

**High-Concentration Polyethylene and Polystyrene Microplastics
Co-exposure Shorten Insect Lifespan and Impose Ecological Risk:
Multi-Omics Evidence from *Drosophila melanogaster***

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1 **Abstract:**

2 Microplastics (MPs) are pervasive environmental pollutants, accumulating in
3 ecosystems and posing a long-term exposure risk to both the entire ecosystem and human
4 health. However, the combined impact of such high doses on insect longevity and the
5 consequent ecological consequences remain understudied. Here we used *Drosophila*
6 *melanogaster* as a model to quantify lifespan shortening under environmentally realistic and
7 extreme concentrations of Polyethylene (PE) and Polystyrene (PS) co-exposures and to
8 unravel the molecular bases of the observed toxicity. Furthermore, we delved into the
9 underlying mechanism through metabolomics and transcriptomics analysis. Our results
10 demonstrated PE and PS MPs co-exposure with greatly high concentrations significantly
11 reduced the lifespan of *Drosophila* and influenced age-related phenotypes such as climbing
12 ability, intestinal barrier and hunger resistance. We found that differential metabolites were
13 engaged in various metabolic pathways, including ABC transporters, alanine, aspartate and
14 glutamate metabolism. Differentially expressed genes (DEGs) were closely related to Toll
15 and Imd signaling pathway and Longevity regulating pathway. Gram-level PE and PS
16 co-exposure triggers immune-metabolic crosstalk failure and represents a realistic terrestrial
17 risk factor for insect longevity. Our data highlight the urgent need to include high-dose
18 microplastic mixtures in terrestrial ecotoxicological risk assessments and biodiversity
19 conservation strategies.

20 **Key words:** high-concentration microplastics, insect lifespan, terrestrial ecotoxicology,

multi-omics, *Drosophila melanogaster*

Synopsis:

Co-exposure to PE and PS MPs with high concentrations induces changes in gene expression and metabolites associated with immune system and energy metabolism in *Drosophila*, thereby affecting their lifespan.

Introduction

Plastic has become an integral part of industrial manufacture and daily lives.^[1] Due to its widespread use and resistance to degradation, plastic accumulates rapidly within ecosystems. The interplay of physical, chemical, and biological processes lead to the disintegration of larger plastic items into microplastics (MPs) and nanoplastics (NPs), defined as particles with at least one dimension less than 5 millimeters. Nowadays, MPs have been detected in various environmental compartments, including soil, atmosphere, water bodies, and even in drinking water and food.^{[2], [3]} A mere one centimeter square of plastic container surface can release MPs in staggering quantities, ranging from millions to billions, following microwave heating or extended storage periods in fridge.^[4] Concentrations of MPs in agricultural soils can reach grams per kilogram, leading to chronic expose for soil-dwelling insects. The pervasive presence of MPs pollution highlights the long-term exposure risks faced by both the entire ecosystem and human beings, as evidenced by the detection of MPs in human whole blood, placenta and fluids.^{[5]-}

^[8] Nonetheless, our understanding of the biological hazards associated with MPs exposure

remains incomplete.^[9]

The biotoxicity of MPs is well-documented in aquatic organism species,^[10-12] with effects including reduced survival, lowered body mass, weakened immunity, diminished reproductive capacity, intestinal dysfunction, behavioral changes metabolic disruption, and heightened oxidative stress.^[13-15] Compared to aquatic systems, the ecological impacts of MPs on terrestrial ecosystems, especially on insect longevity, remain largely overlooked, even though land-based MPs contamination is 4-23 folds higher than in oceans.^{[16], [17]} Evidence suggests that MPs interact with a range of terrestrial organisms, including invertebrates,^[18] fungi,^[19] and pollinating animals. Ingestion or adherence to MPs by these organisms may facilitate their transfer through food chains, potentially affecting ecosystem services and functions.^{[20], [21]}

Drosophila, a well-established terrestrial model organism, is widely used in toxicity studies due to its short and measurable lifespan, which facilitates the quantification of how exposure to high concentrations of MPs reduces longevity. Meanwhile, approximately 75% of human disease-related genes possess functional homologs in *Drosophila*, substantiating the organism's suitability and dependability for modeling human diseases.^[22] To investigate the biotoxicity of MPs on *Drosophila*, various types of MPs have been introduced into the food culture medium, including polystyrene (PS), polyethylene (PE), polyethylene terephthalate (PET), polyamide (PA), and polylactic acid (PLA). The concentrations used ranged from 0.005 mg/L to 25 g/L, and the particle sizes varied from MPs ($\geq 1 \mu\text{m}$) to NPs

(< 1 μ m). PET-MPs and PA-MPs with the concentrations of 10 and 20 g/L could reduce triglyceride, protein and glucose levels in male *Drosophila*, suggesting an effect on energy metabolism.^{[23], [24]} PS was also verified to affect survival rates and alter the ultrastructure of the midgut, ovaries and testes in *Drosophila*.^[25] As a sustainable alternative to traditional plastics, PLA-NPs nonetheless induce structural and molecular disruptions in *Drosophila* larvae.^[26] Additionally, *Drosophila* serves as a promising model for the rapid assessment of MPs mediated toxicity such as cadmium.^[27]

Diverse factors influence the biotoxicity of MPs, including type, particle size, shape, surface charge, exposure concentration and duration, and developmental stage and gender of exposed organisms.^{[21], [28]} However, the toxicological mechanisms associated with these factors remains limited. To clarify the reproductive toxicity mechanism of PS-NPs, transcriptomic analysis was conducted on the ovaries of *Drosophila* following multi-generational exposure. This analysis identified 102 and 208 DEGs in the 1 mg/L and 100 mg/L PS-NPs treatment groups, respectively, compared to the control group.^[29] Similar findings have been observed in the expression of the immune function genes in mussels and oysters.^{[30], [31]}

Currently, researches predominantly examine the effects of short-term exposure to a single type of MPs on the physiology, reproduction and lifespan of *Drosophila*. Although fecundity is also a key component of biological fitness, reflecting an organism's reproductive potential and population sustainability, we prioritize lifespan as our primary

1 endpoint. This is because our study specifically focuses on the aging-inducing effects of
2 long-term high-concentration MPs co-exposure, for which lifespan reduction is the most
3 direct and defining hallmark. We have selected PE and PS as the main co-exposure MPs
4 because the ubiquitous use of PS and PE, their frequent detection in environment and
5 human body. Their prevalence in studies reflect a distribution of PS > PE > PET > PVC.^[32]
6 We hypothesized that irregularly sized and shaped PE and PS MPs can impair the intestinal
7 barrier, and long-term exposure to high concentration PE and PS MPs mixture may affect
8 gene expression and metabolism activity, consequently inducing aging and impacting the
9 lifespan of *Drosophila*. Combined analysis of transcriptome, metabolome and age-related
10 phenotypes was used to explain the potential mechanism of shortened lifespan caused by
11 combined exposure to MPS. The outcomes are expected to bolster our capacity to evaluate
12 the risks posed by MPs.

13 **2. Materials and methods**

14 **2.1 Obtention and characterization of PE and PS MPs**

15 Solid powder of PE and PS with a mesh size of 2000 were purchased from China
16 Petroleum and Chemical Corporation Limited. The morphology of PE and PS MPs were
17 characterized by Scanning Electron Microscope (SEM, Tescan MAGNA, The Czech
18 Republic) and the SEM images were analyzed with ImageJ software to determine the
19 average size of MPs. A Raman spectrometer (Renishaw, UK) was used to identify the types
20 of MPs.

2.2 *Drosophila* strains and PE and PS exposure

In this study, wild type *w¹¹¹⁸* *Drosophila*, obtained from Fungene Biotechnology Co., LTD, were cultured on standard corn medium in an incubator with a relative humidity of 60%, a temperature of 24.5-25.5 °C (constant temperature control, fluctuation range $\pm$ 0.5 °C) and a 12 h light:12 h dark cycle (12L:12D).^[29] This medium contained corn flour, sucrose, brown sugar, agar and yeast.

The study established co-exposure for PE and PS MPs at both low concentrations (10 and 100 mg/L) and high concentrations (10, 20 and 50 g/L) to reflect the multi-pathway, continuous, and accumulative effect of MPs on organisms. To achieve this, a specific, equal mass ratio of mixed PS and PE MPs were added to a solution consisting of 10 ml of pure water and 10 ml of anhydrous ethanol. The mixed MPs solution was sonicated for 30 minutes to ensure uniform distribution. After cooling the medium to 60 °C, the MPs solution was thoroughly mixed with 100 mL of standard corn medium, continuously stirred to integrate. A control group was prepared with standard corn medium containing 10 ml water and 10 ml ethanol. The entire experimental procedure followed the methods of a previous study.^[23]

2.3 PE and PS uptake

A Thermogravimetric Analysis Differential Scanning Calorimetry (TGA/DSC) thermogravimetric analyzer (STA449F5, Netzsch, Germany) couple with a mass spectrometer (QMS 403 Quadro, Netzsch, Germany) were used to analyse PE and PS in the

whole body of *Drosophila*. The body tissue of *Drosophila* in control group, 10 mg/L and 10 g/L PE and PS treatment group were dried at 60 °C and ground into a powder. The dried powder samples of 10 mg were thermally decomposed in a nitrogen atmosphere (50 mL/min). The temperature was increased from 30 to 800 °C at 10 °C/min. All the TGA analyses were blank curve corrected. The mass spectrometer detector, operated in Single Ion Monitoring Mode (SIM), measured the ions m/z 56 for PE and m/z 104 for PS.

To assess the ingestion and distribution of PE and PS MPs in *Drosophila*, we conducted Raman spectroscopic analysis on sliced tissue. The specimens were fixed in 4% paraformaldehyde for over 24 hours, dehydrated through a graded alcohol series, infiltrated with wax, embedded in paraffin blocks, and sectioned to 4 µm thickness. The sections were smoothed in 40 °C warm water, then mounted on slides, dehydrated at 60 °C to remove moisture and wax, and stored at room temperature for analysis.^[25] Using a Renishaw in Via Micro-Raman spectrometer ($\lambda = 532$ nm), we analyzed the particles' distribution in a 120 µm × 120 µm area of the *Drosophila* abdomen via Raman mapping, collecting spectral data from 1600 points at 3 µm intervals. Characteristic peaks at 1296 cm⁻¹ for PE and 1002 cm⁻¹ for PS identified their distribution. ImageJ software determined the average fluorescence intensity in the scanned area.

2.4 Measurement of lifespan-related indicators

2.4.1 Assessment of lifespan

In the lifespan assay, all adult flies were collected within 5-6 hours post-eclosion to

1 ensure uniform age and virgin status. Flies were briefly anesthetized using 0.2-0.5 mL of
2 diethyl ether (10-20 s, sufficient for immobilization without harming survival). Sex was
3 determined based on abdominal morphology: females exhibit a broader, rounded abdomen
4 with interrupted dark stripes, whereas males have a narrower, pointed abdomen with
5 continuous dark stripes. After separation, males and females were housed separately. After
6 separation, male and female flies were housed separately in polypropylene vials (24 mm ×
7 95 mm), with a density of 10 flies per vial to avoid overcrowding. Flies were maintained
8 under the following standardized conditions: temperature 24.5-25.5 °C (fluctuation ±
9 0.5 °C), relative humidity 60% (± 5%), a 12-hour light:12-hour dark cycle (12L:12D)
10 provided by full-spectrum LED strips (simulating natural daylight) inside the incubator, and
11 a calibrated light intensity of 900 lx at the housing level (measured with a lux meter). Each
12 experimental group contained 100 *Drosophila*, with three replicates for reliability. Survival
13 was assessed and recorded daily at the same time, and dead flies were removed immediately.
14 The culture vials were replenished with fresh food every three days to prevent mold growth
15 and nutrient depletion.^[25] The median lifespan was determined by calculating the median
16 survival duration across all flies. The survival rates were analyzed using the log-rank test.

17 **2.4.2 Climbing ability test**

18 After 20 days of rearing, 30 flies under respective exposure conditions were subjected
19 to climbing ability tests. They were gently tapped and vibrated to encourage descent to the
20 test tube's base. We recorded the number of flies that successfully climbed beyond 13 cm

mark within 7 second.^[33] This test was repeated three times per group, with a 30-minute rest between trials to prevent fatigue from affecting performance.

2.4.3 Measurement of adult body weight and length

Adult *Drosophila*, 20-day post-emergence, were carefully collected and placed onto filter paper for the measurement of their weight and length.^[34] Flies were anesthetized using 0.2-0.5 mL of diethyl ether. Body weight was recorded on a Mettler Toledo XS205DU balance (accuracy: 0.01 mg). Body length was measured with a ZOOM-600C stereomicroscope system (Caikang Optical, China) fitted with a calibrated C4 vernier micrometer (0.05 mm per division, total length 5 mm). The experiments were conducted in triplicate to ensure accuracy, with the average values being reported for each parameter.

2.4.4 Evaluation of anti-starvation ability

For the anti-starvation ability test, 20-day-old *Drosophila* of both genders were introduced into a 0.9% agarose medium, which provided hydration without nutritional content.^[35] The vials were maintained under controlled conditions at 25 ± 0.5 °C, $60 \pm 5\%$ relative humidity, and a 12-hour light:12-hour dark cycle. Mortality was recorded daily at a fixed time, and dead flies were promptly removed to limit microbial growth. Each replicate involved 100 flies, and the experiment was repeated three times to ensure reliable results.

2.4.5 Assessment of intestinal barrier function

Following a 20-day exposure, both male and female *Drosophila* were transferred to a medium containing 2.5% brilliant blue (Erioglaucine disodium salt) to evaluate intestinal

barriers integrity. They were divided equally among 10 tubes per group, each containing 10 flies. After 5 days, the percentage of flies exhibiting a blue coloration for each group, referred to as "Smurfs," was determined as an intestinal permeability indicator.^[36]

2.5 Metabolomics Analysis

For the metabolomics study, whole-body *Drosophila* tissue in control and PE+PS group after 20 days feeding were analyzed with triplicates for each group. Samples were extracted with 50% methanol, and analyzed via UPLC-MS on a Vanquish Flex UPLC system (Thermo Fisher Scientific, Bremen, Germany) utilizing an ACQUITY UPLC T3 column (100 mm × 2.1 mm, 1.8 μm, Waters, Milford, USA) for reversed-phase separations at a controlled oven temperature of 35 °C. The mobile phase consisted of solvent A (water, 0.1% formic acid) and solvent B (Acetonitrile, 0.1% formic acid). The flow rate was 0.4 ml/min. Metabolites were identified using a Q-Exactive mass spectrometer (Thermo Scientific) and annotated against Kyoto Encyclopedia of Genes and Genomes (KEGG) and Human Metabolome Database (HMDB) databases with a 10 ppm mass tolerance. Data was preprocessed with XCMS software and analyzed by PCA, t-tests, and PLS-DA, identifying differential metabolites based on VIP, P value, and fold change. We followed established protocols from LC-Bio Technology CO., Ltd (Hangzhou, China) for metabolomic analyses. More details are fully described in the supplementary material.

2.6 Transcriptomic analysis

Total RNA was extracted from the whole-body tissue of *Drosophila* in control and

PE+PS group after 20 days feeding, using the trizol reagent (Thermo Fisher, 15596018). The quality and quantity of the extracted RNA were further validated using a Bioanalyzer 2100 and RNA 6000 Nano LabChip Kit (Agilent, CA, USA, 5067-1511). High-quality RNA, with RNA Integrity Number value exceeding 7.0, was selected for sequencing library construction. mRNA was purified using Dynabeads Oligo (Thermo Fisher, CA, USA) and sequenced on an Illumina Novaseq™ 6000 platform. Transcript expression levels were quantified using StringTie and ballgown package, with DEGs identified through DESeq2 and edgeR software, classified by false discovery rate (FDR) < 0.05 and fold change ≥ 2. These DEGs were analyzed for Gene Ontology (GO) and KEGG pathways enrichment. Transcriptomic analyses also followed the established protocols from LC-Bio Technology CO., Ltd (Hangzhou, China), as shown in the Supporting Material.

2.7 Correlation analysis of transcriptomic and metabolomic

We conducted a correlation analysis utilizing data from both transcriptomic and metabolomic profiles to investigate the relationship between DEGs and identified metabolites. A signed clustering correlation heat map was generated using the OmicStudio platform.

2.8 Statistical analyses

Statistical analyses were performed using GraphPad Prism software, version 7. The lifespan and starvation survivorship data were analyzed using log-rank assays. Differences among groups were assessed employing one-way ANOVA, followed by Tukey's post hoc

HSD test. A $P < 0.05$ was considered significant for all statistical analyses.

3. Results and Discussion

3.1 Characterization and internalization of PE and PS MPs

SEM images demonstrated a uniform dispersion and irregular morphology of PE MPs (Fig. 1A), with an average particle size of $14.55 \pm 5.97 \mu\text{m}$ (Fig. 1C). While PS MPs displayed a more regular shape (Fig. 1B) and a notably smaller average size of $1.86 \pm 0.89 \mu\text{m}$ (Fig. 1D). Raman spectroscopy confirmed their distinct chemical identities by analyzing the unique spectral peaks for PE and PS standard substance (Fig. 1E).

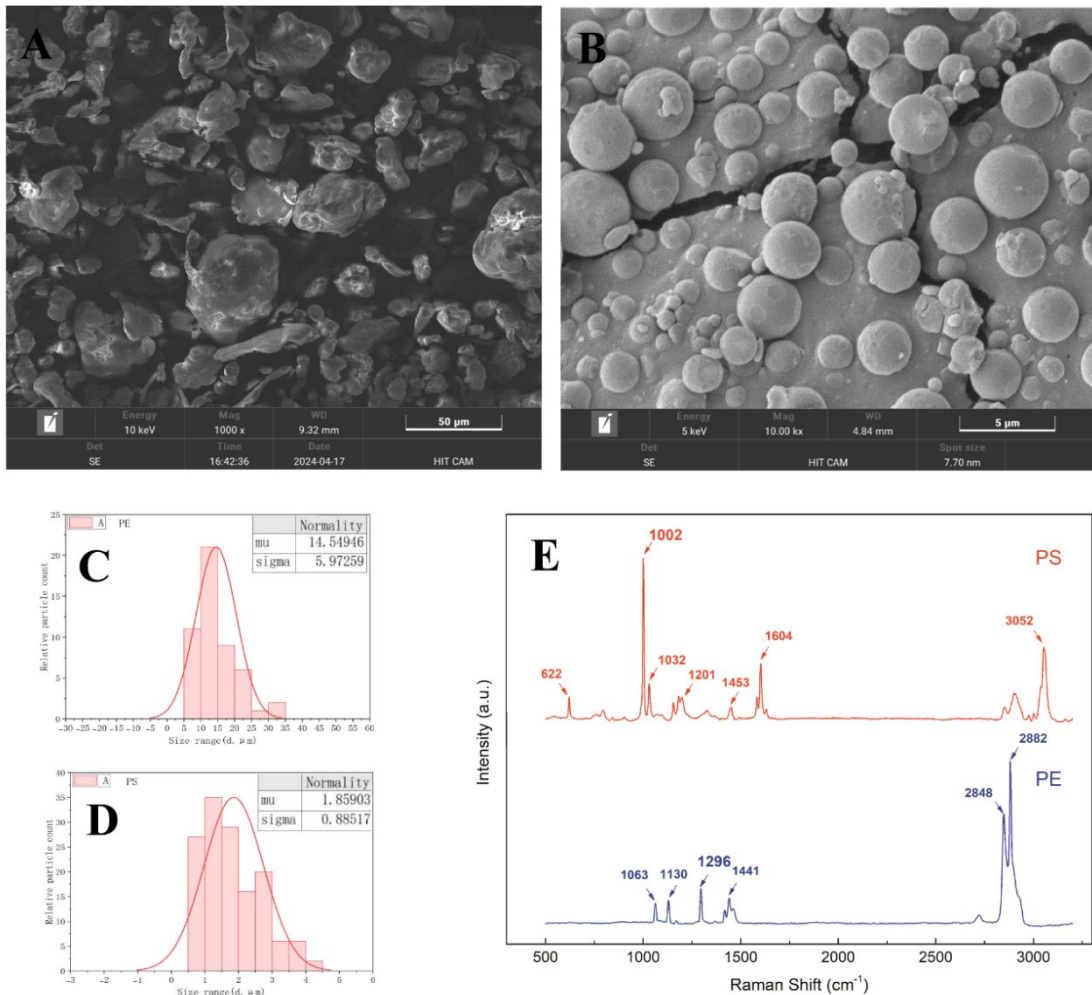


Fig. 1 SEM images of PE-MPs (A) and PS-MPs (B); Histogram of size frequencies of PE-MPs (C) and PS-MPs (D); Raman spectra of PE and PS (E).

The TGA-MS analysis (Fig. S1) demonstrated distinct peak intensities at m/z 56 for PE and at m/z 104 for PS in their respective standard substances. Notably, the signal intensity for both PE and PS in flies cultured in a 10 g/L mixture of PE and PS for 50 days showed a modest increase, while the flies cultured in a 10 mg/L PE and PS exhibited no significant difference from the control group.

To confirm the internalization of MPS, Raman spectroscopy was used for high concentration group. In the randomly selected scanning regions (Fig. 2A to 2C), the size and brightness of Raman signal spot increased with exposure concentration at the characteristic peaks of 1296 cm^{-1} for PE and 1002 cm^{-1} for PS (Fig. 2D to 2I). ImageJ analysis revealed that the average fluorescence intensity for PE across the control, 10 g/L and 50 g/L group were 101.12, 159.45 and 163.23, respectively. For PS, the intensities were 88.39, 156.78 and 171.61, respectively. These results showed that both PE and PS MPs were ingested by *Drosophila*, with ingestion levels rising in tandem with exposure concentration.

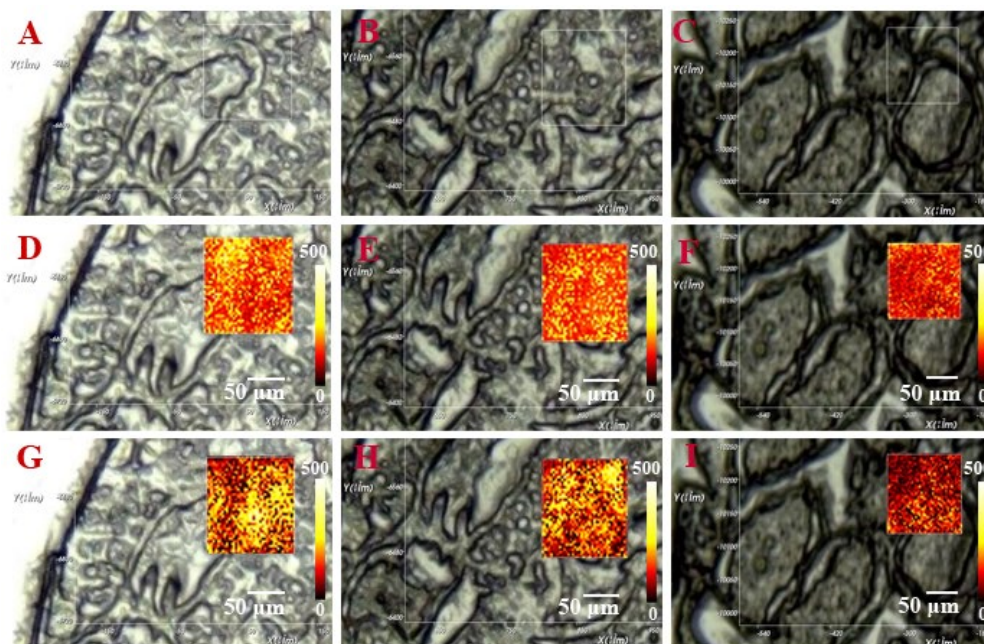


Fig. 2 Raman analysis of ingested PE and PS. Raman mapping test area of different exposure concentrations of 50 g/L (A), 10 g/L (B), and control (C). Raman signal strength at peak 1296⁻¹ for PE of 50 g/L (D), 10 g/L (E), and control (F). Raman signal strength at peak 1002⁻¹ for PS of different exposure concentrations of 50 g/L (G), 10 g/L (H), and control (I).

3.2 Effects of PE and PS MPs on lifespan of *Drosophila*

Long-term exposure diet enriched with high levels of PE and PS MPs substantially reduced the lifespan of *Drosophila*. Exposure to low concentrations of PE and PS MPs at 10 and 100 mg/L did not significantly alter the lifespan of *Drosophila* with median lifespan of 46.94 ± 2.06 and 48.23 ± 1.28 days, respectively. Notably, the slight increase in male lifespan at 100 mg/L may be attributed to a toxicity threshold saturation effect, whereby exposure to this concentration range activates stress defense mechanisms (e.g., enhanced antioxidant enzyme activity or upregulation of DNA repair genes) in male *Drosophila*,

1 helping to maintain metabolic stability and partially counteract the toxic effects. Similar
2 hormesis effect were also discovered in previous reports, where 1 g/L of PET-MPs prolongs
3 the lifespan of male flies.^[37] This low-concentration dynamic is distinct from the robust,
4 linear dose-response lifespan shortening observed at our primary experimental
5 concentrations (10-50 g/L). When subjected to the MPs mixture at concentrations of 10, 20
6 and 50 g/L, the median lifespan of female flies decreased to 32.67 ± 2.31 , 27.33 ± 2.08 , and
7 19.33 ± 3.88 days, respectively, compared with the 51.32 ± 1.00 days in control group. A
8 comparable pattern was noted in male flies, with median lifespan decreasing from $46.53 \pm$
9 0.73 to 23.67 ± 2.31 days as the concentration of MPs increased (Fig. 3A to 3D). It is
10 recognized that *Drosophila*, along with many other insects, exhibit sex-specific responses to
11 a variety of physiological, environmental and ecological stressors.^{[38], [39], [40]} In this study,
12 this sex-specific response to MPs was apparent at the concentration of 10 g/L with females
13 showing a higher survival rate compared to males at equivalent lifespan stages. This result
14 is consistent with the research from Urbisz et al.^[25] However, at higher concentrations of 20
15 and 50 g/L, these sex differences were unobvious. This phenomenon is directly related
16 to the physiological and metabolic characteristics of *Drosophila*. Inherent differences in
17 energy reserve efficiency and immune pathway activity likely grant females greater
18 resilience under low-level stress, whereas the potent toxicity of high concentrations
19 overwhelms these sex-specific tolerance thresholds. Additionally, the mean lifespan of male
20 and female *Drosophila* in 20 g/L of PE and PS exposure decreased by 45.28% and 45.75%

1 compared to control, respectively, which is higher than that of 16.01% and 10.69% caused
2 by 20 g/L PET MPs (2 μ m) exposure.^[37] This indicated that co-exposure to PE and PS had
3 a more pronounced impact on lifespan than exposure to PET alone, which may be related to
4 the type of MPs, because PET is more bioinert as it is often used in food packaging.^{[39], [41]}
5 Another reason could be stem from the synergistic effect of their distinct physicochemical
6 properties. The irregular shape of PE may exacerbate physical damage to the gut epithelium,
7 while the small particle size of PS ($1.86 \pm 0.89 \mu$ m) facilitates tissue barrier penetration.
8 Their combined action results in additive damage, ultimately leading to more severe
9 lifespan shortening, which is fully consistent with our subsequent intestinal barrier function
10 assessment (the Smurf assay).

11 From a real-world exposure perspective, although environmental microplastic
12 concentrations are typically in the microgram to milligram per liter range, they can
13 accumulate over time in pollution hotspots such as landfills and wastewater treatment plants
14 to reach the high concentrations tested here. Thus, our findings provide direct experimental
15 evidence for assessing ecological risks in such environments.

16 Given the pronounced effects on lifespan observed at high concentrations, we focused
17 our subsequent analysis of phenotypic and multi-omics on these exposure groups to further
18 elucidate the mechanistic basis of the observed toxicity.

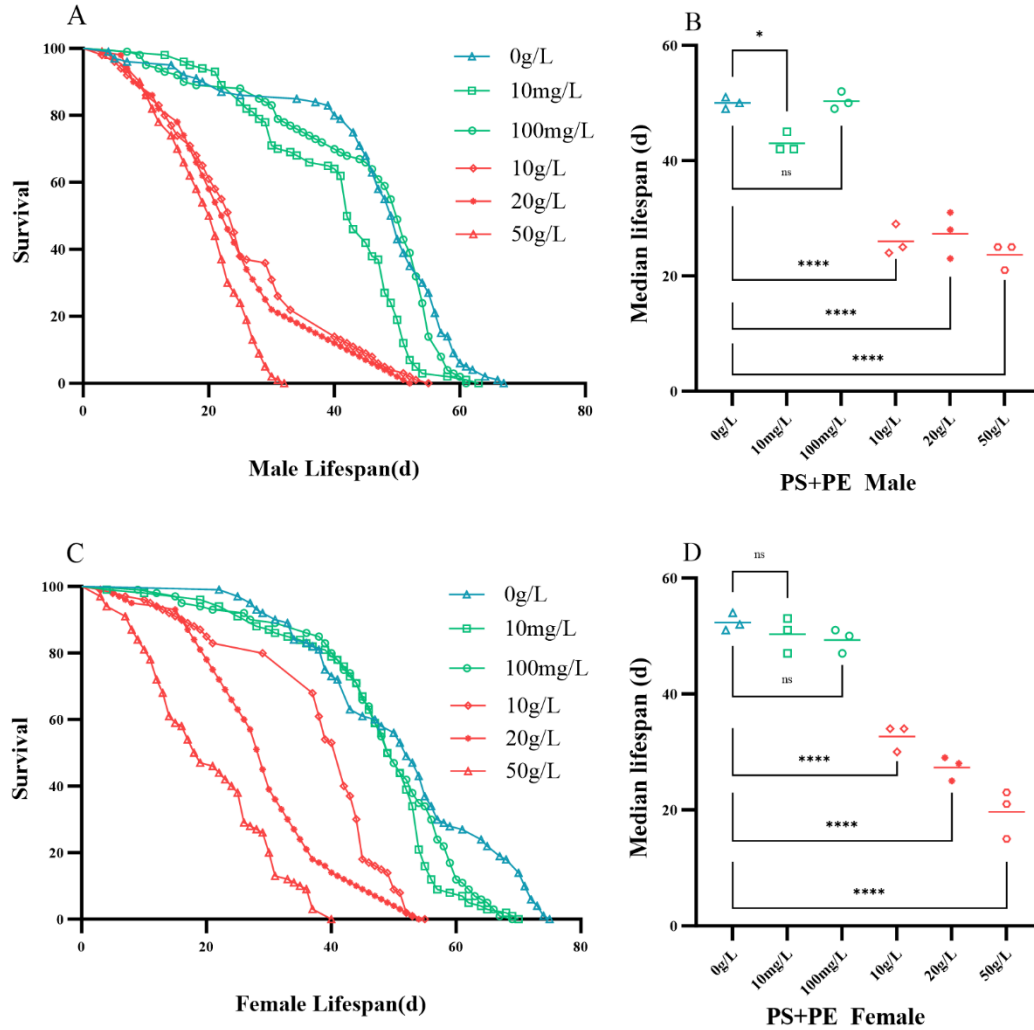


Fig. 3 The effect of PS+PE on the lifespan of *Drosophila*. Statistical analysis for survival curves of male flies (A) and female flies (C) was performed by log-rank test (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). (B, D) Summary of median survival for independent lifespan experiments of male flies (B) and female flies (D). Data are presented as scatter plots and the horizontal line indicates the mean of these three median values. (n=3 independent experiments per group). Statistical analysis was performed by one-way ANOVA with Tukey' post hoc test (n/s, $P > 0.05$, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

3.3 Effects of PE and PS MPs on age-related phenotypes of *Drosophila*

Weight, climbing ability, anti-starvation ability and gut barrier integrity are classic aging phenotypes in *Drosophila*.^[42] Male flies subjected to PS and PE MPs showed a notable reduction in body weight and length, contrasting with the control group, while females displayed an increase (Fig. 4A, B). The climbing assay, a standard measure of *Drosophila*'s spontaneous activity, revealed a dose-dependent reduction in motor function upon exposure to PS and PE MPs (Fig. 4C). The study results reported by Zhang et al. also showed that exposure to PS alone could reduce the climbing ability of *Drosophila*, and this effect was more serious after combined exposure to Cd.^[27] Similarly, the survival curve under starvation conditions indicated a diminished capacity for hunger resistance with increasing MPs concentrations (Fig. 4E, F), and the anti-starvation ability of females was stronger than that of males. Exposure to PET and PA MPs also had similar results, accompanying with a reduced triglyceride, protein and glucose content in males, indicating that energy metabolism of male *Drosophila* was affected by MPs ingestion.^{[23], [24]} In *Drosophila*, lifespan reduction is often associated with intestinal epithelial barrier dysfunction.^[43] The Smurf assay corroborated this, demonstrating a decline in gut barrier integrity post-exposure to PS and PE MPs (Fig. 4D), which was consistent with the previous study.^[27]

The sex-specific phenotypic changes, such as the decreased body weight and length in males while the increased body weight in females, are directly linked to sexually dimorphic

1 regulation of energy metabolism. Transcriptomic data revealed significant downregulation
2 of genes related to energy metabolism (e.g., Pepck2) in males, leading to insufficient energy
3 synthesis and restricted growth. In contrast, females activated compensatory metabolic
4 adjustments through upregulation of stress-response genes such as heat shock protein Hsp70,
5 which helped maintain body weight but at the cost of depleting substantial energy reserves.
6 This also explains why females, despite gaining weight, still exhibited significantly
7 shortened lifespan.

8 The dose-dependent decline in climbing ability directly reflects microplastic-induced
9 impairment of neuromuscular function, corresponding to disruption of the neuroactive
10 ligand–receptor interaction pathway. Similarly, reduced starvation resistance aligns with
11 metabolomic abnormalities in the alanine, aspartate, and glutamate metabolism pathways,
12 which are the core routes for energy-producing substrate synthesis. Crucially, intestinal
13 barrier damage (evidenced by increased "Smurf" flies) emerged as the central node linking
14 exposure to lifespan shortening. The breached gut barrier facilitates particle internalization
15 and triggers immune responses. The resulting competition for cellular resources between
16 immunity and metabolism establishes a vicious cycle of gut damage, immune dysregulation
17 and energy depletion. Taken together, the dose-dependent decline in climbing ability,
18 reduced starvation resistance, and compromised intestinal barrier function paint a coherent
19 picture of physiological decline. This logical cascade is fully consistent with our
20 multi-omics data, collectively demonstrating that high-concentration PE/PS co-exposure

drives accelerated aging through interconnected physiological disruptions.

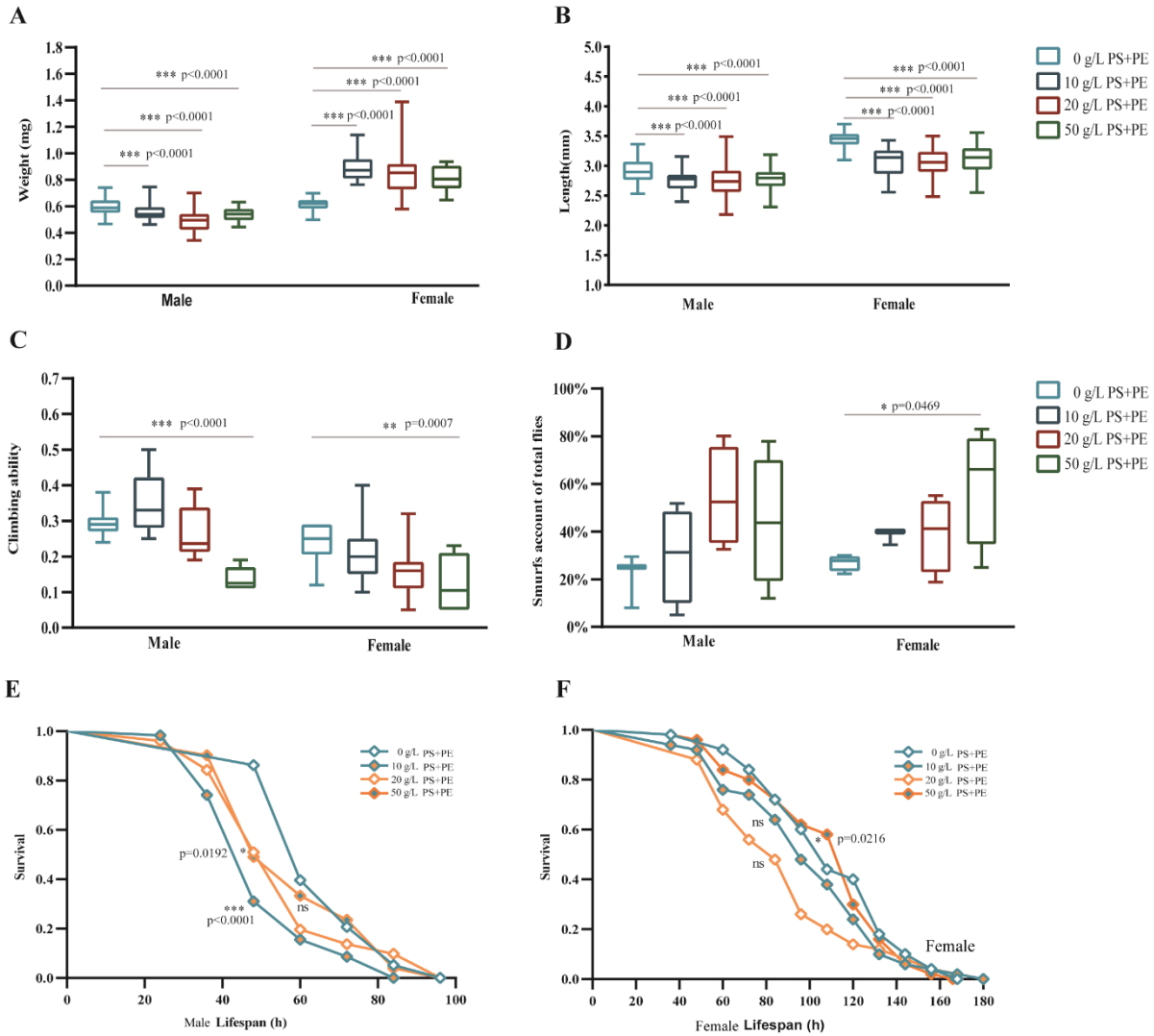


Fig. 4 The effect of different concentrations of PS+PE on *Drosophila* body weight, body length, climbing ability, intestinal barrier, starvation resistance. (A) Effect of PS+PE on the body weight. (B) Effect of PS+PE on the body length. (C) Effect of PS+PE on the climbing ability. (D) Effect of PS+PE on the intestinal barrier. (E, F) Effect of PS+PE on the starvation resistance of flies. (E) Male flies. (F) Female flies. Data are presented as box-and-whisker plots, where the box represents the interquartile range (IQR), the horizontal line inside the box indicates the median, and the whiskers extend to the minimum and

maximum values, analyzed by one-way ANOVA with Tukey' post hoc test. Statistical analysis for survival curves (E, F) was performed by log-rank test (n/s, $P > 0.05$, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$).

3.4 Effects of PE and PS MPs on global metabolomics profiles in the *Drosophila*

The PCA scores plot, with QC samples tightly clustered as purple dots, demonstrated a high stability and reliability of our metabolomics platform (Supporting Material Fig. S2). PLS-DA analysis distinctly segregated the two groups of samples (Fig. S3 A and C). R²_Y and Q² values exceeding the threshold of 0.5 indicated the model's goodness of fit and predictive ability, with specific values of 0.998 and 0.733 for females, and 0.999 and 0.853 for males, respectively. The comparison of R²_Y and Q² values in Fig. S3 B and D, illustrating lower values on the left side versus higher on the right, confirmed the model's comprehensive explanatory and robust predictive capabilities for the observed sample variations.

Metabonomic analysis revealed significant metabolic disparities between the control and PE+PS groups, identifying 24 distinct metabolites in males and 7 in females, as depicted in the clustering heat maps Fig. 5 and Supporting Material Table S1. In males, exposure to PE and PS MPs led to significant changes in organic acids and derivatives, organic heterocyclic compounds, lipid molecules, phenylpropanoids and polyketides, organic oxygen compounds, nucleosides, nucleotides and analogues. Among these, Lysophosphatidylcholine (Lyso PC 18:0, LPC), a glycerophospholipid and lipid-like molecule, is recognized for its anti-inflammatory properties.^[44] LPC containing

1 docosaehaenoic acid at the sn-1 position and the 18:0 LPC can inhibit inflammatory
2 mediator release, such as TNF- α , IL-6, IL- α , IL-1 β , and IL-10.^{[45], [46]} Furthermore, the
3 balance between pro-inflammatory cytokines and anti-inflammatory cytokines is crucial for
4 delaying aging. In females, exposure to PE and PS MPs induced significant alterations in
5 organic heterocyclic compounds, lipid molecules, organic acids and derivatives, as well as
6 nucleosides, nucleotides and analogues. Notably, glutamine, categorized under organic
7 acids and derivative, is intricately linked to the aging process due to its vital role in cell
8 proliferation and survival.^{[47], [48]} Glutamine serves as a precursor for essential molecules
9 including amino acids and nucleotide. It is instrumental in the synthesis of nicotinamide
10 adenine dinucleotide phosphate (NADPH), glutathione (GSH) and adenosine triphosphate
11 (ATP), which are crucial for redox homeostasis and energy supply.^[49] Meanwhile, aging is
12 commonly characterized by energy generation reduction^[50] and redox homeostasis
13 dysfunction.^[51] Therefore, changes of LPE, LPC, L-Glutathione and glutamine may
14 contribute to aging by disrupting energy metabolism.

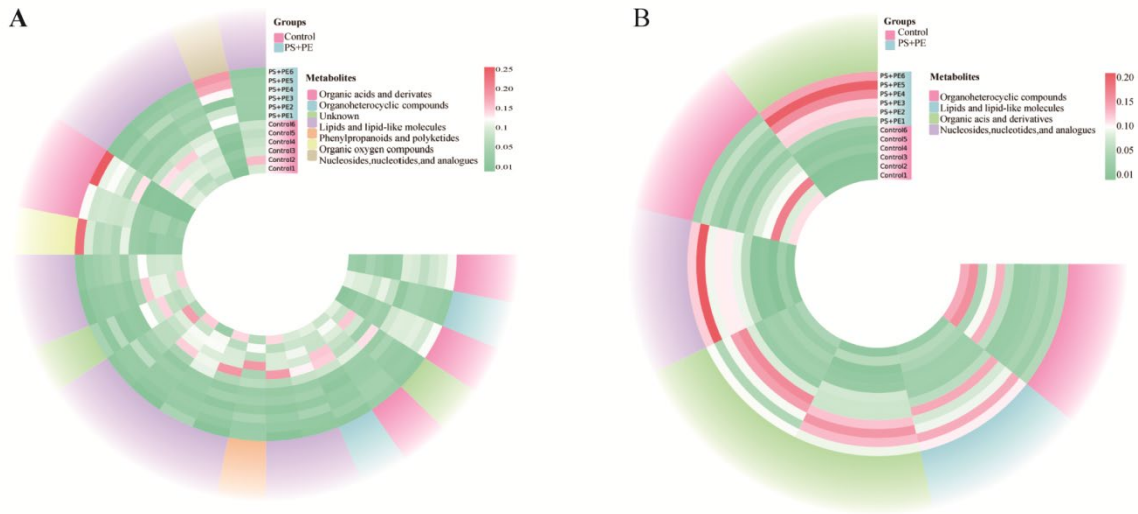


Fig. 5 Heatmaps of differential metabolites compared between control and PS+PE exposure groups in male flies (A) and female flies (B).

Enrichment analysis visualized via bubble diagram revealed distinct KEGG pathways of metabolites enrichments in male and female flies. In males, the most significantly enriched pathways included ABC transporters, alanine, aspartate and glutamate metabolism, and purine metabolism (Fig. 6A). Females showed predominant enrichment in ABC transporters, choline metabolism in cancer, and metabolism of alanine, aspartate, glutamate, cysteine and methionine (Fig. 6B). Notably, ABC transporters were the most enriched pathway in both genders, highlighting their crucial role in insect physiology.^[52] This pathway involves a family of integral membrane proteins essential for the transmembrane transport of various molecules.^[53]

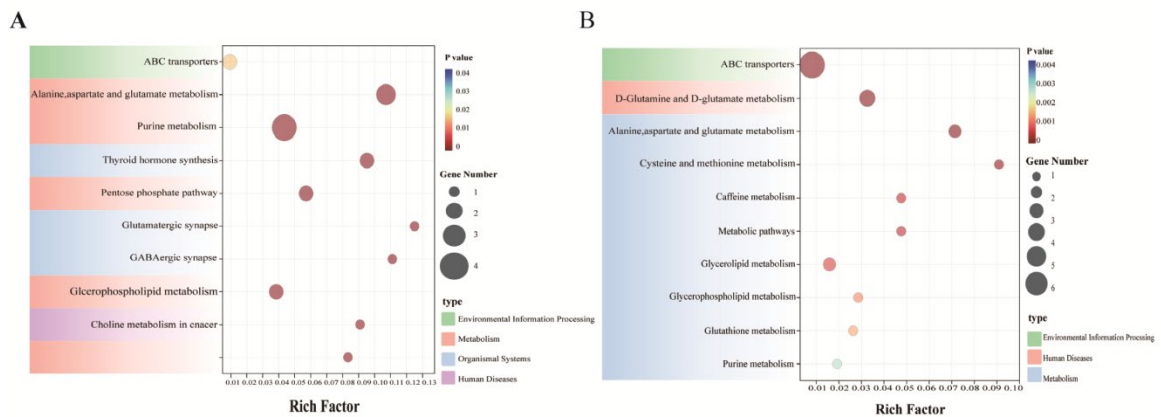


Fig. 6 Metabolic pathways analysis of PS+PE exposure on *Drosophila* for the male flies (A) and female flies (B). The top 10 pathways of significant of the up-regulated and down-regulated DEMs on KEGG. The X-axis was the rich factor, the Y-axis represents the name of the pathway. The bubbles size represents the number of differential expression metabolites involved. The bubbles color indicates the enrichment degree of pathway.

3.5 Effects of PE and PS MPs on global transcriptomics profiles in the *Drosophila*

In our study, we detected significant changes in gene expression profiles, with a total of 286 DEGs in males and 205 DEGs in females, when comparing the PE+PS exposure group to the control group. Notably, the majority of these DEGs were implicated in the regulation of energy metabolism (Fig. 7). Phosphoenopyruvate carboxykinase (PEPCK), a pivotal regulatory enzyme in gluconeogenesis, glyceroneogenesis, serine synthesis, and amino acid metabolism, is stringently regulated at the transcriptional level. [54] UDP-glycosyltransferases (UGTs), a widespread superfamily of enzymes, mediate biotransformation and play crucial roles in detoxifying harmful exogenous chemicals and

1 modulating signaling molecules.^[55] The immune response to MPs may exacerbate energy
2 constraints, diverting energy from growth and reproduction to maintenance, which includes
3 energetically costly cellular processes.^[56] These metabolic and immune disruptions caused
4 by MPs could increase the susceptibility of organism to further stress from pathogens, other
5 pollutants, and environmental factors, potentially affecting lifespan. Heat shock proteins
6 (Hsps) are crucial in regulating protein structure and folding.^[57] They essential for
7 *Drosophila's* stress resistance, including thermal and environmental stressors.^[58] Hsp70, a
8 predominant Hsp family member, serves as a sensitive bioindicator for environmental
9 monitoring due to its role in cellular defense mechanisms under pollutant stress.^[59] In this
10 study, a significant upregulation of the Hsp70 gene in female flies was observed after
11 exposure to PS and PE MPs, indicating that such exposure triggers a stress response. This
12 upregulation is likely a compensatory mechanism to mitigate the stress induced by PE and
13 PS MPs. This is consistent with observations that female *Drosophila* exhibit a greater
14 climbing ability, a enhanced hunger resistance, and a higher survival rate at 20 days
15 compared to male flies. Our findings align with those of Brandts et al., who noted an
16 increase in Hsp70 gene expression in mussels' digestive glands exposed to PS nanoplastic.
17 The digestive gland of mussels are key organs for xenobiotic metabolism and detoxification,
18 widely used as sensitive indicators in aquatic toxicology.^[60] Given proteotoxicity's role in
19 aging, Hsps may enhance *Drosophila's* stress resistance by mediating protein refolding or
20 degradation.^[61]

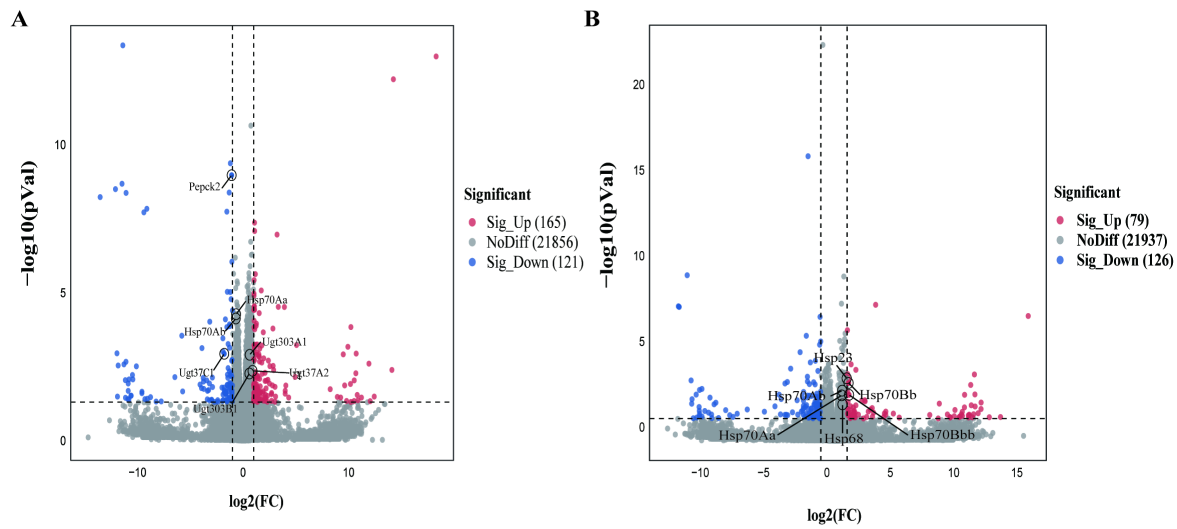


Fig. 7 The effect of PS+PE exposure on global transcriptomics profiles. Volcano plot of distribution trends for differentially expressed genes between control and PS+PE exposure groups. The negative trends for differentially expressed genes between control and PS+PE exposure groups. The negative $\log_{10}$ -transformed P-values of the t-test were plotted against the $\log_2$ -transformed fold change in the control-treatment experiment. Red circle and blue circle, up- and down-regulation of DEGs, respectively. Grey dots indicate that the genes were not differentially expressed. (A) Male flies. (B) Female flies.

These DEGs were categorized across 201 biological processes, 70 cellular components, and 138 molecular functions (Fig. 8). In male flies, KEGG enrichment analysis revealed interference in the Toll and Imd signaling pathways after PE and PS co-exposure, along with perturbations in Lysosome, Neuroactive ligand-receptor interaction, and Drug metabolism - cytochrome P450 pathways. Female flies displayed a distinct enrichment in pathways such as Insect hormone biosynthesis, Longevity regulating pathways - multiple species, Spliceosome, and Autophagy-animal. Network analysis of these pathways indicated that some genes participate in multiple signaling pathways simultaneously (Fig. 8). Notably,

1 in *Drosophila*, humoral immunity is primarily mediated by the Toll and Imd signaling
2 pathway.^[62] The Toll pathway, activated in response to systemic infections, plays a key role
3 in adipose tissue growth, metabolism, and hemocyte activation.^[63] The Imd pathway is
4 essential for bacterial defense,^[64] controlling the expression of most antimicrobial peptides,
5 highlighting its role in immune homeostasis.^[65] The intestinal epithelium is a recognized
6 immunoactive tissue, playing a key role in longevity regulation.^[66] Microplastics can
7 induce an immune response through mechanical damage to the intestinal epithelium, while
8 excessive immune activation can hasten degeneration, cause inflammation, and reduce
9 lifespan.^[43] The Smurf assay results provided strong confirmation of this association.

10 **3.6 Combined analysis of transcriptomics and metabolomics**

11 Combined analysis of transcriptomics and metabolomics has revealed the involvement
12 of multiple DEGs and metabolites in energy metabolism pathways (Fig. 9). A pronounced
13 interconnection between immune and metabolic processes is particularly evident in the gut.
14 Enriched ABC transporters pathway suggests a compensatory stress response where flies
15 upregulate transporter expression to expel microplastics or their metabolites. However, this
16 ATP-intensive process indirectly exacerbates energy metabolic stress, directly linking to the
17 abnormal expression of genes like *Pepck1/2* in the transcriptome. Among the differential
18 metabolites, aberrant levels of the anti-inflammatory mediator LPC disrupt immune
19 homeostasis, leading to chronic inflammation. Concurrently, disrupted glutamine
20 metabolism, a precursor for energy metabolism and the antioxidant system, results in

1 insufficient synthesis of NADPH, GSH, and ATP, triggering oxidative stress and energy
2 deficiency. Together, these drivers contribute to aging phenotypes (shortened lifespan,
3 reduced starvation resistance). In the transcriptome, the upregulation of heat shock protein
4 genes (e.g., Hsp70, Hsp23) represents a critical stress response, repairing protein damage at
5 a heightened metabolic cost. While dysregulation of the Toll/Imd signaling pathway in
6 males compromises innate immunity, corroborating the observed intestinal barrier damage.
7 In summary, microplastics exert toxicity via a chain reaction: Physical damage (gut barrier
8 disruption) – molecular dysregulation (gene/metabolite abnormalities) – physiological
9 functional decline (lifespan/phenotypic alterations), with the interplay between immune and
10 energy metabolism dysregulation serving as the central mechanism. This conclusion is
11 supported by phenotypic, transcriptomic, and metabolomic data, forming a coherent and
12 logical framework.

13 To reinforce the reliability of the omics findings, we have bolstered our conclusions
14 with two lines of validation. First, the consistency of the proposed core mechanisms (energy
15 metabolism disruption, immune activation, oxidative stress) with established literature is
16 supported by independent wet-lab experiments. For instance, Ranjan et al. demonstrated
17 that PE MP exposure upregulates oxidative stress-related genes (e.g., Sod2, Cat) in
18 *Drosophila* via RT-qPCR and ROS assays, ^[67] aligning with our transcriptomic results.
19 Similarly, Jia et al. validated MP-induced intestinal barrier damage through Western blot
20 and immunohistochemistry, ^[68] which corresponds with our Smurf assay and multi-omics

1 associated with immune function and energy metabolism. These insights significantly
2 enhance our comprehension of the molecular mechanisms that underlie the physiological
3 and behavioral impairments observed in *Drosophila* when subjected to MPs exposure.

4 To build upon these findings and address the study's limitations, we outline the
5 following future directions. First, incorporating fecundity measurements will provide a
6 more comprehensive assessment of organismal fitness, thereby refining our understanding
7 of the ecological risks posed by microplastic co-exposure to terrestrial invertebrates. Second,
8 to further validate the omics-based mechanisms proposed here, we plan to procure a
9 *Drosophila* strain with an identical genetic background, meticulously replicate the rearing
10 and exposure conditions, and perform targeted experiments. This will include using
11 RT-qPCR to verify the expression patterns of core differentially expressed genes (e.g., Sod2,
12 Cat, Toll, Pepck2) and employing ELISA to quantify key molecules (e.g., intestinal
13 tight-junction proteins and oxidative stress markers), ensuring the robustness and
14 completeness of the mechanistic framework.

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1 **Author contributions**

2 Yingyu Liu: Data curation, Investigation. Cheng Wang: Formal analysis. Caixia Wang:
3 Writing- Original draft. Longhuan Fu: Investigation. Yunbo Zhang: Writing- Reviewing and
4 Editing. Zhuo Gao: Visualization. Zhugen Yang: Writing- Reviewing and Editing. Fanyu
5 Meng: Conceptualization, Methodology, Supervision.

6 **Data Availability**

7 Data will be made available on request.

References

- [1] Thompson, R.C., Courtene-Jones, W., Boucher, J., Pahl, S., Raubenheimer, K. and Koelmans, A.A. 2024. Twenty years of microplastic pollution research-what have we learned? *Science* 386(6720), eadl2746.
- [2] Vethaak, A.D. and Legler, J. 2021. Microplastics and human health. *Science* 371(6530), 672-674.
- [3] Yang, L., Kang, S., Luo, X. and Wang, Z. 2024. Microplastics in drinking water: A review on methods, occurrence, sources, and potential risks assessment. *Environ Pollut* 348, 123857.
- [4] Hussain, K.A., Romanova, S., Okur, I., Zhang, D., Kuebler, J., Huang, X., Wang, B., Fernandez-Ballester, L., Lu, Y., Schubert, M. and Li, Y. 2023. Assessing the Release of Microplastics and Nanoplastics from Plastic Containers and Reusable Food Pouches: Implications for Human Health. *Environ Sci Technol* 57(26), 9782-9792.
- [5] Leslie, H.A., van Velzen, M.J.M., Brandsma, S.H., Vethaak, A.D., Garcia-Vallejo, J.J. and Lamoree, M.H. 2022. Discovery and quantification of plastic particle pollution in human blood. *Environ Int* 163, 107199.
- [6] Ragusa, A., Svelato, A., Santacroce, C., Catalano, P., Notarstefano, V., Carnevali, O., Papa, F., Rongioletti, M.C.A., Baiocco, F., Draghi, S., D'Amore, E., Rinaldo, D., Matta, M. and Giorgini, E. 2021. Plasticenta: First evidence of microplastics in human placenta. *Environ Int* 146, 106274.
- [7] Chen, C.Y. and Lin, Z. 2024. Exploring the potential and challenges of developing physiologically-based toxicokinetic models to support human health risk assessment of microplastic and nanoplastic particles. *Environ Int* 186, 108617.
- [8] Zuri, G., Karanasiou, A. and Lacorte, S. 2023. Human biomonitoring of microplastics and health implications: A review. *Environ Res* 237(Pt 1), 116966.
- [9] Ramsperger, A., Narayana, V.K.B., Gross, W., Mohanraj, J., Thelakkat, M., Greiner, A., Schmalz, H., Kress, H. and Laforsch, C. 2020. Environmental exposure enhances the internalization of microplastic particles into cells. *Sci Adv* 6(50).
- [10] Purayil, N.C., Thomas, B. and Tom, R.T. 2024. Microplastics - A major contaminant in marine macro algal population: Review. *Mar Environ Res* 193, 106281.
- [11] Yakubu, S., Miao, B., Hou, M. and Zhao, Y. 2024. A review of the ecotoxicological status of microplastic pollution in African freshwater systems. *Sci Total Environ* 946, 174092.
- [12] Yong, C.Q.Y., Valiyaveetil, S. and Tang, B.L. 2020. Toxicity of Microplastics and Nanoplastics in Mammalian Systems. *Int J Environ Res Public Health* 17(5).
- [13] Bhat, R.A.H., Sidiq, M.J. and Altinok, I. 2024. Impact of microplastics and nanoplastics on fish health and reproduction. *Aquaculture* 590, 741037.
- [14] Kumar, V., Umesh, M., Chakraborty, P., Sharma, P., Sarojini, S., Basheer, T., Kaur, K., Pasrija, R. and Barcelo, D. 2024. Origin, ecotoxicity, and analytical methods for microplastic detection in aquatic systems. *TrAC Trends in Analytical Chemistry* 170, 117392.
- [15] Hirt, N. and Body-Malapel, M. 2020. Immunotoxicity and intestinal effects of nano- and microplastics: a review of the literature. *Part Fibre Toxicol* 17(1), 57.
- [16] Wang, K., Li, J., Zhao, L., Mu, X., Wang, C., Wang, M., Xue, X., Qi, S. and Wu, L. 2021. Gut microbiota protects honey bees (*Apis mellifera* L.) against polystyrene microplastics exposure risks. *J*

- Hazard Mater 402, 123828.
- [17] Horton, A.A., Walton, A., Spurgeon, D.J., Lahive, E. and Svendsen, C. 2017. Microplastics in freshwater and terrestrial environments: Evaluating the current understanding to identify the knowledge gaps and future research priorities. *Sci Total Environ* 586, 127-141.
 - [18] Jemec Kokalj, A., Dolar, A., Drobne, D., Škrlep, L., Škapin, A.S., Marolt, G., Nagode, A. and van Gestel, C.A.M. 2022. Effects of microplastics from disposable medical masks on terrestrial invertebrates. *J Hazard Mater* 438, 129440.
 - [19] de Souza Machado, A.A., Kloas, W., Zarfl, C., Hempel, S. and Rillig, M.C. 2018. Microplastics as an emerging threat to terrestrial ecosystems. *Glob Chang Biol* 24(4), 1405-1416.
 - [20] Liang, J., Ji, F., Wang, H., Zhu, T., Rubinstein, J., Worthington, R., Abdullah, A.L.B., Tay, Y.J., Zhu, C., George, A., Li, Y. and Han, M. 2024. Unraveling the threat: Microplastics and nano-plastics' impact on reproductive viability across ecosystems. *Sci Total Environ* 913, 169525.
 - [21] Liu, H.P., Cheng, J., Chen, M.Y., Chuang, T.N., Dong, J.C., Liu, C.H. and Lin, W.Y. 2022. Neuromuscular, retinal, and reproductive impact of low-dose polystyrene microplastics on *Drosophila*. *Environ Pollut* 292(Pt B), 118455.
 - [22] Brandt, A. and Vilcinskis, A. 2013. The Fruit Fly *Drosophila melanogaster* as a Model for Aging Research. *Adv Biochem Eng Biotechnol* 135, 63-77.
 - [23] Zhong, L., Jin, H., Tang, H., Xu, Y., Liu, X. and Shen, J. 2022. Intake of polyamide microplastics affects the behavior and metabolism of *Drosophila*. *Chemosphere* 308(Pt 3), 136485.
 - [24] Shen, J., Liang, B., Zhang, D., Li, Y., Tang, H., Zhong, L. and Xu, Y. 2021. Effects of PET microplastics on the physiology of *Drosophila*. *Chemosphere* 283, 131289.
 - [25] Urbisz, A.Z., Małota, K., Chajec, Ł. and Sawadro, M.K. 2024. Size-dependent and sex-specific negative effects of micro- and nano-sized polystyrene particles in the terrestrial invertebrate model *Drosophila melanogaster*. *Micron* 176, 103560.
 - [26] Alaraby, M., Abass, D., Farre, M., Hernández, A. and Marcos, R. 2024. Are bioplastics safe? Hazardous effects of polylactic acid (PLA) nanoplastics in *Drosophila*. *Sci Total Environ* 919, 170592.
 - [27] Zhang, Y., Wolosker, M.B., Zhao, Y., Ren, H. and Lemos, B. 2020b. Exposure to microplastics cause gut damage, locomotor dysfunction, epigenetic silencing, and aggravate cadmium (Cd) toxicity in *Drosophila*. *Sci Total Environ* 744, 140979.
 - [28] Liang, B., Zhang, D., Liu, X., Xu, Y., Tang, H., Li, Y. and Shen, J. 2022. Sex-specific effects of PET-MPs on *Drosophila* lifespan. *Arch Insect Biochem Physiol* 110(3), e21909.
 - [29] Tu, Q., Deng, J., Di, M., Lin, X., Chen, Z., Li, B., Tian, L. and Zhang, Y. 2023. Reproductive toxicity of polystyrene nanoplastics in *Drosophila melanogaster* under multi-generational exposure. *Chemosphere* 330, 138724.
 - [30] Détrée, C. and Gallardo-Escárate, C. 2018. Single and repetitive microplastics exposures induce immune system modulation and homeostasis alteration in the edible mussel *Mytilus galloprovincialis*. *Fish Shellfish Immunol* 83, 52-60.
 - [31] Gardon, T., Morvan, L., Huvet, A., Quillien, V., Soye, C., Le Moullac, G. and Le Luyer, J. 2020. Microplastics induce dose-specific transcriptomic disruptions in energy metabolism and immunity of the pearl oyster *Pinctada margaritifera*. *Environ Pollut* 266(Pt 3), 115180.
 - [32] Kögel, T., Bjørøy, Ø., Toto, B., Bienfait, A.M. and Sanden, M. 2020. Micro- and nanoplastic toxicity

- on aquatic life: Determining factors. *Sci Total Environ* 709, 136050.
- [33] Qiu, W., Chen, X., Tian, Y., Wu, D., Du, M. and Wang, S. 2020. Protection against oxidative stress and anti-aging effect in *Drosophila* of royal jelly-collagen peptide. *Food Chem Toxicol* 135, 110881.
- [34] Krittika, S. and Yadav, P. 2022. Trans-generational effect of protein restricted diet on adult body and wing size of *Drosophila melanogaster*. *R Soc Open Sci* 9(1), 211325.
- [35] Zhang, Q., Hao, L.C. and Hong, Y. 2020a. Exposure evaluation of diisononyl phthalate in the adults of *Drosophila melanogaster*: Potential risks in fertility, lifespan, behavior, and modes of action. *Comp Biochem Physiol C Toxicol Pharmacol* 238, 108847.
- [36] Rera, M., Bahadorani, S., Cho, J., Koehler, C.L., Ulgherait, M., Hur, J.H., Ansari, W.S., Lo, T., Jr., Jones, D.L. and Walker, D.W. 2011. Modulation of longevity and tissue homeostasis by the *Drosophila* PGC-1 homolog. *Cell Metab* 14(5), 623-634.
- [37] Liang, B., Zhang, D., Liu, X., Xu, Y., Tang, H., Li, Y., Shen, J. 2022. Sex-specific effects of PET-MPs on *Drosophila* lifespan. *Archives of Insect Biochemistry and Physiology*, 110, e21909.
- [38] Parkash, R. and Ranga, P. 2013. Sex-specific divergence for adaptations to dehydration stress in *Drosophila kikkawai*. *J Exp Biol* 216(Pt 17), 3301-3313.
- [39] Shen, J., Liang, B. and Jin, H. 2023. The impact of microplastics on insect physiology and the indication of hormesis. *TrAC Trends in Analytical Chemistry* 165, 117130.
- [40] Jin, H., Xu, Y., Kong, F. and Shen, J. 2024. Chronic exposure to polytetrafluoroethylene microplastics caused sex-specific effects in the model insect, *Drosophila melanogaster* (Diptera: Drosophilidae). *Journal of Economic Entomology* 117(2), 516-523.
- [41] Lee, H.Y., Lee, S.H. and Min, K.J. 2022. The Increased Abundance of Commensal Microbes Decreases *Drosophila melanogaster* Lifespan through an Age-Related Intestinal Barrier Dysfunction. *Insects* 13(2).
- [42] Thiem, J., Viskadourou, M., Gaitanidis, A., Stravopodis, D. J., Strauß, R., Duch, C. and Consoulas, C. 2024. Biological aging of two innate behaviors of *Drosophila melanogaster*: Escape climbing versus courtship learning and memory. *PLOS ONE* 19(4), e0293252
- [43] Guo, L., Karpac, J., Tran, S.L. and Jasper, H. 2014. PGRP-SC2 promotes gut immune homeostasis to limit commensal dysbiosis and extend lifespan. *Cell* 156(1-2), 109-122.
- [44] Hung, N.D., Kim, M.R. and Sok, D.E. 2011. Oral administration of 2-docosahexaenoyl lysophosphatidylcholine displayed anti-inflammatory effects on zymosan A-induced peritonitis. *Inflammation* 34(3), 147-160.
- [45] Huang, L.S., Hung, N.D., Sok, D.E. and Kim, M.R. 2010. Lysophosphatidylcholine containing docosahexaenoic acid at the sn-1 position is anti-inflammatory. *Lipids* 45(3), 225-236.
- [46] Yan, J.J., Jung, J.S., Lee, J.E., Lee, J., Huh, S.O., Kim, H.S., Jung, K.C., Cho, J.Y., Nam, J.S., Suh, H.W., Kim, Y.H. and Song, D.K. 2004. Therapeutic effects of lysophosphatidylcholine in experimental sepsis. *Nat Med* 10(2), 161-167.
- [47] Meynial-Denis, D. 2016. Glutamine metabolism in advanced age. *Nutr Rev* 74(4), 225-236.
- [48] Salabei, J.K., Lorkiewicz, P.K., Holden, C.R., Li, Q., Hong, K.U., Bolli, R., Bhatnagar, A. and Hill, B.G. 2015. Glutamine Regulates Cardiac Progenitor Cell Metabolism and Proliferation. *Stem Cells* 33(8), 2613-2627.
- [49] Xiao, D., Zeng, L., Yao, K., Kong, X., Wu, G. and Yin, Y. 2016. The glutamine- α -ketoglutarate

- (AKG) metabolism and its nutritional implications. *Amino Acids* 48(9), 2067-2080.
- [50] Giezenaar, C., Chapman, I., Luscombe-Marsh, N., Feinle-Bisset, C., Horowitz, M. and Soenen, S. 2016. Ageing Is Associated with Decreases in Appetite and Energy Intake--A Meta-Analysis in Healthy Adults. *Nutrients* 8(1),28.
- [51] Meng, J., Lv, Z., Qiao, X., Li, X., Li, Y., Zhang, Y. and Chen, C. 2017. The decay of Redox-stress Response Capacity is a substantive characteristic of aging: Revising the redox theory of aging. *Redox Biol* 11, 365-374.
- [52] Huang, H., Lu-Bo, Y. and Haddad, G.G. 2014. A *Drosophila* ABC transporter regulates lifespan. *PLoS Genet* 10(12), e1004844.
- [53] Campos, A., Araújo, P., Pinheiro, C., Azevedo, J., Osório, H. and Vasconcelos, V. 2013. Effects on growth, antioxidant enzyme activity and levels of extracellular proteins in the green alga *Chlorella vulgaris* exposed to crude cyanobacterial extracts and pure microcystin and cylindrospermopsin. *Ecotoxicol Environ Saf* 94, 45-53.
- [54] Xiong, Y., Lei, Q.Y., Zhao, S. and Guan, K.L. 2011. Regulation of glycolysis and gluconeogenesis by acetylation of PKM and PEPCK. *Cold Spring Harb Symp Quant Biol* 76, 285-289.
- [55] Ramírez, J., Mirkov, S., House, L.K. and Ratain, M.J. 2015. Glucuronidation of OTS167 in Humans Is Catalyzed by UDP-Glucuronosyltransferases UGT1A1, UGT1A3, UGT1A8, and UGT1A10. *Drug Metab Dispos* 43(7), 928-935.
- [56] Olsen, T.B., Christensen, F.E., Lundgreen, K., Dunn, P.H. and Levitis, D.A. 2015. Coelomic Transport and Clearance of Durable Foreign Bodies by Starfish (*Asterias rubens*). *Biol Bull* 228(2), 156-162.
- [57] Kim, H.J., Hwang, N.R. and Lee, K.J. 2007. Heat shock responses for understanding diseases of protein denaturation. *Mol Cells* 23(2), 123-131.
- [58] Feder, M.E. and Krebs, R.A. 1997. Ecological and evolutionary physiology of heat shock proteins and the stress response in *Drosophila*: complementary insights from genetic engineering and natural variation. *Exs* 83, 155-173.
- [59] Liu, T., Wang, X., You, X., Chen, D., Li, Y. and Wang, F. 2017. Oxidative stress and gene expression of earthworm (*Eisenia fetida*) to clothianidin. *Ecotoxicol Environ Saf* 142, 489-496.
- [60] Brandts, I., Teles, M., Gonçalves, A.P., Barreto, A., Franco-Martinez, L., Tvarijonaviciute, A., Martins, M.A., Soares, A., Tort, L. and Oliveira, M. 2018. Effects of nanoplastics on *Mytilus galloprovincialis* after individual and combined exposure with carbamazepine. *Sci Total Environ* 643, 775-784.
- [61] Hagymasi, A.T., Dempsey, J.P. and Srivastava, P.K. 2022. Heat-Shock Proteins. *Curr Protoc* 2(11), e592.
- [62] Yu, S., Luo, F., Xu, Y., Zhang, Y. and Jin, L.H. 2022. *Drosophila* Innate Immunity Involves Multiple Signaling Pathways and Coordinated Communication Between Different Tissues. *Front Immunol* 13, 905370.
- [63] Myllymäki, H., Valanne, S. and Rämet, M. 2014. The *Drosophila* imd signaling pathway. *J Immunol* 192(8), 3455-3462.
- [64] Mussabekova, A., Daeffler, L. and Imler, J.L. 2017. Innate and intrinsic antiviral immunity in *Drosophila*. *Cell Mol Life Sci* 74(11), 2039-2054.
- [65] Lemaitre, B., Kromer-Metzger, E., Michaut, L., Nicolas, E., Meister, M., Georgel, P., Reichhart, J.M. and

- Hoffmann, J.A. 1995. A recessive mutation, immune deficiency (*imd*), defines two distinct control pathways in the *Drosophila* host defense. *Proc Natl Acad Sci U S A* 92(21), 9465-9469.
- [66] Franceschi, C., Garagnani, P., Parini, P., Giuliani, C. and Santoro, A. 2018. Inflammaging: a new immune-metabolic viewpoint for age-related diseases. *Nat Rev Endocrinol* 14(10), 576-590.
- [67] Ranjan, H., Senthil Kumar, S., Priscilla, S., Swaminathan, S., Umezawa, M. and Sheik Mohideen, S. 2024. Polyethylene microplastics affect behavioural, oxidative stress, and molecular responses in the *Drosophila* model. *Environmental Science: Processes & Impacts* 26(12), 2203-2214.
- [68] Jia, R., Han, J., Liu, X., Li, K., Lai, W., Bian, L., Yan, J. and Xi, Z. 2023. Exposure to polypropylene microplastics via oral ingestion induces colonic apoptosis and intestinal barrier damage through oxidative stress and inflammation in mice. *Toxics* 11(2), 127.



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High-concentration polyethylene and polystyrene microplastics co-exposure shorten insect lifespan and impose ecological risk: multi-omics evidence from *Drosophila melanogaster*

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