Materials and Methods

Drosophila genetics

Flies were raised under standard conditions at 25℃ with 70% humidity. All fly crosses were carried out at 25°C with standard laboratory conditions unless noted otherwise. All strains were obtained from Bloomington *Drosophila* Stock Center (BDSC), the Vienna *Drosophila* RNAi Center (VDRC), Tsinghua Fly Center, or as gifts from colleagues. The following fly strains were used in this study: w^{1118}, *Dopa-GAL4* (targeting DA neurons), *elav-GAL4* (a pan-neuronal driver), and *UAS-mCD8.GFP*. Transgenic flies expressing RNAi targeting *Amy-p*, *Hsp70Ba*, *CecA1*, *Hsp70Aa*, *Drs*, or *Mdr50* were obtained from Tsinghua Fly Center and made based on vector VALIUM20 and the integrate site is attp40 on the 2nd chromosome. Plasmids are microinjected into fly embryos for creating the transgenic flies.

Confocal microscopy and Statistical analysis

Fly adult brains were dissected and fixed in 4% formaldehyde for 40 minutes, then washed with PBT (PBS + 0.1% TX-100) for 3 times and dissected further to remove additional debris in PBS solution. After dissection, images of fly adult brains expressing GFP reporter were acquired by scanning a serial Z-stack of average 25-30 sections, each of 0.4 μm thickness, using Nikon A1 confocal microscope (Tokyo, Japan) with the 40X or 60X objective. The whole brain was positioned so that they can be scanned anteriorly to posteriorly (top to bottom).

For statistical analyses, original, unmodified images were imported into ImageJ (National Institutes of Health), and the intensity threshold for the relevant channel was set so that maximum number of objects were selected without miscounting two adjacent objects into one. The number of animals (n) per genotype analyzed was shown in the designated Figures. All data were imported into GraphPad Prism 8 and shown in column bar graphs. For calculating the statistical significance, two-tailed unpaired t-test or ordinary one-way ANOVA followed by Tukey's multiple comparisons test was used. P value less than 0.05 is considered significant. ns: no significance, $p \geq 0.05$; *: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; ****: $p<0.0001$.

Paraquat analysis

More than 100 adult flies of 3 days old for each genotype were starved for 2 hours (fly food without yeast) and subsequently treated with methyl viologen dichloride in a final concentration of 0.16% (vol/vol). Paraquat solution was added to filter papers in aliquots of 200 μl every 8 hour at 25°C. Flies were transferred to vials with new fly food (no yeast) and new paraquat filter paper every 8 hour. Proportion alive (live/total) in percentage (%) was calculated every 8 hour by counting the number of dead flies at each time point. At least three independent experiments were performed. Data points were drawn in curves using Prism software.

Lifespan analysis

Newly eclosed wild-type w^{1118} flies (1 to 2 days old, n=400 for each genotype) were collected and placed separately in 20 vials (each contains 20 unisex flies). Flies were transferred to new vials with standard fly food every 3 days, and the number of dead flies were counted. All experiments were repeated at least three times, and the percentage of flies survived (live/total, %) was analyzed. Data points were drawn in curves using Prism software.

RNA isolation and Library preparation

Total RNA was extracted using the Trizol reagent according to the manufacturer's protocol. RNA purity and quantification were evaluated using the NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). RNA integrity was assessed using the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA). Then the libraries were constructed using TruSeq Stranded mRNA LT Sample Prep Kit (Illumina, San Diego, CA, USA) according to the manufacturer's instructions. The transcriptome sequencing and analysis were conducted by OE Biotech Co., Ltd. (Shanghai, China).

RNA sequencing and Differentially expressed genes analysis

The libraries were sequenced on an Illumina HiSeq X Ten platform and 150 bp paired-end reads were generated. Raw data (raw reads) of fastq format were firstly processed using Trimmomatic (Bolger et al., 2014) and the low quality reads were removed to obtain the clean reads. The clean reads were mapped using HISAT2 (Kim et al., 2015). FPKM (Roberts et al., 2011) of each gene was calculated using Cufflinks (Trapnell et al., 2010), and the read counts of each gene were obtained by HTSeq-count (Anders et al., 2015). Differential expression analysis was performed using the DESeq (2012) R package. The screening conditions for differentially expressed genes were foldchange > 2 and the significance of false discovery rate (FDR) < 0.05. FDR is the adjusted P-value. Hierarchical cluster analysis of differentially expressed genes (DEGs) was performed to demonstrate the expression pattern of genes in different groups and samples. GO enrichment and KEGG (Kanehisa et al., 2008) pathway enrichment analysis of DEGs were performed respectively using R based on the hypergeometric distribution. The DAVID database was used to classify the functions of all DEGs according to their biological processes (BPs), cellular components (CCs) and molecular functions (MFs). GO terms with a P-value less than 0.05 were considered significantly enriched among the DEGs. An example of the values for the gene Gnmt and CecB is shown below:

Reference:

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