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The nuclear receptor DHR3/Hr46 is required in the blood brain barrier of mature males for courtship

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Short title: Blood brain barrier transcripts and male courtship

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Abstract

The blood brain barrier (BBB) forms a stringent barrier that protects the brain from components in the circulation that could interfere with neuronal function. At the same time, the BBB enables selective transport of critical nutrients and other chemicals to the brain. Many of these processes are still poorly understood. Beyond these functions, another recently recognized function is even less characterized, specifically the role of the BBB in modulating behavior by affecting neuronal function in a sex dependent manner. Notably, signaling in the adult *Drosophila* BBB is required for normal male courtship behavior. Courtship regulation also relies on male-specific molecules in the BBB. Our previous studies have demonstrated that adult feminization of these cells in males significantly lowered courtship. Here, we conducted microarray analysis of BBB cells isolated from males and females. Findings revealed that these cells contain male- and female-enriched transcripts, respectively. Among these transcripts, nuclear receptor DHR3/Hr46 was identified as a male-enriched BBB transcript. DHR3/Hr46 is best known for its essential roles in the ecdysone response during development and metamorphosis. In this study, we demonstrate that DR3/Hr46 is specifically required in the BBB cells of mature males for courtship behavior. The protein is localized in the nuclei of sub-perineurial glial cells (SPG), indicating that it might act as a transcriptional regulator. These data provide a catalogue of sexually dimorphic BBB transcripts and demonstrate a physiological adult role for the nuclear receptor DH3/Hr46 in the regulation of male courtship, a novel function that is independent of its developmental role.

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Author summary

The blood brain barrier very tightly regulates which molecules can enter the brain. This is an important protection for the brain, however, it also complicates communication between molecules in the circulating fluid and the brain. In fly courtship, for example, circulating male-specific products are crucially required for normal courtship. But the neuronal circuits that ultimately control the behavior are inside the brain, separated from these molecules by the blood brain barrier. The mechanisms of this communication are not known. Here we show that the blood brain barrier itself contains sex-specific RNAs and we show that one of them, a nuclear receptor called DHR3, is required in adult males for normal courtship. These findings promise new insight into the communication between blood brain barrier and the brain.

Introduction

It is well established that the two layers of glial cells that tightly surround the nervous system form the *Drosophila* blood brain barrier (BBB)(1). Flies have a non-vascular open circulatory system that distributes the hemolymph. The BBB forms the tight exclusion barrier that is essential to protecting neurons from hemolymph components that could interfere with neuronal function (2, 3). At the same time, the barrier needs to allow selective uptake of nutrients and other molecules needed for

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brain function. The *Drosophila* blood brain barrier (BBB) surrounds the brain like a tight cap. It consists of two layers of glial cells. The outer perineurial glia cells (PG cells) are thought to function as a barrier for large-molecular weight molecules. The inner layer, the subperineurial glia (SPG), is adjacent to the neuronal cell bodies and contains the tight junctions that form the physical barrier (Fig 1A). It has been shown in a number of genetic and functional studies that the barriers in flies and vertebrates share not only structure and function, but also many homologous proteins that ensure their function, as shown in (4). A recent microarray study of isolated BBB cells has expanded on these earlier findings and shown that besides the characteristic barrier proteins, fly and mouse BBB cells share a large number of conserved proteins (5). That study has also provided the first detailed “inventory” of these cells in *Drosophila*. While the barrier and selective uptake functions of the BBB are its most obvious essential function, evidence is starting to accumulate that other physiological processes in BBB cells are contributing to brain function. For example, the G-protein-coupled receptor *moody* is specifically expressed in the subperineurial glial cells (SPG)(6, 7). While the absence of both *moody* isoforms leads to a leaky BBB (6, 7), mutants with only one of the isoforms have intact barriers, but behavioral defects in their response to cocaine and alcohol (6). In addition, *moody*, in a function independent of its function in barrier integrity, is also required in BBB cells for normal male courtship (8). That active signaling processes in BBB cells regulate neuronal output was further indicated by the finding that BBB-specific reductions in the G protein Galpha(o) cause courtship defects, while leaving the barrier integrity intact (8). It has been found that the circulating hemolymph

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contains male-specific factors from the fat body that are needed to ensure normal courtship (9). It is not clear how these factors interact with the male brain circuits that regulate the behavior. Here we examine whether the BBB expresses sex-specific transcripts that might be part of this communication. This would be in agreement with the finding that feminization of the BBB by expression of the feminizing TRA protein specifically in the BBB of adult males results in a significant reduction in male courtship (8). In these experiments, the tightness of the barrier was unaffected, suggesting that specific male transcripts are physiologically participating in courtship control. The identity of these factors and their function is unknown. Here we identify sex-preferentially expressed transcripts in the BBB of males and females and demonstrate that the nuclear receptor DHR3/Hr46, best known for its roles in larval development (10-12), is physiologically required in the BBB of adult mature males to ensure normal male courtship behavior.

Results

A microarray screen identifies male- and female-enriched transcripts in the BBB

In our previous experiments, there was a strong reduction in male courtship when we conditionally feminized adult BBB cells (8). This suggests that feminization disrupts male-specific transcripts that are physiologically required for normal mating behavior. In order to identify these transcripts, we isolated BBB cells from males and females and characterized their transcripts. The Gal4/UAS system was used to mark these cells (13). We expressed the fluorescent protein DsRed in the

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nuclei of SPG cells, using the *moody-Gal4* driver that drives expression in SPG cells (*SPG-Gal4*) (6). As seen in Fig 1B, the large nuclei of the SPG cells were specifically marked. We dissected fly brains and manually removed and collected fluorescent cells. Cells from approximately 50 flies were pooled for each biological replicate, and the RNA of three biological replicas from separate crosses was prepared for each sex. The RNA was subsequently used for microarray analysis by GenUs Biosystems (http://www.genusbiosystems.com/). The results confirmed the presence of sex-preferentially expressed transcripts in the BBB of males and females, respectively. 284 transcripts were identified that were enriched > 2 fold in either males and females (Figures 2C, D). Of those, 112 were male-preferentially expressed (S1 Table). As expected, the male-specifically expressed *rox* RNAs that are required for dosage compensation were highly specific to males. Furthermore, sex-specific *dsx* transcripts were identified because male and female transcripts use different polyA-sites and can thus be identified by microarray (14). An analysis of the GeneOntology of the enriched transcripts is shown in Table S2. Sex specific categories such as dosage compensation and sex determination are well represented, further confirming that the isolated cells are sexually determined. About half of the genes fall into one of these categories. The rest of the genes could not be assigned to a specific category. In addition to identifying sex-preferentially expressed RNAS, the experiment also provided an inventory of RNAs present in the BBB cells. The vast majority of BBB transcripts is equally expressed in males and females. Among them, as predicted for SPG cells, were RNAs that are characteristic of BBB cells (4, 5): RNAs for the junction proteins *sinu* and *neurexin*, for example,

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and the previously characterized SPG transcripts for *moody* and *Mdr65*. The most likely contaminating cells from the dissections would be fat body cells which are in close proximity to the BBB, and neuronal cells. We found very small amounts of the fat body transcript *Lsp-2,* or of the neuronal marker *elav*. They were not preferentially present in males or females, indicating that low amounts of these cells are unlikely to affect the identification of sex-specific transcripts in the BBB.

The nuclear receptor DH3/Hr46 is required in the BBB for courtship

One of the male-enriched BBB RNAs is the transcript for the nuclear receptor *DHR3/Hr46* (Fig 1E). DHR3/Hr46 is an orphan nuclear receptor that is most related to the mammalian ROR receptor (Retinoic acid related orphan receptor). DHR3/Hr46 is a well-described transcriptional regulator of larval developmental processes in response to ecdysone, but no adult functions have been described so far. To examine whether *DHR3/Hr46* is required in the BBB for courtship we conditionally expressed two different *DHR3/Hr46-RNAi* constructs specifically in the BBB of mature adult males and examined their courtship (Fig 2). Male courtship in *Drosophila melanogaster* consists of well-defined stereotyped behavioral steps that can easily be quantified in a courtship index (CI) (15-17). The CI is calculated as the fraction of time the male spends displaying any element of courtship behavior (orienting, following, wing extension, licking, attempted copulation, copulation) within a 10 minute observation period (18). We used the *Gal4/Gal80*^{ts} system to restrict knockdown to mature males (19). Two different *BBB-Gal4* drivers were used to direct expression, the previously described *SPG-Gal4*, and a SPG-cell-specific

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Mdr65-Gal4 driver that was generated in our lab (Fig 2D). The ATP binding cassette (ABC) transporter *Mdr65* has been shown to be specifically expressed in the SPG cells of the BBB (20, 21). Control flies containing a copy of just one of the two respective transgenes were grown, treated and tested in parallel to the knockdown flies as controls. At 18°C, Gal4 is inhibited by Gal80^{ts}, and *DHR3/Hr46-RNAi* is not expressed. At this temperature, all genotypes exhibited normal courtship. In contrast, following induction at 32°C, males expressing *DHR3/Hr46-RNAi* in the BBB had significantly reduced courtship ($p \leq 0.001$) (Figs 2A, B). Reduction was observed with both drivers in combination with either of two different *UAS-DHR3/Hr46-RNAi* constructs. While courtship was reduced, the males were capable of performing all of the steps of courtship, but they did so with lower probability. To eliminate general sickness of the males as a cause for the reduced courtship, we performed a short-term activity assay (22) and found no activity defects in the knockdown flies (Fig 2C). We conclude that *DHR3/Hr46* is required in the BBB of mature males for normal courtship behavior. SPG BBB cells are glial cells. To confirm the glial requirement for DHR3/Hr46 we used the glia-specific driver *repo-Gal4* to drive *UAS-DHR3/Hr46-RNAi* in adult males and observed equally reduced courtship ($p<0.001$) (Fig 3A). As expected, when we expressed *DHR3/Hr46-RNAi* in the BBB with *Mdr-Gal4* in the presence of a glial-expressed Gal80 blocker (*repo-Gal80*) (23) we observed a reversal of the courtship defects. Together our findings demonstrate that *DHR3/Hr46* is needed in the glial SPG cells for normal courtship.

To assess whether *DHR3/Hr46* knockdown affects the integrity of the BBB, we tested the tightness of the BBB by injecting 10kD Texas-Red (TR)-marked Dextran.

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It is well documented that in wildtype animals TR-Dextran will be kept out of the brain and accumulate at the BBB, whereas a leaky BBB would allow entry into the brain (6). As shown in Fig 3E, males expressing *DHR3/Hr46* RNAi have normal BBB barrier function with the dye accumulating at the barrier. These findings indicate that BBB integrity is not compromised in the mutants, giving support to a physiological function for *DHR3/Hr46* in the BBB that is required for normal regulation of courtship.

DHR3/Hr46 and its ligand are present in SPG nuclei

To examine the intracellular distribution of DHR3/Hr46, we used antibodies generated by the Thummel lab (11) to study the protein distribution in SPG cells of mature animals (Fig 4). Indy-GFP was used as a BBB marker; it is expressed in both PG and SPG cells (5). DNA was labeled with DAPI. BBB cells are big flat cells with large polyploid nuclei (24). Anti-DHR3/Hr46 antibody staining detected DHR3/Hr46Hr46 in the cytoplasm and the nucleus of SPG cells (Figs 4 A-D). DHR3/Hr46 is a transcriptional activator in larvae, and its presence in the nucleus of BBB cells is consistent with a transcriptional role in these cells.

DHR3/Hr46 belongs to the family of ligand activated nuclear receptors. And while its ligand is unknown, insertion of the putative ligand binding domain into the Gal4 activation domain results in the transcriptional activation of Gal4 in cells containing the putative ligand. Palanker et al. have shown that this construct recapitulates Hr46 activation in cells where Hr46 transcriptional activity has been observed (25).

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Binding of the putative ligand activates *Gal4*$^{LBD(DHR3)}$ whose activity can then be visualized by a *UAS- reporter*. Importantly, in the construct, the *Gal4*$^{LBD(DHR3)}$ reporter is driven by a *hsp70* heat shock promoter *(hsp70- Gal4*$^{LBD(DHR3)}$*)*. This makes it possible to interrogate the presence of the ligand at a time of choice. We induced the *Gal4*$^{LBD(DHR3)}$ ligand sensor in mature males by exposing the flies to 37 degrees Celsius for one hour and fixed their brains four hours later. We combined *hsp70-Gal4*$^{LBD(DHR3}$ with *UAS-dsRed* to visualize Gal4 activity, and *indy-GFP* for visualization of the BBB. As shown in Figs 4 E-G, dsRed staining is observed in the large nuclei of SPG cells, indicating that the ligand for Hr46 is present in these cells in adult mature males. Together our findings support a scenario in which Hr46 is activated and physiologically needed in the cells of adult males to support normal male courtship behavior.

Discussion

Our microarray screen revealed that the *Drosophila* BBB contains male-enriched transcripts in males, as well as female-enriched transcripts in females. We have previously observed a reduction in male courtship when we conditionally feminized the BBB cells of mature males. Together these findings suggest that sex-specifically enriched transcripts contribute to a “male-specific” state of BBB cells that shapes its physiology and its dynamic interaction with the brain to modulate courtship. The previous feminization experiments were done in mature adult males by conditional induction of the female-specific TRA protein (26) . TRA is a master controller of sex

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determination by virtue of its direct control of the two major sex specific transcription factors DSX and FRU, which in turn control a multitude of genes (27, 28). Non-induced males were normal, demonstrating that it was the acute adult change in transcripts that resulted in disturbed courtship. In the microarray experiments described here we sampled all transcripts that were present in the BBB cells of mature males and females. These animals were of the same age as the flies in the TRA induction studies. Neither males nor females had mating experience. The sex-specific transcripts we identify here therefore likely include transcripts that were affected in the feminization experiment.

We identified a total of 284 sex-preferentially expressed transcripts. It is likely that a number of them are required in the regulation of sex-specific behaviors and that their disruption will affect courtship. Identifying them holds the promise of insight into the physiological processes that underlie BBB-brain communication that is required for normal courtship. However, there will likely also be commonly expressed transcripts that participate in these sex-specific processes as they interact with sex-specific partners or regulators, or respond to sex-specific incoming signals. The majority of identified SPG transcripts are equally expressed in males and females, representing an insight into the overall transcriptional "makeup" of SPG cells of mature males and females. We expect many of them to overlap with the transcripts identified by deSalvo et al. (5). In contrast to our study, their characterization included both layers of the BBB, PG and SPG cells, without distinguishing between males and females.

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DHR3/Hr46 belongs to the nuclear-receptor superfamily that is characterized by the presence of a highly conserved DNA-binding domain (DBD) and a less conserved C-terminal ligand-binding and dimerization domain (LBD). The ligand for DHR3/Hr46 is unknown, but the reporter construct made by Palanker et al. strongly indicates that a ligand exists that binds to the LBD in the receptor (25). In larvae, Palanker et al. observed fairly widespread, but not ubiquitous, activation, including in the fat body, leading to the speculation that DHR3 might have metabolic functions. ROR, the mammalian homologue of DHR3, is known to bind cholesterol and play a role in lipid homeostasis. Flies do not produce cholesterol, but take it up from their diet and it is an important precursor for the steroid ecdysone among other roles. Another nuclear receptor, DHR96 has been shown to bind cholesterol in *Drosophila* and to be essential for cholesterol homeostasis (29), but this does not exclude a role for DHR3/Hr46. Palanker et al. observed strong Gal4^{DHR3LDB} reporter expression in tissues of late third instars, with expression dropping in pre-pupariation, but strong activation was observed again in late pupae. We show here activation of the reporter construct in the BBB of mature adult males. In these experiments, the reporter construct is conditionally induced by a heat pulse in mature males. Thus, the observed activation reflects a "snapshot" of the presence of the putative ligand at that time. The observed activity coincides with the time when knockdown of DHR3 causes a reduction in courtship.

DH3/Hr46 is best known for its essential role in development as an ecdysone effector. It is activated by ecdysone and is a part of an activation cascade in response to ecdysone. It induces another nuclear receptor, Ftz-F1, among numerous other

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genes. Eventually, it acts as a negative feedback regulator to turn off ecdysone-receptor signaling (10-12, 30, 31). DHR3/Hr46 has essential functions during embryogenesis, prior to molts, and at the onset of metamorphosis. To our knowledge, this is the first report of an adult non-developmental role for DHR3/Hr46. Our conditional knockdown experiments demonstrate that its presence in the BBB of mature males is needed for normal courtship. Whether this reflects a role for an ecdysone-induced signaling cascade and transcriptional activation of downstream targets remains to be determined. Data from (32) suggest that ecdysone and the ecdysone receptor (EcR) are present in the BBB. We have likewise found in our screen that *EcR* and *ftz-F1* RNAs are present in SPG cells in a non-sex-specific manner. In analogy to its developmental role, DHR3/Hr46 most likely acts as a transcriptional regulator. Our observation that the Hr46 protein is present in SPG nuclei supports this interpretation. However, in an unexpected finding Montagne et al have identified DHR3 as a S6K interacting protein in late larvae/prepupae (33). Intriguingly, this function required a novel form of DHR3 that did not contain the DNA binding domain, but did require the ligand binding domain. The presence of this altered form of DHR3 increased phosphorylation activity of S6K. This finding, together with the short time scale of the response led the authors to propose an alternative non-genomic role of DHR3, possibly as a mediator of the metabolic state of these cells. We do not know whether this isoform plays a role in courtship and whether DHR3 might have a role that is independent of EcR in the BBB, conceivably in addition to the transcriptional role that is suggested by its presence in the nucleus.

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Taken together, the data presented here demonstrate an adult physiological role for DHR3/Hr46, a nuclear receptor mainly known for its crucial function in development , in the glial cells of the BBB where it is required for the regulation of normal male courtship.

Materials and Methods

Fly stocks

SPG-Gal4/TM3 (6) was a gift from Roland Bainton, UCSF. *tubP-Gal80*^{ts}*/CyO* and *tubP-Gal80t*^{s} */TM3,Sb* flies were a gift from Gregg Roman (University of Mississippi). *DHR3/Hr46* RNAi lines *y*^{1} *v*^{1}*;P{TRiP.JF02542}attP2* (BL 27253) and *y*^{1} *v*^{1}*; P{TRiP.JF02543}attP2* (BL 27254); *w*^{1118}*; P{w[+mC]=UAS-RedStinger (dsRed)}4/CyO* (BL 8546); *w*; P{PTT-GC}IndyYC0017/TM6C, Sb1* (*Indy-GFP*) (BL 50860) were obtained from the Bloomington *Drosophila* stock center (https://bdsc.indiana.edu/). *y, repo-Gal4* on X was a gift from Takeshi Awasaki (University of Massachusetts (23); The *y* mutation was removed by recombination. *w; Pin, repo-Gal80/CyO* flies were a gift from Rob Jackson (Tufts University). Pin was removed by recombination. *w; +; repo-Gal80* flies (23) were a gift from Christian Klämbt (University of Münster). *w*^{1118}*; P{w[+mC]=hs-GAL4-DHR3.LBD* was a gift from Carl Thummel (University of Utah).

Gal80^{ts} *experiments*

tubP-Gal80^{ts} carrying flies and control flies were raised at 18°C. Virgin males were collected at eclosion and kept in individual vials for 5–8 days at 18°C. Flies were

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then placed at 32°C for 24 hours for induction. Following induction, induced and uninduced flies were kept at 25°C overnight prior to courtship assays.

Behavioral assays

The courtship assay and activity assay were performed as previously described (34). In short, males were placed in a plexiglass "mating wheel" (diameter 0.8 cm), together with a 2–4 hrs old *Canton-S* virgin female. The courtship index was calculated as the fraction of time the male spent displaying any element of courtship behavior (orienting, following, wing extension, licking, attempted copulation, copulation) within a 10-minute observation period (18). Short-term activity assays were performed as previously described (22). Individual males were placed into the ''mating wheel'' containing a filter paper with a single line dividing the chamber in half. After 2–3 minutes of acclimation time, the number of times the male crossed the center line within the three-minute observation time was counted. Each graph represents sets of control and experimental genotypes that were grown, collected, aged and tested in parallel. In each behavioral session, equal numbers of all genotypes were tested.

Microarray analysis

To isolate blood-brain barrier cells, flies bearing the *SPG-Gal4* driver were crossed to flies carrying the fluorescent reporter transgene, *UAS-DsRed*. This resulted in the expression of the visible fluorescent marker DsRed to mark the nuclei of BBB cells. Prior to the experiment, both the driver *SPG-Gal4* and the *UAS-DsRed* lines were

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outcrossed with a Cantonized *w*^{1118} strain for 10 generation. The flies were grown in a 25°C incubator under a 12hrs light/12hrs dark cycle. Eclosing males and females were collected and kept in separate groups of 10-15 flies of the same sex under the same conditions for 4 days and then dissected between ZT 5 and ZT 7 to control for levels of cycling transcripts. Equal numbers of males and females originating from the same culture were dissected in each sitting. The brains were dissected in ice-cold 1 X PBS. The dissected brains were then transferred to a new petri dish containing ice-cold 1X PBS within half an hour. Carefully, under the fluorescent microscope, individual and/or groups of blood-brain barrier cells marked with DsRed were isolated manually by using Dumont # 5 SF superfine forceps (Fine Science Tools, Inc). The cells were then immediately transferred to a frozen droplet of Trizol reagent on dry ice and stored in -80°C until further processing. Cells were isolated from at least 50 brains for each genotype. The approximate total number of cells isolated per brain varied from ~60-120. The forceps were cleaned with RNAZap when moving from one genotype to the other.

The isolated BBB cells of male and female flies were provided to GenUs Biosystems (http://www.genusbiosystems.com/) for microarray analysis. A total of 3 biological replicates for males and females were submitted. Cells were lysed in TRI reagent (Ambion) and Total RNA was isolated using phenol/chloroform extraction followed by purification over RNeasy spin columns (Qiagen). The concentration and purity of Total RNA was measured by spectrophotometry at OD260/280 and the quality of the Total RNA sample was assessed using an Agilent Bioanalyzer with the RNA6000 Nano Lab Chip (Agilent Technologies). Labeled cRNA was prepared by linear

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amplification of the Poly (A)+ RNA population within the Total RNA sample. Briefly, 1 µg of Total RNA was reverse transcribed after priming with a DNA oligonucleotide containing the T7 RNA polymerase promoter 5' to a dT(24) sequence. After second-strand cDNA synthesis and purification of double-stranded cDNA, in vitro transcription was performed using T7 RNA polymerase. The quantity and quality of the cRNA was assayed by spectrophotometry and on the Agilent Bioanalyzer. One microgram of purified cRNA was fragmented to uniform size and applied to *Drosophila* (V2) Gene Expression microarray (Agilent Technologies, Design ID 021791) in hybridization buffer. Arrays were hybridized at 37° C for 18 hrs in a rotating incubator, washed, and scanned with a G2565 Microarray Scanner (Agilent Technologies). Arrays were processed with Agilent Feature Extraction software and data was analyzed with GeneSpring GX software (both Agilent Technologies). To compare individual expression values across arrays, raw intensity data from each gene was normalized to the 75th percentile intensity of each array. Genes with values greater than background intensity in all female or all male replicates were filtered for further analysis. Differentially expressed genes were identified with fold change > 2-fold and Welch Ttest, p-value < 0.05.

The data discussed in this publication have been deposited in NCBI's Gene Expression Omnibus and are accessible through GEO Series accession number GSE 157122 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE157122).

<u>Generation of Mdr65-Gal4 transgenic flies</u>

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650 bp of sequence upstream of the *Mdr65* coding sequence was PCR amplified from *CS* genomic DNA using the primers *5'cggaattc*(EcoRI)*TCCATCACTTAGCAAAGCAGACTTCAATC* and 5'cgggatcc(BamH1) *GGTGATGTTTAGTCGGCACTGACGA* and inserted into the *Drosophila* transformation vector *pGATN* to create *Mdr65-Gal4*. In *pGATN*, expression of the yeast transcription factor Gal4 is driven by the inserted promoter sequences (13). Transgenic flies were generated by *Rainbow Transgenic Flies* (https://www.rainbowgene.com/) by P-element mediated insertion. The expression pattern in *Mdr65-Gal4* transgenic lines was examined by crosses to *UAS-dsRed* (nuclear) or *UAS-mcD8-dsRed*.

Immunohistochemistry

Immunohistochemistry on isolated brains was performed as described in Li et al. (35). The DHR3/Hr46 antibody was a gift from Carl Thummel, University of Utah (11) and was used at 1:50 dilution. To visualize BBB cells, flies carrying *Indy-GFP* were used for anti-DHR3/ anti-GFP double staining. *Indy-GFP* marks BBB cells (36).

Antibodies used:

Rabbit anti-DHR3, 1:50 (gift of Carl Thummel, University of Utah (11)); Rabbit anti-RFP (abcam ab62341), 1:200; chicken anti-GFP (abcam ab13970), 1:500; Alexa Fluor 555 goat anti-rabbit (Invitrogen A21429) 1:200; Alexa Fluor 635 goat anti-mouse (Invitrogen A31575) 1:200; Alexa Fluor 488 goat anti-chicken (Thermo Fisher Scientific A-11039).

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Injection of 10kd Dextran-TR to assess the integrity of the BBB was performed as described in Hoxha et al. (8).

Test for presence of DHR3/Hr46 ligand

hs-Gal4$^{LBD(DHR3)}$ flies were crossed to *UAS-dsRed(nuclear); indy-GFP* flies. Progeny were collected at eclosion and kept in small groups of males or females for 4 days. Expression of *hs-Gal4*$^{LBD(DHR3}$ was induced by placing flies in prewarmed food vials at 37°C for one hour, followed by recovery at room temperature for three hours and brain isolation. dsRed expression as a measure of Gal4 activation by DHR3 ligand and GFP (as BBB marker) were assessed by immunohistochemistry.

Statistical Analysis

Two-way analysis of variance (ANOVA) was used to establish overall significance. Post hoc analysis for multiple comparisons was carried out with Tukey (HSD). P values < 0.05 were considered statistically significant. All statistical calculations were done using XLSTAT (Addinsoft, NY, NY) running on Microsoft Excel for Mac (version 16). All ±error bars are standard error of the mean (SEM).

Figure legends

Figure 1. Microarray analysis of isolated SPG cells of the BBB. (A) Schematic of the *Drosophila* Blood Brain Barrier (BBB) (modified from (37). The BBB consists of two layers of glial cells, the outer Perineurial Glia (PG) facing the circulating hemolymph, and the inner Subperineurial Glia (SPG) with septate junctions that

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form the main barrier. The SPG is in contact with the underlying nuclei of the neuronal cortex. (B) Isolated fly brain with SPG cells labeled by nuclear DsRed expression driven by *SPG-Gal4*. Fluorescently marked cells like these from males and females were hand-isolated for RNA extraction. (C) Probes present (above background) in all male or female samples are displayed as normalized to the 75th percentile intensity of each array (19,218 probes). Each spot is the mean of 3 samples from each condition. Black spots =differentially expressed genes (>2Fold, T-test p-value < 0.05, 284 probes). Red/orange=High expression, Yellow=Medium expression, Blue=Low expression. (D) Differentially expressed genes (>2 fold,T-test p-value < 0.05) in Male vs. Female are displayed as normalized to the median value of each probe across six samples (284 probes). The heat map color scale is shown on the right. (E) DHR3/Hr46) is preferentially expressed in males.

Figure 2. Knockdown of DHR3/Hr46 in the BBB of mature males reduces courtship. Graphs show the courtship index CI (fraction of time males spend courting during the observation period) ± SEM (A, B), or the performance of males in a control activity assay (# of line crossings ± SEM) (C) of the indicated genotypes. N= 20. Data were analyzed by ANOVA followed by Tukey multiple comparisons (p<0.05). Indices that are significantly different from the controls are marked by asterisks. *UAS-Hr46i* expression is restricted by the presence of *tubP-Gal80*^{ts} at 18^{o}C (induction -). Placement of 5-day-old males at 32^{o}C for 16-24 hours (induction +) releases the Gal80 inhibition and leads to the expression of RNAi. (A) Expression of two different *UAS-Hr46-RNAi* (1-line 27253 and 2-line 27254) using *SPG-Gal4*

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significantly reduces male courtship. B) Conditional expression of *UAS-Hr46i* (27254) using *Mdr-Gal4* in adult males similarly reduces courtship in comparison to controls. The controls are 1) *+/Gal80*^{ts}*;+/SPG-Gal4* and *+/Mdr-Gal4; +/ Gal80*^{ts}, respectively and *2*) *+; +/UAS-Hr46 RNAi*. (C) The activity of the mutants as measured by number of line crossings is not lower than in control flies. *+/Hr46*$^{(1)}$ control flies have reduced activity after induction that does not correlate with their courtship index. (D) *Mdr-Gal4* expression in SPG cells visualized by expression of *UAS-dsRed* in dissected brain (red). For comparison, *indy-GFP* (green) is expressed in both PG and SPG cells. (E) Blood–brain barrier integrity is not compromised in *Mdr-Gal4/ UAS-Hr46-RNAi* males. Flies were injected with 10 kDa TR-Dextran (red) and dye penetration into or exclusion from the brain was examined by confocal microscopy. The brain nuclei are stained with DAPI. A tight BBB is indicated by the demarcated red line on the surface of the brain indicating exclusion of TR-dextran from the brain of *Mdr-Gal4/ UAS-Hr46-RNAi* males.

Figure 3. Hr46/DHR3 is required in the glial SPG cells for courtship. Graphs show the courtship index CI (fraction of time males spend courting during the observation period) ± SEM. N= 20. Data were analyzed by ANOVA followed by Tukey multiple comparisons ($p<0.05$). Indices that are significantly different from the controls are marked by asterisks. (A) Conditional glial knockdown of *DHR3/Hr46* in mature males using *repo-Gal4; Gal80*^{ts} reduces courtship. (B) The courtship reduction of *Mdr-Gal4* directed *DHR3/Hr46* knockdown can be reversed by Gal80 expression in glial cells directed by *repo-Gal80.*

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Figure 4. DHR3/Hr46 protein is located in SPG nuclei, and a reporter construct indicates that the DHR3/Hr46 ligand is present in the SPG cells of mature males.

(A-D) Anti-DHR3 antibody staining (Red) shows the presence of DHR3 in the nuclei and cytoplasm of SPG cells. Indy-GFP (green) marks BBB cells. Blue: DNA staining (DAPI). (E-G) Activation of the *hs-Gal4*$^{\mathit{LBD}\ \mathrm{DHR3}}$ reporter (25) indicates the presence of the DHR3/Hr46 ligand in SPG cells. *hs-Gal4*$^{\mathit{LBD\ DHR3}}$*/UAS-dsRed/indy-GFP* mature males were heat-shocked to express *Gal4*$^{\mathit{LBD\ DHR3}}$. Following binding of DHR3 ligand, Gal4 initiates transcription at *UAS-dsRed(nuclear).* dsRed can be seen expressed in the characteristic large nuclei of SPG cells (red). *Indy-GFP* expression is used as a BBB marker (green). Blue: DNA staining (DAPI, blue).

S1 Table. Differentially expressed genes in BBB from males vs. females (>2Fold, p<0.05). 284 transcripts were identified that differ by >2Fold (p<0.05). Of those 112 were male-preferentially expressed, 172 were female-preferentially expressed.

S2 Table. Gene Ontology classifications of the 284 genes differentially expressed in BBB Males vs. Females.

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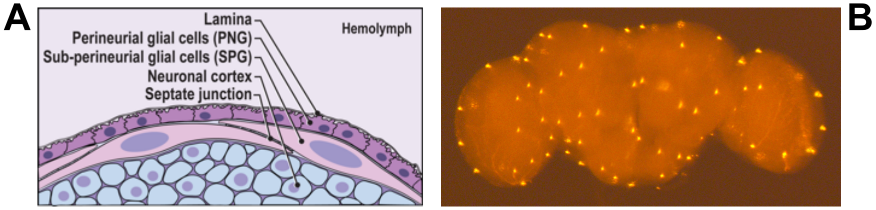
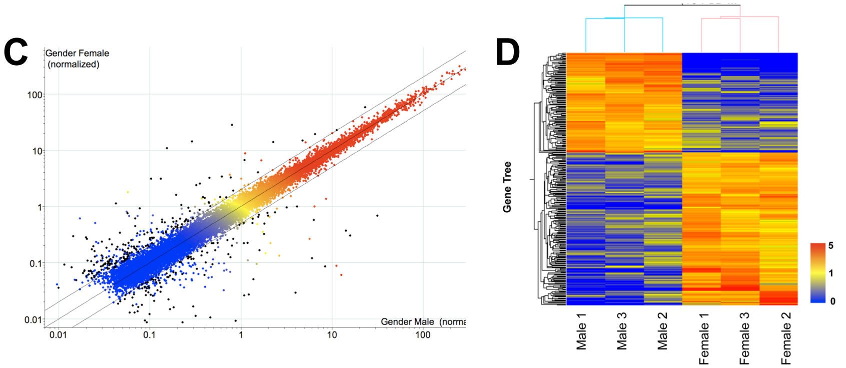
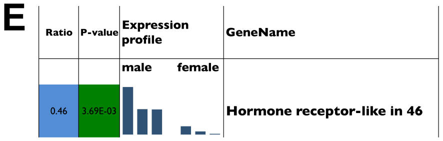
Fig 1



M. de la Flor, modified from Daneman and Barres (2005)





Ratio	P-value	Expression profile	GeneName
		male female	
0.46	3.69E-03		Hormone receptor-like in 46

Fig 2

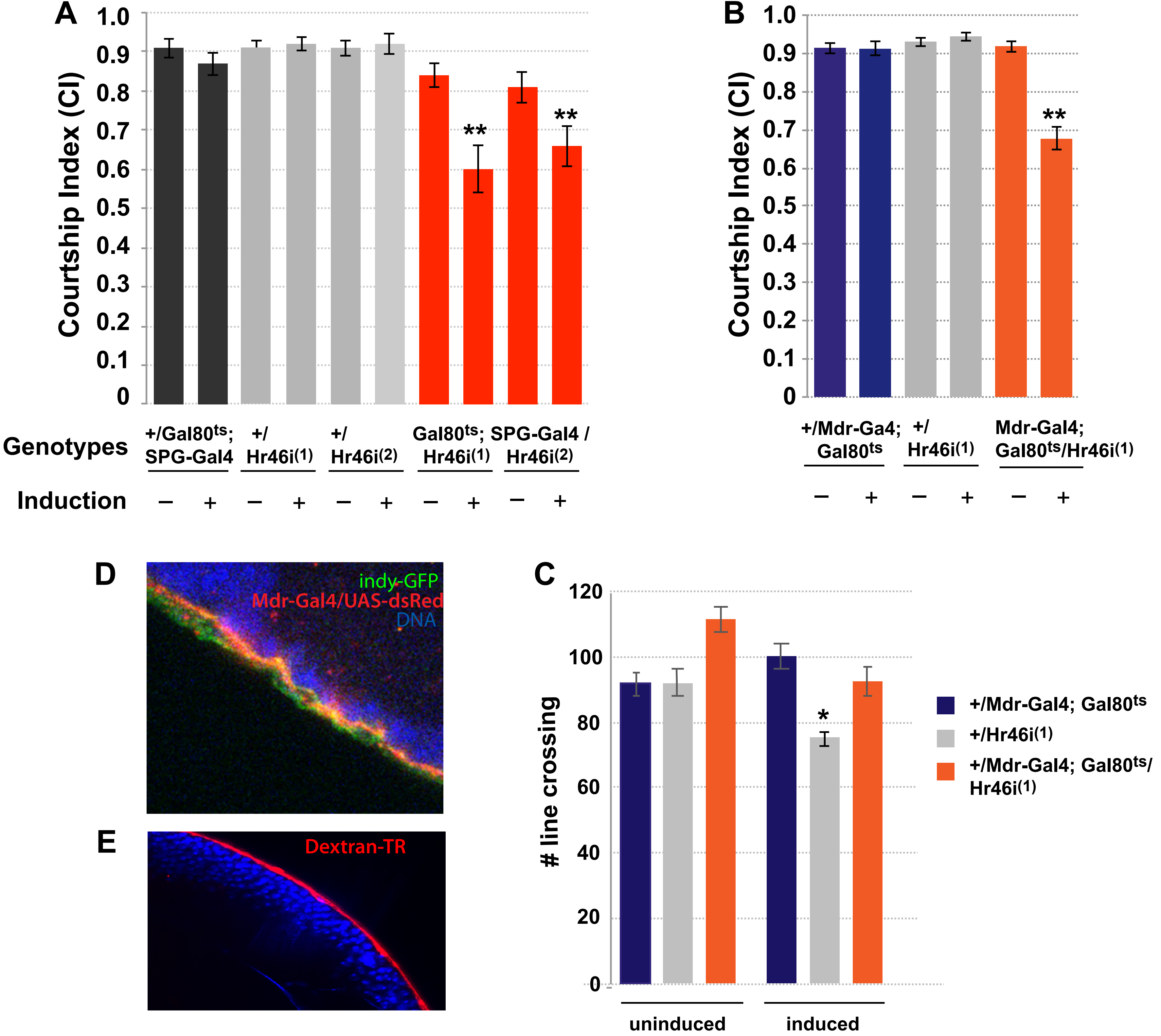

A
Courtship Index (CI)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
**
**
Genotypes
+/Gal80ts; SPG-Gal4
+/ Hr46i(1)
+/ Hr46i(2)
Gal80ts; SPG-Gal4 / Hr46i(1)
Hr46i(2)
Induction
– + – + – + – + – +
B
Courtship Index (CI)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
**
+/Mdr-Ga4; Gal80ts
+/ Hr46i(1)
Mdr-Gal4; Gal80ts/Hr46i(1)
– + – + – +
D
indy-GFP
Mdr-Gal4/UAS-dsRed
DNA
E
Dextran-TR
C
line crossing
120
100
80
60
40
20
0
*
uninduced
induced
+/Mdr-Gal4; Gal80ts
+/Hr46i(1)
+/Mdr-Gal4; Gal80ts/ Hr46i(1)

Fig 3

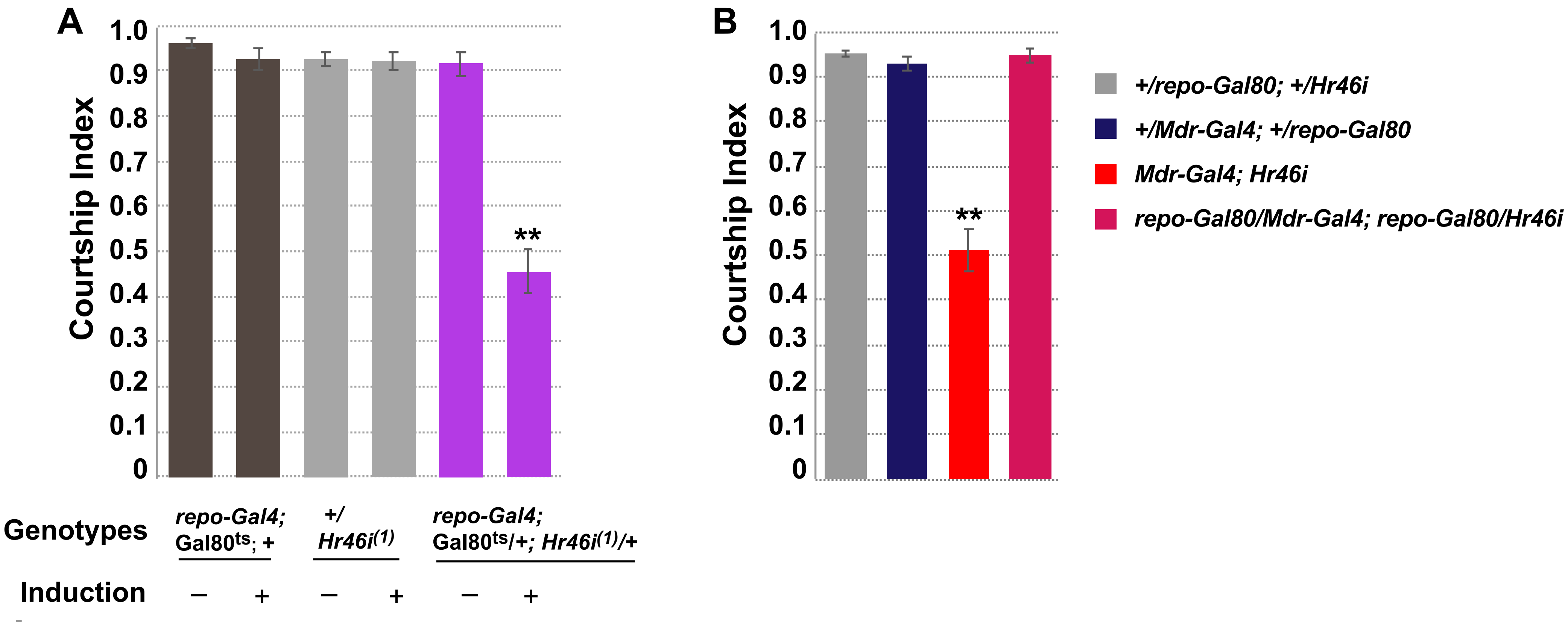
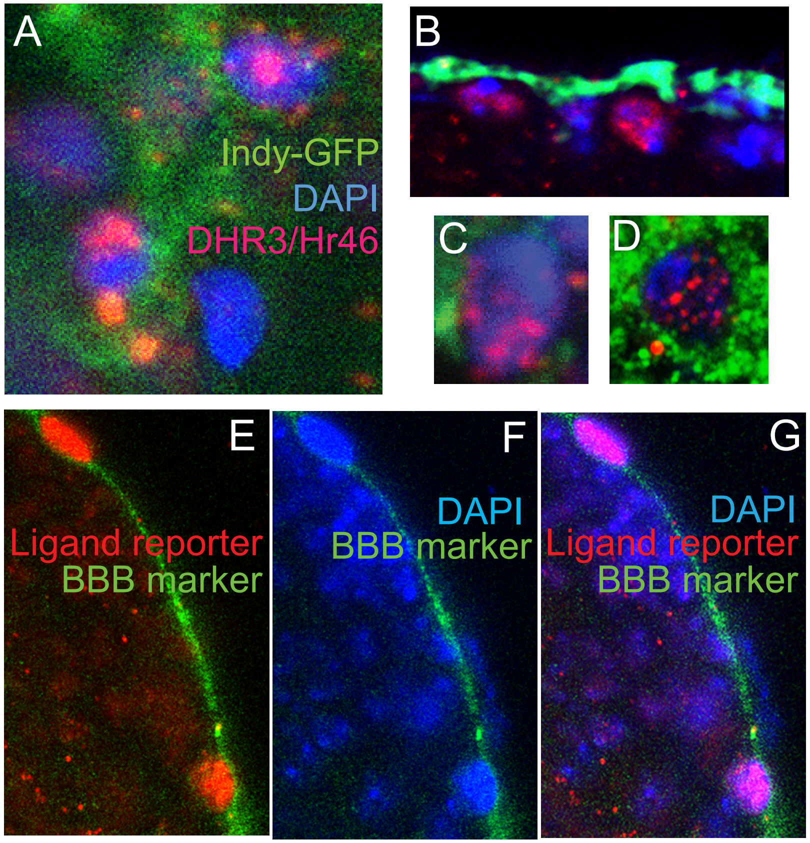

A
Courtship Index
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
**
Genotypes
repo-Gal4; Gal80ts; +
+/ Hr46i(1)
repo-Gal4; Gal80ts/+; Hr46i(1)/+
Induction
−
+
−
+
−
+
B
Courtship Index
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
**
+/repo-Gal80; +/Hr46i
+/Mdr-Gal4; +/repo-Gal80
Mdr-Gal4; Hr46i
repo-Gal80/Mdr-Gal4; repo-Gal80/Hr46i

Fig. 4



A
Indy-GFP
DAPI
DHR3/Hr46
B
C
D
E
Ligand reporter
BBB marker
F
DAPI
BBB marker
G
DAPI
Ligand reporter
BBB marker

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Differentially expressed genes in BBB from males vs. females (>2Fold, p<0.05, 284 probes)

Intensity values are normalized to the 75th percentile intensity of each array.
p-values are based on a Welch T-test.

Systematic	GeneSymbol	PrimaryAccession	UniGeneID	GO	1 Male ormaliz	2 Male ormaliz	3 Male ormalize	4 Female Normaliz	5 Female ormaliz	6 Female ormaliz	Ratio	P-value
A_09_P010076	Yp1	FBtr0071419	FBgn0004045	GO:0003824(	0.06	0.19	0.22	10.56	3.03	18.99	64.58	4.03E-03
A_09_P010081	Yp3	FBtr0073821	FBgn0004047	GO:0003824(	0.09	0.20	0.57	12.21	4.12	26.93	50.84	6.60E-03
A_09_P011441	Yp2	FBtr0071424	FBgn0005391	GO:0003824(	0.42	0.56	1.38	24.59	8.79	51.77	32.48	6.53E-03
A_09_P033016	Cht2	FBtr0072795	FBgn0022702	GO:0004568(	0.08	0.12	0.11	4.38	2.06	2.90	29.31	2.27E-04
A_09_P040561	CG14222	FBtr0074763	FBgn0031043	GO:0008080(	0.01	0.02	0.02	0.26	0.33	0.30	23.02	1.57E-03
A_09_P041791	Cp36	FBtr0071203	FBgn0000359	GO:0005213(	0.05	0.06	0.02	0.20	2.71	0.47	16.77	4.65E-02
A_09_P046876		IP02754	Dm.4788		0.07	0.26	0.06	0.78	7.80	0.74	15.62	4.91E-02
A_09_P047621	Cp7Fb	FBtr0071201	FBgn0014465	GO:0016491(	0.03	0.01	0.02	0.22	0.29	0.28	14.77	4.53E-03
A_09_P017246	Ste:CG33238	FBtr0300087	FBgn0053238	GO:0005634(	0.01	0.03	0.01	0.43	0.16	0.25	14.44	1.21E-03
A_09_P030341	Cp7Fb	FBtr0071201	FBgn0014465	GO:0016491(	0.06	0.06	0.04	0.79	0.73	0.74	13.56	4.65E-07
A_09_P017282	Ste:CG33246	FBtr0300095	FBgn0053246	GO:0005634(	0.06	0.09	0.08	1.29	0.59	0.89	11.61	1.15E-03
A_09_P029356	dhd	FBtr0070749	FBgn0011761	GO:0005634(	0.05	0.36	0.17	1.02	2.62	1.20	10.13	3.45E-02
A_09_P166400		TC241037			0.08	0.11	0.01	0.30	1.18	0.35	9.95	3.19E-02
A_09_P041771	Cp15	FBtr0076572	FBgn0000355	GO:0005213(	0.01	0.08	0.02	0.26	0.44	0.08	9.61	4.68E-02
A_09_P061401	Jon99Fi	FBtr0085652	FBgn0039778	GO:0004252(	0.00	0.00	0.02	0.11	0.03	0.14	9.37	3.58E-02
A_09_P017256	Ste:CG33240	FBtr0300089	FBgn0053240	GO:0005956(	0.02	0.03	0.01	0.27	0.13	0.18	8.52	1.74E-03
A_09_P042086	dsx	FBtr0081760	FBgn0000504	GO:0000122(	0.03	0.03	0.03	0.25	0.19	0.27	7.91	2.26E-05
A_09_P031461	pgc	FBtr0112520	FBgn0016053	GO:0007277(	0.01	0.03	0.03	0.11	0.30	0.11	7.73	6.25E-03
A_09_P119000	CycB	FBtr0071911	FBgn0000405	GO:0000086(	0.02	0.07	0.01	0.18	0.25	0.12	7.63	3.00E-02
A_09_P198615	Hsp70Aa	FBtr0082512	FBgn0013275	GO:0001666(	0.41	0.68	0.25	2.00	2.39	5.36	7.15	7.58E-03
A_09_P017276		TC239464		GO:0005634(	0.26	0.40	0.30	3.68	1.41	2.13	7.05	6.84E-03
A_09_P029571	Hsp70Aa	FBtr0082512	FBgn0013275	GO:0001666(	0.92	1.81	0.54	5.62	5.47	9.03	6.77	1.59E-02
A_09_P063611		LP04080	Dm.11995		0.01	0.04	0.07	0.17	0.19	0.27	6.73	3.90E-02
A_09_P041786	Cp19	FBtr0076573	FBgn0000358	GO:0005213(	0.05	0.05	0.04	0.16	0.53	0.32	6.49	1.73E-02
A_09_P029581	Hsp70Bb	FBtr0082637	FBgn0013278	GO:0001666(	0.34	0.44	0.28	1.52	1.37	4.72	6.21	3.06E-02
A_09_P041876	CycB	FBtr0071911	FBgn0000405	GO:0000086(	0.04	0.09	0.07	0.18	0.61	0.57	5.95	2.66E-02
A_09_P218800		LD23983	Dm.2955		0.02	0.04	0.02	0.08	0.29	0.10	5.94	1.90E-02
A_09_P059675	Hsp70Bbb	FBtr0082636	FBgn0051354	GO:0001666(	2.34	2.95	1.43	9.55	9.25	23.23	5.92	8.96E-03
A_09_P108115	Arpc3B	FBtr0113478	FBgn0065032	GO:0003779(	0.01	0.02	0.01	0.08	0.05	0.12	5.82	9.69E-03
A_09_P041776	Cp16	FBtr0076574	FBgn0000356	GO:0005213(	0.02	0.01	0.04	0.09	0.15	0.14	5.68	3.67E-03
A_09_P113130	CG14075	FBtr0075024	FBgn0036835		0.63	0.72	0.50	3.98	3.05	3.13	5.53	2.48E-06
A_09_P176625		CO186850	Dm.33307		0.03	0.02	0.02	0.13	0.09	0.10	5.33	1.73E-03
A_09_P171040					0.01	0.04	0.02	0.14	0.07	0.10	5.05	6.82E-03
A_09_P119565	dsx	FBtr0081760	FBgn0000504	GO:0000122(	0.03	0.07	0.02	0.22	0.18	0.17	4.88	1.63E-02
A_09_P148580	CHES-1-like	FBtr0071079	FBgn0029504	GO:0003700(	0.25	0.22	0.19	1.20	1.04	0.93	4.81	3.63E-08
A_09_P055091	Muc68D	FBtr0076119	FBgn0036203	GO:0005576(	0.01	0.05	0.01	0.06	0.07	0.14	4.81	4.37E-02
A_09_P149325		TC256165		GO:0002119(	0.08	0.06	0.02	0.20	0.23	0.19	4.71	4.97E-02
A_09_P067431	CG4020	FBtr0070824	FBgn0029821	GO:0005488(	0.11	0.21	0.08	0.48	0.63	0.64	4.64	1.62E-02
A_09_P030336	Cp7Fa	FBtr0100003	FBgn0014464	GO:0003674(	0.07	0.07	0.08	0.36	0.34	0.29	4.57	3.33E-08
A_09_P021206	CG17633	NM_135466	Dm.7694	GO:0004181(	0.02	0.05	0.01	0.16	0.05	0.19	4.51	3.97E-02
A_09_P134245	ovo	FBtr0070738	FBgn0003028	GO:0000122(	0.08	0.16	0.04	0.33	0.29	0.41	4.28	4.71E-02
A_09_P112275	CG4020	FBtr0070824	FBgn0029821	GO:0005488(	0.09	0.22	0.09	0.47	0.56	0.55	4.26	2.43E-02
A_09_P043951	osk	FBtr0081954	FBgn0003015	GO:0007277(	0.03	0.06	0.04	0.14	0.24	0.13	4.20	1.63E-03
A_09_P034456	CG14075	FBtr0075024	FBgn0036835		0.72	0.87	0.71	4.15	2.41	3.26	4.19	1.71E-03
A_09_P212665		BI631263	Dm.6890		0.03	0.06	0.02	0.15	0.10	0.12	4.18	9.97E-03
A_09_P042706	hfw	FBtr0070270	FBgn0001189	GO:0005575(	0.06	0.06	0.06	0.26	0.28	0.21	4.07	5.03E-07
A_09_P040936	r-cup	FBtr0077269	FBgn0031142	GO:0005488(	0.02	0.01	0.03	0.07	0.08	0.06	4.05	7.35E-04
A_09_P054086	CG5804	FBtr0076591	FBgn0035926	GO:0000062(	0.03	0.09	0.05	0.21	0.14	0.33	4.05	2.13E-02
A_09_P078091	CG6133	FBtr0070669	FBgn0026079	GO:0003723(	0.04	0.10	0.12	0.36	0.28	0.32	3.97	2.03E-02
A_09_P010936	yl	FBtr0073897	FBgn0004649	GO:0005509(	0.03	0.07	0.05	0.14	0.35	0.15	3.95	1.68E-02

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Systematic	GeneSymbol	PrimaryAccession	UniGeneID	GO	ormaliz	ormaliz	ormalize	Normaliz	ormaliz	ormaliz	Ratio	P-value
A_09_P190865		TC242599			0.02	0.03	0.02	0.11	0.09	0.10	3.94	8.12E-03
A_09_P030346	Cp7Fc	FBtr0071202	FBgn0014466		0.05	0.05	0.03	0.18	0.22	0.11	3.91	3.03E-03
A_09_P078056	Mipp2	FBtr0070884	FBgn0026060	GO:0003993(	0.12	0.12	0.30	0.53	0.55	0.79	3.82	3.14E-02
A_09_P075131	CG3509	FBtr0082969	FBgn0038252	GO:0000786(	0.03	0.04	0.02	0.07	0.21	0.08	3.79	2.89E-02
A_09_P077971	CG2652	FBtr0070505	FBgn0025838	GO:0005634(	0.23	0.60	0.37	1.47	1.11	1.65	3.77	2.47E-02
A_09_P002691	CG17672	FBtr0110982	FBgn0083978	GO:0003735(	0.36	0.41	0.37	1.23	1.15	2.05	3.75	1.59E-04
A_09_P168703		TC237125			0.25	0.26	0.28	0.93	0.92	0.99	3.61	4.61E-06
A_09_P043966	otu	FBtr0071238	FBgn0003023	GO:0003676(	0.03	0.02	0.03	0.09	0.16	0.07	3.61	1.02E-02
A_09_P178935		TC249774			0.01	0.02	0.02	0.09	0.04	0.06	3.58	7.48E-03
A_09_P169159		TC242617			0.01	0.02	0.02	0.06	0.04	0.05	3.52	8.39E-03
A_09_P040376	Mec2	FBtr0074669	FBgn0030993	GO:0016020(	0.01	0.05	0.02	0.10	0.07	0.08	3.49	4.36E-02
A_09_P180690		TC237960			0.14	0.17	0.13	0.71	0.34	0.51	3.46	1.16E-02
A_09_P019306	Ir25a	FBtr0289979	FBgn0031634	GO:0004970(	0.01	0.01	0.01	0.03	0.02	0.06	3.43	2.73E-02
A_09_P070536	CG6733	FBtr0084352	FBgn0039052	GO:0004046(	0.01	0.04	0.01	0.06	0.05	0.06	3.40	2.82E-02
A_09_P204080		TC248381			0.01	0.05	0.03	0.12	0.08	0.09	3.40	4.72E-02
A_09_P065356	CG7916	FBtr0080549	FBgn0028534		0.02	0.02	0.02	0.09	0.03	0.09	3.39	2.66E-02
A_09_P123280		GH08205	Dm.5529		0.03	0.06	0.01	0.07	0.12	0.09	3.39	3.03E-02
A_09_P078881	XRCC1	FBtr0070763	FBgn0026751	GO:0000012(	0.13	0.13	0.10	0.43	0.41	0.35	3.37	5.93E-06
A_09_P203785		TC253114			0.01	0.01	0.04	0.10	0.04	0.07	3.35	4.11E-02
A_09_P130245	CG32755	FBtr0070862	FBgn0052755	GO:0004252(	0.03	0.02	0.01	0.06	0.07	0.08	3.34	2.33E-02
A_09_P174050		CO180140	Dm.32308		0.02	0.03	0.02	0.08	0.04	0.09	3.32	2.71E-02
A_09_P009321	CG18190	FBtr0273238	FBgn0034403	GO:0005875(	0.03	0.07	0.06	0.10	0.28	0.12	3.25	3.55E-02
A_09_P056706	Ilp3	FBtr0076373	FBgn0044050	GO:0005158(	3.49	4.47	6.18	16.94	12.99	14.85	3.24	3.80E-03
A_09_P014066	CG31904	FBtr0079501	FBgn0260479	GO:0005326(	0.01	0.03	0.02	0.07	0.04	0.08	3.23	3.88E-02
A_09_P184695					0.02	0.05	0.04	0.13	0.08	0.10	3.22	8.26E-03
A_09_P181000		TC238156			0.11	0.11	0.09	0.45	0.30	0.28	3.21	2.10E-03
A_09_P213545		SD02447	Dm.13899		0.06	0.08	0.03	0.25	0.13	0.15	3.16	1.03E-02
A_09_P039541	CG13008	FBtr0074323	FBgn0030780		0.08	0.09	0.06	0.22	0.25	0.23	3.13	7.75E-05
A_09_P038616	CG11674	FBtr0073926	FBgn0030551	GO:0000398(	0.04	0.04	0.02	0.08	0.18	0.09	3.12	1.43E-02
A_09_P051371	5-ربيع الأول	FBtr0070244	FBgn0028550	GO:0003700(	0.04	0.02	0.03	0.08	0.10	0.10	3.12	2.70E-03
A_09_P221105					0.03	0.04	0.03	0.12	0.08	0.13	3.10	1.84E-03
A_09_P113000	Ilp3	FBtr0076373	FBgn0044050	GO:0005158(	4.33	5.69	9.34	21.05	14.60	22.26	3.10	1.40E-02
A_09_P222415	tlk	FBtr0299580	FBgn0086899	GO:0004672(	0.31	0.24	0.27	0.91	0.71	0.87	3.04	1.77E-06
A_09_P188255	CG12826	FBtr0088943	FBgn0033207		0.04	0.04	0.05	0.14	0.16	0.07	3.04	1.97E-02
A_09_P149005		TC236551		GO:0004213(	0.06	0.08	0.06	0.21	0.17	0.19	2.95	2.49E-04
A_09_P191550	Lsd-2	FBtr0110969	FBgn0030608	GO:0005515(	0.26	0.14	0.18	0.54	0.56	0.55	2.93	7.80E-03
A_09_P207745	up	FBtr0073853	FBgn0004169	GO:0005509(	0.06	0.06	0.07	0.28	0.12	0.18	2.92	2.56E-02
A_09_P167837		TC241479			0.03	0.04	0.04	0.10	0.08	0.15	2.91	9.37E-03
A_09_P170820	ImpE1	FBtr0076606	FBgn0001253	GO:0003674(	0.07	0.05	0.03	0.12	0.26	0.10	2.91	4.67E-02
A_09_P110435	pgc	FBtr0112520	FBgn0016053	GO:0007277(	0.04	0.04	0.02	0.11	0.13	0.07	2.90	7.13E-03
A_09_P186555	CG15445	FBtr0077253	FBgn0031161		0.20	0.20	0.23	0.57	0.62	0.62	2.83	1.75E-05
A_09_P064396	yellow-g	FBtr0072902	FBgn0041709	GO:0003674(	0.03	0.07	0.06	0.12	0.19	0.14	2.81	1.22E-02
A_09_P209795	CG15308	CO264762	Dm.25253		0.03	0.04	0.03	0.06	0.07	0.13	2.80	1.94E-02
A_09_P061806	CG2003	FBtr0085871	FBgn0039886	GO:0005316(	0.04	0.03	0.03	0.11	0.07	0.10	2.79	3.22E-03
A_09_P169610		TC243008			0.03	0.02	0.03	0.06	0.05	0.08	2.79	2.15E-03
A_09_P010301	alphaTub67C	FBtr0076393	FBgn0087040	GO:0000280(	0.15	0.19	0.20	0.34	0.82	0.43	2.77	3.91E-02
A_09_P197060		AW940284	Dm.7880		0.05	0.10	0.05	0.21	0.12	0.21	2.76	1.81E-02
A_09_P051331		LD48059			0.99	1.75	1.84	4.51	2.99	4.72	2.71	1.17E-02
A_09_P034446	CG3819	FBtr0075048	FBgn0036833	GO:0003676(	0.03	0.04	0.02	0.08	0.07	0.08	2.69	7.86E-03
A_09_P077296	Ant2	FBtr0073425	FBgn0025111	GO:0005471(	0.41	0.43	0.48	1.05	1.04	1.49	2.69	6.14E-04
A_09_P048456		NP12807819			0.04	0.08	0.04	0.20	0.09	0.13	2.69	2.84E-02
A_09_P224810		TC257397			0.03	0.04	0.04	0.12	0.08	0.09	2.64	5.80E-04
A_09_P226515		LD10495	Dm.1545		0.06	0.07	0.09	0.27	0.15	0.17	2.64	6.98E-03
A_09_P012496	mtrm	FBtr0076613	FBgn0010431	GO:0003674(	0.09	0.12	0.10	0.21	0.40	0.21	2.64	2.44E-02
A_09_P209100		CO304209	Dm.24222		0.11	0.11	0.09	0.24	0.34	0.25	2.63	2.79E-04

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Systematic	GeneSymbol	PrimaryAccession	UniGeneID	GO	ormaliz	ormaliz	ormalize	Normaliz	ormaliz	ormaliz	Ratio	P-value
A_09_P013416	RnrS	FBtr0088046	FBgn0011704	GO:0004748(	0.09	0.13	0.08	0.21	0.36	0.21	2.62	8.54E-03
A_09_P044156	plu	FBtr0086306	FBgn0003114	GO:0003677(	0.03	0.05	0.04	0.11	0.10	0.07	2.60	2.97E-03
A_09_P090090	CR33963	NR_002692	Dm.4329	GO:0003674(	0.04	0.04	0.07	0.15	0.12	0.09	2.60	4.77E-03
A_09_P042801	Hsp27	FBtr0076454	FBgn0001226	GO:0005875(	0.40	0.53	0.40	0.83	1.28	1.37	2.58	4.75E-03
A_09_P038581	CG1368	FBtr0073891	FBgn0030539	GO:0005213(	0.03	0.04	0.04	0.12	0.08	0.08	2.56	1.58E-04
A_09_P162875		EC241822	Dm.34635		0.11	0.11	0.10	0.26	0.30	0.26	2.55	5.63E-05
A_09_P188290		TC242105			0.16	0.14	0.21	0.42	0.56	0.34	2.54	2.52E-03
A_09_P004446	CG34437	FBtr0112738	FBgn0085466		0.03	0.04	0.03	0.10	0.08	0.09	2.54	5.20E-04
A_09_P189675	CG34417	FBtr0112707	FBgn0085446	GO:0003779(	0.13	0.12	0.08	0.33	0.19	0.31	2.54	8.66E-03
A_09_P206870	Hsp27	FBtr0076454	FBgn0001226	GO:0005875(	0.26	0.46	0.33	0.72	1.13	0.76	2.52	8.08E-03
A_09_P184930		TC244055			0.05	0.06	0.06	0.16	0.13	0.16	2.52	9.79E-05
A_09_P180530		TC235712			20.59	22.08	26.37	74.73	49.01	52.11	2.52	1.94E-03
A_09_P153540	Hml	FBtr0075753	FBgn0029167	GO:0005529(	0.09	0.17	0.19	0.47	0.30	0.33	2.51	2.83E-02
A_09_P026966	CG3906	FBtr0072121	FBgn0034871		0.01	0.03	0.02	0.04	0.05	0.08	2.49	1.82E-02
A_09_P009566	stg	FBtr0085397	FBgn0003525	GO:0000086(	0.02	0.02	0.03	0.08	0.06	0.07	2.49	3.57E-03
A_09_P194885	Hsp27	FBtr0076454	FBgn0001226	GO:0005875(	0.45	0.68	0.63	1.04	1.60	1.74	2.47	6.35E-03
A_09_P060721	CG14523	FBtr0085349	FBgn0039612	GO:0004222(	0.04	0.08	0.04	0.14	0.10	0.12	2.47	1.77E-02
A_09_P067096	CG2861	FBtr0070731	FBgn0029728		0.03	0.03	0.03	0.08	0.07	0.06	2.47	4.88E-03
A_09_P067446	CG3011	FBtr0070827	FBgn0029823	GO:0004372(	0.52	1.24	0.70	1.93	1.48	2.35	2.46	4.28E-02
A_09_P017711	Cyp6t1	FBtr0077212	FBgn0031182	GO:0004497(	0.04	0.05	0.08	0.14	0.13	0.14	2.46	3.06E-02
A_09_P111075	CG30345	FBtr0305317	FBgn0050345	GO:0005215(	0.03	0.05	0.04	0.15	0.07	0.10	2.46	1.83E-02
A_09_P205005	Lsd-2	FBtr0110969	FBgn0030608	GO:0005515(	0.35	0.18	0.21	0.57	0.68	0.52	2.45	1.97E-02
A_09_P071556	CG11878	FBtr0084821	FBgn0039310		0.04	0.07	0.05	0.14	0.15	0.09	2.44	8.59E-03
A_09_P210930	CG13056	FBtr0075376	FBgn0040794		0.09	0.14	0.22	0.26	0.33	0.44	2.43	4.89E-02
A_09_P196605	Osi7	FBtr0078597	FBgn0037414		0.02	0.03	0.05	0.11	0.05	0.08	2.41	1.95E-02
A_09_P198270		NP027442			0.08	0.07	0.04	0.12	0.14	0.21	2.40	9.45E-03
A_09_P167565		SD02447	Dm.13899		0.06	0.08	0.07	0.23	0.12	0.17	2.40	9.42E-03
A_09_P012081	Myo61F	FBtr0072672	FBgn0010246	GO:0003774(	0.05	0.06	0.06	0.16	0.09	0.17	2.40	2.87E-02
A_09_P079121	inx7	FBtr0071034	FBgn0027106	GO:0005243(	0.16	0.34	0.24	0.51	0.52	0.64	2.38	4.04E-02
A_09_P066931	CG6414	FBtr0070624	FBgn0029690	GO:0004091(	0.06	0.04	0.03	0.13	0.08	0.08	2.37	8.57E-03
A_09_P172440		AW943916	Dm.31640		0.02	0.02	0.03	0.05	0.04	0.07	2.36	1.06E-02
A_09_P161555		EC264001	Dm.34252		0.03	0.04	0.06	0.13	0.10	0.06	2.36	3.62E-02
A_09_P038241	CG12716	FBtr0073752	FBgn0030439		0.15	0.14	0.13	0.38	0.37	0.25	2.35	4.90E-03
A_09_P039486	CG13012	FBtr0290216	FBgn0030769		0.23	0.19	0.18	0.46	0.51	0.43	2.34	2.61E-05
A_09_P066806	CG3603	FBtr0070558	FBgn0029648	GO:0005488(	0.14	0.23	0.19	0.51	0.39	0.41	2.32	7.59E-04
A_09_P038871	RpL37a	FBtr0074027	FBgn0030616	GO:0003735(	0.19	0.24	0.30	0.47	0.56	0.62	2.31	2.00E-03
A_09_P195970		TC248077			0.06	0.07	0.08	0.24	0.13	0.13	2.30	2.62E-02
A_09_P071976	CG14237	FBtr0085013	FBgn0039428		0.03	0.03	0.02	0.09	0.04	0.06	2.29	4.17E-02
A_09_P000686	His1:CG33804	FBtr0091808	FBgn0053804	GO:0003677(	0.05	0.12	0.10	0.23	0.22	0.14	2.29	3.10E-02
A_09_P166070		TC240284			0.05	0.07	0.05	0.12	0.12	0.14	2.28	1.18E-03
A_09_P031456	nompA	FBtr0089381	FBgn0016047	GO:0005576(	0.04	0.03	0.03	0.08	0.07	0.09	2.27	2.44E-03
A_09_P115600		RH09469	Dm.31030		0.36	0.42	0.60	0.99	0.98	1.07	2.27	1.06E-02
A_09_P025191	CG42326	FBtr0299788	FBgn0259226		0.03	0.03	0.02	0.06	0.04	0.06	2.26	9.66E-03
A_09_P172890		BG637820	Dm.31834		0.14	0.32	0.22	0.63	0.38	0.45	2.25	4.76E-02
A_09_P203140	CG42849	FBtr0304014	FBgn0262096		0.13	0.22	0.20	0.55	0.32	0.35	2.24	1.63E-02
A_09_P196265					0.09	0.10	0.07	0.19	0.19	0.18	2.23	3.09E-04
A_09_P042291	exu	FBtr0086242	FBgn0000615	GO:0000398(	0.04	0.04	0.03	0.07	0.10	0.07	2.23	1.11E-02
A_09_P119495	dl	FBtr0081006	FBgn0260632	GO:0000122(	0.22	0.27	0.22	0.63	0.35	0.64	2.20	3.31E-02
A_09_P063581		IP03442	Dm.27499		0.07	0.10	0.11	0.16	0.23	0.22	2.19	4.37E-03
A_09_P125675	Rrp4	FBtr0072111	FBgn0034879	GO:0000175(	0.02	0.05	0.05	0.09	0.07	0.09	2.18	4.26E-02
A_09_P115535	CG9897	FBtr0071989	FBgn0034807	GO:0004252(	0.04	0.05	0.02	0.09	0.10	0.06	2.15	4.36E-02
A_09_P052731	CG11350	FBtr0073307	FBgn0035552		0.08	0.04	0.04	0.15	0.08	0.12	2.14	2.59E-02
A_09_P035931	CG12582	FBtr0078964	FBgn0037215	GO:0004567(	0.19	0.35	0.24	0.51	0.62	0.51	2.14	2.49E-02
A_09_P125740	CG9293	FBtr0080525	FBgn0032516	GO:0008270(	0.11	0.08	0.13	0.18	0.22	0.26	2.13	5.13E-03
A_09_P069956	CG15497	FBtr0084113	FBgn0038894		0.06	0.08	0.08	0.15	0.14	0.18	2.13	4.97E-05

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Systematic	GeneSymbol	PrimaryAccession	UniGeneID	GO	ormaliz	ormaliz	ormalize	Normaliz	ormaliz	ormaliz	Ratio	P-value
A_09_P186545	Hsp27	FBtr0076454	FBgn0001226	GO:0005875(	0.64	0.83	0.58	1.10	1.57	1.70	2.12	5.17E-03
A_09_P187970					0.17	0.20	0.23	0.57	0.35	0.35	2.09	1.38E-02
A_09_P031116	CycB3	FBtr0084728	FBgn0015625	GO:0000281(	0.04	0.04	0.05	0.08	0.09	0.09	2.09	1.94E-03
A_09_P018421	CG4259	FBtr0077854	FBgn0031389	GO:0004252(	0.05	0.07	0.05	0.17	0.08	0.11	2.08	4.25E-02
A_09_P007436	CG6347	FBtr0087637	FBgn0033874	GO:0004197(	0.05	0.06	0.05	0.10	0.10	0.12	2.08	6.56E-04
A_09_P062806	CG3526	FBtr0070546	FBgn0040355	GO:0005622(	0.06	0.05	0.05	0.16	0.09	0.09	2.06	2.90E-02
A_09_P215595	CG9812	FBtr0306150	FBgn0034860		0.05	0.05	0.04	0.14	0.07	0.11	2.06	3.93E-02
A_09_P114075	CG15579	FBtr0070657	FBgn0040906		0.32	0.31	0.26	0.75	0.57	0.52	2.05	1.11E-03
A_09_P054871	CG7607	FBtr0076163	FBgn0036145		9.86	10.94	12.82	21.54	24.71	21.86	2.03	7.59E-05
A_09_P177560		TC236023			0.11	0.11	0.07	0.23	0.20	0.16	2.03	5.21E-03
A_09_P002011	CG32335	FBtr0273416	FBgn0063667	GO:0005634(	0.04	0.04	0.04	0.08	0.08	0.09	2.00	1.04E-03
A_09_P101550	CG4041	FBtr0300998	FBgn0029736	GO:0004672(	0.17	0.19	0.29	0.42	0.49	0.36	2.00	2.06E-02
A_09_P220545					0.51	0.28	0.43	0.23	0.17	0.19	0.50	3.06E-02
A_09_P059916	CG31462	FBtr0081851	FBgn0051462		0.14	0.08	0.09	0.06	0.06	0.04	0.50	2.36E-02
A_09_P039381	CalpC	FBtr0074226	FBgn0260450	GO:0004198(	0.25	0.27	0.26	0.14	0.12	0.12	0.49	2.39E-03
A_09_P197010	CG34391	FBtr0112634	FBgn0085420		0.13	0.08	0.11	0.04	0.06	0.05	0.49	1.22E-02
A_09_P174340	CG6356	FBtr0084601	FBgn0039178	GO:0008513(	0.95	0.76	1.14	0.41	0.39	0.62	0.49	1.02E-02
A_09_P123675	Drep-2	FBtr0088597	FBgn0028408	GO:0005622(	0.28	0.16	0.19	0.10	0.12	0.08	0.49	1.45E-02
A_09_P118705		TC248180		GO:0005643(	0.09	0.07	0.08	0.06	0.03	0.04	0.49	1.28E-02
A_09_P166290	boi	FBtr0114528	FBgn0040388	GO:0007155(	0.09	0.08	0.05	0.04	0.04	0.03	0.48	1.82E-02
A_09_P067621	CG42340	FBtr0299863	FBgn0259242	GO:0005267(	0.05	0.05	0.06	0.02	0.03	0.03	0.48	1.37E-02
A_09_P180500	CG8709	FBtr0303580	FBgn0033269	GO:0005737(	0.07	0.04	0.06	0.04	0.02	0.03	0.48	3.19E-02
A_09_P197225		NP535325			0.10	0.09	0.12	0.06	0.05	0.04	0.48	4.46E-03
A_09_P040746	CG9576	FBtr0070027	FBgn0031091	GO:0008270(	0.06	0.06	0.10	0.04	0.04	0.03	0.48	2.52E-02
A_09_P145565	norpA	FBtr0301475	FBgn0262738		0.59	0.41	0.43	0.24	0.21	0.22	0.47	1.78E-03
A_09_P211350	CG17838	FBtr0083961	FBgn0038826	GO:0000166(	1.43	1.04	0.75	0.54	0.66	0.33	0.47	4.43E-02
A_09_P131435	odd	FBtr0077557	FBgn0002985	GO:0003676(	0.11	0.08	0.08	0.06	0.05	0.03	0.47	2.58E-02
A_09_P182195	Pka-R1	FBtr0299891	FBgn0259243	GO:0001932(	0.30	0.24	0.25	0.12	0.13	0.13	0.47	7.77E-05
A_09_P104655	Mcm3	FBtr0070762	FBgn0024332	GO:0003677(	0.21	0.19	0.25	0.08	0.11	0.11	0.47	6.97E-04
A_09_P192270		TC247933			0.35	0.23	0.19	0.10	0.11	0.15	0.46	1.57E-02
A_09_P219135					1.91	1.35	1.42	0.79	0.62	0.73	0.46	7.57E-04
A_09_P226155	Hr46	FBtr0306346	FBgn0000448	GO:0003700(	0.36	0.24	0.24	0.15	0.12	0.11	0.46	3.69E-03
A_09_P191475	CG42492	FBtr0300464	FBgn0259994		1.76	1.02	1.24	0.71	0.50	0.60	0.46	1.02E-02
A_09_P000101	D2R	FBtr0091461	FBgn0053517	GO:0001591(	0.13	0.08	0.07	0.03	0.07	0.03	0.46	3.53E-02
A_09_P204390	CG40351	FBtr0113870	FBgn0040022	GO:0000166(	0.12	0.10	0.10	0.05	0.04	0.06	0.45	1.78E-03
A_09_P035096	CG11796	FBtr0078226	FBgn0036992	GO:0003868(	0.03	0.05	0.07	0.02	0.02	0.02	0.45	3.35E-02
A_09_P197265	CG2681	FBtr0070480	FBgn0024997	GO:0004842(	0.50	0.49	0.50	0.24	0.22	0.21	0.45	9.09E-05
A_09_P071831	CG5948	FBtr0084939	FBgn0039386	GO:0006801(	0.10	0.10	0.14	0.05	0.05	0.05	0.44	1.60E-03
A_09_P003491	CG34264	FBtr0306849	FBgn0085293		0.05	0.08	0.13	0.04	0.04	0.03	0.44	1.68E-02
A_09_P164055	CG34306	FBtr0112502	FBgn0085335		0.05	0.03	0.05	0.01	0.02	0.03	0.43	1.37E-02
A_09_P216680	CG42492	FBtr0300464	FBgn0259994		2.06	1.17	1.39	0.79	0.55	0.61	0.43	9.79E-03
A_09_P069291	CG11391	FBtr0083789	FBgn0038732	GO:0003824(	0.19	0.11	0.15	0.08	0.06	0.06	0.43	1.77E-03
A_09_P072621	CG42796	FBtr0303603	FBgn0261929	GO:0004993(	0.10	0.06	0.09	0.02	0.05	0.03	0.42	1.54E-02
A_09_P043891	nod	FBtr0073516	FBgn0002948	GO:0003677(	0.15	0.11	0.11	0.06	0.07	0.03	0.41	1.80E-02
A_09_P193985		TC243529			0.53	0.49	0.63	0.19	0.24	0.25	0.41	2.01E-05
A_09_P220670		TC242940			0.05	0.04	0.05	0.03	0.02	0.01	0.40	4.00E-02
A_09_P078831	l(1)G0045	FBtr0070782	FBgn0026702	GO:0001682(	0.09	0.08	0.09	0.05	0.02	0.03	0.40	3.01E-02
A_09_P053166	CG6602	FBtr0077047	FBgn0035673		0.36	0.45	0.30	0.13	0.10	0.24	0.40	4.60E-02
A_09_P090165	CG4857	FBtr0305180	FBgn0026083		0.38	0.29	0.23	0.12	0.13	0.10	0.40	2.30E-03
A_09_P185440		GH26506	Dm.29317		1.98	1.98	1.87	0.75	0.73	0.82	0.39	2.76E-05
A_09_P011896	GstD5	FBtr0082572	FBgn0010041	GO:0004364(	0.10	0.10	0.15	0.05	0.04	0.05	0.39	1.06E-03
A_09_P063791	CG42492	FBtr0300464	FBgn0259994		1.52	0.98	1.48	0.56	0.41	0.56	0.39	2.43E-03
A_09_P178841	CG40813	FBtr0113934	FBgn0085521		0.11	0.18	0.09	0.06	0.04	0.04	0.39	1.32E-02
A_09_P176495		BP559788	Dm.33278		0.25	0.31	0.28	0.17	0.07	0.10	0.38	4.22E-02
A_09_P224960					0.09	0.11	0.09	0.03	0.03	0.05	0.38	4.00E-03

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A_09_P221790		TC243589			0.26	0.16	0.12	0.08	0.08	0.04	0.38	2.06E-02
A_09_P210670	CG34205	FBtr0112398	FBgn0085234		0.07	0.05	0.08	0.03	0.02	0.03	0.38	1.06E-02
A_09_P035611	CG14567	FBtr0078454	FBgn0037126		0.11	0.11	0.07	0.04	0.03	0.04	0.37	3.20E-03
A_09_P181920		TC238641			0.09	0.21	0.19	0.07	0.07	0.04	0.37	3.44E-02
A_09_P205240		CO332204	Dm.29000		0.43	0.73	0.95	0.31	0.21	0.22	0.37	2.43E-02
A_09_P066711	CG14778	FBtr0070254	FBgn0029580	GO:0005778(	0.37	0.33	0.40	0.12	0.16	0.12	0.36	7.67E-05
A_09_P066186	CG31672	FBtr0077825	FBgn0028952		0.07	0.06	0.07	0.05	0.02	0.02	0.36	4.06E-02
A_09_P090472		SD26211			0.14	0.13	0.20	0.06	0.06	0.05	0.35	8.65E-04
A_09_P203005	Nc73EF	FBtr0075269	FBgn0010352	GO:0004591(	0.12	0.09	0.07	0.03	0.06	0.02	0.35	3.32E-02
A_09_P211035	CG13375	FBtr0300326	FBgn0040370	GO:0003924(	0.17	0.18	0.11	0.07	0.06	0.03	0.35	1.22E-02
A_09_P066466	tyn	FBtr0100135	FBgn0029128	GO:0003674(	2.25	1.79	1.89	0.78	0.61	0.66	0.35	2.07E-06
A_09_P066706	CG14770	FBtr0070213	FBgn0029573		0.21	0.15	0.21	0.07	0.07	0.05	0.34	9.18E-05
A_09_P013826	CG31816	FBtr0080868	FBgn0051816	GO:0003674(	0.10	0.18	0.10	0.06	0.04	0.03	0.34	8.70E-03
A_09_P039951	CG6788	FBtr0074495	FBgn0030880	GO:0005102(	0.32	0.53	0.42	0.22	0.15	0.08	0.33	3.45E-02
A_09_P077961	RhoGAP1A	FBtr0112919	FBgn0025836	GO:0005089(	2.54	2.06	2.47	0.71	0.76	0.89	0.33	2.59E-07
A_09_P225685	CG7852	FBtr0072736	FBgn0035229		0.09	0.06	0.12	0.03	0.01	0.05	0.32	2.69E-02
A_09_P009671	Sxl	FBtr0100206	FBgn0003659	GO:0000166(	0.75	0.73	0.98	0.24	0.28	0.27	0.32	2.38E-05
A_09_P032696	Crg-1	FBtr0070599	FBgn0021738	GO:0003700(	0.46	0.52	0.57	0.22	0.13	0.14	0.31	3.83E-03
A_09_P058106	CG30334	FBtr0087949	FBgn0050334	GO:0003674(	0.05	0.13	0.08	0.02	0.04	0.02	0.31	1.62E-02
A_09_P013211	Cyp4d2	FBtr0070387	FBgn0011576	GO:0004497(	3.66	3.01	2.76	0.92	0.91	1.05	0.31	3.45E-06
A_09_P038441	Ndc80	FBtr0073850	FBgn0030500	GO:0000776(	0.10	0.09	0.09	0.03	0.03	0.02	0.31	8.05E-04
A_09_P171335	CG42492	FBtr0300464	FBgn0259994		0.33	0.18	0.16	0.07	0.07	0.05	0.29	9.35E-03
A_09_P004731					0.07	0.17	0.15	0.04	0.02	0.05	0.29	1.22E-02
A_09_P062881	CG13375	FBtr0070103	FBgn0040370	GO:0003924(	0.23	0.15	0.15	0.05	0.06	0.03	0.29	2.82E-04
A_09_P067481	CG5966	FBtr0070866	FBgn0029831	GO:0004806(	0.06	0.13	0.17	0.02	0.03	0.04	0.28	2.26E-02
A_09_P063846	CG12643	FBtr0071481	FBgn0040942		0.06	0.05	0.09	0.02	0.02	0.01	0.27	1.81E-02
A_09_P072221	CG17189	FBtr0085105	FBgn0039485		0.24	0.65	0.33	0.11	0.08	0.11	0.27	2.29E-02
A_09_P075926	CG17560	FBtr0083330	FBgn0038450	GO:0005488(	1.28	2.41	1.70	0.34	0.32	0.82	0.26	2.29E-02
A_09_P113455	CG17189	FBtr0085105	FBgn0039485		0.48	1.23	0.67	0.15	0.11	0.35	0.24	3.32E-02
A_09_P184910					4.15	4.27	3.90	1.02	0.81	1.15	0.24	2.31E-05
A_09_P042081	dsx	FBtr0081759	FBgn0000504	GO:0000122(	0.37	0.30	0.30	0.10	0.07	0.07	0.24	1.27E-05
A_09_P062741	CG3706	FBtr0070189	FBgn0040342		0.10	0.11	0.12	0.03	0.02	0.02	0.24	6.26E-04
A_09_P036646	CG1077	FBtr0078587	FBgn0037405		0.11	0.10	0.09	0.02	0.05	0.02	0.24	1.33E-02
A_09_P214560		IP11223	Dm.32757		0.08	0.13	0.18	0.04	0.03	0.02	0.23	7.84E-03
A_09_P001411		TC237620			0.05	0.09	0.10	0.02	0.01	0.02	0.22	8.69E-04
A_09_P074216	CG12256	FBtr0082621	FBgn0038002	GO:0004252(	0.44	0.40	1.32	0.19	0.10	0.14	0.22	3.74E-02
A_09_P171190		BP550583	Dm.36834		0.87	1.34	1.00	0.35	0.15	0.16	0.19	9.23E-03
A_09_P047886		GH14214	Dm.33174		1.51	0.99	1.02	0.25	0.17	0.25	0.19	1.00E-04
A_09_P217945	CG11391	FBtr0083789	FBgn0038732	GO:0003824(	0.11	0.05	0.06	0.03	0.01	0.01	0.19	8.97E-03
A_09_P067806	CG4586	FBtr0071003	FBgn0029924	GO:0003995(	0.47	0.50	0.48	0.08	0.11	0.08	0.19	4.91E-05
A_09_P210350		RT01029	Dm.26438		0.06	0.10	0.06	0.02	0.01	0.01	0.18	6.04E-03
A_09_P205860		TC247042			0.07	0.06	0.08	0.01	0.02	0.01	0.17	1.26E-02
A_09_P218595		Y13272	Dm.1882		1.73	2.13	1.49	0.49	0.26	0.22	0.17	6.01E-03
A_09_P174680		CO327868	Dm.32559		0.58	0.84	0.86	0.12	0.10	0.16	0.16	3.98E-05
A_09_P090610		FBtr0300134	Dm.35092		0.09	0.14	0.11	0.01	0.04	0.02	0.16	4.01E-03
A_09_P179511		TC237266			0.05	0.07	0.07	0.01	0.00	0.03	0.14	1.07E-02
A_09_P176280		BP556550	Dm.33227		0.12	0.20	0.15	0.02	0.03	0.02	0.14	3.18E-04
A_09_P047876		GH13568			0.46	0.33	0.39	0.11	0.04	0.03	0.13	1.95E-02
A_09_P077201	CG2709	FBtr0070486	FBgn0024977	GO:0008270(	0.08	0.10	0.12	0.01	0.01	0.02	0.11	2.51E-03
A_09_P193035		SD07644	Dm.169		1.26	1.57	1.94	0.20	0.13	0.17	0.10	1.27E-05
A_09_P191320					0.09	0.10	0.08	0.01	0.00	0.01	0.10	1.22E-02
A_09_P180045		SD26211			5.49	6.27	7.01	0.61	0.50	0.62	0.09	9.20E-10
A_09_P164270		AT14183	Dm.35097		0.14	0.21	0.19	0.02	0.02	0.01	0.09	2.49E-03
A_09_P004671	CG40635	NM_001110696	Dm.36091		0.14	0.18	0.17	0.01	0.01	0.02	0.09	7.38E-06
A_09_P165765		TC240084			0.07	0.10	0.10	0.01	0.00	0.02	0.08	6.73E-03

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A_09_P165825		GH14228	Dm.4050		0.31	0.29	0.29	0.01	0.02	0.02	0.05	4.88E-04
A_09_P144698		TC243186			0.96	1.51	1.62	0.07	0.04	0.04	0.04	3.48E-06
A_09_P101950		FBtr0300162	Dm.35092		0.21	0.31	0.16	0.01	0.00	0.01	0.04	8.89E-04
A_09_P051783		FBtr0300146	Dm.36727		0.31	0.33	0.30	0.01	0.02	0.01	0.03	1.81E-03
A_09_P091170	roX1	NR_002097	Dm.20295	GO:0000805(	15.33	14.59	12.49	0.34	0.32	0.45	0.03	1.08E-07
A_09_P162605	CG13762	FBtr0300708	FBgn0040333	GO:0005262(	1.03	1.23	0.98	0.03	0.02	0.03	0.02	1.11E-04
A_09_P051786		FBtr0300172	Dm.36902		0.91	1.42	1.32	0.03	0.02	0.02	0.02	1.98E-04
A_09_P187380		TC245454			0.38	0.52	0.47	0.01	0.01	0.01	0.02	1.08E-03
A_09_P090105	roX1	NR_002098	Dm.20295	GO:0000805(	7.36	7.68	2.88	0.13	0.12	0.11	0.02	3.94E-03
A_09_P108980	roX1	NR_002098	Dm.20295	GO:0000805(	33.00	31.62	29.20	0.62	0.60	0.82	0.02	4.92E-08
A_09_P091310	roX2	NR_002105	Dm.1443	GO:0000805(	13.00	18.93	15.35	0.37	0.22	0.32	0.02	1.64E-06
A_09_P062696		NP175338			0.81	0.90	0.89	0.01	0.01	0.01	0.01	2.12E-06
A_09_P182115	roX1	NR_002098	Dm.20295	GO:0000805(	10.16	7.33	7.60	0.09	0.11	0.06	0.01	1.06E-05
A_09_P183535		TC246484			3.76	4.40	3.69	0.02	0.03	0.04	0.01	4.52E-07

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Overlap of 284 genes differentially expressed in BBB Males vs. Females with Gene Ontology classifications

Category=the name of the category within the ontology.
Genes in Category=the total number of genes in the genome that have been assigned to the category
Genes in List in Category=the total number of genes that are both in the selected gene list and in the category.
P-value=a hypergeometric p-value without multiple testing corrections.

Biological Process			
Category	**Genes in Category**	**Genes in List in Category**	**p-Value**
GO:16457: dosage compensation complex assembly (sensu Insecta)	13	5	6.77E-08
GO:42714: dosage compensation complex assembly	13	5	6.77E-08
GO:30237: female sex determination	28	6	1.55E-07
GO:35079: polytene chromosome puffing	7	4	2.18E-07
GO:35080: heat shock-mediated polytene chromosome puffing	7	4	2.18E-07
GO:9047: dosage compensation, by hyperactivation of X chromosome	22	5	1.30E-06
GO:7549: dosage compensation	48	6	4.36E-06
GO:42026: protein refolding	15	4	8.05E-06
GO:7296: vitellogenesis	16	4	1.07E-05
GO:19102: male somatic sex determination	6	3	1.41E-05
GO:45496: male analia morphogenesis (sensu Endopterygota)	6	3	1.41E-05
GO:45497: female analia morphogenesis (sensu Endopterygota)	6	3	1.41E-05
GO:7530: sex determination	62	6	1.97E-05
GO:9408: response to heat	128	8	2.06E-05
GO:51084: posttranslational protein folding	19	4	2.22E-05
GO:48086: negative regulation of pigmentation	7	3	2.46E-05
GO:19101: female somatic sex determination	21	4	3.38E-05
GO:30238: male sex determination	8	3	3.91E-05
GO:281: cytokinesis after mitosis	8	3	3.91E-05
GO:9266: response to temperature stimulus	144	8	4.81E-05
GO:48071: sex-specific pigmentation	12	3	1.50E-04
GO:7486: female genitalia morphogenesis (sensu Endopterygota)	13	3	1.93E-04
GO:30540: female genitalia morphogenesis	13	3	1.93E-04
GO:7487: analia morphogenesis (sensu Endopterygota)	13	3	1.93E-04
GO:7079: mitotic chromosome movement towards spindle pole	3	2	2.42E-04
GO:7485: male genitalia morphogenesis (sensu Endopterygota)	15	3	3.03E-04
GO:35263: genital disc sexually dimorphic development	15	3	3.03E-04
GO:45498: sex comb development	15	3	3.03E-04
GO:45934: negative regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolism	348	11	3.15E-04
GO:18993: somatic sex determination	39	4	4.10E-04
GO:19099: female germ-line sex determination	17	3	4.47E-04
GO:18992: germ-line sex determination	18	3	5.33E-04
GO:1666: response to hypoxia	45	4	7.12E-04
GO:7484: genitalia morphogenesis (sensu Endopterygota)	20	3	7.35E-04
GO:19953: sexual reproduction	1413	25	7.67E-04
GO:92: mitotic anaphase B	5	2	7.96E-04
GO:7548: sex differentiation	168	7	8.28E-04
GO:8608: attachment of spindle microtubules to kinetochore	6	2	1.19E-03
GO:51305: chromosome movement towards spindle pole	6	2	1.19E-03
GO:31324: negative regulation of cellular metabolism	420	11	1.48E-03
GO:50832: defense response to fungi	56	4	1.63E-03
GO:45944: positive regulation of transcription from RNA polymerase II promoter	96	5	1.77E-03
GO:86: G2/M transition of mitotic cell cycle	27	3	1.80E-03
GO:9620: response to fungi	59	4	1.97E-03
GO:16481: negative regulation of transcription	312	9	2.10E-03
GO:45892: negative regulation of transcription, DNA-dependent	312	9	2.10E-03
GO:7483: genital disc morphogenesis	29	3	2.22E-03
GO:48070: regulation of developmental pigmentation	30	3	2.45E-03
GO:30730: sequestering of triacylglycerol	9	2	2.80E-03
GO:9892: negative regulation of metabolism	458	11	2.92E-03
GO:7276: gametogenesis	1385	23	2.93E-03
GO:7277: pole cell development	33	3	3.23E-03
GO:50000: chromosome localization	33	3	3.23E-03

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GO:51303: establishment of chromosome localization	33	3	3.23E-03
GO:3: reproduction	1571	25	3.35E-03
GO:45570: regulation of imaginal disc growth	36	3	4.14E-03
GO:31887: lipid particle transport along microtubule	11	2	4.23E-03
GO:30539: male genitalia morphogenesis	37	3	4.48E-03
GO:51338: regulation of transferase activity	74	4	4.50E-03
GO:45859: regulation of protein kinase activity	74	4	4.50E-03
GO:45448: mitotic cell cycle, embryonic	80	4	5.93E-03
GO:7446: imaginal disc growth	82	4	6.47E-03
GO:6950: response to stress	1055	18	6.59E-03
GO:51656: establishment of organelle localization	132	5	6.92E-03
GO:122: negative regulation of transcription from RNA polymerase II promoter	133	5	7.14E-03
GO:16545: male courtship behavior (sensu Insecta), wing vibration	44	3	7.29E-03
GO:45433: male courtship behavior (sensu Insecta), song production	44	3	7.29E-03
GO:46660: female sex differentiation	44	3	7.29E-03
GO:16542: male courtship behavior (sensu Insecta)	45	3	7.76E-03
GO:48065: male courtship behavior (sensu Insecta), wing extension	45	3	7.76E-03
GO:35186: syncytial blastoderm mitotic cell cycle	45	3	7.76E-03
GO:42742: defense response to bacteria	142	5	9.34E-03
Cellular Component			
Category	**Genes in Category**	**Genes in List in Category**	**p-Value**
GO:805: X chromosome	18	5	5.19E-07
GO:803: sex chromosome	20	5	9.26E-07
GO:46536: dosage compensation complex	21	5	1.21E-06
GO:16456: dosage compensation complex (sensu Insecta)	21	5	1.21E-06
GO:5956: protein kinase CK2 complex	15	4	9.29E-06
GO:45495: pole plasm	31	5	9.34E-06
GO:43232: intracellular non-membrane-bound organelle	2013	37	2.31E-05
GO:43228: non-membrane-bound organelle	2013	37	2.31E-05
GO:15630: microtubule cytoskeleton	874	20	1.68E-04
GO:5856: cytoskeleton	1120	23	2.56E-04
GO:5875: microtubule associated complex	700	17	2.76E-04
GO:43229: intracellular organelle	5737	72	5.01E-04
GO:43226: organelle	5737	72	5.01E-04
GO:42600: chorion	20	3	8.17E-04
GO:43234: protein complex	3209	46	8.38E-04
GO:43186: P granule	22	3	1.09E-03
GO:5622: intracellular	7566	86	2.42E-03
GO:19908: nuclear cyclin-dependent protein kinase holoenzyme complex	13	2	6.37E-03
GO:307: cyclin-dependent protein kinase holoenzyme complex	14	2	7.38E-03
GO:30312: external encapsulating structure	45	3	8.58E-03
Molecular Function			
Category	**Genes in Category**	**Genes in List in Category**	**p-Value**
GO:5213: structural constituent of chorion (sensu Insecta)	10	5	9.76E-09
GO:19887: protein kinase regulator activity	93	8	1.18E-06
GO:19207: kinase regulator activity	97	8	1.62E-06
GO:16903: oxidoreductase activity, acting on the aldehyde or oxo group of donors	63	4	1.98E-03
GO:16538: cyclin-dependent protein kinase regulator activity	38	3	4.02E-03
GO:5198: structural molecule activity	843	15	5.06E-03
GO:15245: fatty acid transporter activity	13	2	5.21E-03
GO:5324: long-chain fatty acid transporter activity	13	2	5.21E-03
GO:16620: oxidoreductase activity, acting on the aldehyde or oxo group of donors, NAD or NADP as acceptor	48	3	7.75E-03