Supplementary Methods

1. Fly rearing and developmental staging

Fly stocks (except where specified, the sequenced *D. melanogaster* isogenic *strain* y^1 cn^1 bw^1 sp^1 was used[1]) were reared at 24° C on standard Drosophila medium (http://flystocks.bio.indiana.edu/Fly_Work/media-recipes/media-recipes.htm). To collect larvae and adults, the flies were raised in 250 ml bottles containing 40 ml medium. To aid in staging third instar larvae the medium contained 0.05% bromphenol blue (BPB[2]) and staging was done as described[3].

Synchronized embryos were collected from large population cages (ca. 25 cm x 25 cm x 25 cm; maintained at 24° C on a cycle of 14 h light 10 h dark) from adults that were less than one week old. Following at least one – 2 h pre-lay that preceded timed collections each day, embryos were collected for two hours on three hard egg lay collection plates made in 150 X 15 mm Petri dishes containing a substrate of 3.3% agar, 13% unsulfured molasses, and 0.15% Tegasept. The hard egg lay plates were completely covered with a thin layer of moist yeast paste (Fleischmann's Baker's Dry Yeast) and placed horizontally on a short 1 cm raised Plexiglas bar in the bottom of each cage to avoid crushing flies. Staged embryos were passed through an 850 micron screen and collected on a 75 micron screen to remove adults and yeast paste. Embryos were then dechorionated by treatment with a solution of 50% bleach (3% sodium hypochlorite), 0.2% sodium chloride, and 0.02% Triton-X-100 for five minutes. Embryos were washed twice with 0.2% NaCl, 0.02% Triton buffer and split into two samples. Most of the sample (approximately 95%) was rinsed with de-ionized water in a buchner funnel under mild vacuum, dried briefly, immediately frozen on dry ice and stored at -80˚ C for RNA preparations. The small aliquot was transferred to a clean tube and fixed (0.1 M Pipes (pH 6.9), 2 mM EGTA, 1 mM MgSO4, 4% paraformaldehyde, 0.1% glutaraldehyde and 50% heptane for staging[4]. Samples were shaken for five minutes in the fixative, centrifuged briefly and the aqueous fraction was removed. An equal volume of methanol containing 2 mM EGTA was added and the sample was shaken for five additional minutes. Tissue was washed twice in methanol with 2 mM EGTA and saved at -80˚C for the characterization of developmental stages.

2. Dissection of Organ Systems

To detect rarely expressed and tissue specific RNAs we dissected organ systems from larval, pupal and adult animals. We examined components of the nervous system, from larval and pupal brains and ventral ganglia and from aged 1, 4 and 20-day adult heads (primarily brain) of mated males and virgin and mated females. To interrogate the reproductive system we dissected ovaries from females and testes and accessory glands from males. To study the digestive system we examined larval and pupal salivary glands and larval and aged 1, 4 and 20-day adult midgut, hindgut and malpighian tubules. We dissected larval and pupal fat body the primary metabolic and detoxification organ performing functions analogous to the human liver. To study the epidermis and muscle organ systems, we mass isolated larval imaginal discs adapted from a previously describe approach[2], with modifications detailed below and an aliquot of the sample prep is shown in Supplementary Figure 8. We also dissected larval and aged 1,4 and 20-day adult carcasses, which contain cuticle, epidermis, muscle and oenocytes as well as peripheral neurons. All tissues were stored at -80˚ C immediately after dissection until sufficient material had been collected to permit RNA preparations. A yield of approximately 4 µg total RNA per mg of tissue collected was typical. A cartoon giving the anatomical relationships between the tissues collected is provided in Supp. Fig. 10. Specifics follow:

2A. Larval tissue dissections: Bottles were started with approximately 60 adult OreR flies at 25˚ C. After 5 days, climbing third instar larvae were collected and transferred to a dissecting surface with 1X PBS buffer (Ambion) for dissections. We identified the sex of the larva by the presence of the large clear spherical testes (or smaller ovary) embedded in the white fat body on the lateral sides of the A5 segment. We recorded and collected the tissues with equal representation of each sex. To dissect, the cuticle was torn immediately posterior to the mouth hooks using paired forceps and the larvae were everted as with WPP dissections. The digestive system and fat body were pulled toward the anterior end and away from the cuticle. The digestive system was disconnected from the body immediately anterior to the proventriculus. The salivary glands were collected by pinching them off from the attached fat body. The extensive and reticulated fat body was removed from the carcass and digestive system. The trachea were removed from the digestive system and collected with the carcass. Tissues collected included the gut (fat body removed, Malphigian tubules included), the salivary glands (with as much fat body removed as possible), and carcass (without the guts, salivary glands, fat

body and gonads). Dissections were done concurrently so that all three tissues were collected from a single animal. Male and female tissues were collected in separate tubes and mixed in equal numbers for the RNA preparations.

2B. L3 Imaginal Discs mass preparation: Bulk preparations of imaginal disc tissue were done as previously described[5] with the following modifications. First instar larvae were transferred to ventilated plastic chambers containing seventeen feet of cotton rope saturated in a protein-rich yeast slurry (200 g active dry yeast, 6 oz Gerber's Banana food, 100 ml Grapefruit juice, 50 g ground Special K, 40 g Gerber's Baby Cereal, 20 g Wheat Germ, 1200-1400 ml water) and were allowed to grow until wandering larvae were observed. Larvae were ground with a Kitchen Aid Artisan mixer (Model KSM150SPER) and Kitchen Aid grain mill attachment (Model KGMA) with the plates set to leave about 5% of the total larvae unground. Ringer's solution was replaced with Organ Medium (25 mM β-Glycerol phosphate disodium salt pentahydrate (Fluka 50020), 10 mM KH_2PO_4, 30 mM KCl, 10 mM $MgCl_2$, 3 mM $CaCl_2$, 162 mM sucrose) at all steps. A photograph of the isolated tissues is given (Supplementary Fig. 9).

2C. Fly WPP and 2-day old pupae CNS: Staged WPP and 2-day old pupae were dissected in PBS (phosphate buffered saline). The posterior end of the pupa was removed with two forceps at the A7 abdominal segment. The anterior body of the pupa was removed from the pupal case with forceps. We held each cuticle at the anterior tip and gently teased the body towards the posterior opening with forceps. We pulled the cuticle from the anterior end through the second forceps, holding them nearly closed around the vacated cuticle. This squeezed the body of the pupa out of the cuticle. The yellow eye discs were removed from the brain lobes of the CNS. The connected antennal segment at the anterior margin of the brain was removed. The developing leg and wing disc tissue along with the fat body was removed, and the attached subesophageal ganglion and ring gland were recovered along with the brain. The CNS and ring gland were transferred to a collection tube on dry ice and then stored at -80˚ C until sufficient tissue for RNA isolation was collected.

2D. White pre-pupal salivary gland and fat body: We collected white pre-pupae (WPP) as in Graveley *et al.* [3] and dissected in PBS buffer. We identified the sex of the larva by the presence of the large clear spherical testes (or smaller ovary) embedded in the white fat body on the lateral sides of the A5 segment. We recorded and collected the tissues with equal representation of each sex. We note that the female WPP tend to be larger. We tore the cuticle immediately posterior to the mouth hooks and then everted the WPP by pushing the posterior end inside the body cavity with closed forceps, and finally collected the fat body and salivary glands in separate tubes on dry ice.

2E. Pupal fat body mass preparation: We transferred WPP animals 48 h after staging and resting at 25° C to a 15 ml polycarbonate falcon tube. We added 2 ml of Drosophila Ringers (182 mM KCl, 46 mM NaCl, 3 mM CaCl, 10 mM Tris-HCl pH 7.2) containing 2% Ficoll, and crushed the pupae in the tube to release contents from the cuticles. We added 5 ml of Ringers with 2% Ficoll, mixed with a large bore disposable pipet and filtered through a 100 um screen. The cell suspension was centrifuged at 660xG for 10 minutes at 40°C, and fat body cells were collected from the surface of the buffer and transferred to a 1.5ml eppendorf tube. Cell suspensions were centrifuged at 660xG for 5 minutes at 40° C to remove as much of the buffer from beneath the cells as possible. We froze the fat body cells by placing them on dry ice and stored at -80° C for RNA preparation.

2F. Adult gonads and reproductive tissues: Staged adult flies were anesthetized with CO_2 for 30 minutes or less while dissections were done in PBS (1X; Ambion) for less than 10 minutes each. To dissect/open the abdomen, we pinned down the thorax on either side with a set of surgical steel forceps (size #4 or #5), and pulled the T3 legs posteriorly to remove the overlying cuticle and expose the digestive and reproductive organs. The reproductive tissues were removed and separated from the digestive tract and the cuticle. In the females, the reproductive tissues included the ovaries and their attached oviducts. Due to tearing and mechanical damage during dissection, the oviducts were incompletely recovered. In the males, the reproductive tissues included the testes (generally bright yellow), and the accessory glands (generally translucent, with incomplete recovery of the attached seminal vesicle). We collected the ovaries and oviducts together as a single sample, and separated the testes from the accessory glands for independent RNA isolation and sequencing. These tissues were dissected away from all other attached cells, and then frozen in 1.5 ml tubes submerged in dry ice, and then stored at -80˚ C until sufficient quantities were obtained for RNA purifications.

2G. Adult gut and carcass: Adult flies were staged and anesthetized as for the gonad preparation. The digestive tracts and carcasses were separated after removing the head and discarding. Holding the thorax and pulling the T3 legs posteriorly to expose the digestive and reproductive organs was used to dissect the abdomen. The reproductive tissues were removed and discarded. The digestive tract was separated from the cuticle, adipose tissue (fat body) and other tissues, and then frozen on dry ice. The remaining tissues (without the head and reproductive organs) were frozen on dry ice and designated the carcass. All tissues were stored at -80˚ C until sufficient quantities were obtained for RNA purifications.

2H. Adult head: Isolation of the fly heads was accomplished by placing CO_2-anesthitized adults in a 15 ml conical tube that was then flash frozen in liquid nitrogen for about one minute. The tube was then shaken vigorously for 10 seconds, and tapped on the bench-top. The broken flies were placed in a frozen glass petri dish on dry ice. The frozen severed heads were removed with dissecting forceps and placed in an eppendorf tube on dry ice. Flies were processed in groups of 100 animals per dissection. Isolated tissue was stored at -80˚ C until RNA could be purified from an adequate number of prepared heads. Typically, heads were missing the antennal and maxillary organs, while the mouth-parts were retained.

3. Environmental Perturbations

3A. Heat Shock: Twenty virgin males and 20 virgin females were maintained on standard corn meal agar at 25°C for four days. After four days the 40 adult flies were transferred to clean glass vials and placed in a 36° C water bath (wet heat) and held at 36°C for 1 hour followed by a 30-minute recovery at 25° C prior to freezing in liquid nitrogen. This treatment produced relatively high lethality due to excessive moisture buildup in the vials.

3B. Cold Shock1: Newly eclosed flies were collected, and placed in cornmeal agar food vials containing 20 males and 20 females were and kept at 25° C for 84 hours. Aged, mated flies were transferred to empty glass vials and placed in a micro-cooler water bath containing 10% glycol at 25° C. The temperature was decreased to 0°C at a rate of 0.2°C per minute and then flies were held at 0° C for 9 hours. After the cold treatment flies were transferred to fresh food vials and kept at 25° C for 2 hours for the recovery period. Following recovery flies were placed in 2 ml tubes, flash frozen in liquid nitrogen and stored at -80° C prior to RNA preparations.

3C. Cold Shock 2: Flies were treated as in “Cold Shock 1”, above, except flies were held on food vials for four days. Aged, mated flies were transferred to empty glass vials and placed in a micro-cooler water bath containing 10% glycol at 0° C for two hours. Following the cold shock flies were transferred to fresh food vials and kept at 25° C for 30 minutes for the recovery period. Following recovery flies were placed in 2 ml tubes, flash frozen in liquid nitrogen and stored at -80° C prior to RNA preparations.

3D. Feeding schedule for consumed treatments:

3D1. Treatment schedule for Larvae: For each treatment, approximately 50 (mixed sex) young mated adults were transferred to each fresh food vials and maintained for 12 hours. Vials were cleared and allowed to age 3.5 to 4 days. Vials were then rinsed into a series of sieves using tepid water; feeding third instar larvae were collected form the #40 sieve and transferred to a hard agar plate with a pot of yeast to induce crawling. Prior to reaching the yeast, larvae were captured and 50 larvae were transferred to new food vials containing the treatment of interest (details below), and larvae were allowed to feed for 4 hours. Treated larvae were captured and transferred to 2 ml vials, flash frozen in liquid nitrogen and stored at -80° C prior to RNA preparations. The number of survivors was recorded and the mean lethality calculated for each treatment.

3D2. Treatment schedule for Adults: For each treatment, 40 newly eclosed males and females (1:1) were transferred to fresh food (BDSC corn meal agar) vials and maintained at 25° C for two days. To treat flies, two Kimwipes were folded into a square and put in the bottom of a one-pint glass bottle. Kimwipes were saturated with 4 ml of the treatment solution, (10% sucrose solution and one drop of green vegetable coloring per 50 ml solution, plus the treatment of interest). Harvesting time for adults varied by treatment. Upon harvesting, flies were placed in 2 ml tubes, flash frozen in liquid nitrogen and stored at -80° C prior to RNA preparations.

3D3. Caffeine feeding: Starved larvae (as above) were transferred to food vials containing 1.5 mg/ml caffeine and allowed to feed for 4 h. Adults fed 25 mg/ml caffeine were harvested after 8 h; adults fed 2.5 mg/ml caffeine were harvested after 48 h, and after 24 h an additional 1 ml of treatment solution was dripped onto the Kimwipe. Upon harvesting, flies were placed in 2 ml tubes, flash frozen in liquid nitrogen and stored at -80° C

prior to RNA preparations. For adults, 2.5 mg/ml caffeine is near the LD50 for a 48 h treatment. 25 mg/ml caffeine is 100% lethal after 24 h.

3D4. Copper feeding: Starved larvae were transferred to new food vials containing 0.5 mM $CuSO_4$ and allowed to feed for 12 h. The number of survivors was recorded and the mean lethality calculated for each treatment. Adults were fed with 15 mM $CuSO_4$. After 24 h an additional 1 ml of the treatment solution was dripped onto the Kimwipe. Flies were harvested after 48 h of feeding, placed in 2 ml tubes, flash frozen in liquid nitrogen and stored at -80° C prior to RNA preparations. Adult concentrations were all done at or near the LD50 determined for our feeding method after 48 h. Adults were fed 15 mM copper for 48 h.

3D5. Zinc feeding: Starved larvae were transferred to new food vials containing the 5 mM $ZnCl_2$ and allowed to feed for 12 h. Treated larvae were transferred to 2 ml vials, flash frozen in liquid nitrogen and stored at -80° C prior to RNA preparations. Adults were fed with 4.5 mM $ZnCl_2$. After 24 h an additional 1 ml of the treatment solution was dripped onto the Kimwipe. Flies were harvested after 48 h of feeding, placed in 2 ml tubes, flash frozen in liquid nitrogen and stored at -80° C prior to RNA preparations. Adult concentrations were done at or near the LD50 determined for our feeding method after 48 h. Adults were fed 4.5 mM zinc for 48 h. Zinc appears to cause a neuromuscular defect in both adults and larvae.

3D6. Cadmium feeding: Starved larvae were transferred to new food vials containing 0.05 mM $CdCl_2$ and allowed to feed for 6 or 12 h. Treated larvae were transferred to 2 ml vials, flash frozen in liquid nitrogen and stored at -80° C prior to RNA preparations. Adults were fed with 0.1 mM or 0.05 mM $CdCl_2$. After 24 h an additional 1 ml of solution was dripped onto the Kimwipe. Flies were harvested after 48 h of feeding, placed in 2 ml tubes, flash frozen in liquid nitrogen and stored at -80° C prior to RNA preparations. Adult concentrations were all done at or near the LD50 determined for our feeding method after 48 h. This concentration had a minimal effect on larvae after 6 h. Additionally, two vials of larvae were allowed to complete development and 96% eclosed with no obvious phenotypic abnormalities.

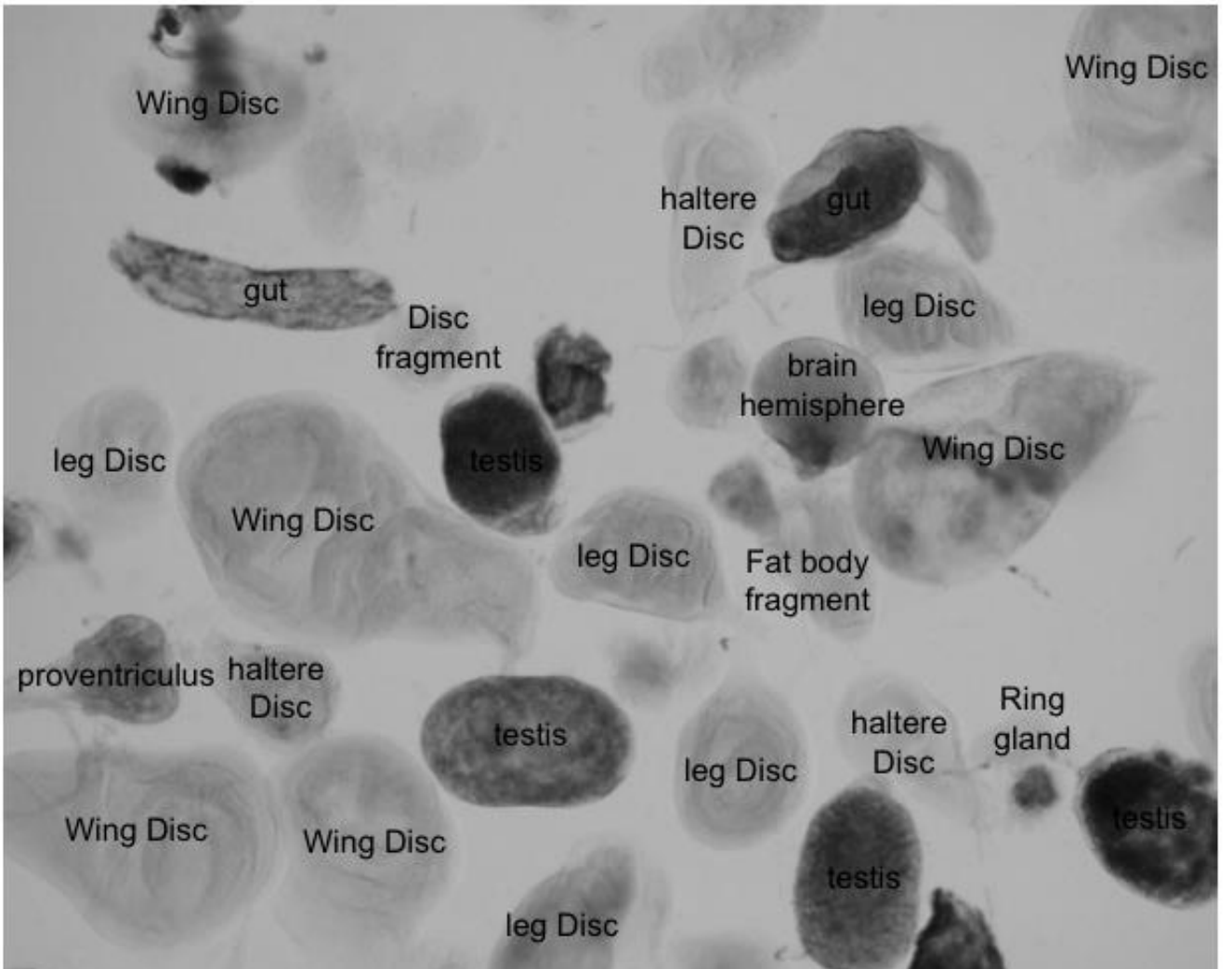
3D7. Paraquat feeding: Two-day-old adults were fed 5 mM paraquat for 48 h, and 3-day-old adults were fed 10 mM paraquat for 24 h. Following the treatment, adult flies were flash-frozen in liquid nitrogen and stored at -80˚ C. Feeding third-instar larvae were transferred to food containing 10 mM paraquat and allowed to feed for 12 h. Following treatment, larvae were collected and flash-frozen in liquid nitrogen and stored at -80˚ C.

3D8. Rotenone feeding: Newly eclosed adults were fed 20 µg/ml rotenone in 10% sucrose continuously for 10 days. Following the treatment adult flies were flash-frozen in liquid nitrogen and stored at -80˚ C. There was no evidence that the adults actually ingested any of the rotenone/sucrose/green dye solution, so we believe that any effect on transcription was likely to be caused by starvation rather than by rotenone itself. Hence we did not sequence RNA from these flies. Feeding third-instar larvae were transferred to food containing either 2 µg/ml or 8 µg/ml rotenone and allowed to feed for 6 h. Following treatment, larvae were collected and flash frozen in liquid nitrogen and stored at -80˚ C.

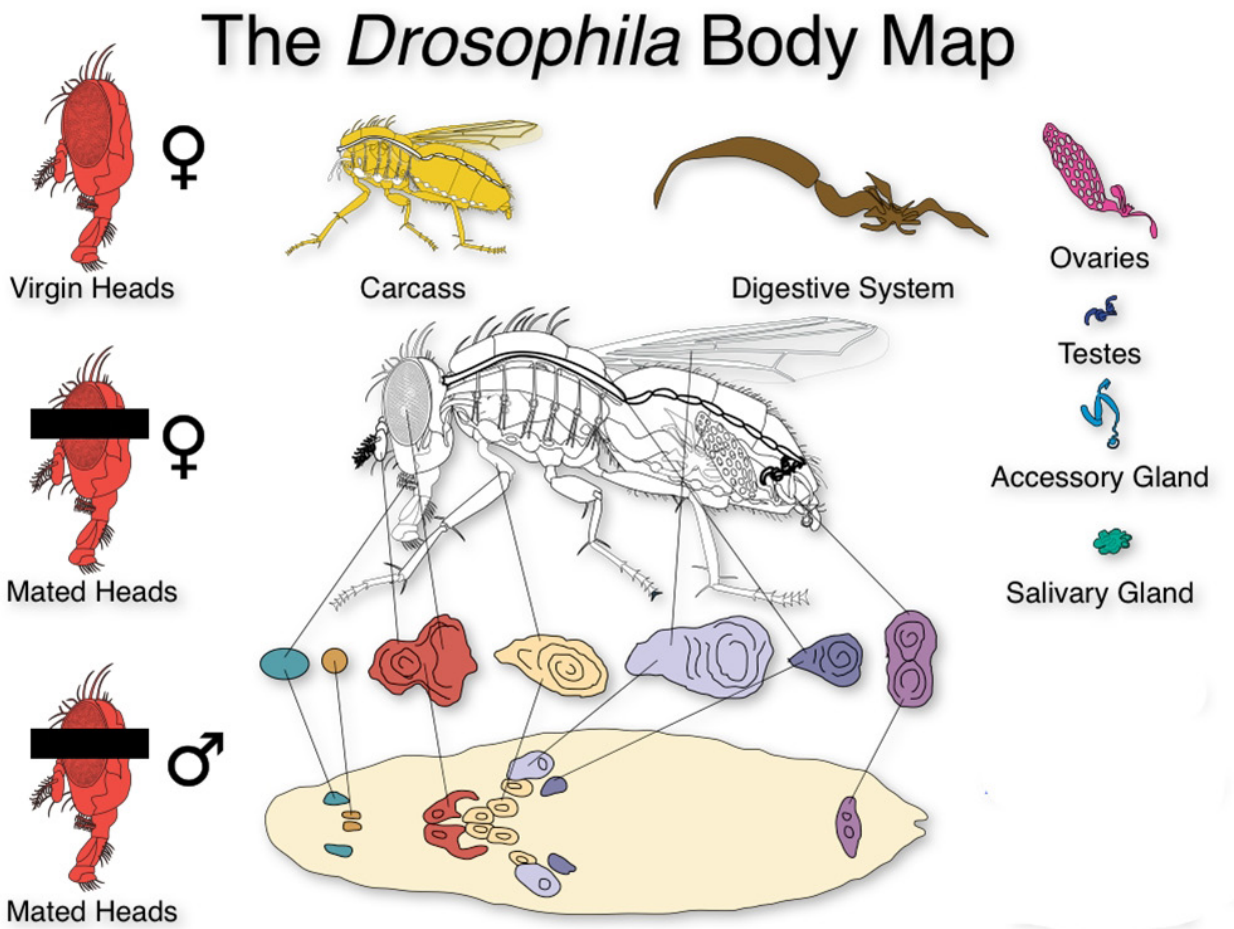
3D9. Resveratrol feeding: Two-day-old adults were fed 100 µM resveratrol in 10% sucrose continuously and samples were harvested at 10 days. Adult flies were flash frozen in liquid nitrogen and stored at -80˚ C.

4. Cell Lines

Each cell line was grown as described at http://dgrc.cgb.indiana.edu/cells according to an individualized protocol.



Supplementary Figure 9 Imaginal Disc mass isolation sample preparation. An image of the imaginal disc mass isolation sample preparation, along with anatomical annotations for the various tissue fragments.



Supplementary Figure 10 Drosophila Body Map. An overview of the tissues collected in the adult and embryo dissections and mass tissue isolations. Maps between the larval progenitor tissues, which were isolated en masse in our sample enriched for Imaginal Discs, and connecting lines indicates corresponding adult tissues.