

A Novel Cell Death Gene Acts to Repair Patterning Defects in *Drosophila melanogaster*

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ABSTRACT Cell death is a mechanism utilized by organisms to eliminate excess cells during development. Here, we describe a novel regulator of caspase-independent cell death, *Mabiki* (*Mabi*), that is involved in the repair of the head patterning defects caused by extra copies of *bicoid* in *Drosophila melanogaster*. *Mabiki* functions together with caspase-dependent cell death mechanisms to provide robustness during development.

ORGANISMS are surprisingly robust to various stresses and perturbations. Elimination of undesirable cells is one mechanism that ensures robust development. For example, compensatory cell death is observed in the expanded prospective head region of embryos from mothers carrying extra (six) copies of *bicoid* (*6xbcd*) in *Drosophila melanogaster*. *bcd* mRNA is localized to the anterior pole of the oocyte, forming an anteroposterior gradient of BCD protein in the embryo (Frohnhöffer and Nüsslein-Volhard 1986; Berleth *et al.* 1988). *6xbcd* embryos show a posterior shift in expression of the downstream genes and the position of the cephalic furrow located near the head/trunk junction. Nevertheless, many embryos still survive to adulthood (Namba *et al.* 1997). More importantly, the final pattern and size of the adult structures are mostly normal (but for an exception, see Busturia and Lawrence 1994). *Drosophila* has repair mechanisms for these patterning defects, and one is cell death in the expanded head region of

embryos. However, the mechanism leading to this compensatory cell death remains largely unexplored.

To identify genes involved in this repair, we first screened a panel of 152 autosomal deficiencies for those with significantly lower viability in *6xbcd* than in normal (*2xbcd*) condition, namely, for haploinsufficient genes in the *6xbcd* condition, and obtained two candidate regions, 29A2-A3 and 34A7-B6 (Supporting Information, Table S1 and Table S2). To complement this haploinsufficiency screen, we performed a microarray expression analysis to identify genes with differential expression between the two conditions at embryonic stage 11–12, when extensive cell death occurs in the expanded head region of *6xbcd* embryos (Table S3 and Table S4; Namba *et al.* 1997). Twelve genes showed more than twofold higher expression in *6xbcd* compared to *2xbcd*. Cross-referencing the genetic positions and changes in gene expression from these two complementary screens allowed us to identify a candidate gene, *CG15479*, involved in the repair. We named this gene *Mabiki* (abbreviated as *Mabi*). The intronless *Mabi* gene contains a 615-bp open reading frame and encodes a potential member of the basic region-leucine zipper (bZIP) family of transcription factors that binds specifically to DNA as dimers (Fassler *et al.* 2002). While it is found only in the Diptera lineage, there are three paralogs, *CG14014*, *CG16813*, and *CG16815*, in the *D. melanogaster* genome (Figure S1).

The higher expression of *Mabi* in *6xbcd* embryos compared to *2xbcd* was validated by real-time quantitative PCR (4.7-fold difference). *Mabi* was expressed throughout the embryo at stage 11, but a stronger signal was

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Expression data from this study have been deposited in the Gene Expression Omnibus (GEO) database under the accession number GSE36675.

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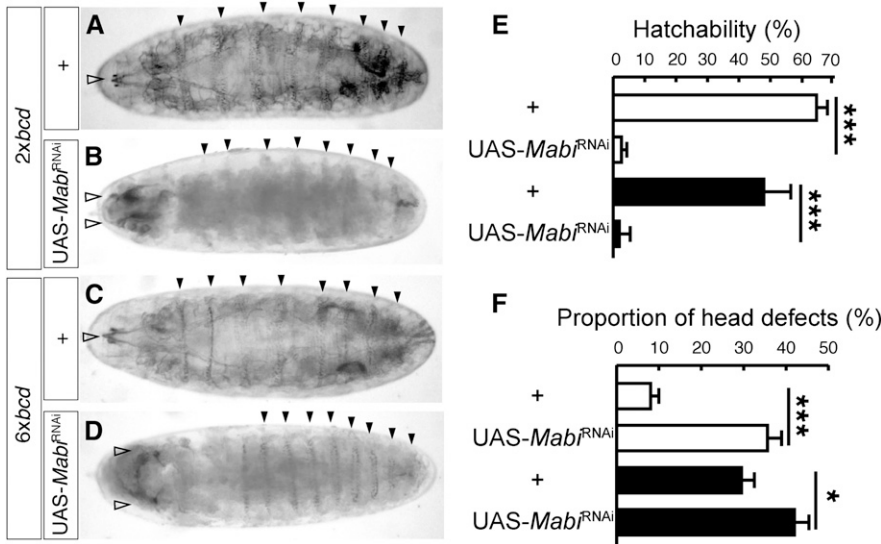


Figure 1 Defects in *Mabi* knockdown embryos. After a 2-hr egg collection and 1-hr incubation at 25°, embryos were heat shocked at 37° for 1 hr and then incubated at 25°. (A and C) *hsp70-GAL4/+* embryos at stage 17. (B and D) Examples of head defects of unhatched *UAS-Mabi^{RNAi}/+; hsp70-GAL4/+* embryos. (A and B) *2xbcd*, and (C and D) *6xbcd* embryos. Small black arrowheads indicate abdominal segments (A1–A8). Large open arrowheads indicate mouth hook. Anterior is to the left in all images. (E) Hatchability (%) of *Mabi* knockdown embryos. (F) Frequency (%) of embryos showing head defects among dead embryos. (E and F) White and black bars represent *2xbcd* and *6xbcd* embryos, respectively. Error bar represents the standard error of the mean of four experiments. * and *** indicate statistical significance at the 5% and 0.1% levels, respectively.

detected in the anterior region in both *2xbcd* and *6xbcd* (Figure S2). At stage 12, a few cells showed *Mabi* expression; however, some *6xbcd* embryos with head defects (3/79) showed strong and broad *Mabi* expression in the head domain.

Since there is no mutant available for *Mabi*, we conducted RNAi knockdown experiments. A 1-hr heat shock during early embryogenesis resulted in embryonic lethality of *UAS-Mabi^{RNAi}; hsp70-GAL4* and the frequency of embryos with head defects (expanded head domains or abnormality in mouth hook formation, or both) was significantly greater in *Mabi* (RNA interference, RNAi) embryos than in control embryos in both *2xbcd* and *6xbcd* conditions (Figure 1), implying that *Mabi* is essential even under normal conditions. While the injection of 449-bp *Mabi* dsRNA molecules also effectively prevented eggs from hatching in both conditions (Pilot *et al.* 2006), the

hatchability of embryos injected with either of two shorter dsRNA molecules was significantly reduced only in *6xbcd* (Figure 2). Together with the observation that the relative viability of *Df(2L)ED784* heterozygotes lacking the *Mabi* gene was reduced in *6xbcd* to about 60% of that in *2xbcd* (Table S1), this result suggests that the development in the *6xbcd* condition depends on higher expression of *Mabi*.

What is more, after a 1-hr heat shock, the number of acridine orange (AO) positive cells was fewer in *UAS-Mabi^{RNAi}; hsp70-GAL4* embryos than in control embryos (Figure 3, A–D), implying a requirement for *Mabi* in cell death. *Mabi* was indeed able to trigger cell death in both embryos and imaginal discs. Heat shock induction of *Mabi* expression in *hsp70-GAL4/UAS-Mabi* embryos produced many AO positive cells (Figure 3, E and F); AO positive cells were also detected in the posterior region of small

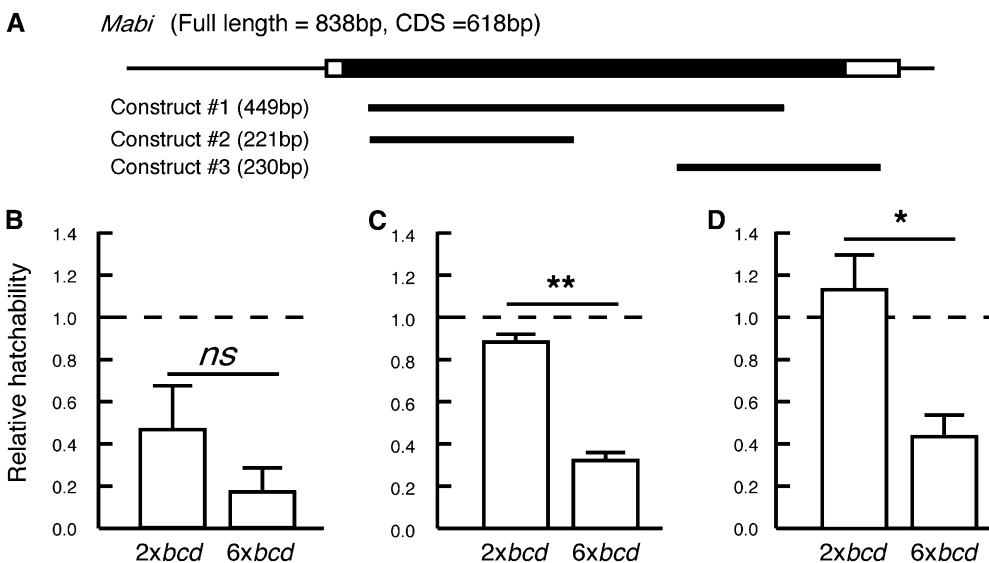


Figure 2 Reduced hatchability of embryos injected with *Mabi* dsRNA. (A) The *Mabi* gene structure (top) and three constructs used for dsRNA-mediated RNAi. The coding region is shown as a black box, while white boxes represent UTRs. All constructs are designed within the coding region. (B–D) Effect of dsRNA injection was assessed by measuring the relative hatchability = (hatchability of *Mabi*-dsRNA injected embryos) / (hatchability of dsRNA-free water injected embryos). Error bar represents the standard error of the mean of four experiments. (B) Construct #1 (449 bp); (C) construct #2 (221 bp); and (D) construct #3 (230 bp). * and ** indicate statistical significance at the 5% and 1% levels, respectively; ns, not significant.

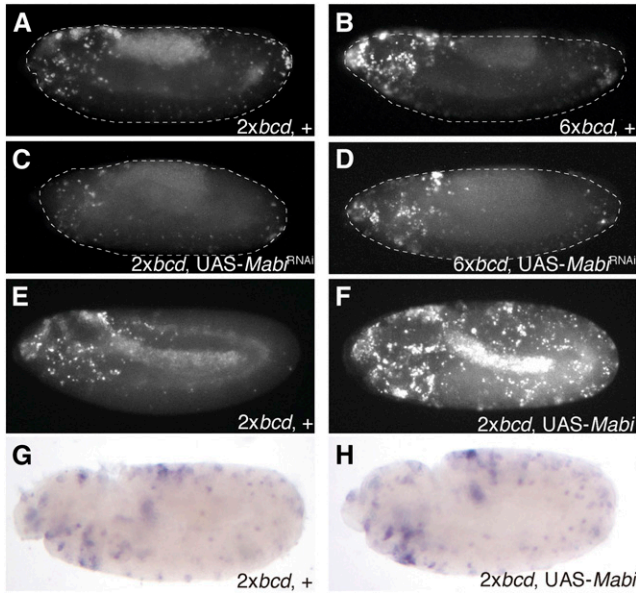


Figure 3 *Mabi* knockdown reduces cell death in embryos, while ectopic expression of *Mabi* induces cell death without activation of *rpr*. (A–D) Acridine orange (AO) staining of stage-12 embryos. (A and B) *hsp70-GAL4/+* and (C and D) *UAS-Mabi^{RNAi/+}; hsp70-GAL4/+*. (A and C) *2xbcd* and (B and D) *6xbcd* embryos. (E–H) AO staining (E and F) and *rpr* expression visualized by whole mount *in situ* hybridization (G and H) in stage-11 embryos. (E and G) *hsp70-GAL4/+* and (F and H) *hsp70-GAL4/UAS-Mabi*. All embryos were heat shocked for 1 hr. Anterior is to the left in all images.

wing discs of *en-GAL4/+; UAS-Mabi/+* larvae and the final size of the posterior region of the wings was reduced by 40% (Figure S3).

It has been shown that cell death in *6xbcd* embryos involves the proapoptotic *reaper* (*rpr*) gene, which triggers the canonical cell death pathway through caspase activation (Namba *et al.* 1997; Bangs and White 2000). However, *rpr* expression was not altered in the *hsp70-GAL4/UAS-Mabi* embryos (Figure 3, G and H). Consistent with this observation, coexpression of *p35* did not rescue the eye defects observed in *ey-GAL4/+; UAS-Mabi/+* (Figure 4). Thus, cell death induced by *Mabi* is likely to be caspase independent. In addition, unlike *p53*, ectopic expression of *Mabi* by the *glass-multimer-reporter* (*GMR*)-*GAL4* driver did not affect eye size (Figure 4). *Mabi* presumably requires an as-yet-undefined factor or factors to induce cell death.

Here, we describe the identification and characterization of *Mabi*, a novel regulator of cell death that is involved in the elimination of excessive cells in the expanded head region of *6xbcd* embryos. The findings suggest that elevated expression of *rpr* (Namba *et al.* 1997) and the ensuing caspase-dependent cell death are not sufficient to repair head patterning in the conditions with elevated concentrations of *bcd*. Both the caspase-dependent and independent cell death pathways act to confer developmental robustness in *6xbcd* conditions.

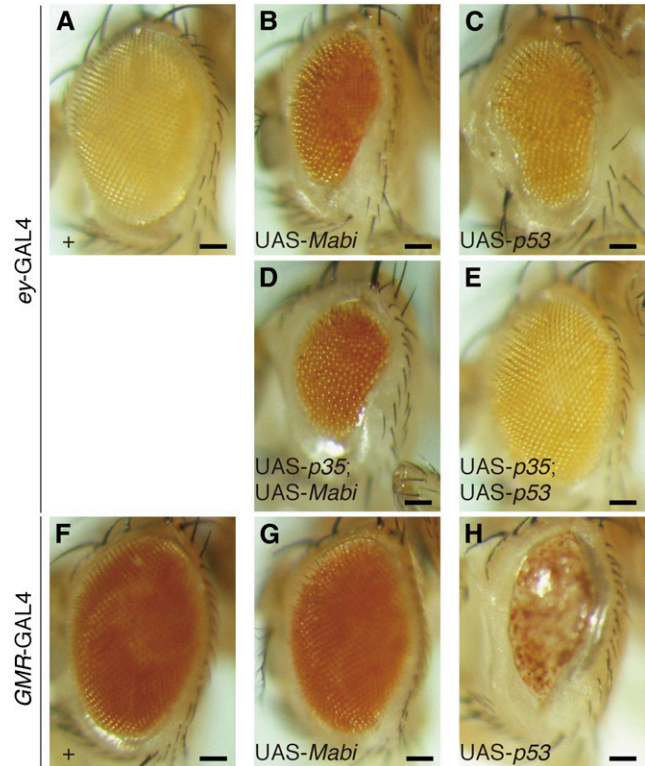


Figure 4 Effects of ectopic expression of the *Mabi* gene in eye imaginal discs. *Mabi* or *p53* are ectopically expressed by *ey-GAL4* (A–E) or *GMR-GAL4* (F–H). (A) *ey-GAL4/+*, (B) *ey-GAL4/+; UAS-Mabi/+*, (C) *ey-GAL4/+; UAS-p53/+*, (D) *ey-GAL4/+; UAS-Mabi/UAS-p35*, (E) *ey-GAL4/+; UAS-p53/UAS-p35*, (F) *GMR-GAL4/+*, (G) *GMR-GAL4/+; UAS-Mabi/+*, and (H) *GMR-GAL4/+; UAS-p53/+*. Bar gives the relative scale.

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	<i>Mabi</i>	<i>cg14014</i>	<i>cg16813</i>	<i>cg16815</i>
<i>D. melanogaster</i> ^a				
<i>D. sechellia</i> ^a	1	1	1	1
<i>D. simulans</i> ^a	1	1	2	1
<i>D. yakuba</i> ^a	1	1	1	1
<i>D. erecta</i> ^a	1	1	1	1
<i>D. ananassae</i> ^a	1	1	1	1
<i>D. pseudoobscura</i> ^a	1	1	1	1
<i>D. persimilis</i> ^a	1	1	1	1
<i>D. willistoni</i> ^a	1	1	1	1
<i>D. virilis</i> ^a	1	1	1	1
<i>D. mojavensis</i> ^a	1	1	1	1
<i>D. grimshawi</i> ^a	1	1	2	2
<i>Episyrphus balteatus</i> ^b	1	-	-	-
<i>Megalotia abdita</i> ^b	2	-	-	-
<i>Clogmia albipunctata</i> ^b	-	-	-	-
<i>Culex quinquefasciatus</i> ^a	2	-	-	-
<i>Anopheles gambiae</i> ^a	4	-	-	-
<i>Aedes aegypti</i> ^a	1	-	-	-
<i>Bombyx mori</i> ^a	-	-	-	-
<i>Tribolium castaneum</i> ^a	-	-	-	-

Figure S1 Evolutionary origins of *Mabi* paralogous genes. The data are from OrthoDB (referred as ^a; Waterhouse *et al.* 2011) and diptex (referred as ^b; Jiménez-Guri *et al.* 2013) databases. The topology of the phylogenetic tree is from Jiménez-Guri *et al.* (2013). While a *Mabi* homolog could not be identified from any species outside the Diptera, there are three paralogs, *cg14014*, *cg16813* and *cg16815*, in the *D. melanogaster* genome, which arose after the divergence of *Drosophila* and *Episyrphus* (hoverfly) approximately 60 MYA (Wiegmann *et al.* 2011). Two of the paralogs, *cg16813* and *cg16815*, are closely linked to *Mabi*. However, the overall amino acid identity of the three paralogs to *Mabi* is low (*cg14014*, 34.5%; *cg16813*, 34.9%; and *cg16815*, 39.7%), and sequence homology was not detected at the nucleotide level. The number gives the gene copy number, where each copy was assigned exclusively to one of the four genes. – indicates the absence of the gene.

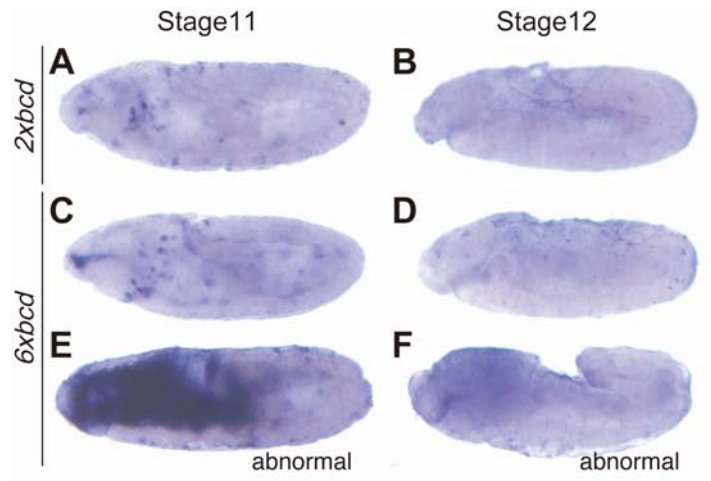


Figure S2 *Mabi* expression in stage-11 and -12 embryos, visualized by whole-mount *in situ* hybridization. (A and B) Embryos from *2xbcd* mother. (C-F) Embryos from *6xbcd* mothers. (A, C and E) stage 11, and (B, D and F) stage 12. (E and F) *6xbcd* embryos showing abnormal morphology. Anterior is to the left in all images.

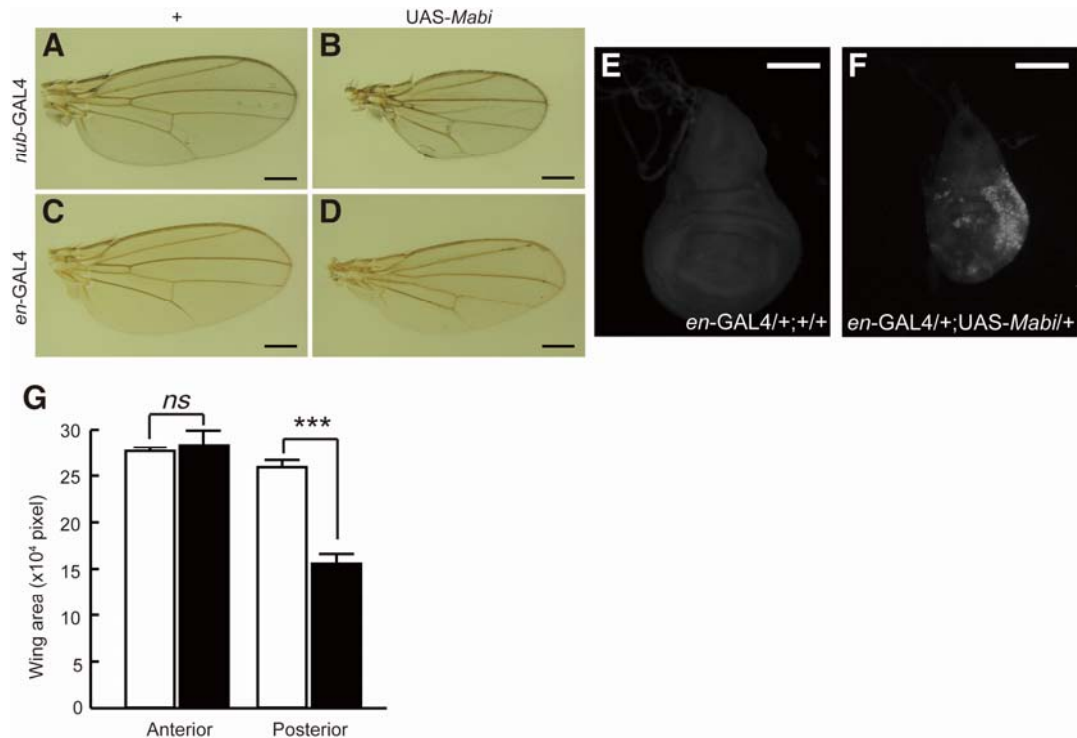


Figure S3 Cell death and reduction in wing size by *Mabi* overexpression. *Mabi* was expressed using either the *nub-GAL4* (B) or the *en-GAL4* driver (D), which are compared with controls shown in (A) and (C), respectively. The bar indicates the relative scale. (E and F) AO staining of wing disc. Posterior is to the right. (E) *en-GAL4/+*, and (F) *en-GAL4/+; UAS-Mabi/+*. (G) Relative size of posterior wing region (posterior to the fourth longitudinal vein) was reduced in *en-GAL4/+; UAS-Mabi/+*. Asterisk indicates statistical significance by student's *t* test at the 0.1% level, and *ns* indicates not significant. Error bar represents the standard error of the mean of five wings.

File S1

Methods

A highly inbred *y w* strain (TT16 sib-mated for more than 100 generations) was used as the control strain with two copies of *bcd* gene (*2xbcd*). *bcd⁺¹⁸* strain carries two *P[bcd]* inserts on the X chromosome (Berleth *et al.* 1988; provided by Claude Desplan). The second and third chromosomes of the *bcd⁺¹⁸* strain were replaced by those of TT16 and the resultant strain was used in subsequent experiments for the *6xbcd* condition where the mothers have six copies of *bcd* genes. For screening for haplo-insufficient genes in the *6xbcd* condition, females of TT16 (*2xbcd*) and *bcd⁺¹⁸* (*6xbcd*) were crossed with males of each deficiency strain. At least five flies of each sex were used for crosses. These parental flies were transferred to new vials every 24 hours for five days. The numbers of F₁ progeny carrying a deficiency and those carrying the balancer chromosome were counted and tested for heterogeneity between the *2xbcd* and *6xbcd* conditions (χ^2 -test) for each sex separately. To confirm significant results ($P < 0.05$ in both sexes), such deficiencies were retested in two additional replicates.

For microarray expression analysis, we crossed TT16 males with TT16 females to obtain *2xbcd* embryos and with *bcd⁺¹⁸* females to obtain *6xbcd* embryos. In both crosses, eggs were collected within 90 minutes and incubated for 8 hours at 25°C. After discarding unfertilized eggs under stereomicroscope, we extracted RNA from more than 100 eggs using the RNeasy Mini Kit (Qiagen) followed by DNase I digestion. We used the GeneChip One-Cycle Target Labeling and the Control reagents (Affymetrix) for labeling, and the GeneChip Hybridization, Wash, and Stain kit and the Affymetrix Drosophila Genome 2.0 arrays (Affymetrix; 15,223 probes) for hybridization. Fluidics and scanning were performed following Affymetrix protocols, and the relative expression level of each probe set was analyzed by the Statistical Expression algorithm implemented in the GeneChip Operating System (GCOS, Affymetrix) software package. We carried out paired (*2xbcd* vs. *6xbcd*) microarray experiments in three replicates using independent RNA samples.

We performed real-time quantitative PCR using a Thermal Cycler Dice® Real Time System (TP800 from TaKaRa-bio) and SYBR® Premix Ex Taq™ II (Perfect Real Time, TaKaRa-bio). Total RNA from embryos was prepared as described in the microarray experiment. cDNA was synthesized using the PrimeScript® RT reagent Kit (Perfect Real Time, TaKaRa-bio). *actin57B* was used as an internal control for normalizing the expression levels. Primers used were *Mabi*-F, CCACCGCTAGTATCCTCAA; *Mabi*-R, GGACGTATCTAAGCATGCTCT; *bcd*-F, CGCTCATCGAAAAGCTTCA; *bcd*-R, CATCCGCACCCACATCAC; *actin57B*-F, CGTGTCATCCTTGTTTCGAGA; and *actin57B*-R, ACCGCGAGCGATTAACAAGTG.

Mabi and *rpr* expression in embryos were studied by whole-mount *in situ* hybridization. We cloned the *Mabi* and *rpr* genes into a TOPO PCR4 vector (Invitrogen) using primer pairs GATTACGAAGAGCGGCAGAT and TGCTTAACCTTTAAGGCATT, and AGCCTCGGAGCACTACTCAA and TGTGCTTGTCCAAACTCAG, respectively. M13 forward and reverse primers were used to make template DNA for *in vitro* RNA transcription. Antisense DIG-RNA probe was generated with T7 RNA polymerase in buffer containing DIG RNA labeling Mix (Roche) and RNase inhibitor (Roche) at 37°C for 3 hours. The RNA was then purified by RNeasy Kit (Qiagen), quantified by optical density at 260 nm, and checked by agarose gel electrophoresis. The probe was denatured at 90°C for 2 min before use. Hybridization was carried out at 55°C for 16 hours. We used anti-DIG-AP Fab fragments (Roche) and NBT/BCIP staining solution (Roche). Embryos were photographed with an El-Einsatz Axiophot microscope (ZEISS) and a CCD camera, ProgRes® MF cool (JENOPTIK).

We knockdowned *Mabi* expression by using *CG15479*^{KK106905} (*UAS-Mabi*^{RNAi}; Vienna Drosophila RNAi Center) or by direct injection of *Mabi* double-stranded RNA (dsRNA). For the latter, we amplified the *Mabi* coding sequence by using KOD-Plus DNA polymerase (Toyobo) and the following primers containing the T7 promoter sequence (5'-GCTTCTAATACGACTCACTATAG-3') in either the forward or reverse primer:

Sense449bp-T7-F, 5'-T7seq-ATCGCCACAAAAAGTTCGAC-3';
 Sense449bp-R, 5'-ACTCTGTTGCGGCGATCTTGA-3';
 Antisense449bp-F, 5'-ATCGCCACAAAAAGTTCGAC-3';
 Antisense449bp-T7-R, 5'-T7seq-ACTCTGTTGCGGCGATCTTGA-3';
 Sense230bp-T7-F, 5'-T7seq-GATGACCTGCAGATCGAACA-3';
 Sense230bp-R, 5'-GGACGTATCTAAGCATGCTCCT-3';
 Antisense230bp-F, 5'-GATGACCTGCAGATCGAACA-3';
 Antisense230bp-T7-R, 5'-T7seq-GGACGTATCTAAGCATGCTCCT-3';
 Sense221bp-T7-F, 5'-T7seq-ATCGCCACAAAAAGTTCGAC-3';
 Sense221bp-R, 5'-TTGGTAGGAAACGCTTGAGG-3';
 Antisense221bp-F, 5'-ATCGCCACAAAAAGTTCGAC-3'; and
 Antisense221bp-T7-R, 5'-T7seq-TTGGTAGGAAACGCTTGAGG-3'.

PCR products were purified using the QIAquick PCR Purification kit (QIAGEN). Transcription from the T7 promoters was performed with Promega Ribomax kits (Promega), and then dsRNA were diluted to a concentration of about 1 µg/µl with DEPC-treated water. Embryos from *2xbcd* and *6xbcd* females were collected for 40 minutes at 25°C, dechorionated in 50% bleach, and washed with fresh water. Twenty embryos were aligned on a slide glass and

covered with silicon oil (KF-96 series produced from Shin-Etsu Silicone). Embryos were then injected with *Mabi* dsRNA or dsRNA-free DEPC-treated water as a control. The injected embryos were allowed to develop at 25°C in a moist chamber and the number of hatched larvae was counted after 26 hours. About 160 embryos were injected in a single experiment (40 embryos for each of the following four groups: a *Mabi* dsRNA- and water-injected embryos in the *2xbcd* and *6xbcd* conditions) and this experiment was repeated 4 times for each construct.

We generated a transgenic fly strain expressing *Mabi* under GAL4 control as follows. We PCR-amplified DNA fragments containing the whole *Mabi* protein coding sequence with KOD-Plus DNA polymerase (Toyobo) and a pair of primers, CGGAATTCGGATTACGAAGAGCGGCAGAT (*EcoRI-Mabi-F*) and GCTCTAGAGCTGCTTAACCTTTAAGGCATTT (*XbaI-Mabi-R*). The amplified fragments were cloned into the pBluescript II SK(+) vector (Stratagene), verified by DNA sequencing, and then subcloned into pUASTattB (Bischof *et al.* 2007) by using *EcoRI* and *XbaI* sites. The resultant construct was injected into $y^1 M\{vas-int.Dm\}ZH-2A w^*$; $M\{3xP3-RFP.attP\}ZH-86Fb$ (Bloomington Drosophila Stock Center at Indiana University) embryos, which express phiC31 integrase specifically in germline cells and contain the phiC31 genomic destination site attP at 86F8 on the third chromosome (Groth *et al.* 2004; Bateman *et al.* 2006; Bischof *et al.* 2007).

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Table S1 Relative viability of deficiency heterozygotes from 6xbcd mothers to those from 2xbcd mothers

Strain	Replicate 1	Replicate 2	Replicate 3
<i>Df(2L)ED3</i>	(1.07, 1.18)		
<i>Df(2L)ED21</i>	(0.82, 1.29)		
<i>Df(2L)ED62</i>	(0.90, 1.11)		
<i>Df(2L)ED94</i>	(0.37***, 0.63**)	(0.53**, 0.67*)	(0.82, 0.80)
<i>Df(2L)ED105</i>	(0.87, 1.08)		
<i>Df(2L)ED136</i>	(0.69*, 1.20)		
<i>Df(2L)ED206</i>	(0.91, 1.02)		
<i>Df(2L)ED243</i>	(1.01, 0.95)		
<i>Df(2L)ED247</i>	(0.82, 0.98)		
<i>Df(2L)ED250</i>	(0.69, 0.83)		
<i>Df(2L)ED334</i>	(1.25, 0.91)		
<i>Df(2L)ED385</i>	(0.81, 1.02)		
<i>Df(2L)ED489</i>	(0.57**, 0.92)		
<i>Df(2L)ED501</i>	(0.72, 0.94)		
<i>Df(2L)ED548</i>	(0.94, 0.73*)		
<i>Df(2L)ED578</i>	(0.69*, 0.70*)	(0.50**, 0.61*)	(0.58**, 0.67**)
<i>Df(2L)ED629</i>	(0.71, 0.72)		
<i>Df(2L)ED647</i>	(0.77, 0.84)		
<i>Df(2L)ED680</i>	(0.87, 1.00)		
<i>Df(2L)ED690</i>	(0.69*, 0.93)		
<i>Df(2L)ED700</i>	(1.10, 0.93)		
<i>Df(2L)ED729</i>	(1.34*, 1.26)		
<i>Df(2L)ED746</i>	(1.09, 1.06)		
<i>Df(2L)ED761</i>	(0.81, 0.93)		
<i>Df(2L)ED775</i>	(0.88, 0.87)		
<i>Df(2L)ED779</i>	(0.87, 0.63***)		
<i>Df(2L)ED784</i>	(0.53***, 0.68***)	(0.59***, 0.68*)	(0.53***, 0.66**)
<i>Df(2L)ED793</i>	(0.69*, 0.82)		
<i>Df(2L)ED1054</i>	(0.78, 0.83)		
<i>Df(2L)ED1102</i>	(0.78, 0.77)		
<i>Df(2L)ED1109</i>	(0.88, 0.93)		
<i>Df(2L)ED1161</i>	(1.29, 1.27)		
<i>Df(2L)ED1196</i>	(0.40***, 0.92)		
<i>Df(2L)ED1202</i>	(0.82, 0.81)		
<i>Df(2L)ED1272</i>	(0.40***, 0.71)		
<i>Df(2L)ED1303</i>	(0.65***, 0.73)		
<i>Df(2L)ED1315</i>	(1.17, 1.10)		
<i>Df(2L)ED1378</i>	(0.81, 0.93)		
<i>Df(2L)ED1473</i>	(0.70, 1.17)		
<i>Df(2L)ED4559</i>	(0.72, 0.96)		
<i>Df(2L)ED5878</i>	(1.08, 0.84)		
<i>Df(2L)ED7762</i>	(0.62***, 0.83)		
<i>Df(2L)ED7853</i>	(0.81, 1.10)		
<i>Df(2L)ED8142</i>	(0.98, 1.40*)		
<i>Df(2L)ED8185</i>	(0.82, 1.07)		
<i>Df(2L)ED12527</i>	(0.71, 1.20)		
<i>Df(2R)ED1552</i>	(0.56*, 0.89)		
<i>Df(2R)ED1618</i>	(0.76, 0.97)		
<i>Df(2R)ED1673</i>	(0.74*, 0.81)		
<i>Df(2R)ED1715</i>	(0.82, 0.86)		
<i>Df(2R)ED1725</i>	(0.52**, 0.94)		
<i>Df(2R)ED1735</i>	(0.83, 0.99)		
<i>Df(2R)ED1742</i>	(0.78, 0.91)		
<i>Df(2R)ED1791</i>	(0.69, 0.63*)		
<i>Df(2R)ED2155</i>	(0.65, 1.00)		
<i>Df(2R)ED2219</i>	(0.80, 0.76)		
<i>Df(2R)ED2308</i>	(0.91, 1.14)		

<i>Df(2R)ED2354</i>	(0.68*, 0.71*)	(0.57*, 0.53**)	(0.84, 0.79)
<i>Df(2R)ED2426</i>	(0.98, 0.80)		
<i>Df(2R)ED2436</i>	(0.88, 1.15)		
<i>Df(2R)ED2457</i>	(0.70*, 0.86)		
<i>Df(2R)ED3181</i>	(1.00, 0.98)		
<i>Df(2R)ED3610</i>	(0.49***, 1.20)		
<i>Df(2R)ED3683</i>	(0.72, 0.73)		
<i>Df(2R)ED3728</i>	(0.34*, 0.41**)	(0.87, 0.75)	
<i>Df(2R)ED3791</i>	(0.86, 0.78)		
<i>Df(2R)ED3923</i>	(0.81, 1.06)		
<i>Df(2R)ED3952</i>	(0.88, 1.04)		
<i>Df(2R)ED4061</i>	(0.89, 1.05)		
<i>Df(2R)ED4071</i>	(0.54**, 0.95)		
<i>Df(3L)ED201</i>	(1.05, 1.07)		
<i>Df(3L)ED207</i>	(1.31, 1.45)		
<i>Df(3L)ED210</i>	(0.85, 1.01)		
<i>Df(3L)ED211</i>	(1.29, 0.93)		
<i>Df(3L)ED217</i>	(0.79, 0.97)		
<i>Df(3L)ED220</i>	(0.77, 1.04)		
<i>Df(3L)ED223</i>	(0.69, 0.76)		
<i>Df(3L)ED224</i>	(0.64*, 0.96)		
<i>Df(3L)ED225</i>	(0.81, 0.86)		
<i>Df(3L)ED229</i>	(0.85, 0.96)		
<i>Df(3L)ED230</i>	(1.42, 1.16)		
<i>Df(3L)ED4079</i>	(0.67***, 0.63***)	(0.93, 1.03)	
<i>Df(3L)ED4256</i>	(0.99, 1.07)		
<i>Df(3L)ED4287</i>	(0.70, 1.08)		
<i>Df(3L)ED4288</i>	(1.07, 1.02)		
<i>Df(3L)ED4293</i>	(1.20, 1.23)		
<i>Df(3L)ED4341</i>	(1.04, 1.06)		
<i>Df(3L)ED4408</i>	(1.47*, 1.16)		
<i>Df(3L)ED4421</i>	(1.42, 1.14)		
<i>Df(3L)ED4457</i>	(1.36, 0.88)		
<i>Df(3L)ED4470</i>	(1.41, 0.83)		
<i>Df(3L)ED4475</i>	(0.80, 0.99)		
<i>Df(3L)ED4483</i>	(1.58*, 1.23)		
<i>Df(3L)ED4486</i>	(0.95, 0.74)		
<i>Df(3L)ED4502</i>	(0.81, 0.92)		
<i>Df(3L)ED4543</i>	(0.82, 0.78)		
<i>Df(3L)ED4674</i>	(1.06, 1.07)		
<i>Df(3L)ED4685</i>	(0.76, 0.63*)		
<i>Df(3L)ED4710</i>	(0.75, 0.69*)		
<i>Df(3L)ED4782</i>	(1.09, 1.12)		
<i>Df(3L)ED4786</i>	(0.94, 0.89)		
<i>Df(3L)ED4858</i>	(0.59, 0.58*)		
<i>Df(3L)ED4978</i>	(1.19, 1.15)		
<i>Df(3L)ED5017</i>	(1.28, 1.13)		
<i>Df(3R)ED2</i>	(1.13, 1.53*)		
<i>Df(3R)ED5071</i>	(0.17***, 0.56**)	(1.28, 1.01)	
<i>Df(3R)ED5138</i>	(1.18, 1.13)		
<i>Df(3R)ED5147</i>	(1.16, 1.20)		
<i>Df(3R)ED5156</i>	(1.37, 0.86)		
<i>Df(3R)ED5177</i>	(0.92, 0.98)		
<i>Df(3R)ED5187</i>	(0.76, 1.04)		
<i>Df(3R)ED5196</i>	(1.42, 0.97)		
<i>Df(3R)ED5197</i>	(1.06, 1.01)		
<i>Df(3R)ED5230</i>	(0.83, 1.04)		
<i>Df(3R)ED5327</i>	(0.78, 0.92)		
<i>Df(3R)ED5330</i>	(0.85, 0.71)		

<i>Df(3R)ED5339</i>	(0.72, 0.83)	
<i>Df(3R)ED5416</i>	(0.93, 0.70)	
<i>Df(3R)ED5429</i>	(0.88, 0.98)	
<i>Df(3R)ED5474</i>	(1.41*, 0.89)	
<i>Df(3R)ED5518</i>	(0.94, 1.44*)	
<i>Df(3R)ED5610</i>	(1.07, 1.20)	
<i>Df(3R)ED5612</i>	(1.14, 0.88)	
<i>Df(3R)ED5623</i>	(1.12, 1.02)	
<i>Df(3R)ED5644</i>	(0.75, 0.78)	
<i>Df(3R)ED5664</i>	(1.20, 0.96)	
<i>Df(3R)ED5705</i>	(1.12, 1.07)	
<i>Df(3R)ED5780</i>	(0.27***, 0.66)	
<i>Df(3R)ED5807</i>	(0.93, 0.59*)	
<i>Df(3R)ED5815</i>	(0.94, 0.73)	
<i>Df(3R)ED5938</i>	(0.78, 0.80)	
<i>Df(3R)ED6025</i>	(1.04, 1.03)	
<i>Df(3R)ED6058</i>	(1.23, 1.05)	
<i>Df(3R)ED6076</i>	(0.88, 1.01)	
<i>Df(3R)ED6085</i>	(1.26, 0.95)	
<i>Df(3R)ED6096</i>	(0.40**, 0.63*)	(0.69, 0.92)
<i>Df(3R)ED6103</i>	(0.62, 0.89)	
<i>Df(3R)ED6220</i>	(1.02, 0.85)	
<i>Df(3R)ED6232</i>	(0.89, 0.82)	
<i>Df(3R)ED6235</i>	(1.07, 0.79)	
<i>Df(3R)ED6255</i>	(0.95, 0.74)	
<i>Df(3R)ED6265</i>	(1.03, 1.49)	
<i>Df(3R)ED6310</i>	(1.41, 1.38)	
<i>Df(3R)ED6316</i>	(1.40, 1.39)	
<i>Df(3R)ED6332</i>	(1.00, 0.75)	
<i>Df(3R)ED6346</i>	(1.32, 1.21)	
<i>Df(3R)ED6361</i>	(0.98, 1.95***)	
<i>Df(3R)ED6362</i>	(0.69, 0.90)	
<i>Df(3R)ED7665</i>	(1.56, 1.15)	
<i>Df(3R)ED10257</i>	(1.30, 1.40)	
<i>Df(3R)ED10566</i>	(1.19, 1.10)	
<i>Df(3R)ED10845</i>	(0.87, 1.23)	

Relative viability is obtained by dividing the proportion of deficiency heterozygotes (the number of deficiency heterozygotes / the number of balancer heterozygotes) in *6xbcd* by that in *2xbcd*. Statistical significance (χ^2 -test) for each sex is given in parentheses (male and female). *ns*, $P \geq 0.05$; *, $P < 0.05$; **, $P < 0.01$; and ***, $P < 0.001$.

Table S2 Candidate genes obtained from deficiency screening and microarray expression data

	Microarray data (Replicate 1, Replicate 2, Replicate 3)
Region 29A2-A3	
<i>wol</i> (cg7870)	NC, NC, NC
<i>Scgα</i> (cg7851)	NC, I, NC
cg7840	NC, NC, I
cg7830	NC, NC, NC
Region 34A2-B6	
<i>DnaJ-H</i> (cg9828)	NC, NC, NC
<i>Sir2</i> (cg5216)	NC, NC, NC
<i>Sfmbt</i> (cg16975)	NC, NC, NC
cg5439	NC, NC, NC
cg5287	NC, NC, NC
cg31849	NC, NC, NC
cg5458	NC, NC, NC
cg16974	NC, NC, NC
<i>Edem2</i> (cg5682)	NC, NC, NC
cg16972	NC, NC, NC
<i>Pect</i> (cg5547)	NC, NC, NC
cg15482	NC, NC, NC
<i>snRNA:U2:34ABa</i> (cr31850)	-
<i>kek4</i> (cg9431)	NC, NC, NC
<i>snRNA:U2:34ABb</i> (cr31854)	-
cg9426	NC, NC, NC
cg5705	NC, NC, NC
<i>Ski6</i> (cg15481)	NC, NC, NC
cg16812	NC, NC, NC
cg15480	NC, I, I
cg16813	NC, NC, NC
cg16815	I, NC, NC
<i>Prosa6T</i> (cg5648)	NC, NC, NC
<i>Mabi</i> (cg15479)	I, I, I
cg5867	NC, NC, I
cg5945	NC, NC, NC
<i>snRNA:U2:34ABc</i> (cr33788)	-
<i>snRNA:U5:34A</i> (cr31853)	-
cg16820	NC, NC, NC
cg31728	NC, NC, NC
cg31851	NC, NC, NC
cg31730	NC, NC, NC
cg6043	NC, NC, I
cg31848	NC, NC, NC
cg6108	NC, NC, NC
<i>Drep-4</i> (cg9414)	NC, NC, NC
cg6116	NC, NC, NC
cg31729	NC, NC, NC
cg15639	NC, NC, NC
cg16824	NC, NC, NC
cg16825	NC, NC, NC
cg16970	NC, NC, NC

I, up-regulated in *6xbcd* with a statistical significance of $P < 0.002$; and NC, not significant

Table S3 Up-regulated genes in 6xbcd condition

Probe ID ^a	Gene ^b	Fold change in expression ^c				Molecular function ^d
		Rep.1	Rep.2	Rep.3	Ave.	
1622906_at	<i>cg9027</i>	1.15	1.41	2.14	1.57	Superoxide dismutase activity
1623028_at	<i>cg14872</i>	1.32	1.23	3.48	2.01	Binding
1623035_at ^{§, §§}	<i>NaPi-T (cg10207)</i>	3.25	3.25	4.00	3.50	High affinity inorganic phosphate, sodium symporter activity
1623039_at	<i>cg3409</i>	1.15	1.23	1.23	1.20	Monocarboxylic acid transmembrane transporter activity
1623173_at	<i>cg10013</i>	2.64	3.03	1.74	2.47	
1623246_at [§]	<i>cg6475</i>	2.14	1.62	3.73	2.50	Glucuronosyltransferase activity
1623256_at	<i>GstE1 (cg5164)</i>	1.15	1.52	1.41	1.36	Glutathione transferase activity
1623258_at	<i>cg12493</i>	1.23	2.64	2.14	2.00	siRNA binding
1623427_at	<i>cg7179</i>	1.23	1.41	2.83	1.82	
1623474_at ^{§, §§}	<i>Mabi (cg15479)</i>	2.30	4.00	4.29	3.53	
1623519_at	<i>esc (cg14941)</i>	1.23	1.23	1.15	1.20	Histone methyltransferase activity (H3-K27 specific), protein binding, histone methyltransferase activity
1623521_at	<i>tobi (cg11909)</i>	1.52	1.32	1.32	1.38	Alpha-glucosidase activity
1623632_s_at [§]	<i>cg43693</i>	1.32	1.74	1.74	1.60	
1623706_at	<i>cg9498</i>	1.15	1.15	2.83	1.71	Transferase activity, transferring phosphorus-containing groups
1623713_at [§]	<i>cg18581</i>	1.23	3.73	2.64	2.53	
1623714_at ^{§§}	<i>cg8620</i>	2.14	2.14	5.66	3.31	
1623732_at	<i>Npc2e (cg31410)</i>	1.52	2.30	2.46	2.09	Sterol binding
1623787_at [§]	<i>LKR (cg7144)</i>	1.52	2.46	3.25	2.41	Saccharopine dehydrogenase (NAD+, L-lysine-forming) activity
1623825_s_at [§]	<i>cg17018</i>	1.62	1.32	1.87	1.60	Nucleic acid binding
1623849_s_at [§]	<i>cg6726</i>	1.52	1.41	2.14	1.69	Aminoacylase activity
1623900_a_at	<i>Mal-B2 (cg14935)</i>	1.52	1.15	1.52	1.39	Alpha-glucosidase activity
1623903_at ^{§, §§}	<i>cg18473</i>	2.14	2.46	2.64	2.41	Aryldialkylphosphatase activity
1624150_at	<i>FKBP59 (cg4535)</i>	1.32	1.15	1.07	1.18	Protein binding
1624290_at	<i>cg4752</i>	1.15	2.00	2.46	1.87	5-oxoprolinase (ATP-hydrolyzing) activity
1624312_at	<i>cg3326</i>	1.87	1.62	2.14	1.88	ATPase activity
1624325_at	<i>pip (cg9614)</i>	1.07	1.62	1.32	1.34	Sulfotransferase activity
1624362_at [§]	<i>Nplp4 (cg15361)</i>	1.52	2.83	2.46	2.27	Neuropeptide hormone activity
1624531_s_at	<i>Glut4EF (cg34360)</i>	1.15	1.32	1.87	1.44	Zinc ion binding
1624623_at	<i>cg43332</i>	1.32	2.30	1.87	1.83	
1624638_a_at	<i>cg4365</i>	1.23	1.07	1.32	1.21	Hydroxyacylglutathione hydrolase activity
1624692_at	<i>cg6830</i>	1.07	1.23	1.41	1.24	Transferase activity, transferring phosphorus-containing groups
1624695_at	<i>cg3999</i>	1.23	1.74	1.87	1.61	Glycine dehydrogenase (decarboxylating) activity
1624752_at	<i>Prestin (cg5485)</i>	1.07	2.30	1.32	1.56	High affinity sulfate transmembrane transporter activity
1624818_s_at [§]	<i>Sply (cg8946)</i>	1.15	1.52	1.32	1.33	Sphinganine-1-phosphate aldolase activity
1625022_a_at ^{§, §§}	<i>cg6125</i>	2.83	2.83	4.92	3.53	High affinity sulfate transmembrane transporter activity
1625023_a_at	<i>nAcR6-21C (cg11822)</i>	1.32	1.32	1.23	1.29	Acetylcholine-activated cation-selective channel activity
1625053_at	<i>cg16898</i>	1.62	1.15	3.48	2.09	Transferase activity, transferring phosphorus-containing groups
1625077_at	<i>cg30265</i>	2.14	1.74	1.74	1.88	
1625114_at	<i>Cyp310a1 (cg10391)</i>	1.23	1.23	1.52	1.33	Electron carrier activity
1625140_at	<i>NtR (cg6698)</i>	1.23	1.32	1.23	1.26	Excitatory extracellular ligand-gated ion channel activity

1625143_at [§]	<i>Cpr65Ea (cg8640)</i>	1.87	1.87	39.40	14.38	Structural constituent of chitin-based cuticle
1625174_at	<i>cg15067</i>	1.87	1.87	5.28	3.00	
1625265_at [§]	<i>cg9119</i>	1.52	1.32	1.23	1.36	
1625321_a_at	<i>mtg (cg7549)</i>	1.41	1.52	1.87	1.60	Chitin binding
1625369_at	<i>cg10877</i>	1.52	1.62	1.41	1.52	Catalytic activity
1625382_at [§]	<i>Osi6 (cg1151)</i>	1.87	1.15	1.41	1.48	
1625439_at	<i>Tsp29Fa (cg9494)</i>	1.07	1.62	4.92	2.54	
1625481_a_at	<i>retn (cg5403)</i>	1.07	1.15	1.23	1.15	Transcription regulatory region sequence-specific DNA binding, repressing transcription factor binding, DNA binding
1625503_at [§]	<i>cg32425</i>	1.62	1.32	1.32	1.42	
1625559_a_at	<i>cg30217</i>	1.32	1.07	2.83	1.74	
1625592_s_at	<i>cg11307</i>	1.07	1.15	1.62	1.28	Transferase activity, transferring glycosyl groups
1625657_at [§]	<i>cg32335</i>	1.87	1.87	1.87	1.87	
1625683_at	<i>cg5112</i>	1.32	1.32	1.15	1.26	Fatty acid amide hydrolase activity
1625744_at	<i>GstE6 (cg17530)</i>	1.15	1.32	1.23	1.23	Glutathione transferase activity
1625745_at	<i>cg31643</i>	1.23	1.23	1.32	1.26	Protein kinase activity
1625759_at	<i>Ku80 (cg18801)</i>	1.32	1.52	1.23	1.36	ATP-dependent DNA helicase activity, single-stranded DNA-dependent ATP-dependent DNA helicase activity
1625762_s_at	<i>cg32189</i>	2.14	1.23	1.52	1.63	
1625925_at	<i>His2A:cg31618 (cg31618)</i>	1.23	1.15	1.07	1.15	DNA binding
1625949_at	<i>Gpdh (cg9042)</i>	1.32	1.07	1.32	1.24	Glycerol-3-phosphate dehydrogenase [NAD+] activity
1626048_at	<i>HLHmy (cg8333)</i>	1.15	1.15	1.23	1.18	Transcription factor binding, sequence-specific DNA binding
1626264_at	<i>cg9883</i>	1.23	1.41	1.74	1.46	
1626285_at	<i>cg13806</i>	1.52	1.87	1.62	1.67	Chitin binding
1626324_at [§]	<i>Cyp309a1 (cg9964)</i>	1.32	3.48	2.46	2.42	Electron carrier activity
1626499_at	<i>GATAe (cg10278)</i>	1.32	1.41	1.07	1.27	Sequence-specific DNA binding RNA polymerase II transcription factor activity
1626536_at	<i>cg6776</i>	1.32	1.62	2.64	1.86	Glutathione dehydrogenase (ascorbate) activity, transferase activity, transferring sulfur-containing groups
1626566_at	<i>cg12262</i>	1.07	1.15	1.52	1.25	Acyl-CoA dehydrogenase activity
1626577_at	<i>cg15556</i>	1.07	3.48	1.74	2.10	G-protein coupled receptor activity
1626606_at ^{§, §§}	<i>blanks (cg10630)</i>	2.64	2.64	2.83	2.70	siRNA binding
1626732_at	<i>cg30217</i>	1.62	2.46	1.74	1.94	
1626755_at [§]	<i>cg30085</i>	1.32	1.23	1.32	1.29	
1626857_at	<i>cg4408</i>	1.07	1.15	1.74	1.32	Metalloproteinase activity
1626893_at	<i>Gasp (cg10287)</i>	1.07	1.23	1.52	1.27	Chitin binding, structural constituent of peritrophic membrane
1626984_at [§]	<i>Gld (cg1152)</i>	1.32	1.87	2.64	1.94	Glucose dehydrogenase activity
1627067_at	<i>CanB2 (cg11217)</i>	1.41	1.41	1.62	1.48	Protein binding
1627109_at	<i>Hsc70-5 (cg8542)</i>	1.32	1.23	1.07	1.21	Unfolded protein binding
1627134_at	<i>ApepP (cg6291)</i>	1.07	1.32	1.74	1.38	Aminopeptidase activity
1627176_at	<i>cg31431</i>	1.23	1.23	1.23	1.23	Fibroblast growth factor-activated receptor activity
1627204_at	<i>cg4749</i>	1.32	1.15	1.07	1.18	
1627302_at	<i>Fmo-2 (cg3174)</i>	1.15	1.15	1.23	1.18	Monooxygenase activity

1627343_a_at [§]	<i>cg5535</i>	1.41	1.52	1.41	1.45	Amino acid transmembrane transporter activity, cationic amino acid transmembrane transporter activity
1627647_at [§]	<i>cg3940</i>	2.00	2.30	3.48	2.59	Carbonate dehydratase activity
1627793_s_at	<i>cg9134</i>	1.23	1.41	1.87	1.50	Sugar binding
1627834_a_at	<i>aret (cg31762)</i>	1.23	1.23	1.41	1.29	Protein binding, RNA binding, mRNA 3'-UTR binding
1628017_a_at	<i>me31B (cg4916)</i>	1.23	1.15	1.15	1.18	RNA binding
1628067_s_at	<i>dyl (cg15013)</i>	1.52	1.41	1.74	1.56	Structural constituent of chitin-based cuticle
1628083_at	<i>cg5036</i>	1.23	2.14	2.64	2.00	GTPase activator activity
1628084_at	<i>BobA (cg12487)</i>	1.23	1.15	1.23	1.20	
1628099_at	<i>bor (cg6815)</i>	1.41	1.41	1.07	1.30	Nucleoside-triphosphatase activity, ATP binding
1628121_at [§]	<i>Osi14 (cg1155)</i>	1.41	1.32	2.00	1.58	
1628237_s_at [§]	<i>cg10602</i>	1.74	1.62	1.41	1.59	Leukotriene-A4 hydrolase activity
1628257_s_at [§]	<i>PHGPx (cg12013)</i>	1.41	1.41	1.41	1.41	Peroxidase activity
1628271_at	<i>cg3609</i>	1.07	1.23	1.52	1.27	Nucleotide binding, oxidoreductase activity
1628320_at	<i>cg9471</i>	1.32	1.15	1.32	1.26	NADPH dehydrogenase activity, biliverdin reductase activity
1628334_at [§]	<i>m4 (cg6099)</i>	1.62	1.41	2.00	1.68	
1628398_at [§]	<i>cg5853</i>	1.32	1.32	1.32	1.32	ATPase activity coupled to transmembrane movement of substances, transporter activity
1628602_a_at	<i>cg9248</i>	1.32	1.32	1.52	1.38	Catalytic activity
1628655_at	<i>cg4476</i>	1.23	1.32	2.30	1.62	Neurotransmitter transporter activity, potassium:amino acid symporter activity
1628835_at	<i>TwidE (cg14534)</i>	1.07	3.48	4.00	2.85	Structural constituent of chitin-based cuticle
1628922_s_at	<i>veg (cg6657)</i>	1.32	1.15	1.32	1.26	Mannosyltransferase activity
1629014_s_at	<i>cg2604</i>	1.23	1.23	1.32	1.26	Nucleotide binding, oxidoreductase activity
1629055_a_at	<i>Got2 (cg4233)</i>	1.32	1.41	3.03	1.92	L-aspartate:2-oxoglutarate aminotransferase activity
1629061_s_at	<i>Hsp22 (cg4460)</i>	1.62	1.62	1.32	1.52	
1629093_at	<i>cg14960</i>	1.41	1.74	1.15	1.43	
1629095_a_at	<i>Men (cg10120)</i>	1.32	1.32	1.07	1.24	Malate dehydrogenase (oxaloacetate-decarboxylating) (NADP+) activity
1629150_at	<i>cg17337</i>	1.32	1.23	1.23	1.26	Metallopeptidase activity, dipeptidase activity, tripeptidase activity
1629153_s_at	<i>cg5991</i>	1.15	1.23	1.32	1.23	Phosphatidylserine decarboxylase activity
1629181_at	<i>cg33494</i>	1.15	1.87	1.62	1.55	
1629271_at	<i>cg10444</i>	1.23	1.15	1.23	1.20	Sodium-dependent multivitamin transmembrane transporter activity
1629398_at	<i>cg10383</i>	1.41	1.32	1.32	1.35	Binding, hydrolase activity acting on ester bonds
1629431_at	<i>cg18747</i>	1.32	1.74	2.46	1.84	
1629464_a_at	<i>cg32473</i>	1.23	1.23	1.23	1.23	Aminopeptidase activity
1629469_s_at	<i>cg10960</i>	1.07	1.15	1.23	1.15	Glucose transmembrane transporter activity
1629515_at	<i>Pdk (cg8808)</i>	1.07	1.32	1.23	1.21	Pyruvate dehydrogenase (acetyl-transferring) kinase activity

1629518_at [§]	<i>cg2493</i>	1.41	1.32	1.32	1.35	Serine-type carboxypeptidase activity
1629614_at	<i>cg31549</i>	1.07	1.15	1.41	1.21	Oxidoreductase activity
1629642_a_at [§]	<i>cg17664</i>	1.41	2.46	2.83	2.23	Water channel activity, channel activity
1629643_at	<i>cg42816</i>	1.52	1.52	6.06	3.03	
1629693_at	<i>cg15080</i>	1.52	1.07	1.52	1.37	
1629732_at [§]	<i>cg8791</i>	2.46	1.74	2.30	2.17	High affinity inorganic phosphate:sodium symporter activity
1629740_at [§]	<i>His1:cg31617 (cg31617)</i>	1.23	1.41	1.74	1.46	DNA binding
1629778_s_at [§]	<i>cg5130</i>	1.62	2.00	2.00	1.87	Zinc ion transmembrane transporter activity
1629869_s_at [§]	<i>Fatp (cg7400)</i>	1.62	1.62	1.87	1.71	Long-chain fatty acid transporter activity
1629886_s_at	<i>for (cg10033)</i>	1.15	1.15	1.32	1.21	cGMP-dependent protein kinase activity
1629919_at	<i>Cpr100A (cg12045)</i>	1.07	2.00	14.93	6.00	Structural constituent of chitin-based cuticle
1629964_at	<i>Nufip (cg4076)</i>	1.23	1.23	1.23	1.23	mRNA binding
1630019_s_at	<i>Tmhs (cg12026)</i>	1.07	1.41	1.23	1.24	
1630020_at	<i>cg13041</i>	1.07	2.00	32.00	11.69	
1630056_at	<i>cg32054</i>	1.23	1.41	2.14	1.60	Transmembrane transporter activity
1630202_a_at	<i>cg8632</i>	1.07	1.32	1.52	1.30	Nucleotide binding, cation transmembrane transporter activity
1630212_at [§]	<i>cg2065</i>	1.62	1.23	1.74	1.53	Nucleotide binding, oxidoreductase activity
1630266_at	<i>Ilp4 (cg6736)</i>	1.32	1.07	1.15	1.18	Insulin receptor binding
1630324_at	<i>wcd (cg7989)</i>	1.23	1.15	1.23	1.20	
1630433_at	<i>skap (cg11963)</i>	1.32	1.23	1.52	1.36	Succinate-CoA ligase (ADP-forming) activity
1630466_at	<i>l(2)k09022 (cg10805)</i>	1.41	1.23	1.07	1.24	Binding
1630502_at	<i>ana (cg8084)</i>	1.32	1.32	1.15	1.26	Growth factor activity
1630503_at	<i>cg6045</i>	1.62	2.00	2.00	1.87	Xanthine dehydrogenase activity
1630575_at	<i>cg1907</i>	1.23	1.07	1.23	1.18	Oxoglutarate:malate antiporter activity, transmembrane transporter activity
1630621_at	<i>cg15212</i>	1.23	1.87	84.45	29.18	
1630688_at [§]	<i>Hsp83 (cg1242)</i>	1.32	1.15	1.32	1.26	ATPase activity coupled
1630801_at	<i>Gsc (cg2851)</i>	1.41	1.32	1.07	1.27	RNA polymerase II core promoter proximal region sequence-specific DNA binding transcription factor activity involved in negative regulation of transcription, protein heterodimerization activity, protein homodimerization activity
1630857_s_at	<i>NTPase (cg3059)</i>	1.23	1.15	1.15	1.18	Guanosine-diphosphatase activity, uridine-diphosphatase activity
1630933_at [§]	<i>cg6738</i>	1.41	1.74	2.00	1.72	Aminoacylase activity
1630968_at [§]	<i>cg13907</i>	1.41	1.41	1.52	1.45	Secondary active monocarboxylate transmembrane transporter activity
1631153_at	<i>osk (cg10901)</i>	1.62	1.52	2.83	1.99	Hydrolase activity
1631237_s_at [§]	<i>cg13384</i>	1.52	1.87	1.74	1.71	Amino acid transmembrane transporter activity
1631249_at	<i>cg12713</i>	1.23	1.23	1.23	1.23	
1631291_at	<i>Ect4 (cg43119)</i>	1.23	1.62	1.32	1.39	Binding

1631321_s_at [§]	<i>His1:cg33840</i> (<i>cg33840</i>)	1.23	1.32	1.52	1.36	DNA binding
1631333_s_at ^{§,§§}	<i>Adh</i> (<i>cg3481</i>)	2.83	4.00	18.38	8.40	Protein homodimerization activity, alcohol dehydrogenase (NAD) activity, acetaldehyde dehydrogenase (acetylating) activity
1631354_x_at	<i>cg13679</i>	1.07	2.30	22.63	8.67	Phosphoribosylaminoimidazolecarboxamide formyltransferase activity, IMP cyclohydrolase activity
1631392_at	<i>cg11089</i>	1.32	1.23	1.07	1.21	
1631463_a_at ^{§,§§}	<i>bcd</i> (<i>cg1034</i>)	3.03	3.03	4.92	3.66	Sequence-specific DNA binding transcription factor activity, translation repressor activity, protein binding, mRNA 3'-UTR binding, sequence-specific distal enhancer binding RNA polymerase II transcription factor activity
1631513_at	<i>cg30000</i>	1.32	1.15	1.52	1.33	Glycogen phosphorylase activity, pyridoxal phosphate binding, protein homodimerization activity
1631620_at	<i>GlyP</i> (<i>cg7254</i>)	1.23	1.41	1.87	1.50	
1631626_at	<i>aub</i> (<i>cg6137</i>)	1.23	1.23	1.41	1.29	piRNA binding, RNA binding
1631628_s_at	<i>Prx2540-2</i> (<i>cg11765</i>)	1.41	1.41	1.07	1.30	Thioredoxin peroxidase activity
1631785_at	<i>gwl</i> (<i>cg7719</i>)	1.32	1.07	1.41	1.27	Protein serine/threonine kinase activity
1631817_a_at	<i>Got1</i> (<i>cg8430</i>)	1.23	1.32	1.52	1.36	L-aspartate:2-oxoglutarate aminotransferase activity
1631821_at	<i>Sr-CI</i> (<i>cg4099</i>)	1.41	1.23	1.32	1.32	Scavenger receptor activity, polysaccharide binding
1631946_at	<i>cg7173</i>	1.52	1.41	1.23	1.39	Ran GTPase binding
1631954_at	<i>Ranbp9</i> (<i>cg5252</i>)	1.15	1.15	1.23	1.18	
1631973_at	<i>Ote</i> (<i>cg5581</i>)	1.23	1.07	1.23	1.18	
1631994_a_at ^{§,§§}	<i>Mocs1</i> (<i>cg33048</i>)	2.30	2.46	2.46	2.41	4 iron (4 sulfur cluster binding), metal ion binding, catalytic activity
1632080_s_at	<i>Cpr67Fa2</i> (<i>cg18349</i>)	1.07	1.52	6.50	3.03	Structural constituent of chitin-based cuticle
1632146_a_at	<i>cg5508</i>	1.32	1.32	1.23	1.29	Glycerol-3-phosphate O-acyltransferase activity
1632188_at	<i>Hexo1</i> (<i>cg1318</i>)	1.07	1.23	1.15	1.15	Beta-N-acetylglucosaminidase activity
1632228_at	<i>cg6733</i>	1.23	1.87	1.07	1.39	Aminoacylase activity
1632238_at	<i>cg13248</i>	1.07	1.52	2.14	1.58	Amino acid transmembrane transporter activity, cationic amino acid transmembrane transporter activity
1632273_at	<i>cg5789</i>	1.07	1.52	1.52	1.37	Xenobiotic-transporting ATPase activity, ATPase activity coupled to transmembrane movement of substances, transporter activity
1632383_at	<i>Art1</i> (<i>cg6554</i>)	1.23	1.07	1.15	1.15	Protein-arginine omega-N asymmetric methyltransferase activity, histone methyltransferase activity (H4-R3 specific), protein-arginine omega-N monomethyltransferase activity, histone methyltransferase activity

1632525_at	<i>cg9925</i>	1.23	1.62	2.14	1.67	Zinc ion binding
1632526_s_at	<i>Nc73EF (cg11661)</i>	1.15	1.23	1.32	1.23	Oxoglutarate dehydrogenase (succinyl-transferring) activity
1632623_at ^{§, §§}	<i>Cyt-b5-r (cg13279)</i>	3.03	2.14	2.83	2.67	Electron carrier activity
1632664_at	<i>Osi12 (cg1154)</i>	1.52	2.14	1.07	1.58	
1632675_s_at	<i>cg5455</i>	1.15	1.41	1.32	1.29	Catalytic activity
1632690_a_at	<i>cg13049</i>	1.07	1.52	6.06	2.88	
1632699_s_at	<i>Adh (cg3481)</i>	1.23	1.62	2.14	1.67	Protein homodimerization activity, alcohol dehydrogenase (NAD) activity, acetaldehyde dehydrogenase (acetylating) activity
1632945_at [§]	<i>Msp-300 (cg42768)</i>	1.32	2.30	2.83	2.15	Actin binding
1632958_a_at [§]	<i>cg42365</i>	1.41	1.74	1.32	1.49	
1632966_at	<i>cg8213</i>	2.30	1.32	1.32	1.65	Serine-type endopeptidase activity
1633002_at	<i>salt (cg2196)</i>	1.32	1.74	1.52	1.53	Sodium:iodide symporter activity
1633048_at [§]	<i>cg8193</i>	2.14	2.14	1.62	1.97	Monophenol monooxygenase activity
1633124_at	<i>cg6026</i>	2.83	2.64	1.32	2.26	
1633131_s_at	<i>cg32165</i>	1.23	1.15	1.23	1.20	Binding, protein transporter activity
1633400_at	<i>cue (cg12086)</i>	1.15	1.15	1.32	1.21	Low-density lipoprotein receptor activity
1633425_at ^{§, §§}	<i>Smvt (cg2191)</i>	2.83	3.03	4.29	3.38	Sodium-dependent multivitamin transmembrane transporter activity
1633473_s_at	<i>Ald (cg6058)</i>	1.15	1.41	1.62	1.40	Fructose-bisphosphate aldolase activity
1633488_at	<i>aay (cg3705)</i>	1.32	1.23	1.32	1.29	Phosphoserine phosphatase activity
1633503_at	<i>cg12171</i>	1.23	1.07	1.52	1.27	Oxidoreductase activity acting on CH-OH group of donors
1633645_at	<i>cg42565</i>	1.15	1.52	1.74	1.47	
1633696_at [§]	<i>TM4SF (cg11303)</i>	1.41	2.14	2.00	1.85	
1633718_at [§]	<i>cg5355</i>	1.32	1.23	1.23	1.26	Serine-type endopeptidase activity
1633775_at	<i>wry (cg31665)</i>	1.07	1.87	3.03	1.99	Notch binding
1633778_at [§]	<i>gb (cg6070)</i>	1.32	1.32	1.32	1.32	Amino acid transmembrane transporter activity
1633870_at	<i>cg9436</i>	1.32	1.15	1.52	1.33	Alditol:NADP+ 1-oxidoreductase activity
1634002_at	<i>cg13315</i>	1.23	1.07	1.74	1.35	
1634011_at	<i>HLHmδ (cg8328)</i>	1.15	1.15	1.32	1.21	Sequence-specific DNA binding
1634084_at [§]	<i>cg4213</i>	1.87	2.46	1.52	1.95	
1634086_a_at	<i>faf (cg1945)</i>	1.41	1.87	1.74	1.67	Ubiquitin-specific protease activity
1634096_at [§]	<i>Aldh (cg3752)</i>	1.23	1.62	3.48	2.11	Aldehyde dehydrogenase (NAD) activity, acetaldehyde dehydrogenase (acetylating) activity
1634112_a_at	<i>cg5174</i>	1.07	1.15	1.15	1.12	Catalytic activity, pyridoxal phosphate binding
1634185_at	<i>cg32053</i>	1.41	1.32	1.32	1.35	Transmembrane transporter activity
1634197_at	<i>cg11576</i>	1.23	1.52	1.52	1.42	
1634314_s_at	<i>ldh (cg7176)</i>	1.15	1.23	1.52	1.30	Isocitrate dehydrogenase (NADP+) activity
1634336_at	<i>bam (cg10422)</i>	1.62	1.23	2.00	1.62	Nucleic acid binding translation repressor activity
1634546_at	<i>Tig (cg11527)</i>	1.07	1.41	1.15	1.21	Binding
1634568_at	<i>Cpr67Fa2 (cg18349)</i>	1.41	1.87	55.72	19.67	Structural constituent of chitin-based cuticle
1634702_at [§]	<i>cg7227</i>	1.32	1.41	1.32	1.35	Scavenger receptor activity
1634762_a_at	<i>cg15096</i>	1.32	1.62	2.83	1.92	High affinity inorganic phosphate:sodium symporter activity
1634786_at	<i>lectin-28C (cg7106)</i>	1.52	6.96	1.74	3.41	Galactose binding

1634950_at	<i>Mat89Ba (cg12785)</i>	1.15	1.23	1.15	1.18	
1634961_s_at	<i>Vha68-2 (cg3762)</i>	1.07	1.32	1.32	1.24	Hydrogen-exporting ATPase activity (phosphorylative mechanism)
1634989_at	<i>Acon (cg9244)</i>	1.32	1.15	1.23	1.23	Aconitate hydratase activity
1635044_at	<i>Hsp26 (cg4183)</i>	1.32	1.52	1.62	1.49	Protein binding
1635070_at	<i>cg9953</i>	1.23	1.07	1.32	1.21	Serine-type carboxypeptidase activity
1635083_at	<i>nrm (cg43079)</i>	1.07	1.41	2.30	1.59	
1635089_at	<i>tun (cg8253)</i>	1.15	1.32	1.41	1.29	Hydrolase activity in linear amides acting on carbon-nitrogen (but not peptide) bonds
1635191_at	<i>Tsp42Ed (cg12846)</i>	1.15	1.87	1.87	1.63	
1635256_s_at	<i>cg1275</i>	1.07	1.23	1.74	1.35	Electron carrier activity
1635263_at [§]	<i>cg11825</i>	1.52	1.62	1.52	1.55	
1635271_at [§]	<i>cg30431</i>	2.00	1.52	2.00	1.84	Nucleic acid binding
1635362_at	<i>cg13654</i>	1.15	1.87	1.87	1.63	
1635380_at [§]	<i>cg30413</i>	1.52	1.52	4.00	2.34	
1635390_s_at	<i>cg9331</i>	1.15	1.15	1.87	1.39	NAD binding, oxidoreductase activity acting on the CH-OH group of donors (NAD or NADP as acceptor)
1635398_at	<i>Scp2 (cg14904)</i>	1.23	1.52	1.62	1.46	GTPase activity
1635449_s_at	<i>Dip-B (cg9285)</i>	1.23	1.62	2.00	1.62	Tripeptidyl-peptidase activity, dipeptidyl-peptidase activity
1635461_at	<i>cg3362</i>	1.15	1.32	1.32	1.26	5'-nucleotidase activity, magnesium ion binding
1635468_a_at	<i>cg7730</i>	1.74	1.15	2.14	1.68	
1635494_at [§]	<i>proPO-A1 (cg42639)</i>	2.14	2.46	1.52	2.04	Catechol oxidase activity, monophenol monooxygenase activity
1635510_at ^{§, §§}	<i>cg34025</i>	10.56	8.57	16.00	11.71	
1635512_at	<i>cg11893</i>	1.15	2.00	1.52	1.55	Transferase activity (transferring phosphorus-containing groups)
1635666_at	<i>His2B:cg17949 (cg17949)</i>	1.32	1.15	1.15	1.21	
1635715_at [§]	<i>His4:cg31611 (cg31611)</i>	1.32	1.52	1.52	1.45	DNA binding
1635745_a_at [§]	<i>cg4389</i>	1.41	1.23	1.23	1.29	long-chain-3-hydroxyacyl-CoA dehydrogenase activity, long-chain-enoyl-CoA hydratase activity
1635771_a_at	<i>cg42669</i>	1.15	1.32	1.41	1.29	
1635789_at	<i>cg42533</i>	1.15	1.41	1.32	1.29	GTP binding, GTPase binding, guanyl-nucleotide exchange factor activity
1635800_at	<i>cg5431</i>	1.07	1.62	3.48	2.06	Tyrosine-ester sulfotransferase activity
1635808_s_at [§]	<i>Dic1 (cg8790)</i>	1.23	1.52	1.41	1.39	Inorganic phosphate transmembrane transporter activity, sulfate transmembrane transporter activity, malate transmembrane transporter activity, thiosulfate transmembrane transporter activity, oxaloacetate transmembrane transporter activity, succinate transmembrane transporter activity
1635812_at [§]	<i>cg16965</i>	1.52	1.32	1.32	1.38	Alpha,alpha-trehalase activity
1635817_at	<i>cg11131</i>	1.15	1.32	17.15	6.54	
1635848_at	<i>Inos (cg11143)</i>	1.23	1.15	1.32	1.23	Inositol-3-phosphate synthase activity

1635900_at [§]	<i>Thor (cg8846)</i>	1.32	1.32	1.32	1.32	Eukaryotic initiation factor 4E binding
1635937_at [§]	<i>cg4500</i>	2.14	1.74	1.74	1.88	Long-chain fatty acid-CoA ligase activity
1636097_at	<i>TwdID (cg14243)</i>	1.32	2.30	11.31	4.98	Structural constituent of chitin-based larval cuticle
1636125_a_at	<i>grsm (cg7340)</i>	1.52	1.41	1.15	1.36	Aminopeptidase activity
1636325_at	<i>Osi7 (cg1153)</i>	1.62	1.52	1.23	1.46	
1636398_at	<i>cg10086</i>	1.32	2.14	3.25	2.24	
1636410_at	<i>cg3505</i>	1.41	1.23	3.03	1.89	Serine-type endopeptidase activity
1636468_a_at	<i>cg5033</i>	1.23	1.15	1.07	1.15	Ribonucleoprotein complex binding
1636474_at	<i>Vha100-5 (cg12602)</i>	1.07	1.23	3.48	1.93	Hydrogen-exporting ATPase activity (phosphorylative mechanism)
1636510_a_at	<i>Lsd-1 (cg10374)</i>	1.41	1.07	1.52	1.33	
1636516_at [§]	<i>cg32189</i>	2.64	1.74	3.48	2.62	
1636672_at [§]	<i>Brd (cg3096)</i>	1.32	1.32	1.52	1.38	
1636674_at [§]	<i>cg11407</i>	3.03	1.41	2.64	2.36	Long-chain fatty acid transporter activity
1636676_at	<i>cg2150</i>	1.15	2.14	3.48	2.26	
1636718_s_at	<i>cg4502</i>	1.23	1.41	1.32	1.32	Acid-amino acid ligase activity
1636728_at	<i>Ptr (cg11212)</i>	1.32	1.23	1.41	1.32	Receptor activity
1636764_at ^{§, §§}	<i>cg31075</i>	3.48	3.25	2.64	3.12	Aldehyde dehydrogenase (NAD) activity
1636774_at	<i>Cpsf160 (cg10110)</i>	1.23	1.23	1.15	1.20	Protein binding
1636842_at	<i>cg8460</i>	1.23	1.15	1.32	1.23	Chitinase activity, cation binding
1636886_at	<i>spn-E (cg3158)</i>	1.15	1.15	1.74	1.35	Helicase activity, ATP-dependent RNA helicase activity
1636943_s_at	<i>Spn5 (cg18525)</i>	1.23	1.23	1.41	1.29	Serine-type endopeptidase inhibitor activity
1636947_at	<i>bbg (cg42230)</i>	1.23	1.23	1.07	1.18	
1636979_at	<i>cg7912</i>	1.32	1.52	1.52	1.45	High affinity sulfate transmembrane transporter activity
1637109_s_at	<i>cg3961</i>	1.15	1.62	1.07	1.28	Long-chain fatty acid-CoA ligase activity
1637129_at [§]	<i>GstE3 (cg17524)</i>	1.41	1.32	1.15	1.29	Glutathione transferase activity
1637292_at	<i>Esp (cg7005)</i>	1.32	1.15	1.15	1.21	Secondary active sulfate transmembrane transporter activity
1637366_at	<i>nimC4 (cg16876)</i>	1.32	1.23	1.07	1.21	
1637407_at [§]	<i>cg32243</i>	1.62	1.52	1.41	1.52	
1637444_s_at	<i>pug (cg4067)</i>	1.32	1.52	1.62	1.49	Methylenetetrahydrofolate dehydrogenase (NADP+) activity, formate-tetrahydrofolate ligase activity, methenyltetrahydrofolate cyclohydrolase activity
1637562_at	<i>TwdIT (cg5812)</i>	1.07	2.46	2.46	2.00	Structural constituent of chitin-based cuticle
1637569_s_at	<i>cg13465</i>	1.15	1.15	1.32	1.21	
1637765_at	<i>cg14147</i>	1.07	1.41	6.96	3.15	
1637826_at [§]	<i>cg10623</i>	1.87	1.62	2.00	1.83	Selenocysteine methyltransferase activity
1637847_at	<i>Mdh1 (cg5362)</i>	1.15	1.15	1.32	1.21	L-malate dehydrogenase activity
1638020_at	<i>cg10467</i>	1.23	1.52	1.74	1.50	Aldose 1-epimerase activity
1638057_at	<i>cg18765</i>	1.32	1.32	1.52	1.38	
1638120_at	<i>cg1927</i>	1.15	1.32	1.52	1.33	
1638131_s_at	<i>5-HT1A (cg16720)</i>	1.32	2.30	4.92	2.85	G-protein coupled amine receptor activity, serotonin receptor activity
1638154_at	<i>cg2187</i>	1.32	1.52	2.00	1.61	Sodium:iodide symporter activity
1638201_at	<i>26-29-p (cg8947)</i>	1.07	1.74	1.41	1.41	Cysteine-type endopeptidase activity
1638457_at	<i>cg18347</i>	1.32	1.15	1.32	1.26	Transmembrane transporter activity

1638554_at	<i>cg4115</i>	1.07	1.23	1.41	1.24	Binding
1638558_a_at	<i>Snap25 (cg40452)</i>	1.15	1.23	2.30	1.56	SNAP receptor activity
1638587_at	<i>tor (cg1389)</i>	1.23	1.52	1.87	1.54	Protein tyrosine kinase activity
1638652_at [§]	<i>cg40006</i>	1.32	1.41	1.62	1.45	
1638811_at	<i>Sug (cg7334)</i>	1.15	1.23	1.52	1.30	
1638869_at [§]	<i>Cpr51A (cg10112)</i>	1.62	2.00	17.15	6.92	Structural constituent of chitin-based cuticle
1638892_at	<i>cg5397</i>	1.23	2.14	1.74	1.71	Sterol O-acyltransferase activity
1638933_at	<i>mus304 (cg7347)</i>	1.41	1.52	1.52	1.48	ATP-dependent DNA helicase activity
1638948_at	<i>slam (cg9506)</i>	1.07	1.32	2.00	1.46	
1638973_s_at	<i>cg11149</i>	2.00	1.62	2.83	2.15	N-acetyllactosaminide beta-1,3-N-acetylglucosaminyltransferase activity
1639092_at	<i>cg5880</i>	1.23	1.32	1.41	1.32	Protein-cysteine S-palmitoyltransferase activity
1639110_at [§]	<i>Slc45-1 (cg4484)</i>	1.87	2.83	3.73	2.81	Sucrose transmembrane transporter activity
1639251_at [§]	<i>Trf4-2 (cg17462)</i>	1.32	1.52	2.64	1.82	DNA-directed DNA polymerase activity
1639297_at	<i>cg9568</i>	1.07	1.41	2.46	1.65	
1639359_at [§]	<i>cg31974</i>	1.87	1.41	2.46	1.91	Transferase activity (transferring phosphorus-containing groups)
1639391_at	<i>cg17109</i>	1.41	1.87	3.73	2.34	Aminoacylase activity
1639415_at [§]	<i>Tps1 (cg4104)</i>	1.23	1.23	1.23	1.23	Alpha,alpha-trehalose-phosphate synthase (UDP-forming) activity
1639471_s_at [§]	<i>muskelin (cg8811)</i>	1.32	1.32	1.87	1.50	
1639480_at	<i>Cat (cg6871)</i>	1.32	1.15	1.23	1.23	Catalase activity
1639513_at	<i>cg5214</i>	1.15	1.41	1.23	1.26	Dihydrolipoyllysine-residue succinyltransferase activity
1639660_s_at	<i>cg10550</i>	1.07	1.32	1.62	1.34	Transferase activity (transferring phosphorus-containing groups)
1639741_at [§]	<i>HLHm5 (cg6096)</i>	1.52	1.74	2.00	1.75	RNA polymerase II core promoter proximal region sequence-specific DNA binding transcription factor activity involved in negative regulation of transcription, sequence-specific DNA binding, protein heterodimerization activity, protein homodimerization activity
1639853_at	<i>cg3509</i>	2.00	1.07	1.74	1.60	DNA binding
1639896_at	<i>cg43085</i>	1.07	1.62	1.41	1.37	
1639923_at	<i>cg30502</i>	1.23	1.52	1.23	1.33	Heme binding, oxidoreductase activity
1639953_at	<i>osk (cg10901)</i>	1.74	1.62	1.74	1.70	Hydrolase activity
1640065_at	<i>GstE7 (cg17531)</i>	1.07	1.23	1.41	1.24	Glutathione transferase activity
1640239_at	<i>rig (cg30149)</i>	1.32	1.23	1.62	1.39	Protein binding
1640264_at	<i>cg34376</i>	1.23	1.41	1.32	1.32	
1640296_a_at [§]	<i>Optix (cg18455)</i>	1.41	1.62	1.32	1.45	Sequence-specific DNA binding
1640360_at	<i>IM2 (cg18106)</i>	1.41	1.07	4.59	2.36	
1640451_at	<i>cg10863</i>	1.23	1.52	1.62	1.46	Alditol:NADP+ 1-oxidoreductase activity
1640466_s_at	<i>cg6543</i>	1.41	1.07	1.32	1.27	Enoyl-CoA hydratase activity
1640489_at	<i>cg18522</i>	1.23	1.15	2.46	1.61	2 iron (2 sulfur cluster binding), iron ion binding, electron carrier activity, oxidoreductase activity acting on CH-OH group of donors, flavin adenine dinucleotide binding
1640541_at [§]	<i>faf (cg1945)</i>	1.52	1.62	1.62	1.59	Ubiquitin-specific protease activity

1640590_at [§]	<i>cg32195</i>	1.52	1.41	1.74	1.56	Transferase activity (transferring phosphorus-containing groups)
1640593_at	<i>cg14570</i>	1.32	1.62	2.64	1.86	
1640650_at [§]	<i>lrp-1B (cg6342)</i>	1.32	1.23	1.74	1.43	Iron-responsive element binding, aconitate hydratase activity
1640720_a_at	<i>cg14872</i>	1.23	1.41	2.64	1.76	Binding
1640734_a_at	<i>cg16718</i>	1.41	1.32	1.52	1.42	
1640754_at	<i>bmm (cg5295)</i>	1.23	1.15	1.23	1.20	Triglyceride lipase activity
1640755_at	<i>Cyp6a8 (cg10248)</i>	1.07	1.23	1.74	1.35	Alkane 1-monooxygenase activity
1640835_a_at	<i>Gdh (cg5320)</i>	1.07	1.23	1.52	1.27	Glutamate dehydrogenase [NAD(P)+] activity, glutamate dehydrogenase (NAD+) activity, identical protein binding
1640862_a_at	<i>csul (cg3730)</i>	1.23	1.07	1.32	1.21	Methyltransferase activity, protein methyltransferase activity
1640898_at	<i>DNasell (cg7780)</i>	1.23	1.15	1.23	1.20	Deoxyribonuclease II activity
1640917_at	<i>cg15706</i>	1.15	1.32	1.23	1.23	
1640918_at	<i>ome (cg42280)</i>	1.15	1.32	1.32	1.26	Dipeptidyl-peptidase activity
1640928_at [§]	<i>Mdh2 (cg7998)</i>	1.32	1.32	2.64	1.76	Malate dehydrogenase activity
1640929_at	<i>cg7564</i>	1.32	1.23	1.15	1.23	
1641038_at	<i>cg10650</i>	1.15	1.32	1.52	1.33	
1641108_at	<i>cg17928</i>	1.52	1.52	1.52	1.52	Oxidoreductase activity acting on paired donors with oxidation of a pair of donors resulting in the reduction of molecular oxygen to two molecules of water, heme binding
1641125_at	<i>cg1942</i>	1.74	1.32	1.52	1.53	Transferase activity (transferring acyl groups other than amino-acyl groups)
1641148_a_at	<i>AcCoAS (cg9390)</i>	1.23	1.23	1.74	1.40	Acetate-CoA ligase activity
1641199_a_at	<i>cg1544</i>	1.23	1.74	1.62	1.53	Oxoglutarate dehydrogenase (succinyl-transferring) activity
1641280_at [§]	<i>Ance-4 (cg8196)</i>	1.41	1.41	1.87	1.56	Metallopeptidase activity, peptidyl-dipeptidase activity
1641393_at	<i>Cpr65Eb (cg8638)</i>	1.07	2.00	2.64	1.90	Structural constituent of chitin-based cuticle
1641443_at	<i>cg5418</i>	2.46	2.14	1.62	2.08	
1641575_at	<i>Cad87A (cg6977)</i>	1.15	1.32	1.07	1.18	Calcium ion binding
1641645_at	<i>cg5402</i>	1.41	1.74	9.19	4.11	
1641647_at	<i>Galt (cg9232)</i>	1.32	1.52	1.32	1.38	UDP-glucose:hexose-1-phosphate uridylyltransferase activity
1641671_at	<i>cg2183</i>	1.23	1.32	2.00	1.52	
641677_at	<i>slbo (cg4354)</i>	1.15	1.74	1.32	1.40	RNA polymerase II regulatory region sequence-specific DNA binding

We selected genes significantly up-regulated in *6xbcd* compared to *2xbcd* in three different ways. First, we selected genes with a change in expression that was consistently in the same direction in all three replicates, computed the Fisher's combined probability of three replicates (Sokal and Rohlf 1995), and listed 344 genes significantly up-regulated in *6xbcd* at the false discovery rate (FDR) of 0.001 in this table. Second, we listed 87 genes significantly up-regulated in *6xbcd* in all three replicates (GCOS, $P < 0.002$ for each replicate; marked by [§]). Finally, we listed 12 genes showing more than a two-fold difference in expression between *6xbcd* and *2xbcd* in all three replicates (marked by ^{§§}): *cg34025* (average fold difference = 11.71), *Adh (cg3481; 8.40)*, *bcd (cg1034; 3.66)*, *Mabi (cg15479; 3.53)*, *cg6125 (3.53)*, *NaPi-T (cg10207; 3.50)*, *Smtv (cg2191; 3.38)*, *cg31075 (3.12)*, *blanks (cg10630; 2.70)*, *Cyt-b5-r (cg13279; 2.67)*, *cg18473 (2.41)*, and *Mocs1 (cg33048; 2.41)*. Because the X chromosome differs between *2xbcd* and *6xbcd* embryos, we excluded X-linked genes from the analysis.

^a Probes on the Affymetrix chip.

^{b, d} From fly base, version FB2012_01 (<http://flybase.org/>).

^c Relative expression level in *6xbcd* compared to that in *2xbcd*.

Table S4 Down-regulated genes in 6xbcd condition

Probe ID ^a	Gene ^b	Fold change in expression ^c				Molecular function ^d
		Rep.1	Rep.2	Rep.3	Ave.	
1622907_at	<i>cg5189</i>	0.87	0.81	0.81	0.83	
1622923_at	<i>wrapper</i> (<i>cg10382</i>)	0.76	0.87	0.66	0.76	
1623000_at [§]	<i>cg33093</i>	0.54	0.76	0.57	0.62	Oxidoreductase activity acting on paired donors with incorporation or reduction of molecular oxygen (2-oxoglutarate as one donor and incorporation of one atom each of oxygen into both donors)
1623191_at	<i>Rab9</i> (<i>cg9994</i>)	0.66	0.76	0.66	0.69	GTPase activity
1623243_at	<i>cg5656</i>	0.62	0.93	0.38	0.64	Alkaline phosphatase activity
1623261_at	<i>RabX4</i> (<i>cg31118</i>)	0.87	0.93	0.71	0.84	GTPase activity
1623378_at	<i>Oli</i> (<i>cg5545</i>)	0.87	0.87	0.81	0.85	Sequence-specific DNA binding transcription factor activity
1623453_at	<i>Nf-YB</i> (<i>cg10447</i>)	0.81	0.81	0.87	0.83	Sequence-specific DNA binding
1623459_at	<i>Spn4</i> (<i>cg9453</i>)	0.66	0.87	0.66	0.73	Serine-type endopeptidase inhibitor activity
1623594_at	<i>blow</i> (<i>cg1363</i>)	0.93	0.71	0.76	0.80	
1623605_a_at	<i>cbt</i> (<i>cg4427</i>)	0.87	0.87	0.81	0.85	Sequence-specific DNA binding
1623643_s_at [§]	<i>ldgf3</i> (<i>cg4559</i>)	0.50	0.81	0.71	0.67	Imaginal disc growth factor receptor binding
1623728_at	<i>cg13689</i>	0.81	0.71	0.87	0.80	
1623896_a_at	<i>cg34404</i>	0.87	0.87	0.76	0.83	
1624069_at [§]	<i>cg7296</i>	0.81	0.71	0.57	0.70	
1624195_at	<i>sug</i> (<i>cg3850</i>)	0.81	0.76	0.62	0.73	Zinc ion binding, nucleic acid binding
1624269_at	<i>gkt</i> (<i>cg8825</i>)	0.81	0.87	0.87	0.85	Phosphoric diester hydrolase activity
1624759_s_at	<i>Antp</i> (<i>cg1028</i>)	0.81	0.87	0.66	0.78	Sequence-specific DNA binding, sequence-specific distal enhancer binding RNA polymerase II transcription factor activity
1624841_s_at	<i>cg13585</i>	0.81	0.93	0.76	0.83	
1624854_s_at [§]	<i>Lis-1</i> (<i>cg8440</i>)	0.76	0.66	0.62	0.68	ATPase activity (coupled), dynein binding, enzyme regulator activity
1624857_at	<i>Fis1</i> (<i>cg17510</i>)	0.81	0.76	0.76	0.78	Binding
1624859_at	<i>rpr</i> (<i>cg4319</i>)	0.76	0.87	0.81	0.81	Phospholipid binding, protein homodimerization activity
1624873_at	<i>kar</i> (<i>cg12286</i>)	0.76	0.87	0.71	0.78	Monocarboxylic acid transmembrane transporter activity
1624907_at [§]	<i>Tsp42Ee</i> (<i>cg10106</i>)	0.76	0.87	0.76	0.80	
1625122_at	<i>Obp99c</i> (<i>cg7584</i>)	0.71	0.87	0.71	0.76	Odorant binding
1625215_s_at	<i>nrv2</i> (<i>cg9261</i>)	0.93	0.81	0.81	0.85	Sodium:potassium-exchanging ATPase activity, cation transmembrane transporter activity
1625276_a_at	<i>Eip71CD</i> (<i>cg7266</i>)	0.93	0.71	0.81	0.82	Peptide-methionine-(S)-S-oxide reductase activity

1625411_at	<i>cg42730</i> or <i>tectonic</i> (<i>cg42731</i>)	0.93	0.87	0.62	0.81	
1625434_at	<i>cg3270</i>	0.71	0.76	0.81	0.76	Oxidoreductase activity
1625563_s_at	<i>cg14253</i>	0.76	0.66	0.76	0.73	
1625688_at	<i>cg6293</i>	0.62	0.81	0.71	0.71	L-ascorbate:sodium symporter activity
1625875_at	<i>cg10635</i>	0.81	0.81	0.76	0.79	Chaperone binding
1626018_a_at	<i>crol</i> (<i>cg14938</i>)	0.93	0.81	0.71	0.82	Zinc ion binding, nucleic acid binding
1626044_at	<i>cg42575</i>	0.81	0.93	0.81	0.85	Phosphate ion transmembrane transporter activity
1626058_at	<i>lbl</i> (<i>cg6570</i>)	0.87	0.87	0.76	0.83	Sequence-specific DNA binding transcription factor activity
1626059_at	<i>knrl</i> (<i>cg4761</i>)	0.87	0.87	0.71	0.82	Sequence-specific DNA binding transcription factor activity, ligand-dependent nuclear receptor activity
1626086_at [§]	<i>cg10621</i>	0.76	0.81	0.71	0.76	Selenocysteine methyltransferase activity
1626144_at [§]	<i>cg7763</i>	0.71	0.66	0.71	0.69	Binding
1626150_at	<i>tsh</i> (<i>cg1374</i>)	0.87	0.87	0.81	0.85	RNA polymerase II core promoter proximal region sequence-specific DNA binding transcription factor activity involved in negative regulation of transcription
1626152_at	<i>Ror</i> (<i>cg4926</i>)	0.76	0.93	0.76	0.82	Transmembrane receptor protein tyrosine kinase activity
1626263_at	<i>cg7510</i>	0.66	0.81	0.62	0.70	
1626556_at	<i>Arpc3A</i> (<i>cg4560</i>)	0.87	0.81	0.81	0.83	Actin binding
1626773_s_at	<i>cg43164</i>	0.81	0.76	0.76	0.78	Binding
1626856_at	<i>cg12560</i>	0.71	0.93	0.71	0.78	N-acetyltransferase activity
1627007_at [§]	<i>cg4306</i>	0.35	0.71	0.33	0.46	Gamma-glutamylcyclotransferase activity
1627312_at	<i>RpL12</i> (<i>cg3195</i>)	0.81	0.81	0.76	0.79	Structural constituent of ribosome
1627334_at [§]	<i>Tsp39D</i> (<i>cg8666</i>)	0.71	0.71	0.66	0.69	
1627419_at	<i>His4r</i> (<i>cg3379</i>)	0.81	0.87	0.87	0.85	DNA binding
1627453_s_at	<i>spi</i> (<i>cg10334</i>)	0.93	0.87	0.81	0.87	Gurken receptor binding, epidermal growth factor receptor binding
1627564_s_at	<i>Smg5</i> (<i>cg8954</i>)	0.76	0.93	0.87	0.85	
1627582_a_at [§]	<i>Tret1-1</i> (<i>cg30035</i>)	0.57	0.66	0.54	0.59	Glucose transmembrane transporter activity
1627719_at	<i>gol</i> (<i>cg2679</i>)	0.81	0.87	0.71	0.80	Zinc ion binding
1627768_at	<i>cg12384</i>	0.87	0.87	0.76	0.83	
1627838_at	<i>uzip</i> (<i>cg3533</i>)	0.71	0.87	0.87	0.82	Axon guidance, axonal fasciculation
1627856_at	<i>Hus1-like</i> (<i>cg2525</i>)	0.81	0.81	0.93	0.85	
1627888_at	<i>cg6672</i>	0.87	0.87	0.87	0.87	Zinc ion transmembrane transporter activity
1627961_a_at [§]	<i>cg18507</i>	0.76	0.81	0.57	0.71	
1628075_at	<i>olf186-M</i> (<i>cg14489</i>)	0.71	0.87	0.81	0.80	
1628150_a_at [§]	<i>cg9449</i>	0.44	0.66	0.54	0.54	Acid phosphatase activity
1628268_at [§]	<i>cg18249</i>	0.62	0.76	0.66	0.68	

1628275_at	<i>Trl (cg33261)</i>	0.87	0.87	0.76	0.83	Sequence-specific DNA binding transcription factor activity, protein binding, transcription factor binding, sequence-specific DNA binding, chromatin binding, DNA binding
1628637_at	<i>ubl (cg3450)</i>	0.93	0.76	0.81	0.83	
1628646_at	<i>cg18507</i>	0.76	0.71	0.62	0.69	
1628678_at [§]	<i>cg13920</i>	0.71	0.81	0.71	0.74	
1628726_s_at	<i>Rgl (cg8865)</i>	0.81	0.81	0.87	0.83	Ral guanyl-nucleotide exchange factor activity
1628743_at	<i>glec (cg6575)</i>	0.87	0.87	0.81	0.85	Carbohydrate binding
1628828_s_at	<i>Oscillin (cg6957)</i>	0.76	0.87	0.81	0.81	Glucosamine-6-phosphate deaminase activity
1628984_s_at [§]	<i>HmgZ (cg17921)</i>	0.81	0.76	0.71	0.76	DNA binding
1628987_at	<i>cg5869</i>	0.87	0.76	0.87	0.83	Actin binding
1629324_at [§]	<i>cg6870</i>	0.50	0.66	0.62	0.59	Electron carrier activity
1629363_at	<i>Acyp2 (cg18505)</i>	0.87	0.76	0.76	0.80	Acylophosphatase activity
1629702_a_at	<i>ab (cg4807)</i>	0.93	0.87	0.76	0.85	Sequence-specific DNA binding transcription factor activity
1629879_at	<i>Nnf1a (cg13434)</i>	0.87	0.76	0.81	0.81	
1630145_s_at	<i>Tsp42Ea (cg18817)</i>	0.87	0.87	0.76	0.83	
1630206_at [§]	<i>CS-2 (cg7464)</i>	0.66	0.71	0.66	0.68	Chitin synthase activity
1630223_at	<i>skl (cg13701)</i>	0.87	0.87	0.76	0.83	
1630348_a_at [§]	<i>cg5059</i>	0.81	0.87	0.71	0.80	
1630476_s_at	<i>nahoda (cg12781)</i>	0.81	0.93	0.81	0.85	
1630540_at	<i>yellow-e3 (cg17045)</i>	0.87	0.81	0.81	0.83	
1630577_at	<i>cg17378</i>	0.57	0.54	0.81	0.64	
1630700_at [§]	<i>cg43064</i>	0.50	0.62	0.57	0.56	
1630985_at	<i>cg30441</i>	0.71	0.66	0.66	0.68	
1631046_s_at	<i>cg42748</i>	0.87	0.81	0.76	0.81	Protein phosphatase 1 binding
1631114_a_at	<i>srpk79D (cg11489)</i>	0.71	0.93	0.62	0.75	Protein kinase activity
1631208_at	<i>cg15525</i>	0.87	0.76	0.81	0.81	
1631330_at	<i>cg3408</i>	0.87	0.81	0.76	0.81	
1631402_s_at	<i>Hr39 (cg8676)</i>	0.76	0.81	0.76	0.78	RNA polymerase II core promoter proximal region sequence-specific DNA binding, ligand-dependent nuclear receptor activity
1631635_at [§]	<i>cg7294</i>	0.76	0.66	0.66	0.69	
1631652_at	<i>cg7713</i>	0.87	0.87	0.81	0.85	
1631761_at [§]	<i>cg31313</i>	0.57	0.62	0.57	0.59	Cysteine-type endopeptidase inhibitor activity
1631804_s_at	<i>PQBP1 (cg11820)</i>	0.93	0.76	0.76	0.82	
1631865_at [§]	<i>cg13064</i>	0.76	0.76	0.44	0.65	
1632011_at	<i>MESK4 (cg31447)</i>	0.71	0.87	0.71	0.76	
1632082_at	<i>Ndg (cg12908)</i>	0.87	0.93	0.87	0.89	Calcium ion binding
1632213_s_at	<i>CR31292</i>	0.81	0.81	0.76	0.79	Hydrolase activity acting on ester bonds

1632245_a_at	<i>Spn4 (cg9453)</i>	0.76	0.87	0.71	0.78	Serine-type endopeptidase inhibitor activity
1632345_at	<i>cg8353</i>	0.76	0.81	0.87	0.81	Cytidine deaminase activity
1632390_at	<i>cer (cg10460)</i>	0.87	0.81	0.87	0.85	Cysteine-type endopeptidase inhibitor activity
1632399_at	<i>cg34007</i>	0.93	0.81	0.66	0.80	
1632457_s_at [§]	<i>mam (cg8118)</i>	0.76	0.81	0.71	0.76	Transcription coactivator activity
1632464_at [§]	<i>Smg6 (cg6369)</i>	0.66	0.87	0.76	0.76	Ribonuclease activity
1632496_at	<i>cg42382</i>	0.71	0.81	0.81	0.78	
1632515_a_at [§]	<i>cg18619</i>	0.71	0.76	0.76	0.74	Protein dimerization activity
1632802_at [§]	<i>Jhl-26 (cg3767)</i>	0.50	0.66	0.50	0.55	Transferase activity (transferring phosphorus-containing groups)
1632855_at	<i>cg7352</i>	0.93	0.71	0.76	0.80	
1632901_s_at	<i>c(3)G (cg17604)</i>	0.76	0.76	0.81	0.78	
1632907_a_at	<i>cg13204</i>	0.87	0.81	0.57	0.75	DNA binding
1632977_at	<i>Poxn (cg8246)</i>	0.81	0.87	0.66	0.78	Sequence-specific DNA binding transcription factor activity
1633145_at	<i>PGRP-LF (cg4437)</i>	0.62	0.87	0.66	0.72	Peptidoglycan binding
1633200_at [§]	<i>cg9449</i>	0.54	0.66	0.57	0.59	Acid phosphatase activity
1633210_at	<i>cg10674</i>	0.93	0.87	0.81	0.87	
1633401_s_at [§]	<i>Cyp12d1-p (cg30489)</i>	0.62	0.50	0.62	0.58	Electron carrier activity
1633540_at	<i>cg8147</i>	0.87	0.54	0.81	0.74	Alkaline phosphatase activity
1633572_at	<i>cg42336</i>	0.71	0.76	0.81	0.76	
1633626_at	<i>cg14104</i>	0.76	0.76	0.81	0.78	
1633789_at [§]	<i>Hand (cg18144)</i>	0.76	0.71	0.66	0.71	RNA polymerase II core promoter proximal region sequence-specific DNA binding transcription factor activity involved in positive regulation of transcription
1633841_at	<i>yellow-e2 (cg17044)</i>	0.81	0.62	0.76	0.73	
1633904_at	<i>scrib (cg43398)</i>	0.81	0.93	0.76	0.83	Protein binding
1633946_at [§]	<i>cg31955</i>	0.35	0.54	0.50	0.46	Binding
1634031_at	<i>Best2 (cg10173)</i>	0.87	0.76	0.76	0.80	Anion channel activity
1634039_at [§]	<i>nerfin-1 (cg13906)</i>	0.87	0.87	0.76	0.83	Zinc ion binding, nucleic acid binding
1634127_a_at	<i>cg13029</i>	0.87	0.81	0.76	0.81	Protein-cysteine S-palmitoyltransferase activity
1634164_at	<i>HSPC300 (cg30173)</i>	0.81	0.71	0.81	0.78	
1634238_a_at	<i>cg9628</i>	0.87	0.76	0.66	0.76	
1634291_at	<i>cg42863</i>	0.76	0.93	0.81	0.83	
1634396_at	<i>cg3303</i>	0.81	0.81	0.71	0.78	Serine-type endopeptidase activity
1634409_at	<i>cg31775</i>	0.71	0.87	0.76	0.78	
1634443_a_at	<i>Rab1 (cg3320)</i>	0.81	0.76	0.81	0.79	GTPase activity
1634479_at		0.76	0.62	0.71	0.69	
1634628_at	<i>cg30392</i>	0.81	0.87	0.81	0.83	Glycolipid transporter activity, glycolipid binding
1634684_at	<i>cg7272</i>	0.71	0.93	0.76	0.80	
1634794_at	<i>cg10924</i>	0.71	0.93	0.76	0.80	Phosphoenolpyruvate carboxykinase (GTP) activity

1634848_at	<i>SPE (cg16705)</i>	0.62	0.87	0.66	0.72	Serine-type endopeptidase activity
1635026_at [§]	<i>tap (cg7659)</i>	0.81	0.76	0.76	0.78	Sequence-specific DNA binding transcription factor activity
1635144_at	<i>klg (cg6669)</i>	0.76	0.87	0.62	0.75	
1635260_at	<i>Stam (cg6521)</i>	0.81	0.87	0.87	0.85	JAK pathway signal transduction adaptor activity
1635300_at	<i>VhaM9.7-a (cg1268)</i>	0.76	0.76	0.81	0.78	Hydrogen-exporting ATPase activity (phosphorylative mechanism)
1635450_a_at	<i>smp-30 (cg7390)</i>	0.66	0.81	0.50	0.66	
1635522_a_at	<i>santa-maria (cg12789)</i>	0.76	0.81	0.66	0.74	Scavenger receptor activity
1635551_at	<i>cg8386</i>	0.87	0.81	0.81	0.83	
1635588_at	<i>cg14977</i>	0.93	0.87	0.76	0.85	
1635639_a_at	<i>Eip74EF (cg32180)</i>	0.57	0.71	0.93	0.74	RNA polymerase II core promoter proximal region sequence-specific DNA binding transcription factor activity involved in positive regulation of transcription
1635725_a_at	<i>Unc-115b (cg31332)</i>	0.87	0.93	0.81	0.87	Zinc ion binding
1635751_at	<i>cg32069</i>	0.81	0.76	0.87	0.81	
1635939_a_at	<i>cg9641</i>	0.87	0.81	0.76	0.81	
1636149_at	<i>cg31705</i>	0.76	0.87	0.93	0.85	
1636165_at	<i>cg31002</i>	0.76	0.87	0.76	0.80	Glucuronosyltransferase activity
1636311_at	<i>Gpdh (cg9042)</i>	0.76	0.62	0.93	0.77	Glycerol-3-phosphate dehydrogenase [NAD+] activity
1636353_s_at	<i>cg8112</i>	0.81	0.93	0.71	0.82	Sterol O-acyltransferase activity
1636440_at [§]	<i>Sip1 (cg10939)</i>	0.81	0.81	0.81	0.81	
1636736_s_at	<i>cg31274</i>	0.66	0.87	0.87	0.80	
1636843_a_at [§]	<i>cg10512</i>	0.66	0.71	0.62	0.66	Oxidoreductase activity
1636856_at	<i>Vhl (cg13221)</i>	0.76	0.76	0.81	0.78	Protein binding, ubiquitin-protein ligase activity
1636942_at [§]	<i>cg8498</i>	0.81	0.87	0.76	0.81	Fatty-acyl-CoA binding
1636960_a_at	<i>RanBPM (cg42236)</i>	0.93	0.93	0.81	0.89	Ran GTPase binding
1636973_at	<i>smp-30 (cg7390)</i>	0.66	0.76	0.47	0.63	
1637008_at	<i>cg14687</i>	0.81	0.71	0.62	0.71	Myosin light chain binding
1637063_at [§]	<i>cg33099</i>	0.71	0.81	0.71	0.74	Gibberellin 20-oxidase activity
1637154_at [§]	<i>cg7470</i>	0.71	0.71	0.71	0.71	Delta1-pyrroline-5-carboxylate synthetase activity, glutamate 5-kinase activity, glutamate-5-semialdehyde dehydrogenase activity
1637173_at	<i>cg34010</i>	0.76	0.87	0.93	0.85	
1637255_a_at	<i>α-Est7 (cg1112)</i>	0.66	0.81	0.57	0.68	Carboxylesterase activity
1637499_s_at [§]	<i>cg5953</i>	0.57	0.71	0.66	0.65	
1637750_at	<i>Sox21b (cg32139)</i>	0.87	0.76	0.62	0.75	Sequence-specific DNA binding transcription factor activity, DNA bending activity
1637778_a_at	<i>cg1969</i>	0.93	0.87	0.76	0.85	Glucosamine 6-phosphate N-acetyltransferase activity

1637813_at	<i>abd-A</i> (<i>cg10325</i>)	0.93	0.81	0.76	0.83	Sequence-specific DNA binding transcription factor activity, sequence-specific DNA binding, sequence-specific distal enhancer binding RNA polymerase II transcription factor activity
1637939_at	<i>Spn43Aa</i> (<i>cg12172</i>)	0.81	0.81	0.54	0.72	Serine-type endopeptidase inhibitor activity
1638038_at	<i>cg4335</i>	0.71	0.87	0.57	0.72	Gamma-butyrobetaine dioxygenase activity
1638051_at [§]	<i>cg17323</i>	0.71	0.71	0.66	0.69	Glucuronosyltransferase activity
1638060_at	<i>cg10077</i>	0.87	0.76	0.76	0.80	RNA helicase activity
1638092_a_at	<i>Arpc3A</i> (<i>cg4560</i>)	0.87	0.76	0.81	0.81	Actin binding
1638220_at	<i>cg16888</i>	0.76	0.57	0.50	0.61	
1638360_at	<i>Oatp26F</i> (<i>cg31634</i>)	0.87	0.81	0.71	0.80	Organic anion transmembrane transporter activity
1638400_at	<i>cg8503</i>	0.87	0.81	0.71	0.80	Histone deacetylase binding
1638498_s_at [§]	<i>cg1600</i>	0.66	0.76	0.62	0.68	Nucleotide binding, oxidoreductase activity, zinc ion binding
1638562_a_at [§]	<i>Cyp6d5</i> (<i>cg3050</i>)	0.62	0.87	0.62	0.70	Electron carrier activity
1638592_at	<i>dnd</i> (<i>cg6560</i>)	0.71	0.81	0.57	0.70	GTPase activity, GTP binding
1638634_at	<i>cg32448</i>	0.81	0.76	0.76	0.78	
1638669_at	<i>cg11395</i>	0.50	0.93	0.87	0.77	
1638912_s_at	<i>Hrb98DE</i> (<i>cg9983</i>)	0.76	0.93	0.81	0.83	Sequence-specific DNA binding
1639069_at [§]	<i>Cyp12d1-d</i> (<i>cg33503</i>)	0.66	0.54	0.57	0.59	Electron carrier activity
1639109_a_at	<i>CRMP</i> (<i>cg1411</i>)	0.81	0.93	0.76	0.83	Dihydropyrimidinase activity
1639144_a_at	<i>Tace</i> (<i>cg7908</i>)	0.81	0.87	0.66	0.78	Metalloendopeptidase activity
1639186_at	<i>cg7918</i>	0.87	0.57	0.87	0.77	G-protein coupled acetylcholine receptor activity
1639355_s_at	<i>Fs(2)Ket</i> (<i>cg2637</i>)	0.93	0.81	0.76	0.83	Protein transmembrane transporter activity
1639530_at	<i>cg14528</i>	0.54	0.81	0.76	0.70	Metalloendopeptidase activity
1639545_a_at	<i>Awh</i> (<i>cg1072</i>)	0.93	0.76	0.81	0.83	Sequence-specific DNA binding transcription factor activity
1639619_a_at	<i>ZnT35C</i> (<i>cg3994</i>)	0.71	0.93	0.76	0.80	Zinc ion transmembrane transporter activity, metal ion transmembrane transporter activity
1639625_at	<i>Vps25</i> (<i>cg14750</i>)	0.93	0.81	0.81	0.85	
1639834_at	<i>cg14291</i>	0.87	0.87	0.76	0.83	N-sulfoglucosamine sulfohydrolase activity
1639856_at	<i>cg5727</i>	0.87	0.81	0.87	0.85	
1639863_at	<i>cg9698</i>	0.87	0.71	0.66	0.75	Procollagen-proline 4-dioxygenase activity
1639892_at	<i>Sodh-1</i> (<i>cg1982</i>)	0.62	0.66	0.93	0.74	L-idoitol 2-dehydrogenase activity
1639906_a_at	<i>cg7759</i>	0.76	0.87	0.54	0.72	Histone deacetylase binding
1639934_at	<i>cg31272</i>	0.81	0.87	0.66	0.78	Lipase activity, transporter activity
1640129_at [§]	<i>slp1</i> (<i>cg16738</i>)	0.76	0.81	0.76	0.78	Sequence-specific distal enhancer binding RNA polymerase II transcription factor activity
1640228_at	<i>cg11658</i>	0.76	0.93	0.71	0.80	
1640289_a_at	<i>cg40439</i>	0.76	0.71	0.76	0.74	

1640337_a_at	<i>Ank2</i> (<i>cg42734</i>)	0.87	0.87	0.71	0.82	Structural constituent of cytoskeleton, cytoskeletal protein binding
1640386_at [§]	<i>wbl</i> (<i>cg7225</i>)	0.81	0.71	0.76	0.76	
1640429_a_at	<i>cg6145</i>	0.87	0.87	0.81	0.85	NAD+ kinase activity
1640472_at	<i>CR42862</i>	0.76	0.87	0.81	0.81	
1640515_s_at	<i>lqf</i> (<i>cg8532</i>)	0.93	0.87	0.76	0.85	
1640775_a_at [§]	<i>Nmdmc</i> (<i>cg18466</i>)	0.38	0.66	0.57	0.54	Methylenetetrahydrofolate dehydrogenase (NADP+) activity, methenyltetrahydrofolate cyclohydrolase activity
1641004_a_at	<i>cg3164</i>	0.81	0.93	0.76	0.83	ATPase activity coupled to transmembrane movement of substances, transporter activity
1641143_s_at	<i>tsl</i> (<i>cg6705</i>)	0.71	0.93	0.76	0.80	Torso binding
1641167_s_at	<i>Vdup1</i> (<i>cg7047</i>)	0.87	0.87	0.71	0.82	
1641192_at	<i>cg14523</i>	0.66	0.81	0.81	0.76	Metalloendopeptidase activity
1641304_s_at	<i>cg9801</i>	0.81	0.87	0.71	0.80	Catalytic activity
1641342_at	<i>cag</i> (<i>cg12346</i>)	0.87	0.81	0.87	0.85	DNA binding
1641390_at [§]	<i>dpr19</i> (<i>cg13140</i>)	0.81	0.76	0.71	0.76	
1641446_s_at [§]	<i>cg5171</i>	0.57	0.62	0.54	0.58	Trehalose-phosphatase activity
1641448_a_at	<i>Dg</i> (<i>cg18250</i>)	0.87	0.76	0.62	0.75	Protein binding
1641496_a_at	<i>grass</i> (<i>cg5896</i>)	0.57	0.81	0.57	0.65	Serine-type endopeptidase activity
1641589_s_at [§]	<i>Abd-B</i> (<i>cg11648</i>)	0.87	0.81	0.76	0.81	Sequence-specific DNA binding transcription factor activity, RNA polymerase II distal enhancer sequence-specific DNA binding
1641596_at	<i>cg17343</i>	0.81	0.81	0.81	0.81	

As in Table S3, this table lists 216 genes whose changes in expression were consistently in the same direction in all three replicates and were significantly down-regulated in *6xbcd* compared to *2xbcd* at the false discovery rate (FDR) of 0.001. Forty-eight genes (marked by [§]) were significantly down-regulated in *6xbcd* in all three replicates (GCOS, $P < 0.002$ for each replicate). None of the down-regulated genes exhibited more than a two-fold reduction in *6xbcd* across three replicates. Because the X chromosome differs between *2xbcd* and *6xbcd* embryos, we excluded X-linked genes from the analysis.

^a Probes on the Affymetrix chip.

^{b, d} From fly base, version FB2012_01 (<http://flybase.org/>).

^c Relative expression level in *6xbcd* compared to that in *2xbcd*.