic organs or tissues: larval/adult midgut, larval Malpighian tubules, adult fat body. |
|     |        |   |                | Within | CG1265 | Expression at moderate levels in the following post-embyronic organs or tissues: larval/adult midgut, larval Malpighian tubules, adult fat body. |
### Supplementary Table 2. Continued.

| Entry | ID | Description | Details |
|-------|----|-------------|---------|
| 144   | roo(1)32 | X | 13430948-14840146 | **upstream** | pgdy | Expression at high levels in the following post-embryonic organs or tissues: adult testis. |
| 145   | roo(1)42 | X | 10618066-10612537 | **downstream** | m7G6A:U514B | No FlyAlts data available because no Affy2 ProbeSet aligns to an exon of m7G6A:U514B. |
| 146   | roo(1)43 | X | 16234756-16243854 | **upstream** | Dp1 | Expression at moderate levels in the following post-embryonic organs or tissues: larval central nervous system, adult ovary. Expression at moderate levels in the following post-embryonic organs or tissues: adult head, adult eye, adult central nervous system, adult crop, larval/adult midgut, larval/adult hindgut, larval Malpighian tubules, adult heart, larval/adult fat body, larval/adult salivary gland, larval trachea, adult male accessory gland, larval/adult carcass. |
| 147   | roo(1)62 | X | 1885158-18859762 | **downstream** | Dieldrin | No FlyAlts data available because an Affy2 ProbeSet aligns to an exon of CG1329. |
| 148   | roo(1)88 | X | 20707008-20715352 | **upstream** | CO5393 | Expression at moderate levels in the following post-embryonic organs or tissues: adult testis. |
| 149   | roo(1)90 | X | 20932946-20933373 | **upstream** | CG1518 | Expression at moderate levels in the following post-embryonic organs or tissues: larval carcass. Expression at moderate levels in the following post-embryonic organs or tissues: larval midgut. |
| 150   | roo(1)716 | X | 21304786-21424793 | **downstream** | CG4476 | Expression at high levels in the following post-embryonic organs or tissues: adult head, adult eye, adult central nervous system, adult crop, larval/adult midgut, larval/adult hindgut, larval Malpighian tubules, adult heart, larval/adult fat body, larval/adult salivary gland, larval trachea, adult male accessory gland, larval/adult carcass. |
| 151   | roo(1)4286 | X | 22198109-22465374 | **upstream** | CG04045 | Expression at moderate levels in the following post-embryonic organs or tissues: adult midgut. |

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**Note:** The table continues with similar entries for other entries.

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**Reference:** Mamillapalli et al.
### Supplementary Table 3.

| roo associated with genes expressed in testis/ovary | roo associated with genes expressed in other tissues | roo associated with genes not expressed in adult tissue/expression data not available |
|----------------------------------------------------|-----------------------------------------------------|----------------------------------------------------------------------------------|
| roo{1}[2][gl][52]                                  | roo{2}283                                           | roo{4}14147                                                                       |
| roo{1}281                                           | roo{2}284                                           | roo{4}11598                                                                       |
| roo{1}311                                           | roo{2}2620                                          | roo{4}11665                                                                       |
| roo{1}grk[2]                                        | roo{2}dmn[3]                                        | roo{4}1708                                                                        |
| roo{1}grk[3]                                        | roo{2}5613                                          | roo{4}989                                                                         |
| roo{1}grk[4]                                        | roo{2}315                                           | roo{4}11653                                                                       |
| roo{1}mus[20][S]                                    | roo{2}1706                                          | roo{4}992                                                                         |
| roo{1}326                                           | roo{2}319                                           | roo{4}1582                                                                        |
| roo{1}339                                           | roo{2}330                                           | roo{4}1265                                                                        |
| roo{1}371                                           | roo{2}366                                           | roo{4}1378                                                                        |
| roo{1}Mhc[4]                                        | roo{2}495                                           | roo{4}1379                                                                        |
| roo{1}402                                           | roo{2}501                                           | roo{4}11411                                                                       |
| roo{1}676                                           | roo{2}784                                           | roo{4}11701                                                                       |
| roo{1}419                                           | roo{2}785                                           | roo{4}1434                                                                        |
| roo{1}spir[183]                                     | roo{2}813                                           | roo{4}25                                                                         |
| roo{1}508                                           | roo{2}828                                           | roo{4}123                                                                         |
| roo{1}521                                           | roo{2}1769                                          |                                                                                 |
| roo{1}3250                                          | roo{2}854                                           |                                                                                 |
| roo{1}668                                           | roo{2}862                                           |                                                                                 |
| roo{1}764                                           | roo{2}896                                           |                                                                                 |
| roo{1}775                                           | roo{2}903                                           |                                                                                 |
| roo{1}670                                           | roo{2}scny[roo]                                    |                                                                                 |
| roo{1}601                                           | roo{2}944                                           |                                                                                 |
| roo{1}602                                           | roo{2}952                                           |                                                                                 |
| roo{1}793                                           | roo{2}958                                           |                                                                                 |
| roo{1}796                                           | roo{2}982                                           |                                                                                 |
| roo{1}806                                           | roo{2}1020                                          |                                                                                 |
| roo{1}815                                           | roo{2}1039                                          |                                                                                 |
| roo{1}816                                           | roo{2}11583                                         |                                                                                 |
| roo{1}707                                           | roo{2}3796                                          |                                                                                 |
| roo{1}850                                           | roo{2}1392                                          |                                                                                 |
| roo{1}866                                           | roo{2}1410                                          |                                                                                 |
| roo{1}867                                           | roo{2}1426                                          |                                                                                 |
| roo{1}868                                           | roo{2}1430                                          |                                                                                 |
| roo{1}883                                           | roo{2}1445                                          |                                                                                 |
| roo{1}898                                           | roo{2}1460                                          |                                                                                 |
| roo{1}911                                           | roo{2}4733                                          |                                                                                 |
| roo{1}927                                           | roo{2}113                                           |                                                                                 |
| roo{1}936                                           | roo{2}134                                           |                                                                                 |
| roo{1}Hn[3]                                         | roo{2}139                                           |                                                                                 |
| roo{1}65                                            | roo{2}138                                           |                                                                                 |
| roo{1}69                                            | roo{2}141                                           |                                                                                 |
| roo{1}74                                            | roo{2}1631                                          |                                                                                 |
| roo{1}95                                            | roo{2}189                                           |                                                                                 |
| roo\{1010 | roo\{v\[36f| | roo\{1011 | roo\{1018 | roo\{1034 | roo\{1043 | roo\{1055 | roo\{1059 | roo\{1076 | roo\{5834 | roo\{2355 | roo\{1189 | roo\{dx[D | roo\{1267 | roo\{1290 | roo\{1348 | roo\{1359 | roo\{1388 | roo\{1395 | roo\{1405 | roo\{1421 | roo\{alpha4GT2[1 | roo\{1425 | roo\{1429 | roo\{1455 | roo\{1458 | roo\{17 | roo\{20 | roo\{w[bf | roo\{w[sp1 | roo\{28 | roo\{37 | roo\{53 | roo\{1649 | roo\{57 | roo\{78 | roo\{lz[L] | roo\{96 | roo\{100 | roo\{132 | roo\{143 | roo\{186 | roo\{193 |