Nutrition-dependent juvenile hormone sensitivity promotes flight muscle degeneration during the aphid dispersal-reproduction transition

Yu Bai[1,2], Xiao-Jin Pei[1], Ning Ban[4], Nan Chen[1], Su-Ning Liu[1], Sheng Li[1,*], and Tong-Xian Liu[3,*]

[1]Guangdong Provincial Key Laboratory of Insect Development Biology and Applied Technology, Institute of Insect Science and Technology, School of Life Sciences, South China Normal University, Guangzhou, 510631, China

[2]State Key Laboratory of Crop Stress Biology for Arid Areas and Key Laboratory of Integrated Pest Management on the Loess Plateau of Ministry of Agriculture, Northwest A&F University, Yangling, 712100, China

[3]Institute of Entomology, Guizhou University, Guiyang, 550025, China

[4]Key Lab of Integrated Crop Pest Management of Shandong province, College of Plant Health and Medicine, Qingdao Agricultural University, Qingdao, 266109, China

[*]Corresponding authors: TXL, tx.liu@gzu.edu.cn; SL, lisheng@scnu.edu.cn

Summary statement

TORC1-mediated tissue-specific JH sensitivity and thus JH signaling induces flight muscle degeneration by blocking energy supply in winged aphids.

SUMMARY

In insects, the loss of flight typically involves a dispersal-reproduction transition, but the underlying molecular mechanisms remain poorly understood. In the parthenogenetic pea aphid, *Acyrthosiphon pisum*, winged females initiate flight muscle degeneration after flight and feeding on new host plants. Similarly, topical

application of juvenile hormone (JH) mimic to starved aphids also induces flight muscle degeneration. We found that feeding preferentially up-regulated expression of the JH receptor gene *Met* and a JH-inducible gene *Kr-h1* in the flight muscles, and thus tissue-specifically enhanced JH sensitivity and signaling. RNAi knockdown of *Kr-h1* prevented flight muscle degeneration. Likewise, blocking nutritional signals by pharmacological inhibition of the target of rapamycin complex 1 (TORC1) impaired JH sensitivity of the flight muscles in feeding aphids, and subsequently delayed muscle degeneration. RNA-seq analysis revealed that enhanced JH signaling inhibited transcription of genes involved in the TCA cycle, most likely resulting in energy supply reduction, mitochondrial dysfunction and muscle fiber breakdown. This study shows that nutrient-dependent hormone sensitivity regulates developmental plasticity in a tissue-specific manner, emphasizing a relatively underappreciated mechanism of hormone sensitivity in modulating hormone signaling.

Key words: Flight muscle; developmental plasticity; juvenile hormone sensitivity; TCA cycle; mitochondria

INTRODUCTION

Dispersal is an evolutionary driver that fundamentally shapes the distribution, abundance and diversity of insects and plays a key role in their persistence in heterogeneous habitats (Weigang and Kisdi, 2015). However, dispersal is associated with high energetic costs (Bonte et al., 2012) that require trade-offs with other physiological activities such as reproduction (Guo et al., 2016). After locating a suitable colonization environment, insects of dispersal are able to integrate external and internal environmental signals, and maintain a systemic energy balance while shifting resources toward the production of offspring instead of flight (Zera and Denno, 1997; Zhang et al., 2019).

A life-history trade-off exists between flight capability and reproduction in many wing-dimorphic insects (Guerra, 2011). Aphids can develop either without or with wings. Unwinged aphids remain on the host plant and feed and reproduce. Winged aphids are induced by environmental cues, such as crowding and reduced nutritional quality. When a winged aphid molts into the adult form, the aphid does not feed before flight. After the aphid flies to a new host plant and begins feeding, the flight muscles (which represent a valuable nutritional resource) degenerate, and presumably freed resources are shunted to developing embryos (Brisson, 2010; Johnson, 1959; Kobayashi and Ishikawa, 1993). Thus, muscle degeneration is an adaptive strategy to maximize reproductive success. It was previously shown that topical application of JH induced flight muscle degeneration that resembled feeding-dependent degeneration (Kobayashi and Ishikawa, 1994). Nevertheless, the regulatory mechanisms underlying the flight muscle degeneration in aphids when flight is unnecessary remains poorly understood.

Hormonal signals commonly integrate environmental cues (such as nutrition) to mediate a range of developmental processes (Nijhout, 1999; Beldade et al., 2011; Simpson et al., 2011; Johnson et al., 2014; Zhang et al., 2019). Besides regulating metamorphosis and reproduction (Jindra et al., 2013; Roy et al., 2018), JH plays key roles in regulating developmental plasticity in insects, including dispersal-reproduction trade-offs (Gotoh et al., 2014; Reiff et al., 2015; Miura, 2018). Target of rapamycin complex 1 (TORC1) is a well conserved nutrient (mainly amino acids) sensor in insect fat body, with a prominent role in linking systemic nutritional conditions to local cellular physiology in peripheral tissues (Colombani et al., 2003; Géminard et al., 2009; Howell and Manning, 2011; Li et al., 2019b). Eukaryotic initiation factor 4E binding protein (4EBP) is a key TORC1 target, and the TORC1-mediated phosphorylation of 4EBP is regarded as an indicator of TORC1 activity (Hietakangas and Cohen, 2009). Recent studies have also implicated the TORC1 pathway in the stimulation of JH biosynthesis via upregulation expression of juvenile hormone acid methyltransferase (*JHAMT*) (Maestro et al., 2009; Pérez-Hedo et al., 2013; Lu et al., 2016; Zhu et al., 2020), which encodes a rate-limiting enzyme for JH

biosynthesis and is expressed specifically in the corpora allata located in insect heads (Shinoda and Itoyama, 2003; Wen et al., 2015). In addition to systemic biosynthesis, gene expression levels of other molecules required for JH signaling, such as JH receptor *Met* (Konopova and Jindra, 2007), also affect JH regulatory efficacy. JH/Met induces the expression of the JH primary-response gene *Kr-h1,* which conservatively reflects JH signal (Jindra et al., 2013; Li et al., 2019a).

To examine how nutrition and JH interact to regulate flight muscle degeneration, we focused on the winged female morph of pea aphids. We report that TORC1 mediates nutritional signaling and enhances the expression of *Met* and *Kr-h1* and thus JH sensitivity specifically in the flight muscles, leading to the restriction of energy supplies and the induction of flight muscle degeneration in winged aphids. Our results highlight hormone sensitivity as an important tissue-specific regulatory mechanism of developmental transitions in insect life history.

RESULTS

Feeding and JH application induce mitochondrial disruption and flight muscle fiber breakdown

Cage studies indicated that newly-molted winged female aphids remained static for 24 h (resting mode). Females then flew toward light and maintained an active state within a 16-h photoperiod (flight mode). Females that successfully located plants for colonization then initiated feeding (settling mode), which resulted in fully distended abdomens by 48–60 h (colonization mode). By 72–84 h post-eclosion, nearly all females began to produce nymphs (larviposition mode) (Figs. 1A–A'').

Examining the flight muscles by transmission electron microscope (TEM) showed that 24-h-old females in flight mode possessed large, square mitochondria that were highly abundant between muscle fibers (Figs. 1B, C, D and S1A). The flight muscles of females in colonization mode (48 h) contained irregularly shaped mitochondria with condensed matrices but exhibited no obvious defects on the muscle fibers (Figs. 1B', C', D and S1B). Subsequently, muscle fibers were severely

degenerated with few mitochondria present in larviposition mode females (72 h) (Figs. 1B'', C'', D and S1C). Until 96 h after eclosion, feeding aphids degenerated almost all the flight muscles (Figs. 1B''', C''', D and S1D). In contrast, when winged females were starved after eclosion, the flight muscle fibers remained morphologically intact over a 96-h observation period although mitochondria shifted from square to a circular morphology (Figs. 1E–F''' and S2A–D). We further noted that muscle degeneration of winged female aphids induced by feeding was limited to the flight muscles but not the leg muscles, where JH signaling (gene expression levels of *Met* and *Kr-h1*) slightly decreased in feeding aphids compared with that in starved aphids (Fig. S3A and B).

We then examined whether exogenous JH plays a role similar to feeding in inducing morphological changes in the flight muscles during starvation. Topical application of methoprene (a JH mimic) significantly induced *Kr-h1* expression in the flight muscles at 6–48 h after treatment during starvation (Fig. 2A). Methoprene application also induced flight muscle degeneration, similarly mitochondrial disruption occurred earlier than muscle fiber breakdown (Figs. 2B–F and S4A–D). Although methoprene induced *Kr-h1* expression in the leg muscles, the induction efficiencies were significantly lower than those in the flight muscles at 12 h after application (Fig. S5A). Notably, methoprene application did not significantly induce leg muscle degeneration (Fig. S5B). It is likely that feeding tissue-specifically activates JH signaling and induces flight muscle degeneration in winged female pea aphids.

Feeding-dependent JH sensitivity induces flight muscle degeneration tissue specifically

We compared the transcriptional dynamics of genes with roles in JH biosynthesis and signaling between winged morph aphids that were fed or starved. We firstly employed RNA-seq analysis of the heads to compare transcriptional changes of all the genes involved in JH biosynthesis between winged aphids in "flight" (24 h post-eclosion) and "colonization" mode (60 h post-eclosion). The results showed that there were no

significant expression changes of the whole JH synthetic genes in the heads between the two statuses (Fig. S6A). The pea aphid may have four *JHAMT* homologs, yet there are no clear evidences showing which gene(s) is the functional *JHAMT* homolog(s). *JHAMT1*, *JHAMT2* and *JHAMT3* were annotated in the transcriptome, and we further verified their expression levels using qPCR under the starvation vs. feeding regime. The relative transcript abundance of *JHAMT1–3* in the heads of fed vs starved winged females was similar up to 72 h post-eclosion (flight muscle degeneration had already initiated at this time) but thereafter diverged post 72 h, with transcript abundance of *JHAMT2* increasing in feeding aphids while *JHAMT3* decreasing in starved aphids and significant differences of *JHAMT* are observed until 96 h post-eclosion once compared feeding with starvation (Fig. 2G–I). Conversely, relative transcript abundance of *Met* and *Kr-h1* were significantly higher in the thoraxes (almost filled with the flight muscles) of fed versus starved females by 48–96 h post eclosion (Figs. 2J and 2K).

The temporal expression pattern of key genes of JH pathway implied that the up-regulated expression of JH intracellular receptor gene *Met* resulted in the increase of JH signaling even though JH synthetic genes were not significantly up-regulated during the colonization of winged aphids. Given these outcomes, we further explored the spatial expression of *Met* and *Kr-h1*. We compared *Met* and *Kr-h1* expression in the heads, thoraxes, ovaries, fat body, and legs versus the flight muscles of winged females at 60 h post eclosion under starved or fed condition. Results showed that each gene was preferentially expressed in the thoraxes and flight muscles of the fed winged aphids, especially *Met* (Fig. 2L and 2M).

Temporal and spatial expression pattern of JH biosynthesis and signaling genes demonstrated that the transcription of JH nuclear receptor gene *Met* was tissue-specifically activated in the flight muscles of winged aphid during the process of colonization. It is reasonable to assume that abundant intracellular receptors contribute to receive JH more sensitively and subsequently induce JH nuclear signal. To verify the hypothesis, we topically applied JH mimic on the winged aphids of colonization mode and detected the expression levels of *Kr-h1* using qPCR in various tissues at 12

h after the treatment. The results showed that the *Kr-h1*-induction efficiency of JH in the flight muscles was significantly higher than that in any other tissues of feeding winged aphids (Fig. 2N).

Subsequently, we employed RNA interference (RNAi) to decrease the feeding-induced gene expression levels of *Met* and *Kr-h1*. RNAi-*Met* was not achieved in the pea aphid, while RNAi-*Kr-h1* resulted in an approximately 50% reduction in gene expression in the flight muscles of the feeding winged aphids at 48 h after the treatment (Fig. 3A). Importantly, RNAi-mediated knockdown of *Kr-h1* delayed mitochondrial disruption and muscle fiber breakdown compared with the control aphids (Figs. 3B–D and S7A and B).

TORC1-mediated nutritional signaling stimulates JH sensitivity in the fight muscles

As shown above, JH sensitivity-induced flight muscle degeneration depends on feeding (nutrition). The TORC1 pathway couples nutrition with JH biosynthesis in many insects (Maestro et al., 2009; Pérez-Hedo et al., 2013; Lu et al., 2016; Zhu et al., 2020). We then assessed whether TORC1 activity was also associated with nutrition-dependent JH sensitivity and flight muscle degeneration in the process of aphid's flight-reproduction life-history transition.

Limited by RNAi efficiency, we used the TOR protein inhibitor rapamycin (Edwards and Wandless, 2007). About 60% rapamycin-treated aphids died intensively at 72 h after the treatment (Fig. 3E). As expected, rapamycin reduced TORC1 activity in whole body excluding embryos as evidenced by reduced 4EBP phosphorylation at 36, 60 and 72 h after the treatment, respectively (Fig. 3F). Rapamycin treatment tissue-specifically decreased the expression of *Met* and *Kr-h1* in the flight muscles while having no effect on *JHAMT1–3* expression in the heads at 36 h after the treatment (Fig. 3G). Until 60 hours, the TORC1 pathway began to participate in the regulation of *JHAMT* expression. The expression levels of *JHAMT2* were significantly down-regulated resulted from rapamycin treatment at this time point (Fig. 3H), when most flight muscles of the control aphids which already got into

larviposition mode had degenerated. TEM analysis further showed that rapamycin significantly inhibited flight muscle degeneration, whereas methoprene application to rapamycin-treated aphids rescued *Kr-h1* expression and flight muscle degeneration at 48 h after the treatment (Fig. 3I–M and S8A–C). Thus, TORC1-mediated nutritional signaling promotes JH sensitivity and signaling in the flight muscles and caused muscle degeneration.

Feeding and JH application suppress the TCA cycle and activate the proteasome pathway in the flight muscles

We conducted two parallel RNA-seq experiments to further explore the molecular mechanism of JH-induced flight muscle degeneration. In the two groups of experiments, JH signals were activated by feeding and artificial JH application in starvation, respectively (Figs. 4A, D). PCA (principal component analysis) results showed high consistency between biological replicates of each treatment (Fig. S9A). RNA-seq analysis of the flight muscles identified 5160 differentially expressed genes between "flight" and "colonization" mode females (Fig. 4B) with the top pathways enriched in the KEGG aggregation analysis listed in Table S4. A number of genes that involved in the proteasome, protein processing and export, autophagy, and phagosome pathway were significantly up-regulated, while other genes regulated the citrate cycle (TCA cycle) of 2-oxocarboxylic acid or carbohydrate metabolism and oxidative phosphorylation, mainly responsible for releasing energy (ATP), were significantly down-regulated in the females of "colonization" mode once compared those with the females of "flight" mode (Fig. 4C, H). Seven of the differentially expressed genes involved in the TCA cycle were further validated by qPCR (Fig. S9B).

A total of 1282 genes were differentially expressed in the flight muscles between methoprene-treated and control females at 24 h after the treatment (Fig. 4E). KEGG aggregation analysis revealed the significantly up- and downregulated pathways (Table S5), which again included a number of genes with functions in the proteasome, protein processing and export, and autophagy pathway were significantly up-regulated, while other genes involved in the TCA cycle 2-oxocarboxylic acid

metabolism were significantly down-regulated in the flight muscles of methoprene-treated females when compared those with the acetone-treated females (Fig. 4F, H and S9C). In addition, heatCluster analysis of 5160+1282 differentially expressed genes showed that JH application and feeding resulted in almost consistent transcriptional changes in the flight muscles (Fig. 4G). RNA-seq experiments indicated that JH signaling inhibited energy supply in the flight muscles of winged aphids. We then determined that the ATP levels in the flight muscles remained almost steady at 24–72 h post eclosion in starved aphids, whereas feeding and JH application decreased the ATP levels (Figs. 4I, J), which could contribute to flight muscle degeneration. In conclusion, nutrition-dependent JH sensitivity promotes flight muscle degeneration by suppressing ATP supply, and activating proteasome pathway in the flight muscles during the aphid dispersal-reproduction transition.

DISCUSSION

First, this study emphasizes hormone sensitivity as a key regulatory mechanism of developmental events in insects. Hormone sensitivity usually refers to the expression levels of hormone receptor and its associated proteins as well as the ability to respond to a hormone in a target tissue (Tang et al., 2011). During the colonization of winged aphids, nutrition-dependent gene expression levels of JH nuclear receptor *Met* thus JH sensitivity in the flight muscles quickly up-regulated, which initiates flight muscle degeneration. We here demonstrate that TORC1-mediated nutritional signaling preferentially upregulates the expression of *Met*, leading to a tissue-specific increase of JH signaling (i.e. *Kr-h1* expression level) in the flight muscles (Figs. 1–3). The variation in hormone sensitivity between tissues could be a general regulatory mechanism underlying tissue-specific responses to hormones. Shingleton et al. (2005) also observed organ-specific sensitivity to insulin receptor signaling during development in *Drosophila*. Another example is the extreme growth of beetle horns, which are highly sensitive to changes in nutrition via the insulin signaling (Emlen et al., 2012) and to sex differentiation via JH signaling (Gotoh et al., 2014). It is known

that TORC1-mediated nutritional signaling plays roles in the stimulation of JH biosynthesis (Maestro et al., 2009; Pérez-Hedo et al., 2013; Lu et al., 2016; Zhu et al., 2020). There was no significant up-regulation of the whole JH biosynthesis genes at the beginning of flight muscle degeneration. Replication of the *JHAMT* gene in pea aphid genome had occurred, while only *JHAMT2* was regulated by the TORC1 pathway at larviposition mode (Fig. 3). In addition, the gene expression level of *JHAMT2* was the highest among *JHAMT* genes, and *JHAMT2* tissue-specifically expressed in the heads (Fig. S6A). Thus, we deduce *JHAMT2* homolog is the functional *JHAMT* in the pea aphid. Nevertheless, in response to nutrition availability during the dispersal-reproduction transition of winged pea aphids, JH sensitivity in the flight muscles certainly plays a more important role than JH biosynthesis for flight muscle degeneration.

Second, this study proves that JH promotes the dispersal-reproduction life history transition of female winged aphids (Fig. 2). In addition to the experimental data presented, blocking JH biosynthesis with precocene II activates flight behavior, thus most of the precocene-treated aphids flew away from the host plants, and did not show any feeding behavior, thus without flight muscle degeneration (Figs. S6B, C; Kobayashi and Ishikawa, 1994). Nevertheless, stable JH biosynthesis is necessary for colonization behavior and the subsequent dispersal-reproduction transition of winged aphids. JH has been the main subject of endocrine regulation of dispersal-reproduction transitions in diverse insect species (Zhang et al., 2019). In response to environmental cues, such as nutrition availability, insects possess the ability to maintain metabolic homeostasis (Koyama et al., 2013; Johnson et al., 2014; Li et al., 2019b). Increasing evidence indicates that JH plays essential roles in the regulatory network of energy metabolism, with diverse and even opposite roles depending on the developmental stage or physiological process in different insect species (Tian et al., 2010; Xu et al., 2013; Hou et al., 2015; Wang et al., 2017). Both dispersal and reproduction are costly, and loss of flight in stable habitats with available nutrition allows parthenogenetic winged aphids to shift resources toward production of offspring away from flight. The combined data in this study suggest that a JH-induced

tissue-specific reduction in energy supply mediates this transition in aphids (Fig. 4). It is worth mentioning that the mechanisms underlying the loss of flight are quite different between male pea aphids, whose wing dimorphisms are under genetic control (Li et al., 2020), and parthenogenetic females. The cricket *G. firmus* should be an ideal model to verify whether the molecular mechanism that we discovered in the pea aphid is conserved in the regulation of flight muscle degeneration in other insect species (Vellichirammal et al., 2014) and, moreover, to overcome the limitation of low-efficiency RNAi in the pea aphid.

Third, this study reveals mitochondria might as an important target organelle of JH signaling in the target tissue cells. Mitochondria are the primary energy-generating system in most eukaryotic cells. Mitochondrial dysfunction has pleiotropic effects on disease, aging, and development (Chan, 2006). In almost all TEM analyses, we observed malformed mitochondria and mitochondrial matrix condensation, which precedes muscle fiber breakdown (Figs. 1–3). Notably, mitochondrial pathology is also a major causative factor for muscle degeneration in Parkinson's disease, as well as the earliest manifestation of muscle degeneration (Greene et al., 2003). Impairment of the α-ketoglutarate dehydrogenase complex of the TCA cycle and respiratory chain complex I of the respiratory chain induces a decrease in ATP production and oxidative stress (Berndt et al., 2013), which could decrease the mitochondrial DNA content and trigger mitochondrial disruption and thus trigger apoptosis (Jaiswal et al., 2015; Tait and Green, 2010). Transcriptome analysis showed that JH suppresses gene expression in the TCA cycle pathway (Fig. 4), which might result in mitochondrial disruption and subsequent muscle fibers breakdown. We had only partially verified above hypothesis by applying inhibitors of the TCA cycle and oxidative phosphorylation limited to strong toxicity of systemic application of the chemicals.

In summary, TORC1-mediated nutritional signaling promotes JH sensitivity and thus JH signaling specifically in the flight muscles of winged aphids. The increased JH signaling inhibits the TCA cycle to reduce energy supply and activates the proteasome pathway, resulting in mitochondrial disruption and muscle fiber breakdown in the fight muscles (Fig. 5). This study highlights that tissue-specific

hormone sensitivity is an important regulatory mechanism of developmental transitions in insect life history.

Materials and methods

Insects and experimental apparatus

Wingless morphs of the pea aphid, *Acyrthosiphon pisum*, derived from a long-established parthenogenetic clone, were kept at low density. The aphids were maintained on broad bean seedlings in a climate chamber at 18°C and ~70% relative humidity with a 16:8 h (light:dark) photoperiod. Winged morphs were induced under high-density conditions as described by Guo et al. (2016). The offspring produced during a 24-h period were retained and further screened on the basis of visible wing primordia at the third instar stage. Newly emerged adults within 12 h were collected to harvest winged aphids with synchronized development as described by Kobayashi and Ishikawa (1994). In the case of starvation, winged aphids were cultured in a petri dish containing filter paper. In the case of feeding, newly emerged or specific treated winged aphids were cultured within our experimental cages for colonization (Fig. S10A).

Juvenile hormone pathway genes annotation and orthologue identification

To search for pea aphid orthologues of JH-related genes involved in biosynthesis and signaling, we performed Blast analysis using protein sequences of JH-related genes of *Drosophila melanogaster* with the *A. pisum* genome in NCBI (http://blast.ncbi.nlm.nih.gov/). The sequences with significant lower E-values or predicted by automated computational analysis as presupposed gene were chosen as the putative pea aphid orthologue (*HMGS1*, hydroxymethylglutaryl-CoA synthase 1, GeneID: 100165154; *HMGS2*, hydroxymethylglutaryl-CoA synthase 2, GeneID: 100161670; *HMGR*, 3-hydroxy-3-methylglutaryl-coenzyme A reductase, GeneID: 100165462; *MVD*, diphosphomevalonate decarboxylase, GeneID: 100574505; *IPPI*, isopentenyl diphosphate isomerase, GeneID: 100166744; *FALD*, farnesol

dehydrogenase, GeneID: 100160714; *JHAMT1*, juvenile hormone acid O-methyltransferase, GeneID: 100569254; *JHAMT2*, juvenile hormone acid O-methyltransferase-like, GeneID: 100160278; *JHAMT3*, juvenile hormone acid methyltransferase, GeneID: 100166857; *JHAMT4*, juvenile hormone acid O-methyltransferase-like, GeneID: 100572117; *CYP15A1*, methyl farnesoate epoxidase-like, GeneID: 100162751; *Met*, circadian locomoter output cycles protein kaput, GeneID: 100573780; *Kr-h1*, zinc finger X-linked protein ZXDB, GeneID: 100159203). RNA-seq of the heads was employed to further estimate the functional *JHAMT* homolog of pea aphid. The candidate sequences of *Met* and *Kr-h1* were and further verified by RT-PCR and re-sequencing. Briefly, the first strand cDNA was synthesized using PrimeScript™ II 1st Strand cDNA Synthesis Kit (Takara, Dalian, China). Target specific primers (Table. S1) were synthesized to amplify the complete coding sequences (CDS) using the first-strand cDNA as templates, and the high-fidelity PrimeSTAR GXL DNA Polymerase reagent (Takara, Dalian, China) was used in our amplification experiments according to the supplier's protocol. PCR-amplified fragments were directly purified and supplied for sequencing. The ORF Finder (http://www.bioinformatics.org/sms2/orffind.html), online tools ExPASy (https://web.expasy.org/translate/) and SMART (http://smart.embl-heidelberg.de) were used to predicted ORF, translate and identify conserved functional domains (Fig. S10B and C) based on our resequencing results, respectively. The sequenced complete coding sequences (CDS) of *Met-A* (OM371056), *Met-B* (OM371057), *Kr-h1-A* (OM371058), *Kr-h1-B* (OM371059) were uploaded to NCBI. In addition, we performed RNAi-*Kr-h1* at nymph stage of the pea aphid to further validate *Kr-h1* orthology. RNAi-*Kr-h1* at 3rd nymph stage resulted in wing abnormal post eclosion but no pre-metamorphosis phenotype (Fig. S10D).

Chemicals, topical application and microinjection

The JH mimic methoprene (Sigma, St. Louis, MO, USA), the corpus allatum inhibitor precocene II (Yuanye Bio-Technology, Shanghai, China) and the TOR inhibitor rapamycin (Selleck, Shanghai, China) were used in this study. Methoprene (1 mg/mL)

and procence (50 mg/mL) solution was prepared with acetone, and 100 nL solution was topically applied to the aphid pronotum via a Nanoject II injector (Drummond Scientific, Broomall, PA, USA). The aphids in flight mode were treated with methoprene, and the treated aphids were subjected to starvation or feeding for JH induction or rescue experiment respectively; methoprene application on the femur served as a parallel control of JH induction experiment. Besides, the aphids in colonization mode were treated with methoprene and the treated aphids were subjected to feeding for another JH induction experiment. Rapamycin was first dissolved in DMSO and further diluted to 2 nM with 0.01 M PBS, and 100 nL of the solution was delivered into the abdomen via microinjection. The aphids in flight mode were treated with rapamycin or procence via the Nanoject II injector apparatus, while the aphids were placed in the feeding cages after the treatment. At least 30 aphids were treated for each group every time.

Quantitative real-time PCR and RNA sequencing

Quantitative real-time PCR (qPCR) were employed to detect the changes of JH biosynthesis and signaling levels in the process of *A. pisum* winged adult colonization or after chemical/dsRNA treatment. The spatial expression profiles of *Met* and *Kr-h1* were determined using tissues from the heads (30 individuals for one biological replicate), thoraxes (10 individuals), ovaries (10 individuals), fat body (20 individuals), flight muscles (10 individuals), and legs (50 individuals) of winged adults at feeding or starvation 60 h after eclosion. The temporal expression profiles of *JHAMT*, *Met* and *Kr-h1* at different developmental stages of winged adults subjected to feeding or starvation conditions post eclosion were determined using head, thorax and thorax samples respectively. For methoprene topical application experiment, starved winged aphids were treated at 24 h post eclosion and the thoraxes were excised from the specimen at 2, 6, 12, 24, and 48 h after the treatment; feeding winged aphids were treated at 48 h post eclosion and the heads, thoraxes, ovaries, fat body, flight muscles, and legs were excised from the specimen at 12 h after the treatment for qPCR analysis. For RNAi and rapamycin application experiments, the heads were

excised for *JHAMT* detection and the flight muscles or fat body were excised for *Met* and *Kr-h1* detection at specific point in time. Three independent biological replicates were performed in all qPCR analysis. All of the samples were stored in RNAiso Plus (Takara) at −80°C until use. Following total RNA isolation and reverse transcription, qPCR was performed on an IQ-5 Real-time system (Bio-Rad, Hercules, CA, USA). *A. pisum* elongation factor 1 alpha (*EF1a*) and ribosomal protein S20 (*Rps20*) were quantified as housekeeping genes for normalization (Chen et al., 2016; Ishikawa et al., 2012). The Ct values normalized against the means of *EF1a* and *Rps20* standard values were obtained to calculate the quantitative variation of target genes using a mathematical model of Pfaffl (2001) and the REST 2009 Software (Qiagen, Hilden, Germany). Gene-specific qPCR primers (target the common region of transcript variants) with appropriate amplification efficiency were screened by cDNA gradient based standard curves (Table. S2).

Four groups of flight muscle samples and two groups of head samples (Each group contains three independent biological replicates) were prepared for RNA sequencing (RNA-Seq). In feeding condition, winged adults in “flight” and “colonization” mode were subjected to flight muscle (48 h post-eclosion) or head (60 h post-eclosion) sampling. In starvation condition, “acetone” and “methoprene” treated aphids were subjected to flight muscle sampling at 24 h after the treatment. Each flight muscle or head sample collected from at least 30 aphids were prepared for RNA extraction. An aliquot of 1000 ng of total mRNA from each sample was used to construct libraries. Single-end, 1x50 sequencing was performed on an Illumina HiSeq 2500 at the Novogene Sequencing Facility. The genome sequence of *A. pisum* was downloaded from NCBI (https://www.ncbi.nlm.nih.gov/genome/?term=pea+aphid), as was the annotation file. The clean reads were mapped to the genome using GSNAP to estimate the expression level of all of the transcripts (Wu et al., 2005). Gene expression levels were estimated by fragments per kilobase of transcript per million fragments mapped. Differential expression analysis of two groups was performed using the DESeq R package (1.10.1). Genes with an adjusted P-value (False Discovery Rate, FDR) < 0.05 found by DESeq were designated as differentially

expressed. We used KOBAS (Mao et al., 2005) software to test the statistical enrichment of differentially expressed genes in KEGG pathways. The entire RNA-seq datasets are available online (PRJNA828046).

RNAi experiments

Gene specific primers (Table S3) were designed to amplify a 307 bp fragments (common regions of isoform A and B) from *Kr-h1*. A 193 bp fragment of the *Mus musculus* lymphotoxin A (*Lta*) was also cloned to serve as an unrelated control gene. The validated plasmid and primers containing T7 RNA polymerase promoter were subjected to PCR amplification to produce DNA templates used for dsRNA synthesis. DsRNA synthesis and purification were performed using a T7 RiboMAX™ Express RNAi System (Promega, Madison, WI, USA), according to the manufacturer's instructions. The concentration of the purified dsRNA was quantified at 260 nm using a NanoDrop 2000c spectrophotometer (Thermo Scientific, Waltham, MA, USA), and was adjusted to 6 μg/μL with RNase free Water. Newly emerged aphids were subjected to 24 h of fasting prior to dsRNA microinjection. They were anesthetized with carbon dioxide, and 150 nL of specific dsRNA was injected into aphid hemolymph using a Nanoject II injector apparatus. The dsRNA treated aphids were placed in the feeding cages after for colonization. At least 30 aphids were treated for each group every time.

Transmission electron microscopy

Newly collected muscle tissues were fixed in 2.5% glutaraldehyde at 4°C overnight followed by thorough washing with PBS. The specimens were then postfixed in 0.5% osmium tetroxide for 2 h, dehydrated in an ethanol gradient, and finally embedded in Epon resin. Longitudinal sections (70 nm) of muscle fibers were prepared with a UC7 ultramicrotome (Leica, Solms, Germany), stained with Reynold's lead citrate, and visualized on an HT7700 transmission electron microscope (Hitachi, Tokyo, Japan) to observe the breakage degree of muscle fibers and the morphology of mitochondria. Quantitative analysis of the damaged muscle fibers was performed by ImageJ.

Western blot analysis

For each sample, 30 aphids without embryos were lysed directly for total protein extraction. Proteins were separated by 12% SDS-PAGE and transferred to a PVDF membrane. The loaded membrane was then blocked with 5% BSA in Tris-buffered saline supplemented with 1% Tween-20 (TBST, pH 8.0) for 1 h at room temperature and incubated with 1:1000 diluted primary antibodies (phospho-4EBP, Thr37/46, #9459S; Tubulin, Beyotime Biotechnology, China) at 4°C overnight. The primary antibodies specifically bind to target proteins. Then, incubated with goat anti-rabbit IgG-HRP secondary antibody (1:5000 dilution) (Boster, Wuhan, China) for 1 h at room temperature. The immune signal was visualized by the ECL chemiluminescent substrate (Boster, Wuhan, China), and images were captured on a ChemiDoc MP System (Bio-Rad, Hercules, CA, USA). Quantitative analysis of the gray value was performed by ImageJ.

ATP assay

ATP levels in the flight muscles were determined using an ATP Assay Kit (Fluorometric) (Cat# ab83355, Abcam, Cambridge, UK). A standard curve was established from a pure ATP gradient according to the manufacturer's instructions. In each biological replicate, approximately 10 mg of the aphid thoraxes was homogenized in 2 N perchloric acid (PCA), and the diluted supernatant was neutralized with 2 M KOH. These deproteinized samples and ATP reaction mixes were successively added into a black 96-well plate, and the reaction was incubated at room temperature for 30 min. The fluorescence readings were measured by an Infinite 200 microplate reader (Tecan, Männedorf, Switzerland) with the excitation/emission setting at 535/587 nm. The mean fluorescence intensity was determined by comparison to a standard curve.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

S.Li and T.X.Liu designed and conceived the study. Y.B., X.J.Pei, N.B., N.C. and S.N.Liu performed experiments and analyzed data. S.Li, T.X.Liu and Y.B. wrote the manuscript.

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Data availability

The entire RNA-seq datasets are available online (PRJNA828046).

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Figures

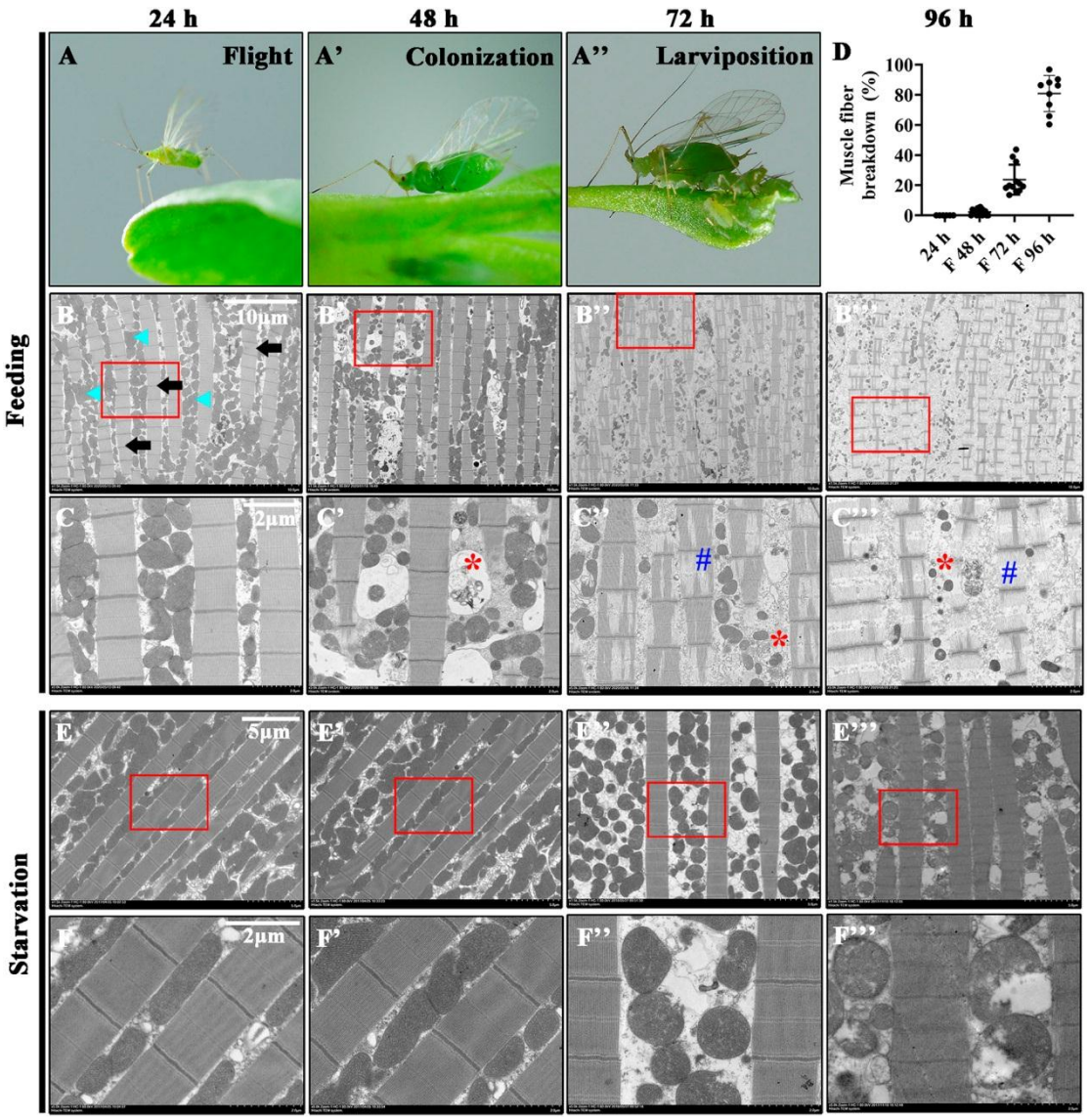

Figure 1. Histological observation of the flight muscles in winged *A. pisum* morphs under feeding and starved conditions post eclosion. Under experimental feeding conditions, the aphids present a typical flight (A), colonization (A'), and larviposition (A'') status at 24, 48, and 72 h post eclosion, respectively. Transmission electron microscopy (TEM) observations of the flight muscles from 24 h to 96 h under feeding (B–B''') or starvation (E–E''') conditions. Detailed magnifications of the sections in the red box highlighting muscle fibers and mitochondria in panels B–B''' and E–E''' are shown in C–C''' and F–F''', respectively. Bule arrowheads indicate

mitochondria; black arrows indicate muscle fibers. "*" indicates disrupted mitochondrial; "#" indicates broken muscle fibers. (D) Quantitative statistics of muscle fiber breakdown: percentage of "blank muscle fiber area" in total muscle fiber area. Three, six, six, and three aphids were examined at 24, 48, 72 h and 96 h, respectively. Individual representative phenotypic image is exhibited here. Other available TEM samples with representative phenotype in each status are presented in figures 1 and 2 of Supplementary Materials. More than two-thirds of the aphids in each status display representative characteristics.

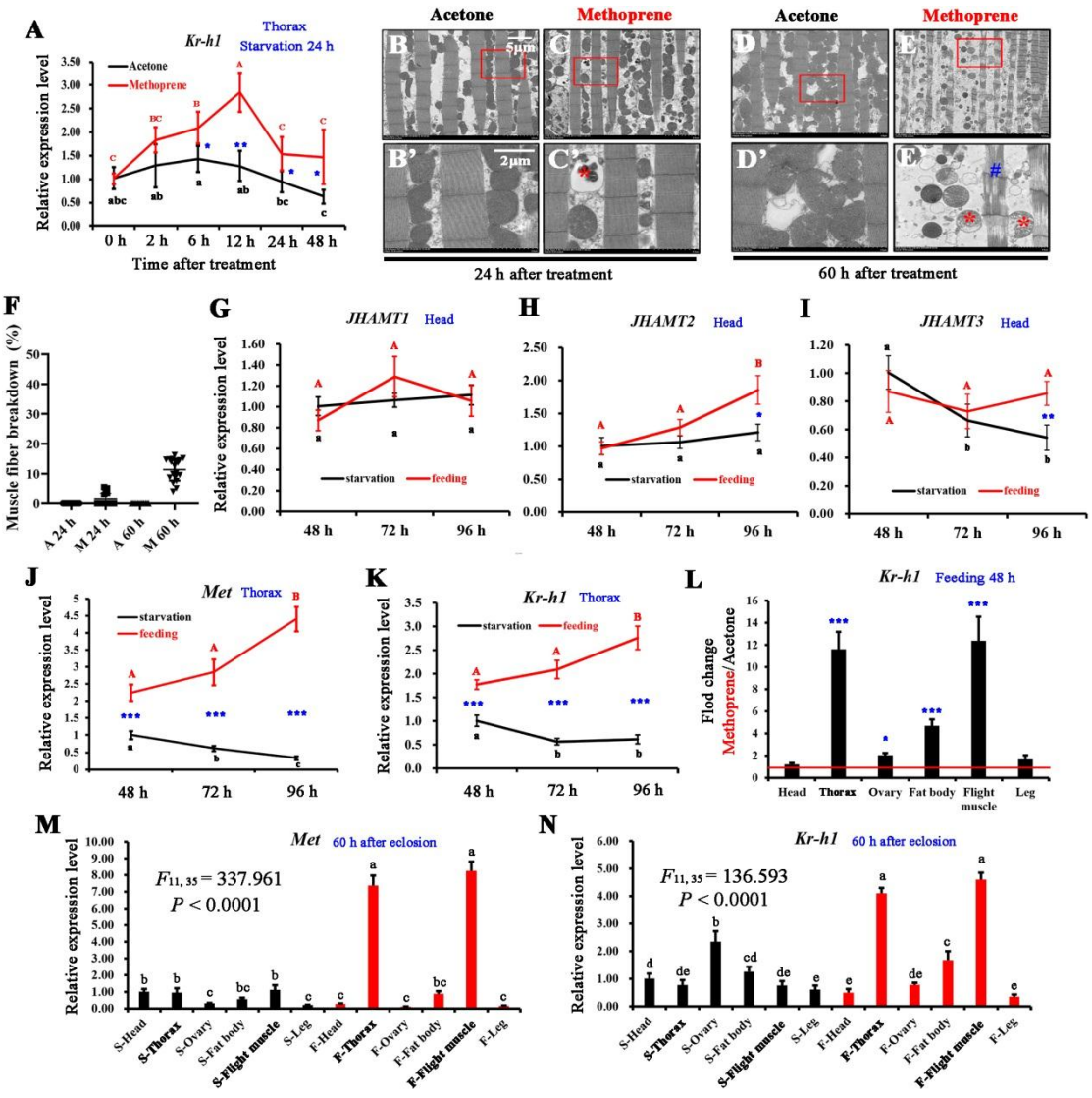

Figure 2. Feeding tissue-specifically stimulates JH sensitivity and signaling in the flight muscles of winged *A. pisum*. (A) Effect of methoprene application (100 ng/aphid) on *Kr-h1* expression in the thoraxes under starvation condition. Methoprene was applied at 24 h post eclosion, and the thoraxes were dissected at various time points after the treatment for qPCR analysis. The data are presented as the mean ± s.e.m. from 3 biological replicates, and each replicate contains 10 aphids. Student's t-test was used to compare the differences between starvation and feeding groups at the same time point (*P < 0.05, **P < 0.01). Different letters indicate statistically significant differences between various time points within starvation or feeding group using LSD multiple comparisons (P < 0.05). (B–E) TEM observation of the flight muscles at 24 and 60 h post methoprene treatment under starvation

condition. Muscle fibers and mitochondria in the red box in panels B–E are magnified in B'–E', respectively, to show the degeneration of mitochondria (*) and muscle fibers (#). (F) Quantitative statistics of muscle fiber breakdown: percentage of "blank muscle fiber area" in total muscle fiber area (A: acetone; M: methoprene), n = 5 for A 24 h, 10 for M 24 h, 5 for A 60 h, and 10 for M 60 h. Individual representative phenotypic image is exhibited here. Other available TEM samples with representative phenotype in each group are presented in figures 3A–D of Supplementary Materials. 8 and 7 of the aphids in methoprene treatment at 24 and 60 h display these characteristics. Transcriptional dynamics of *JHAMT1* (G), *JHAMT2* (H) and *JHAMT3* (I) in the heads, *Met* (J) and *Kr-h1* (K) in the thoraxes of fed and starved aphids at 48–96 h post eclosion. (L) Effect of methoprene application (100 ng/aphid) on *Kr-h1* expression in different body parts or tissues of the fed aphids. Methoprene was applied at 48 h post eclosion, and the tissues were dissected at 12 h after the treatment for qPCR analysis. Relative expression levels of *Met* (M) and *Kr-h1* (N) in different body parts or tissues of fed (F) and starved (S) aphids at 60 h post eclosion. Above qPCR data are presented as the mean ± s.e.m. from 3 biological replicates (each replicate contains 10 aphids for the thoraxes or flight muscles, 30 aphids for the heads, 10 aphids for the ovaries, 20 aphids for the fat body, and 50 aphids for the legs). Differences between 2 groups were determined by Student's t-test (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$), and differences among 3 or more groups were determined by LSD multiple comparisons (significant differences were indicated different letters, $P < 0.05$).

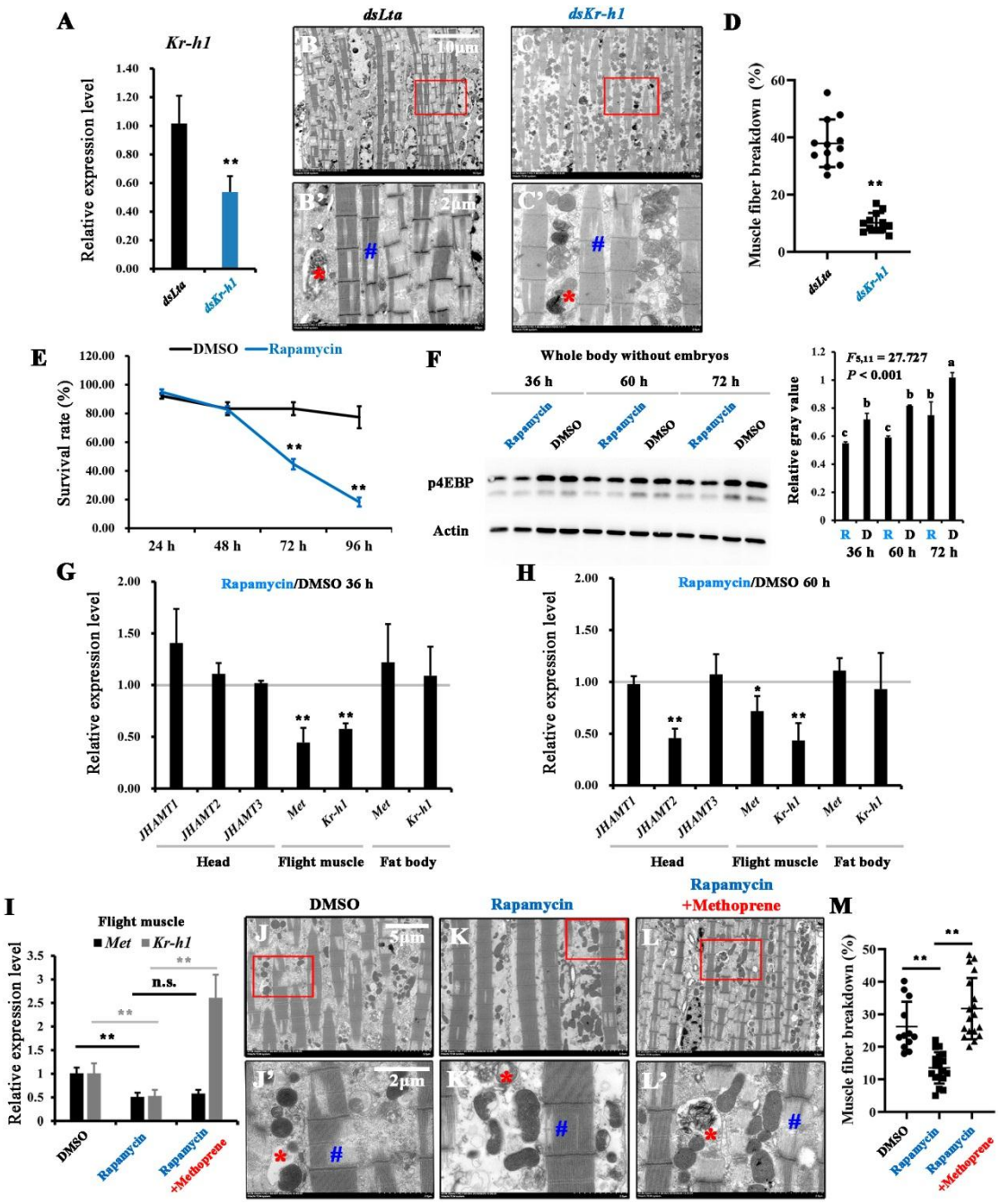

Figure 3. TORC1 pathway-dependent JH sensitivity and signaling induces flight muscle degeneration in winged *A. pisum*. (A) Effect of *Kr-h1* RNAi knockdown on *Kr-h1* transcripts. Injection of dsRNA was performed at 24 h after eclosion, and the flight muscles were dissected 48 h post injection for qPCR detection. (B, C) TEM observation of the flight muscles of *dsLta*- and *dsKr-h1*-injected aphids at 48 h after dsRNA injection. Detailed magnifications of the muscle fibers and mitochondria (red box) are shown in B' and C', respectively. "*" and "#" are used to

indicate mitochondrial disruption and disrupted muscle fibers, respectively. (D) Quantitative evaluation of the breakdown degree of muscle fibers: percentage of "blank muscle fiber area" in the total muscle fiber area. 10 aphids were examined in each treatment. Individual representative phenotypic image is exhibited here. Other available TEM samples with representative phenotype in each treatment are presented in figures 4E and F of Supplementary Materials. 50% of the aphids in *dsKr-h1* treatment display representative characteristics. (E) Survival rates (calculated for at least 90 aphids) of rapamycin (R) and DMSO (D) treated aphids at 24–96 h after the treatment. Effect of rapamycin treatment on phosphorylation levels of 4EBP at 36, 60 and 72 h after the treatment (F) and mRNA levels of *JHAMT1*, *JHAMT2*, *JHAMT3*, *Met* and *Kr-h1* at 36 h (G) and 72 h (H) after the treatment. Total proteins extracted from the whole body without embryos were used for western blot analysis. The heads were dissected for analyses of the expression of *JHAMT*. The flight muscles and fat body were dissected for qPCR analyses of the JH signaling genes, *Met* and *Kr-h1*. (I) Response of the *Met* and *Kr-h1* transcript to methoprene application following rapamycin injection at 48 h post the treatment. Injection of rapamycin (2 nM, 100 nL) and methoprene (1 mg/mL, 100 nL) application were performed at 24 h post eclosion. All qPCR data are presented as the mean ± s.e.m. from 3 biological replicates. *P < 0.05, **P < 0.01 (Student's *t*-test). Histological changes of the flight muscles from DMSO-treated (J) aphids, rapamycin-treated (K) aphids, and methoprene-rescued aphids (L) at 48 h after the treatment. Detailed magnifications of muscle fibers and mitochondria (red box) are shown in J'–L'. (M) Quantitative statistics of muscle fiber breakdown: percentage of "blank muscle fiber area" in total muscle fiber area (n = 5 for DMSO, 10 for rapamycin and 10 for rapamycin + methoprene). Individual representative phenotypic image is exhibited here. Other available TEM samples with representative phenotype in each group are presented in figure 5 of Supplementary Materials. 60% of the chemical treated aphids display representative characteristics.

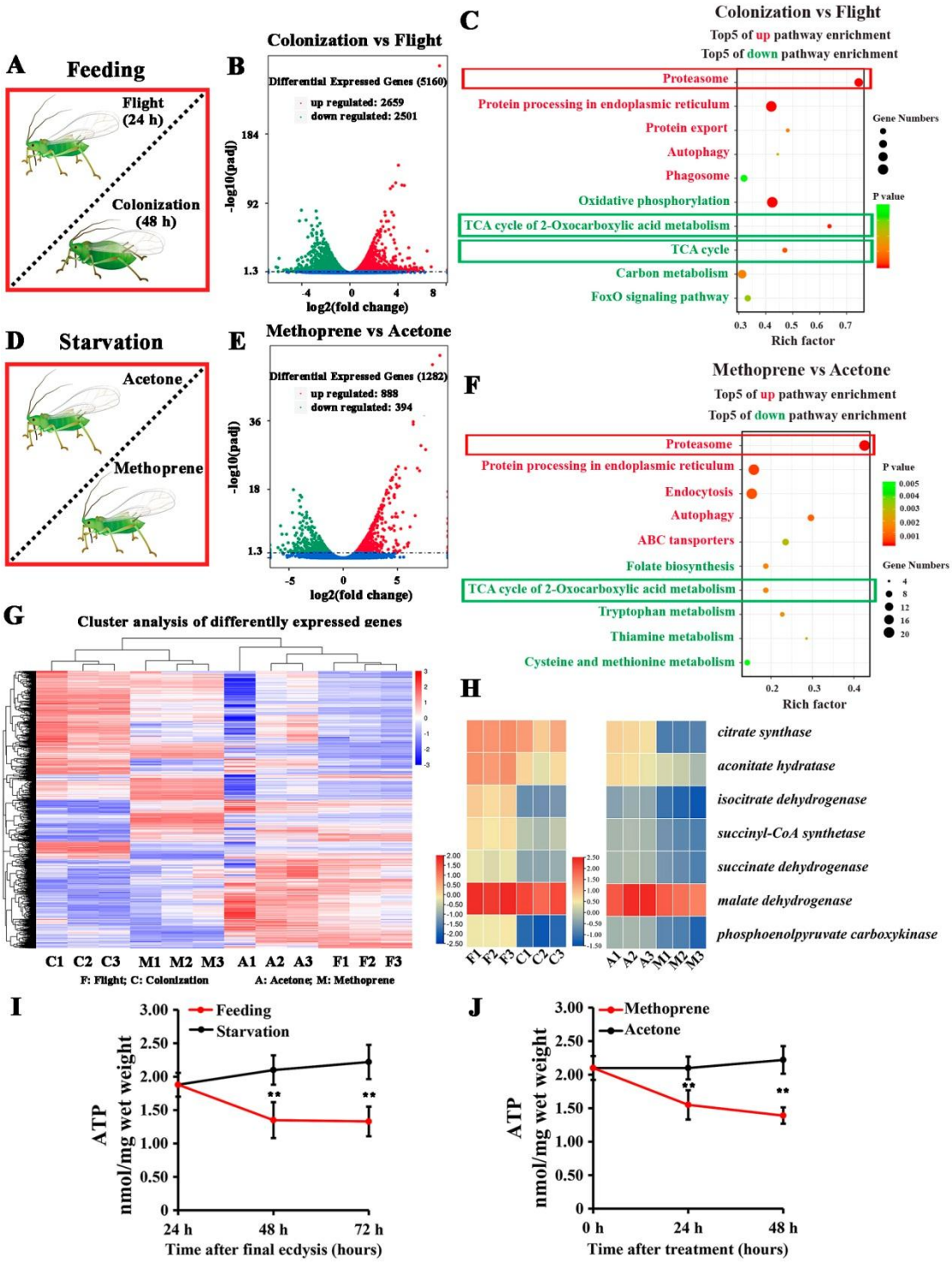

Figure 4. Comparison transcriptomes between integral and feeding or exogenous JH induced degenerating flight muscles of winged *A. pisum*. RNA-seq was performed based on two parallel experimental designs: the flight muscles of winged aphids in "colonization" vs "flight" mode (A), and the flight muscles of "methoprene" vs "acetone" treated aphids under starvation condition were

sampled at 24 h after treatment (D) for comparative RNA-seq analysis. Newly collected aphids were subjected to 24 h of fasting prior to methoprene application (100 ng/aphid). Three independent biological samples (30 aphids were pooled to generate 1 biological replicate) were used for each experimental group to conduct differential expression analysis, and to produce the volcano plots (B, E). KEGG enrichment of functional and signal pathways with respect to up-regulated (red letters) and down-regulated (green letters) gene sets generated from “colonization” vs “flight” (C) and “methoprene” vs “acetone” (F) differential expression analysis, respectively. Heat maps representing the global transcriptional scenery of the whole differentially expressed genes (G) and the genes involved in the TCA cycle (H) in the flight muscles of “colonization”, “flight”, “methoprene” and “acetone” aphids. (I) Temporal dynamics of ATP levels in the flight muscles of fed and starved aphids from 24 to 72 h post eclosion. (J) Effect of methoprene application on ATP levels in the flight muscles of aphids under starvation conditions.

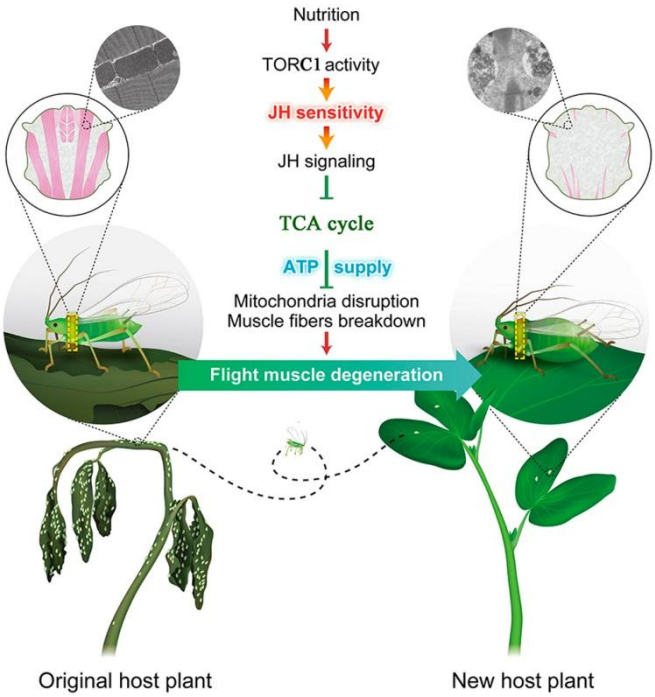

Figure 5. Schematic diagram of the regulation mechanism underlying flight muscle degeneration in winged *A. pisum*. The TORC1 pathway preferentially enhances JH sensitivity in the flight muscles, and the enhanced JH signaling blocks ATP supply via inhibiting the TCA cycle. Shortage of energy supply most likely result in both mitochondria disruption and muscle fibers breakdown which contribute to the eventual degeneration of the flight muscles in winged aphids.

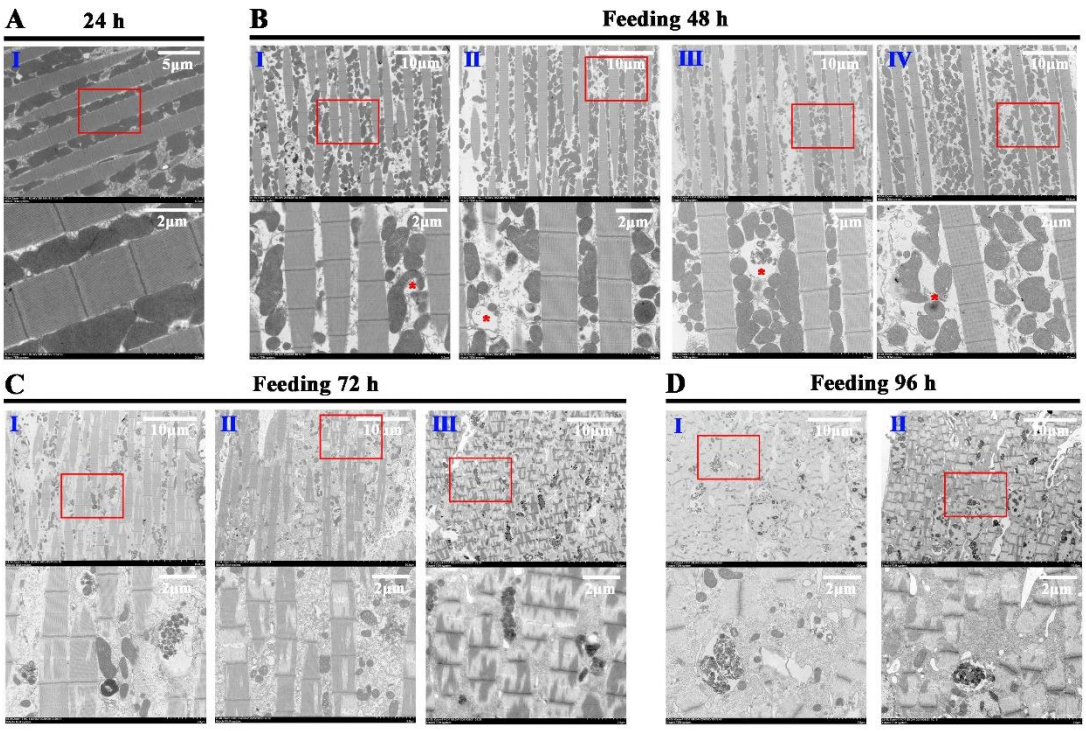

Fig. S1. Feeding induces flight muscle degeneration in winged female *A. pisum*. Transmission electron microscopy (TEM) observations of the flight muscles from 24 h to 96 h post eclosion under feeding conditions (A–D). Detailed magnifications of the sections in the red box highlighting muscle fibers and mitochondria below the corresponding picture. "*" indicates disrupted mitochondrial. I–IV represents biological replications.

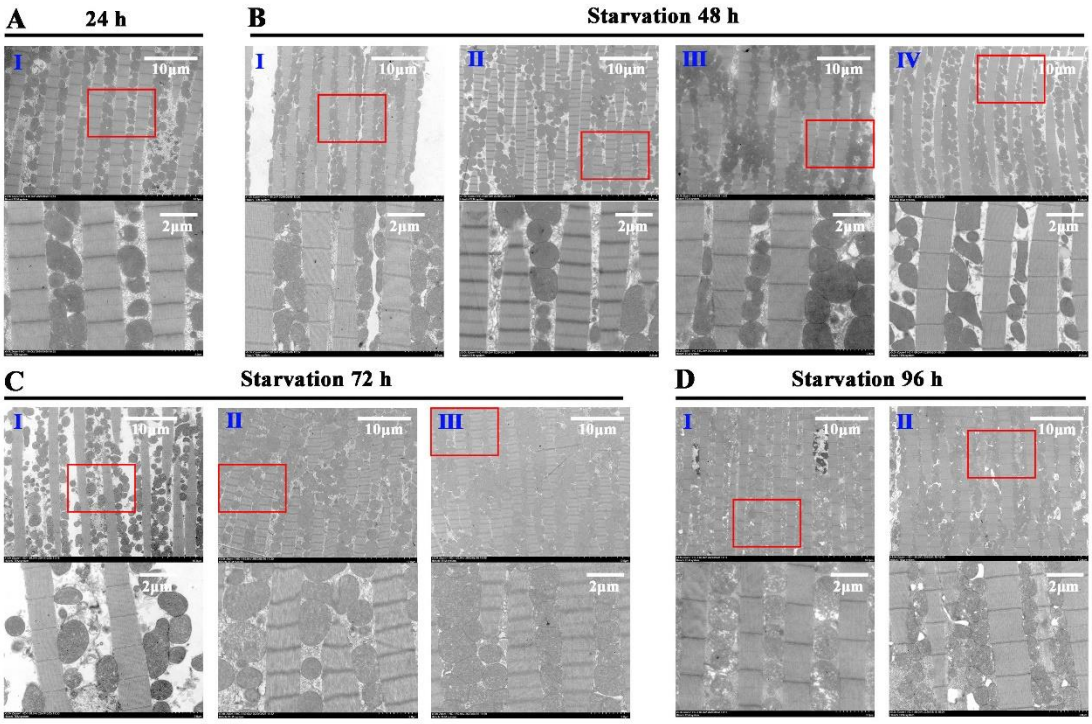

Fig. S2. Flight muscle degeneration would not occur during starvation post eclosion in winged female *A. pisum*. TEM observations of the flight muscles from 24 h to 96 h post eclosion under starvation (A–D). Detailed magnifications of the sections in the red box highlighting muscle fibers and mitochondria below the corresponding picture. I–IV represents biological replications.

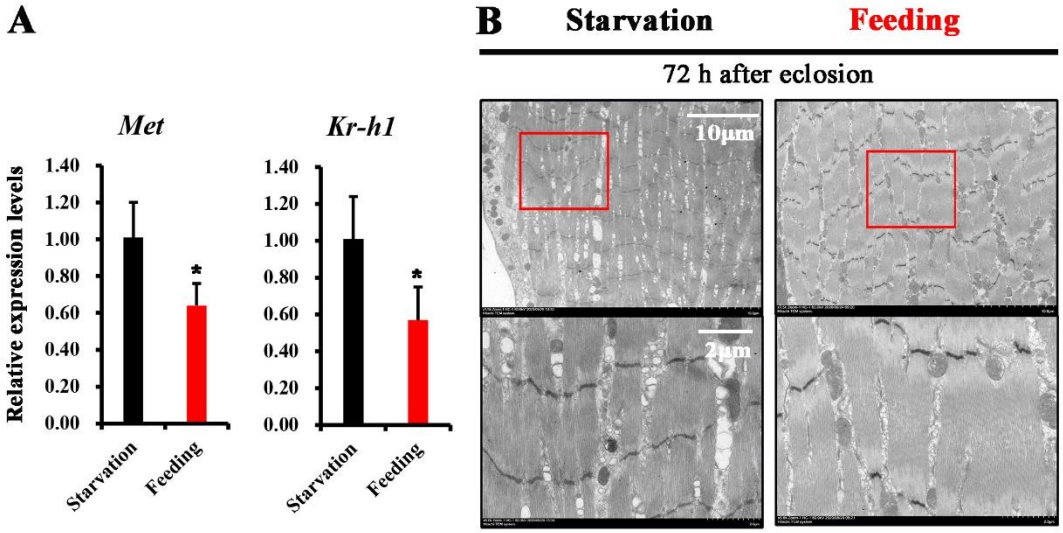

Fig. S3. Feeding-induced muscle degeneration would not occur in the leg muscles during winged aphid colonization. (A) Relative expression levels of *Met* and *Kr-h1* in the legs of fed and starved aphids at 72 h post eclosion. The data are presented as the mean ± s.e.m. from 3 biological replicates, and each replicate contains 50 aphids. Student's t-test was used to compare the differences between starvation and feeding groups (*$P < 0.05$). (B) Longitudinal ultrathin sections of leg muscles in winged female aphids under feeding 72 h and starvation 72 h post eclosion identified by TEM.

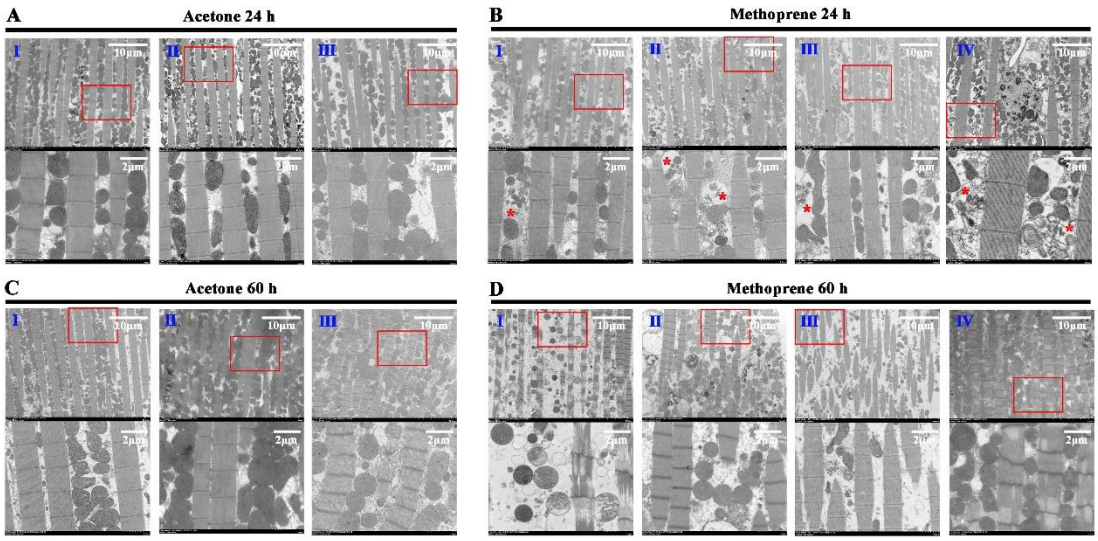

Fig. S4. JH application induces mitochondrial disruption and flight muscle fiber breakdown during starvation in winged female *A. pisum*. TEM observations of the flight muscles at 24 h (A and B) and 60 h (C and D) after methoprene treatment under starvation. Detailed magnifications of the sections in the red box highlighting muscle fibers and mitochondria below the corresponding picture. "*" indicates disrupted mitochondrial. I–IV represents biological replications.

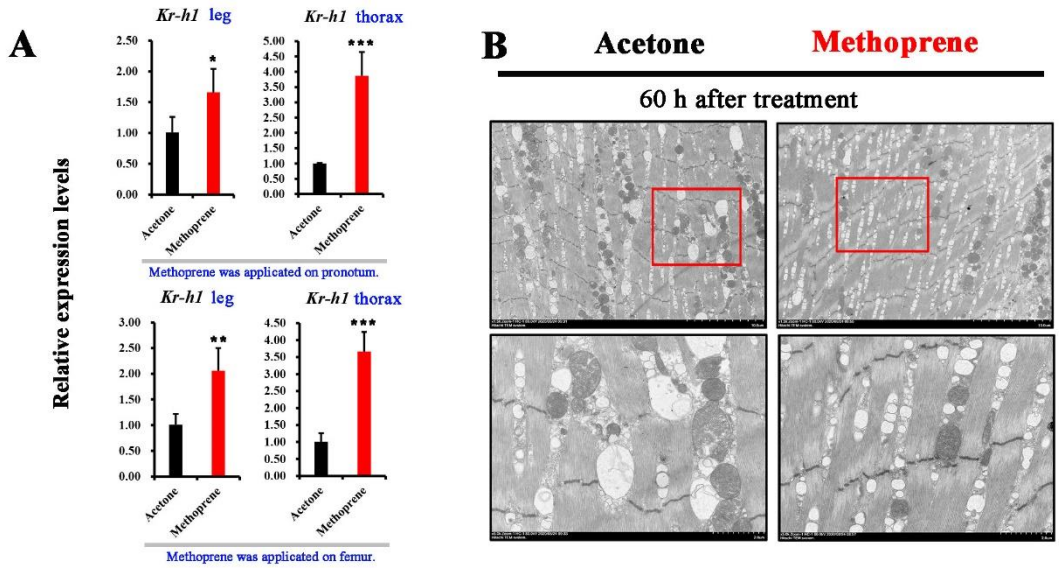

Fig. S5. JH application would not induce mitochondrial disruption and leg muscle fiber breakdown during starvation in winged female *A. pisum*. (A) Relative expression levels of *Kr-h1* in the legs and thoraxes of starved aphids at 12 h post methoprene treatment. Methoprene (100 ng/aphid) was applied at 24 h post eclosion, and the aphids were subjected to starvation. qPCR data are presented as the mean ± s.e.m. from 3 biological replicates (each replicate contains 10 aphids for the thoraxes or 50 aphids for the legs). Differences between 2 groups were determined by Student's t-test (*P < 0.05, ***P < 0.001). (B) Longitudinal ultrathin sections of leg muscles in the control and methoprene-treated aphids at 60 h after the treatment identified by TEM.

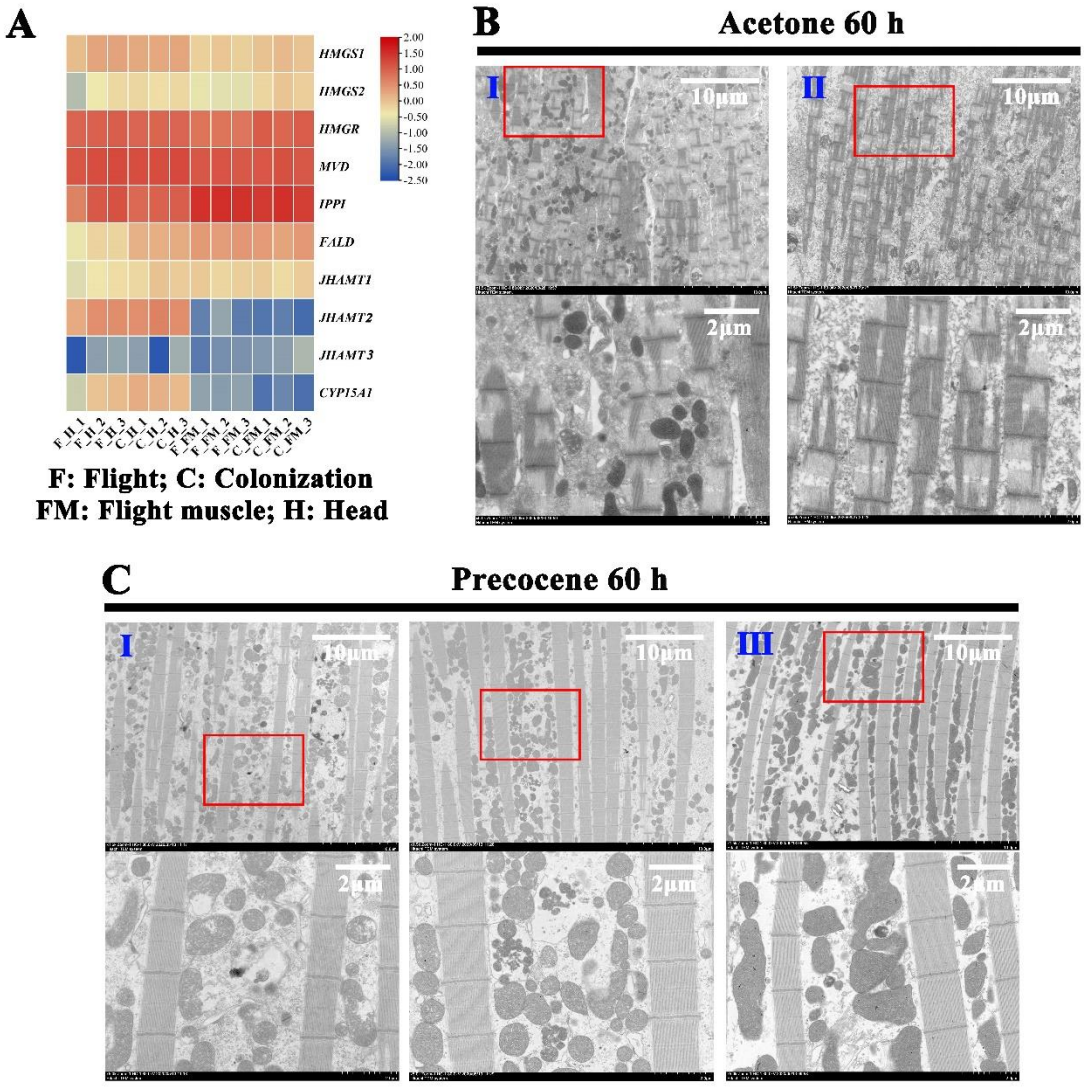

Fig. S6. JH biosynthesis level is relatively stable during colonization of winged aphids, which is necessary to colonization behavior. (A) Heat map representing the transcriptional changes (expression data from the RNA-seq of head and flight muscle) of genes involved in JH biosynthesis between "flight" and "colonization" aphids. (B and C) Effects of precocene II on histological changes of the flight muscles at 60 h after the treatment. Precocene (5 μg/aphid, dissolved in acetone) was topically applicated on the aphids 24 h post eclosion, and the treated aphids were placed in the feeding cages. I–III represents biological replications. Precocene inhibits colonization behavior of the winged aphids.

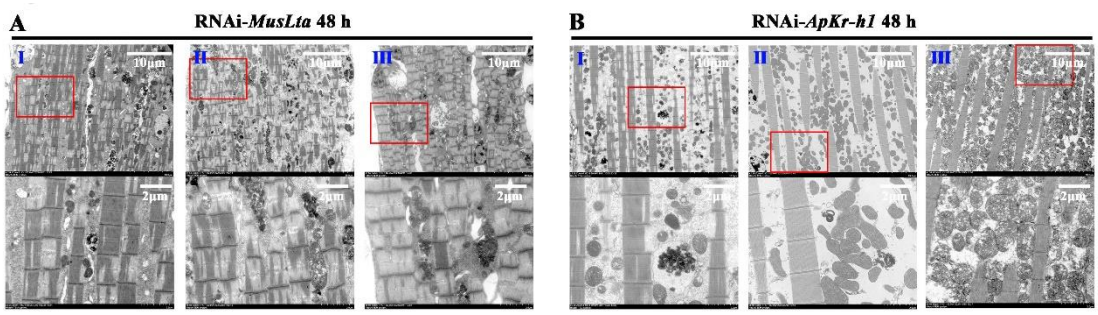

Fig. S7. RNAi-knockdown of *Kr-h1* delays flight muscle degeneration in winged *A. pisum*. TEM observation of the flight muscles of ds*Lta*- (A) and ds*Kr-h1*-injected (B) aphids at 48 h after dsRNA injection. Injection of dsRNA (6 μg/μL, 150 nL/aphid) was performed at 24 h after eclosion, and the treated aphids were placed in the feeding cages. I–III represents biological replications.

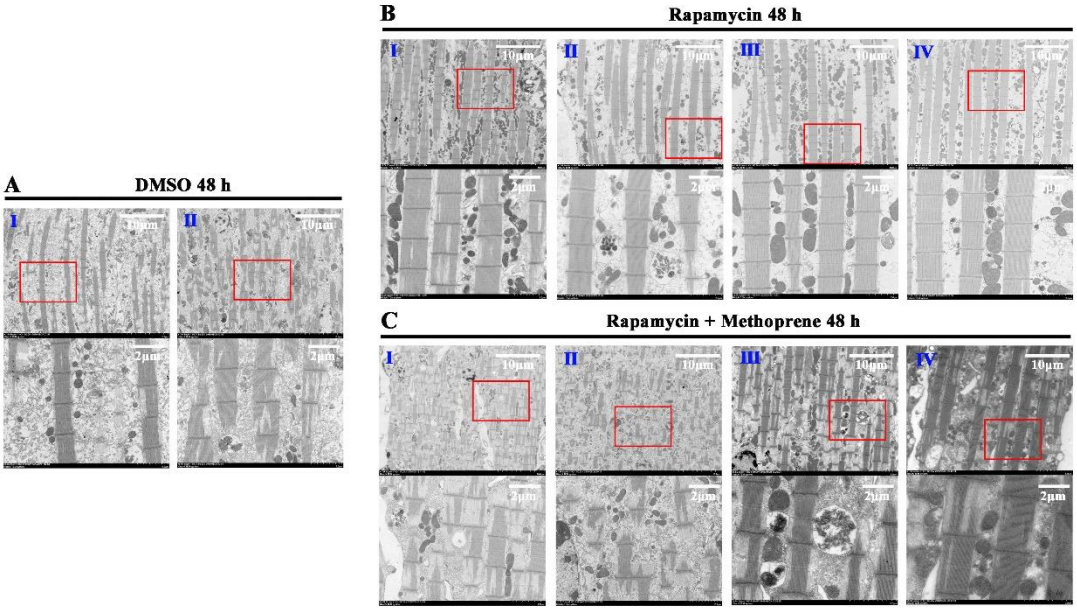

Fig. S8. Suppressing the TORC1 pathway by rapamycin inhibitor delays flight muscle degeneration in winged *A. pisum*. Histological changes of the flight muscles from DMSO-treated (A) aphids, rapamycin-treated (B) aphids, and methoprene-rescued aphids (C) at 48 h after the treatment. Rapamycin injection (2nM, 100 nL/aphid) and methoprene application (100 ng/aphid) were performed at 24 h after eclosion, and the treated aphids were placed in the feeding cages. I–IV represents biological replications.

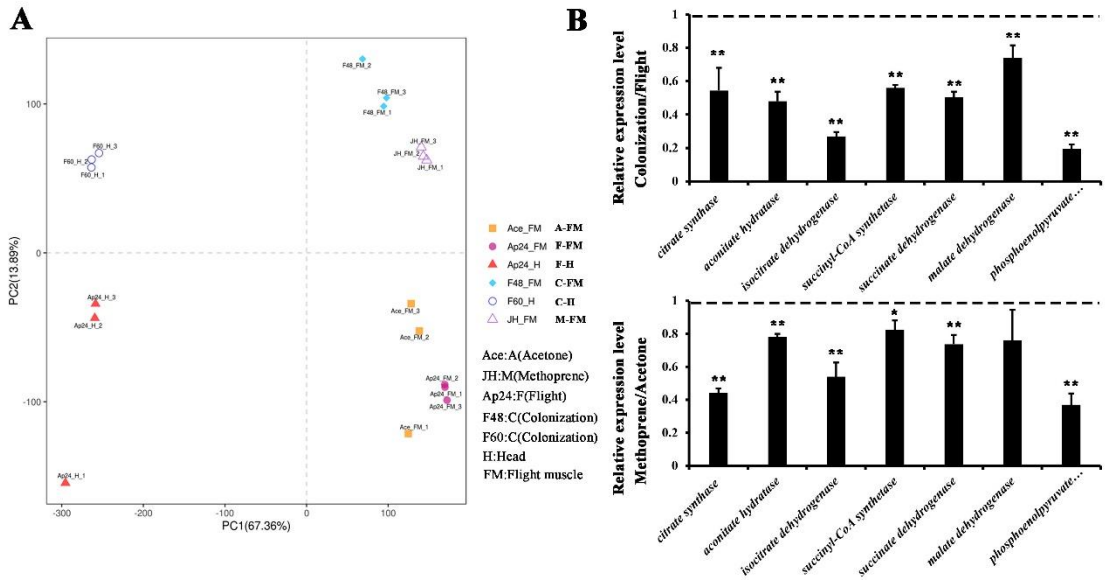

Fig. S9. Feeding and JH application down-regulates genes involved in the TCA cycle in the flight muscles of winged female *A. pisum*. (A) Principal component analysis (PCA) analysis of the whole RNA-seq samples involved in this study. (B and C) Transcriptome verification of selected genes involved in carbohydrate metabolism using qPCR. (B) Gene expression ratio between the aphids which are in the state of colonization and flight regarding the genes involved in the TCA cycle in the thoraxes. (C) Gene expression ratio of methoprene to acetone treated aphids regarding those same genes in the thoraxes at 24 h after the treatment. Differences between 2 groups were determined by Student's t-test (*P < 0.05, **P < 0.01). (CS, citrate synthase, geneID: 100162475; AH, aconitate hydratase, geneID: 100167414; ICDH, isocitrate dehydrogenase, geneID: 100161204; SCS, succinyl-CoA synthetase, geneID: 100168788; SDH, succinate dehydrogenase, geneID: 100165770; MDH, malate dehydrogenase, geneID: 100145825; PEPCK, phosphoenolpyruvate carboxykinase, geneID: 100160700)

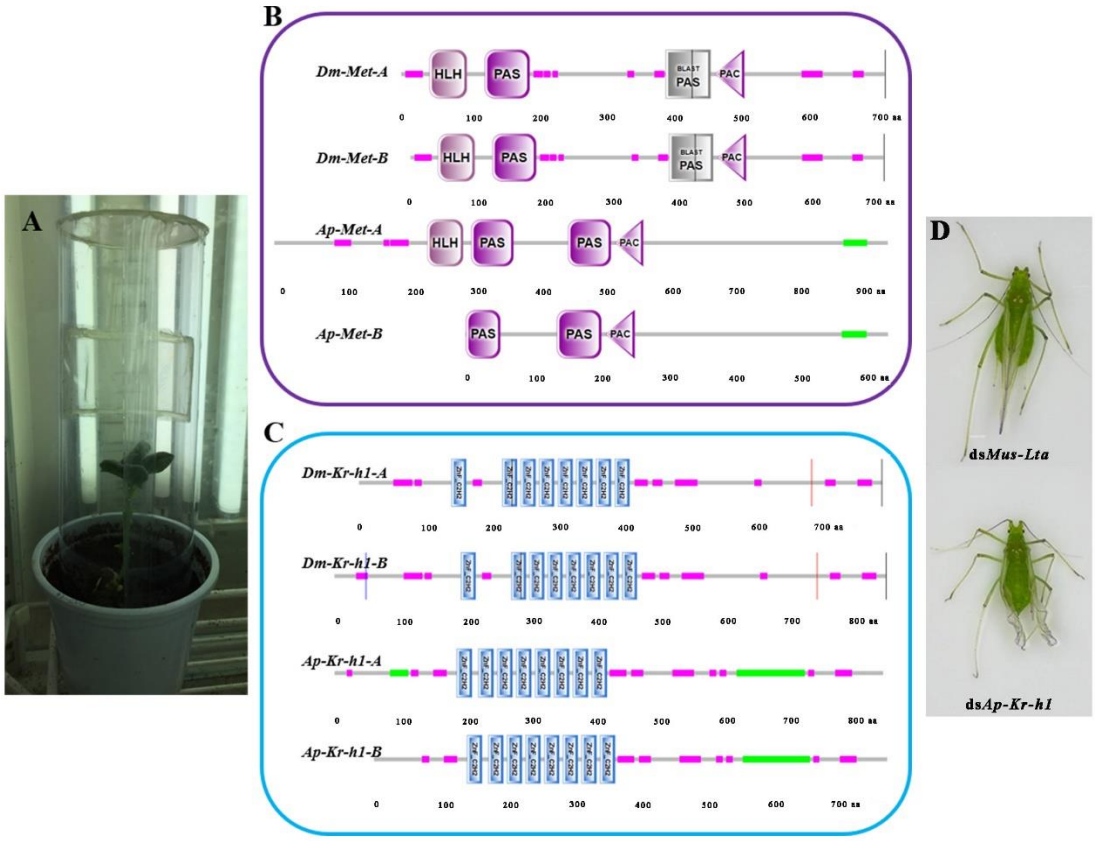

Fig. S10. The experimental devices and domain structure analysis of *ApMet* and *ApKr-h1* protein. (A) Cages supplied for the winged aphids to colonize and feed. Same as *Drosophila melanogaster*, *ApMet* and *ApKr-h1* have two transcriptional isoforms, respectively, named *ApMet* A/B and *ApKr-h1* A/B. Protein sequences are deduced by the ExPASy translation tool, and domains are predicted by the SMART tool. (B) Conserved domains of *DmMet* and *ApMet. Met* belongs to the basic helix–loop–helix (bHLH)-Per-Arnt-Sim (PAS) family of transcription factors. However, *ApMet* B lakes HLH domain. (C) Conserved domains of *DmKr-h1* and *ApKr-h1*. Both *DmKr-h1* A/B and *ApKr-h1* A/B contain eight C2H2-type zinc fingers (Znf) domains. (D) Phenotype of RNAi-*ApKr-h1*. Application of dsRNA was performed at 3rd nymph stage, and wing abnormal phenotype was obtained post eclosion.

Table S1. List of primers utilized in orthologue verification.

Fragment (bp)	Forward (5' to 3')	Reverse (5' to 3')
Ap-Met-A	AAGCTACCGGTGCAGTGTTG	CGTCAATCATATGCCTCAAGTGAAA
Ap-Met-B	AACCTATTCGCCTTTAATACGGAAA	CGTCAATCATATGCCTCAAGTGAAA
Ap-Kr-h1-A	CTTCAGTAGGCTGTTGACTGTTG	TTGTATAATGTGTACGTACGTTGCG
Ap-Kr-h1-B	TCGCACACTGTTCGTTATTTTTATT	TAATGTGTACGTACGTTGCGAAAAA

Table S2. List of primers utilized in qPCR.

gene	Forward (5' to 3')	Reverse (5' to 3')	Efficiency
q*Ap-JHAMT1*	CCATTAGTTGAGTTGTATAAATGT ATGGATAC	AATGTCTTTTATTTCATCTGGTGA ATACAC	*E* = 0.991
q*Ap-JHAMT2*	CAAGGCTGGATTTCGTATCA	TCATCTACAGCTCTGACGG	*E* = 0.985
q*Ap-JHAMT3*	TGCGGTTCTTCTTATTCGCTC	ATTAAGCAAGGCACCGACTTT	*E* = 0.844
q*Ap-Met*	TGCATCTGAACCTGGAATTG	ATCTTCAGTAACATCTTCCAATGT	*E* = 0.998
q*Ap-Kr-h1*	CGGTAGCGATAAGGAGAACA	GTCAGACAGCTGAAGCAATAC	*E* = 0.993
q*Ap-CS*	ACCACGAGGGTGGTAATGTA	CCATACCGGCACTGAATGAA	*E* = 0.990
q*Ap-AH*	CCAGACAAATTGAACGCCAG	AGCAATGACAACCGATCCAT	*E* = 0.995
q*Ap-ICDH*	AGGTCTAACACCCAGTGGAA	CGGCTATATCTGGAGCAGTTC	*E* = 0.989
q*Ap-SCS*	GTGGTGCTACTGCTTCTCAA	ATAGCACACACTTTGGGGTC	*E* = 0.991
q*Ap-SDH*	GGTGTGGGATGCTGGTAAAT	GCTACCGACAATCACACCAT	*E* = 0.997
q*Ap-MDH*	GTTCGGTCTAACAGATTCATTGC	CCGCCGATAACAGGAACATT	*E* = 0.992
q*Ap-PEPCK*	ACATGCTGATCTTAGGCGTG	CATCATCGCCATGTTGGTCT	*E* = 0.994
Ap-EF1α	TAGGAGGTATTGGAACAGTCC	TGTTTGCTGGTGCGAAAA	*E* = 1.030
q*Ap-rps20*	ATCAAAAGAGGCACAAAATCCGT	GCAATCATCTCGGAGCACAC	*E = 0.983*

Table S3. List of primers utilized in RNAi.

Fragment (bp)	Forward (5' to 3')	Reverse (5' to 3')
dsAp-Kr-h1 (307)	T7+AATGGTTCGGGAAGACGATG	T7+TTGGTGTCGACGAGTGATTG
dsMus-Lta (193)	T7+CACCCTCTCCACGAATTG	T7+TAGAAGATGCTGCTGTTTCA

T7 RNA polymerase promoter sequence: TAATACGACTCACTATAGGG.

Table S4. List of top pathways enriched in KEGG aggregation analysis from the comparative transcriptome of "colonization" vs "flight".

KEGG pathway	Background genes	**Up**-regulated genes	***P***-value
Proteasome	47	35	$2.59e^{-8}$
Protein processing in endoplasmic reticulum	126	35	$1.17e^{-5}$
Protein export	27	13	0.01099
Autophagy	27	12	0.02227
Phagosome	72	23	0.03952
KEGG pathway	Background genes	**Down**-regulated genes	***P***-value
Oxidative phosphorylation	125	53	$4.53e^{-6}$
2-Oxocarboxylic acid metabolism	22	14	0.00098
Citrate cycle (TCA cycle)	34	16	0.00461
Carbon metabolism	112	35	0.01178
FoxO signaling pathway	63	21	0.02693

Table S5. List of top pathways enriched in KEGG aggregation analysis from the comparative transcriptome of "methoprene" vs "acetone".

KEGG pathway	Background genes	**Up**-regulated genes	***P***-value
Proteasome	47	20	$1.05e^{-9}$
Protein processing in endoplasmic reticulum	126	20	0.00043
Endocytosis	130	20	0.00062
Autophagy	27	8	0.00089
ABC transporters	34	8	0.00310
KEGG pathway	Background genes	**Down**-regulated genes	***P***-value
Folate biosynthesis	32	6	0.00154
Carbon metabolism	32	6	0.00154
2-Oxocarboxylic acid metabolism	22	5	0.00184
Thiamine metabolism	14	4	0.00266
Cysteine and methionine metabolism	42	6	0.00520