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The microRNA pathway activation in insect cell model upon *Autographa californica* multiple nucleopolyhedrovirus infection

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Abstract: Background: The immune system of insects exerts fundamentally different antiviral mechanisms than mammals. MicroRNAs (miRNAs) play vital roles in developing insect antiviral immunity. MiRNAs expression profiles of insects changed significantly during baculovirus infection. **Methods:** Differential expression profiles of miRNAs in *Spodoptera frugiperda* were monitored by next-generation sequencing (NGS) and RT-qPCR during *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) infection. The transcription levels of genes were detected by RT-qPCR. The 50% tissue culture infective dose (TCID$_{50}$) endpoint dilution assay was used to determine the proliferation of progeny virus. Results: NGS revealed that 49 miRNAs were differentially expressed in Sf9 cells, and 10 of them were significantly up- or down-regulated. Though RT-qPCR analysis, we observed the similar trends for the expression patterns of significantly differentially expressed miRNAs from NGS. Moreover, the transcription levels of core genes, *Exportin5*, *Dicer1*, and *Argonaute1*, in miRNA biogenesis pathways were significantly increased after AcMNPV infection. For five selected miRNAs, miR-34-5p could regulate the proliferation of baculovirus progeny virus and energy metabolism. **Conclusion:** The miRNAs biogenesis pathway in Sf9 cells plays an important role and may be stimulated to resist AcMNPV infection. This work provides evidence for the molecular mechanism of baculovirus-insect interaction and offers novel ideas and directions for green pest control technology.

Introduction

MicroRNAs (miRNAs) are evolutionarily conserved, short (19~25 nucleotides) noncoding RNAs that regulate the post-transcriptional silencing of target genes (Lee *et al.*, 1993; Reinhart *et al.*, 2000). Based on the canonical pathway, a miRNA gene is transcribed by RNA polymerase II in the nucleus to produce the primary miRNA (pri-miRNA) transcript, which contains one or more stem-loop structures; pri-miRNA is cleaved by Drosha (a ribonuclease III enzyme) into hairpin precursor miRNA (pre-miRNA), and then it is translocated into the cytoplasm by exportin-5. In the cytoplasm, pre-miRNA is cleaved by dicer, producing a short duplex RNA molecule (Lee *et al.*, 2004; Thakur *et al.*, 2022; Bartel, 2004). Two dicer proteins have been identified in insects: dicer-1 is mainly involved in miRNA biogenesis, and dicer-2 produces siRNAs (Lee *et al.*, 2004). The miRNA duplex triggers the formation of the RNA-induced silencing complex (RISC), and Argonaute protein (Ago) is the critical component of RISC. There are two Ago proteins: Ago1 for miRNA and Ago2 for siRNAs; however, recent research indicates that miRNAs can also be loaded into Ago2 complexes, especially in insects (Yang and Lai, 2011). When the miRNA duplex becomes related to the RISC, one of the strands is degraded, and the other forms the mature miRNA (Yang and Lai, 2011). MiRNAs have been reported in plants and animals, even their virus. MiRNA expression profiles of various insects and diverse functions, such as development and host-virus interactions, were found (Monsanto-Hearne and Johnson, 2018; Shrivastava *et al.*, 2022).

Baculoviruses are large double-stranded DNA viruses that infect insects. *Autographa californica* multiple nucleopolyhedroviruses (AcMNPV) is the archetypal species of the alphabaculovirus. *Spodoptera frugiperda* is one of the most important pests for many crops, such as corn, soybeans, and sorghum, especially for maize and cotton in America (Johnson, 1987). It has recently invaded the African and Asian continents and first appeared in Yunnan Province, China, in January 2019 (Li *et al.*, 2020). The interaction between baculoviruses and their insect hosts is

complex and varied. After infecting the hosts, both baculoviruses and the insect hosts change in a series of ways to respond to this state. Baculoviruses affect apoptosis, cell cycle, and development of the host (Zhou et al., 2004) and acquire beneficial genes from the host genome for its replication (Thiem, 2009). The hosts also respond to the virus infection simultaneously by regulating the gene expression of baculoviruses, activating some proteins that resist viruses and immune pathways, such as miRNAs pathways (Thiem, 2009; Kemp et al., 2013; Liu et al., 2015; Zhao et al., 2022b).

Based on previous studies, we tried to reveal the role of the miRNA biogenesis pathway in the response of hosts to baculoviruses and to find miRNAs that regulate baculovirus replication. Dubey et al. (2017) reported that miR-2b expression was up-regulated during chikungunya virus (CHIKV) infection in Aedes aegypti. The ubiquitin-related modifier (URM) transcript is negatively regulated by miR-2b to control CHIKV replication (Dubey et al., 2017). The expression level of bantam-3p was increased after AcMNPV infection in Sf9 cells. Virus DNA replication was decreased in the presence of bantam-3p mimic and increased in the presence of bantam-3p inhibitor (Shi et al., 2016). In this study, miRNA expression profiles of Sf9 cells were detected by next-generation sequencing (NGS) during AcMNPV infection. We revealed the miRNA expression in insect cells during baculovirus infection and the critical roles of the miRNA pathways in virus-host interaction. Further, we found the vital function of miR-34-5p, which was down-regulated after AcMNPV infection, in regulating viral replication.

Materials and Methods

Cell line and viruses

S. frugiperda IPLB-Sf21-AE colonial isolate 9 (Sf9) cells were cultured in a serum-free insect cell culture medium (SFX-Insect, HyClone, US) at 27°C. Recombinant baculovirus AcMNPV-enhanced green fluorescent protein (EGFP), which was constructed by inserting the egfp gene under the locus of the p10 promoter of AcMNPV, was used to infect Sf9 cells. Sf9 cells were infected with five multiplicity of infection (MOI) AcMNPV-EGFP, and were harvested at 72 h post-infection (h p.i.).

Next-generation sequence analysis of Sf9-encoded miRNAs

Sf9 cells were infected with AcMNPV-EGFP and were used for the sequencing of small RNAs (sRNAs). Total RNA was extracted from Sf9 cells, and the small RNA libraries were further sequenced using Illumina Hiseq 2500 as per Illumina protocols by Sangon Biotechnology (Shanghai, China). The reads having lengths between 17–34 bp were considered.

Mature miRNA sequences and pre-miRNA sequences of all available insect species were obtained from the miRBase v22.1 database. The 17–35 bp reads were mapped against known mature sequences of S. frugiperda, and those reads satisfying the set criteria were separated and named based on their subject annotation. The unannotated sRNAs to known sequences of S. frugiperda were mapped to other insects' miRNAs in miRBase. The highly homologous miRNAs to other insects were considered to be known miRNAs.

The remaining sequences were subjected to novel miRNAs, which were predicted by miRDeep2 v2.0.0.8 (Friedländer et al., 2012). The potential of novel miRNA precursors and sequences to form a hairpin structure was also considered by miRDeep2, and the miRDeep2 score was used to measure the authenticity of novel miRNAs. The name of the novel miRNAs was abbreviated with '-' ligature species name, 'novel', number, 'mature' or 'star', for example, 'sfr-novel-1-mature'.

MiRNAs expression from NGS was calculated after normalizing the reads. The samples were normalized by calculating reads per million of total sRNA reads (RPM) and were used for comparing the relative abundance of specific miRNAs. The miRNAs in AcMNPV-infected cells were further profiled against mock cells to identify the differentially expressed miRNAs upon AcMNPV-EGFP infection by edgeR (Zhou et al., 2014). The expression value of RMP after normalization was >1 as the filtering condition, and the miRNAs with low expression levels were filtered out.

MiRNAs target genes prediction was performed by the Miranda algorithm (Fahlgren et al., 2007). The threshold parameter set in the prediction process was S ≥ 150, ΔG ≤ −30 kcal/mol, and demanded strict 5′ seed pairing. S refers to single-residue-pair match scores in the matching area, ΔG is Gibbs free energy of miRNA: mRNA binding formation. 3′ untranslated region (3′UTRs) of S. frugiperda were supplied from transcriptome sequencing data (National Center for Biotechnology Information Sequence Read Archive (SRA) accession numbers: SRP075752 (Sf9 cells), SRP075795 (Sf9 cells infected by AcMNPV-EGFP), which have been published by our laboratory (Wei et al., 2017). The targets of selected down- or up-regulated miRNAs were further subjected to analysis by clusterProfiler for Gene Ontology enrichment and KEGG pathway, keeping the threshold P-value < 0.05.

Differential expression of miRNAs analysis by reverse transcription qPCR (RT-qPCR)

The significantly differentially expressed miRNAs from NGS were verified by RT-qPCR. Total RNA was isolated using RNAiso Plus (Takara, Dalian, China). cDNA of mature miRNAs was synthesized by the tailing reaction method using miRNA First Strand cDNA Synthesis kits (Sangon Biotechnology Corp, Shanghai, China). The U6 RNA was included as the reference. The stem-loop RT-qPCR method is widely used to detect and quantify mature miRNAs. For cDNA synthesis of miRNA by stem-loop RT-qPCR method, total RNA was reverse transcribed in combination with the specific stem-loop reverse transcription primer of each miRNA. The 5S rRNA was reverse transcribed by random primer and included as the reference for the respective sample. The sequence of all primers is listed in Suppl. Table 1.

The transcription level of protein genes in the miRNAs biogenesis pathway was validated by reverse transcription qPCR

Total RNA was isolated using RNAiso Plus (Takara Biotechnology, Dalian, China). The cDNA was synthesized using an EasyScript First Strand cDNA Synthesis SuperMix (Transgen, Beijing, China) with oligo dT primer, and the β-

actin was used as the endogenous reference gene. The qPCR was conducted with PerfectStart[TM] Green qPCR SuperMix (Transgene, Beijing, China). The $2^{-\Delta\Delta Cq}$ method was used to calculate the relative level of miRNA or mRNA. The sequence of all primers is listed in Suppl. Table 1.

Transfection and infection

miR-11-3p, miR-2a-3p, miR-34-5p, miR-92a-3p, and miR-278-3p mimics and inhibitors were chemically synthesized and modified by Sangon Biotechnology (Shanghai, China). The negative control of mimic (mimic NC) and inhibitor (inhibitor NC) were provided by Sangon Biotechnology. The mimics and inhibitors were used to transfect into Sf9 cells by TransIT-Insect Transfection Reagent (Mirus, USA), following the manufacturer's instructions. Sf9 cells were infected with 5 MOI AcMNPV-EGFP at 8 h after transfection with 5 nM miRNA mimic, 40 nM miRNA inhibitor, 5 nM mimic NC, and 40 nM inhibitor NC, respectively.

Proliferation analysis of progeny virus

Sf9 cells were infected with AcMNPV-EGFP after transfecting with miRNA mimics or inhibitors. The supernatants containing the budded virions (BVs) were harvested and serially diluted 10^{-1}–10^{-8} times. The proliferation of progeny virus was determined using the 50% tissue culture infective dose (TCID$_{50}$) endpoint dilution assay (Emery, 1992; Feng *et al.*, 2012). The process is described below. The Sf9 cells were infected with the diluted viruses. The experiment with each concentration gradient was repeated eight times at least. After 5–7 days, the number of green fluorescence in each experimental group was counted, then the TCID$_{50}$ of each group was calculated.

Determination of glucose consumption, lactic acid content, and ATP content

Supernatants in multiple groups were obtained to determine the contents of glucose and lactic acid using the Glucose content determination Kit and Lactic acid determination Kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the protocol of the manufacturers. Sf9 cells pellets were obtained to determine the content of ATP using the ATP content determination Kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), and the protein concentrations were determined with BCA Protein Assay Kit (Solarbio Life Sciences, Beijing, China). The glucose mass in the culture medium was calculated according to the following formula:

Glucose mass (mg) = glucose content (mmol/L) × medium volume (L) × glucose molar mass (180 mg/mmol)

The glucose mass in the fresh culture medium was subtracted from the glucose mass in the medium of the treatment group to obtain glucose consumption.

Statistical analyses

Statistical analyses were performed by SPSS software. Data were shown as mean ± SD from at least three independent experiments. The averages of the treatment and the control groups were compared using a bidirectional unpaired Student's *t*-test. *P*-value < 0.05 was set to be statistically significant (*P < 0.05, **P < 0.01).

Results

sRNAs of Sf9 cells by next-generation sequencing

We sequenced small RNA libraries generated from uninfected Sf9 cells and AcMNPV-EGFP-infected Sf9 cells by Illumina Hiseq 2500. In total, 26176613 reads were generated from all libraries. After discarding low-quality and junk sequences, 3610334 and 4121022 unique reads without redundant sequences were obtained in size range of 17–35 bp in mock and AcMNPV-EGFP-infected Sf9 libraries, respectively (Table 1).

The length distributions of the total small RNA in size ranged from 17 to 35 nt and showed two prominent peaks. One peak was located between 19–22 nt, which is the typical length of mature miRNAs and siRNAs. However, we finally failed to find the miRNA or siRNA encoded by AcMNPV. The other peak was located between 27–30 nt, which may be considered the size of P-element induced wimpy testis (PIWI)-interacting RNAs (piRNAs) (Zharikova and Mironov, 2016). The number of miRNAs and piRNAs represented about 50% and 40% of the total sRNA reads, respectively, which were preponderant (Fig. 1a).

Identification of known miRNAs and novel miRNAs

We detected 91 known miRNAs in all libraries. The details are shown in Suppl. Table S2. The top ten known miRNAs with the highest abundance were bantam-3p, miR-11-3p, miR-2b-3p, miR-2a-3p, miR-10482-5p, miR-2755-3p, miR-2c-3p, miR-92b, miR-10458-3p, miR-10483-5p, and some of them were in concurrence with recent reports (Mehrabadi *et al.*, 2013; Kakumani *et al.*, 2015).

The miRDeep2 v2.0.0.8. analysis revealed that 104 novel mature miRNAs were identified as novel miRNAs (Suppl. Table S3). A higher miRDeep score means a higher degree of authenticity for the novel precursor miRNAs, by extension, a higher degree of authenticity for novel miRNAs (Friedländer *et al.*, 2008). Therefore, in Suppl. Table S3, the authenticity of novel miRNAs with higher miRDeep scores is greater than that with lower miRDeep scores, and the authenticity of each novel miRNA needs further experimental verification, such as the northern blot.

Not only the novel miRNAs but also some known miRNAs have homology with other insect species. More than 70% of homologous miRNAs were found in *Bombyx mori*, almost 60% in *Tribolium castaneum*, 50% in *Manduca sexta* and *Drosophila melanogaster*, 44% in *Heliconius*

TABLE 1

Sequencing statistics of mock and AcMNPV-EGFP-infected Sf9 libraries

Sample	Raw date[1]	Total reads[2]	Unique reads[3]	GC percentage
Mock	13254719	10150788	3610334	43.87%
AcMNPV-EGFP-infected	12921894	10813118	4121022	47.05%

Note: [1] The initial reads obtained from Illumina sequencing were named as raw data. [2] Raw data were taken for adapter trimming and filtering of bad quality data and reads less than 17 bp. The remaining sequences were named as total reads. [3] Total reads without redundant sequences were named as unique reads.
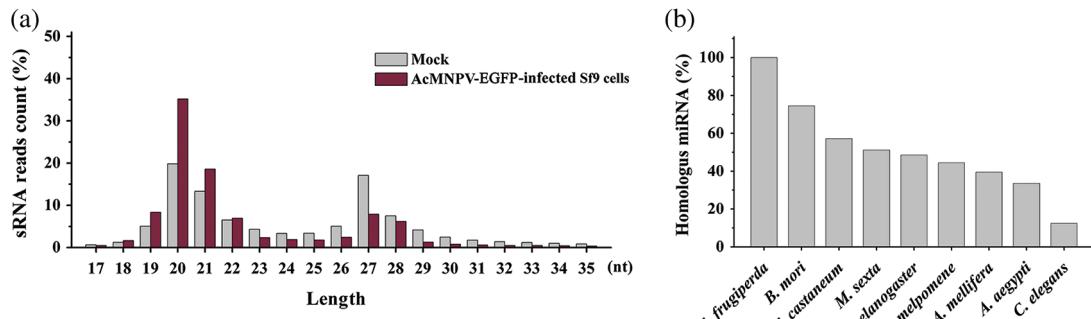
FIGURE 1. Length distribution of small RNA reads and conservation of homologous miRNAs in all libraries. (a) Length distribution of small RNA reads in mock and AcMNPV-EGFP-infected Sf9 libraries; (b) Percentage of miRNAs in Sf9 cell with homologous miRNAs in *B. mori*, *T. castaneum*, *M. sexta*, *D. melanogaster*, *H. melpomene*, *A. mellifera*, *A. aegypti* and *C. elegans*.

melpomene, 39% in *Apis mellifera*, 33% in *Aedes aegypti*, only 12% in *Caenorhabditis elegans* (Fig. 1b). miRNAs in *B. mori* and *T. castaneum* were more similar to these in *S. frugiperda* than other insects.

Differential expression of Sf9 miRNAs upon AcMNPV-EGFP infection
The result of NGS revealed 49 miRNAs differentially expressed in Sf9 cells ($P < 0.05$) (Fig. 2a). Based on P-value < 0.05 and the absolute value of $\log_2$ fold change >1 (difference times >2), we identified five significantly up-regulated miRNAs, which were miR-10461a-5p, miR-275-3p, miR-10453-5p, miR-10484-3p, miR-2765-5p; and five down-regulated miRNAs, including miR-263b-5p, miR-10499b-5p, bantam-5p, miR-745-5p, miR-79-5p. The differential expression level of miRNAs suggested that they might play important roles in host-viruses interaction.

Target gene prediction, GO enrichment, and KEGG pathway analyses
MiRNAs regulate target gene expression through the level of complementary binding between miRNAs sequence and their target mRNA. MiRNAs mainly bind to the 3′UTR of the target mRNA. According to the principle, the differentially expressed miRNAs mentioned above were analyzed for their putative targets by the Miranda algorithm. The predicted targets were involved in a broad range of biological processes. The significant GO terms and KEGG pathways target genes are shown in Figs. 2b and 2c. The most significantly enriched GO terms included nucleus (GO: 0005634) and cytoplasm (GO: 0005737), protein ubiquitination (GO: 0016567), etc. It is worth noting that positive regulation of gene silencing by miRNA (GO: 2000637) in biological process terms was included in GO enrichment. The most significantly enriched KEGG pathways included pathways in the insulin signaling pathway (ko04910), tight junction (ko04530), p53 signaling pathway (ko04115), etc. These results showed that miRNAs played vital roles in host-virus interaction by altering the expression of target genes, and these potential target genes were involved in a variety of biological processes.

Validation of differential expression miRNAs by RT-qPCR
Nine significantly differentially expressed miRNAs (excluding bantam-5p, a miRNA star of bantam) and some other miRNAs (miR-252-5p, miR-92a-3p, miR-92b, miR-11-3p, bantam-3p, miR-2a-3p, miR-34-5p, miR-278-3p) from NGS (Fig. 2a) were verified by RT-qPCR analysis. We observed a similar trend for the expression patterns of these miRNAs measured by RT-qPCR when compared with that in the miRNA NGS data (Fig. 3a). However, miR-278-3p was down-regulated, which was different from that of NGS. miR-34-5p, whose expression was down-regulated in Sf9 cells during AcMNPV-EGFP infection, became the focus of the follow-up studies.

Core genes in the miRNAs biogenesis pathway were regulated by AcMNPV-EGFP
As shown in Figs. 3b~3e, the relative transcription level of *Exportin5* increased from 12 to 72 h p.i.; the transcription level of *Ran* remained almost unchanged until 72 h p.i.; the transcription level of *Dicer1* was increased at 12, 48, and 72 h p.i.; the transcription level of *Argonaute1* significantly increased from 24 to 72 h p.i. These results demonstrated miRNA biogenesis pathway in Sf9 cells was activated during AcMNPV-EGFP infection.

miRNA-34-5p suppressed the replication of progeny virus
According to the above results, the miRNA biogenesis pathway in Sf9 cells was activated to resist AcMNPV infection. MiRNAs play essential roles in insect-baculovirus interaction. However, which miRNAs play key roles in insect-baculovirus interactions? We selected five miRNAs, which are highly expressed in Sf9 cells and are sensitive to AcMNPV infection, and investigated their regulatory effects on the replication of AcMNPV. They were miR-11-3p, miR-2a-3p, miR-34-5p, miR-92a-3p, and miR-278-3p, respectively. Different synthetic miRNAs mimics and inhibitors were used to regulate the expression level of their respective miRNAs in Sf9 cells. The RT-qPCR analysis results showed that miRNAs expression level was significantly up and down-regulated in Sf9 cells by their mimics and inhibitors transfection until 72 h p.i. (Suppl. Fig. S1, Fig. 4b). As shown in Suppl. Figs. S1b, S1c and S1d, in miR-2a-3p, miR-92a-3p, and miR-278-3p mimics and inhibitors groups, the infectious BVs levels exhibited similar to AcMNPV-EGFP groups. It means that miR-2a-3p, miR-92a-3p, and miR-278-3p did not affect the proliferation of BVs. The proliferation levels in the AcMNPV-EGFP, miR-11-3p mimic, and miR-11-3p
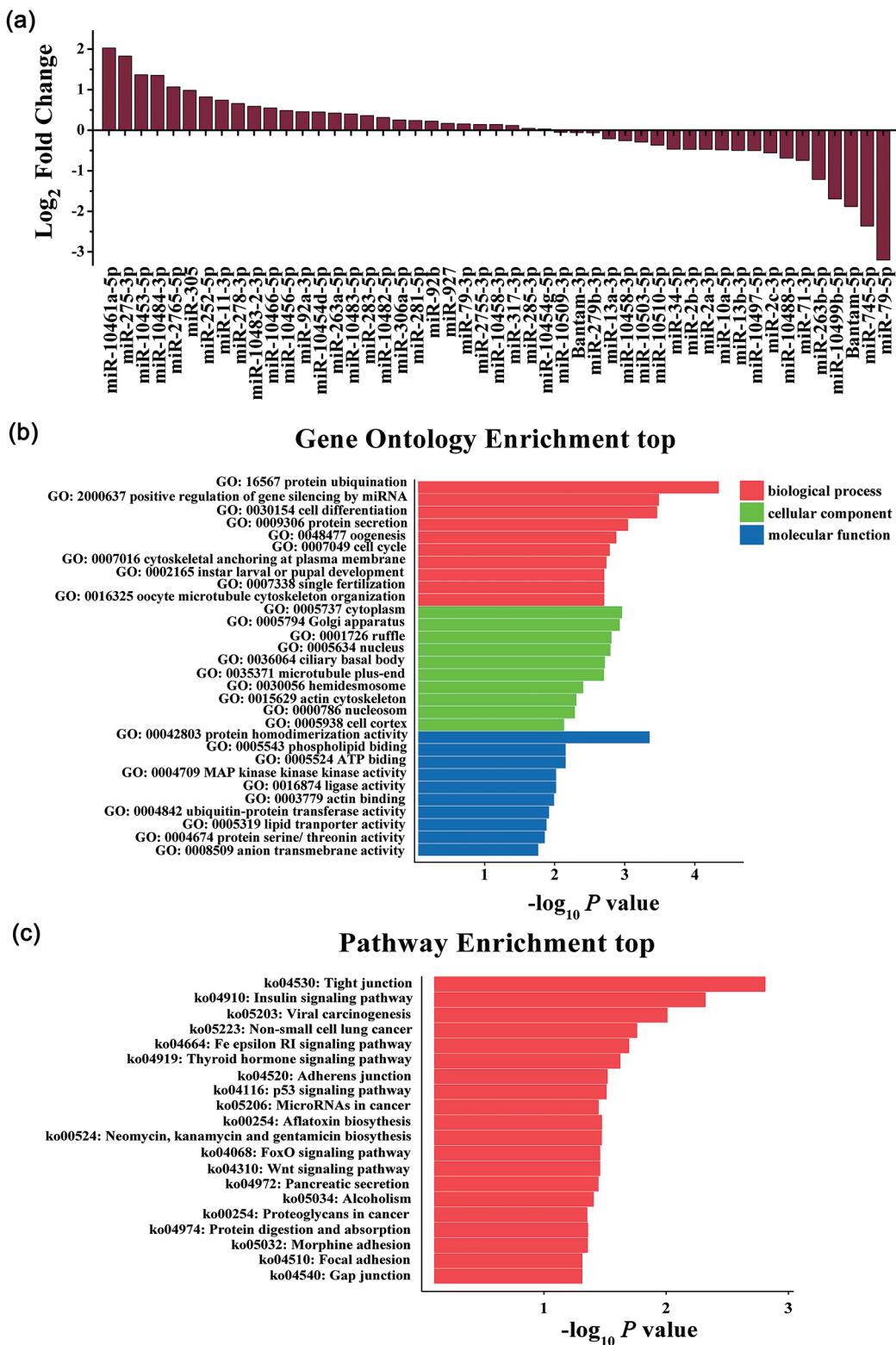
FIGURE 2. Differential expression of miRNAs detected by NGS and GO enrichment and KEGG pathway analysis of target genes. (a) Log_2 Fold change of miRNAs identified in mock and AcMNPV-EGFP infected Sf9 cells by NGS; (b) GO enrichment analysis. Bar charts represented the top 10 of each Gene Ontology term targeted by miRNAs; (c) KEGG pathway analysis. Bar charts represented the top 20 KEGG pathways targeted by miRNAs.

inhibitor groups showed no significant difference at 24 and 72 h p.i. However, at 48 h p.i., the proliferation level of progeny virus in the miR-11-3p mimic group was higher than that in the other two groups (Suppl. Fig. S1a). It implied that miR-11-3p exerted a particular effect on the BV proliferation.

In these five miRNAs, we focused on miR-34-5p. In the miR-34-5p mimic treatment group, the BVs titer showed a distinct down regulation compared to AcMNPV-EGFP group at 24, 48, and 72 h p.i. In contrast, the proliferation levels of progeny virus in the miR-34-5p inhibitor group
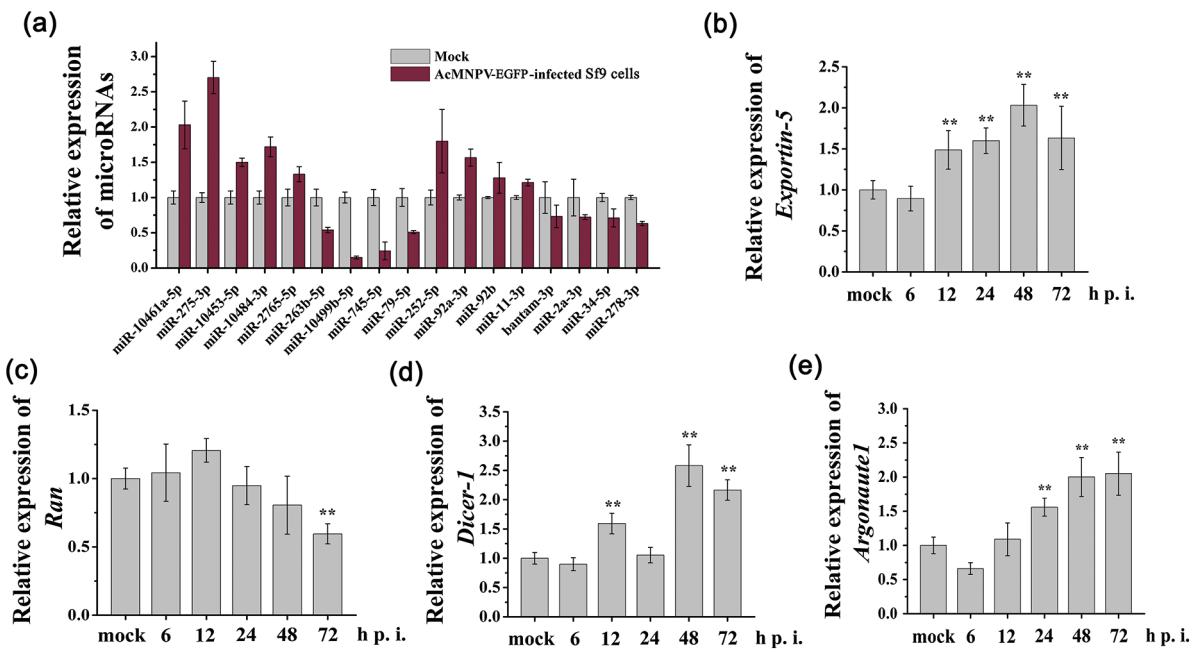
FIGURE 3. RT-qPCR analysis of relative expression of miRNAs and core genes in miRNAs biogenesis pathway. (a) miRNAs from the sequencing data were verified; (b~e) Relative transcription levels of core genes in miRNAs biogenesis pathway were verified. The asterisks indicated that the *t*-test showed a signifcant difference relative to the control (**$P < 0.01$).

were increased compared to AcMNPV-EGFP group (Fig. 4a). These phenomena demonstrated that miR-34-5p inhibited the proliferation of progeny virus. When Sf9 cells were infected by AcMNPV, a series of changes were stimulated in the host, including the differential expression of some miRNAs. miR-34-5p inhibits the infectivity of progeny virus, while the virus inhibits the expression of miR-34-5p for its proliferation.

miR-34-5p suppressed the glucose metabolism in host cells
Baculovirus growth relies heavily on host resources. The utilization and distribution of energy in the host can influence viral replication (Feng *et al.*, 2021). Glucose is the most critical energy source of insect cells in culture, and baculovirus uses the host's glucose to provide energy for its replication through the tricarboxylic acid (TCA) cycle (Kamen *et al.*, 1996; Bernal *et al.*, 2009; Lin *et al.*, 2020). The glucose consumption in the medium was increased significantly during AcMNPV-EGFP infection. At the same time, the ATP content of Sf9 cells showed similar trend to the glucose consumption during AcMNPV-EGFP infection, which was increased significantly (Suppl. Fig. S2). Overexpression of miR-11-3p increased the glucose consumption compared with the AcMNPV-EGFP group, and ATP content and lactic acid accumulation were also increased, although they were still higher than in uninfected group. Down regulation of miR-11-3p showed the opposite effects (Suppl. Fig. S2a). Overexpression of miR-2a-3p inhibited glucose consumption compared with the AcMNPV-EGFP group, and lactic acid accumulation was also decreased. Down regulation of miR-2a-3p showed the opposite effect (Suppl. Fig. S2b). For miR-92a-3p and miR-278-3p, there was no significant difference among mimic, inhibitor, and AcMNPV-EGFP groups in terms of the glucose consumption, ATP generation and lactic acid accumulation (Suppl. Figs. S2c and S2d). These phenomena

suggested that miR-11-3p and miR-2a-3p affected the energy metabolism of host cells during AcMNPV infection.

Significantly, overexpression of miR-34-5p inhibited the glucose consumption and ATP content compared with AcMNPV-EGFP group, although the glucose consumption was still higher than in mock group. And down regulation of miR-34-5p showed the opposite effect (Figs. 4c and 4d). Compared with the AcMNPV-EGFP and mock groups, down regulation of miR-34-5p increased lactic acid accumulation, although the accumulation was still higher than that in mock group (Fig. 4e). These phenomena suggested that overexpression of miR-34-5p inhibited glucose utilization by virus, while down regulation of miR-34-5p contributed to lactic acid accumulation.

Discussion

MiRNAs have been discovered in many insects, including *B. mori*, *D. melanogaster*, *A. aegypti*, etc. (He *et al.*, 2008; Monsanto-Hearne and Johnson, 2018). Moreover, the expression levels of host miRNAs could be altered during virus infection, and the differential expression of the miRNAs represented one aspect of host antiviral responses (Kong *et al.*, 2018). In this study, the length distributions of the total sRNA in size 17–35 nt showed two major peaks. One peak, located between 19–22 nt, was considered to be mature miRNA or siRNA because of the matched size. Theoretically, these miRNA or siRNA contain both host Sf9 cell encoded miRNA and AcMNPV encoded miRNA. According to the method (Singh *et al.*, 2010), we used the BLASTn program to map the unique reads with the AcMNPV genome, 8626 unique reads from infected Sf9 cells mapped onto the AcMNPV genome. At present, five miRNAs encoded by AcMNPV have been found, which are AcMNPV-miR-1, AcMNPV-miR-2, AcMNPV-miR-3,
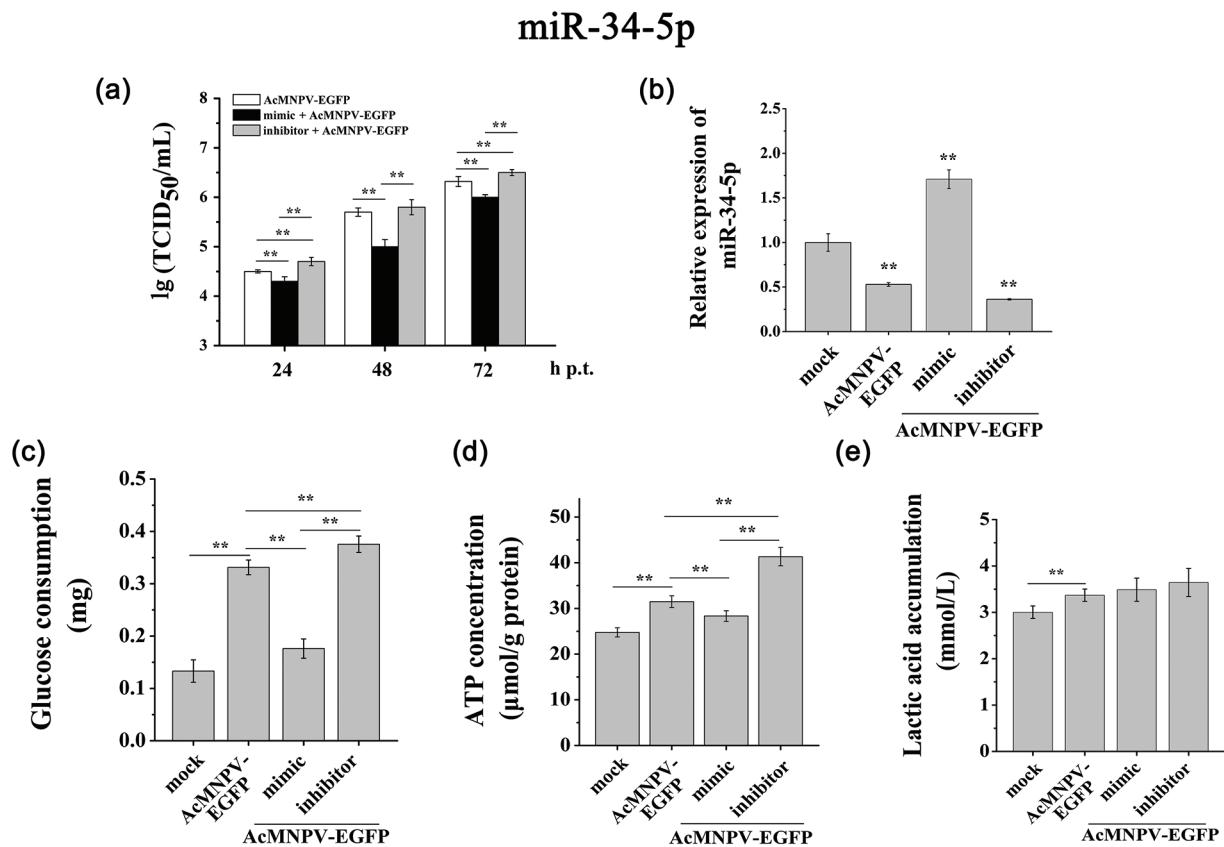
miR-34-5p

FIGURE 4. miR-34-5p regulated the proliferation of AcMNPV-EGFP and the energy metabolism of host cells. (a) 50% tissue culture infective dose ($TCID_{50}$) end-point dilution assay; (b) RT-qPCR analysis of the expression of miR-34-5p after transfection with miR-34-5p mimic and inhibitor; (c~e) Effects of miRNAs on energy metabolism in host Sf9 cells. The effect of miR-34-5p mimic and inhibitor on the glucose consumption (c), ATP content (d) and lactic acid accumulation (e) in host Sf9 cells. The asterisks indicated that the t-test showed a significant difference relative to the control (**$P < 0.01$).

AcMNPV-miR-4, and AcMNPV-miR-5, respectively. Among them, the functions of AcMNPV-miR-1, 3, and 4 have been clarified, respectively (Zhu *et al.*, 2013; Zhu *et al.*, 2016; Jiao *et al.*, 2019; Wang *et al.*, 2021; Zhao *et al.*, 2022a). However, we did not find the miRNA or siRNA encoded by AcMNPV, including these five known AcMNPV-miRs. At the same time, due to the low copy number of miRNA encoded by AcMNPV, we are looking for novel methods to find them. The other peak between 25–30 nt corresponds to the size of piRNAs. PiRNAs have several functions, such as regulation of transposon expression and transposition, regulation of host genes' expression, the influence on epigenetic mechanisms, histone modifications, and DNA methylation (Zharikova and Mironov, 2016; Kolliopoulou *et al.*, 2019). Moreover, piRNAs have other structural features, such as a bias for U at position 1 and a 'ping-pong' signature (Vodovar *et al.*, 2012). Therefore, whether sRNAs in the other peak of 25–30 nt are true piRNAs needs further study and proof. Here, we just focused on miRNAs encoded by Sf9 cells.

We detected 91 known miRNAs and 104 novel miRNAs in sRNA libraries. Upon AcMNPV-EGFP infection, miRNAs showed differential expression. The result revealed 49 miRNAs to be differentially expressed in Sf9 cells. These differentially expressed miRNAs are not the same as the report from Mehrabadi *et al.* (2013). They identified 116 known miRNAs in Sf9 cells, and many miRNAs were differentially expressed. However, reads from deep sequencing were mapped to *B. mori* genome sequences as a primary source of reference because the *S. frugiperda* genome was not available until 2013 (Mehrabadi *et al.*, 2013). In this study, the *S. frugiperda* genome (ZJU_Sfru_1.1), *S. frugiperda* transcriptome (SRP075752) (Wei *et al.*, 2017), and *S. frugiperda* miRNA in miRbase were selected to be primary references. This is very important, even though the genomes of *S. frugiperda* and *B. mori* have high homology. AcMNPV can infect *S. frugiperda* but has a lower capacity to infect *B. mori*. Therefore, this work can accurately and effectively reflect the roles of miRNAs in the response of *S. frugiperda* to AcMNPV infection.

Forty-nine differentially expressed miRNAs were obtained by NGS, 10 of which were significantly differentially expressed. We selected 17 of them for qPCR validation. Except for miR-278-3p, the expression trend of other miRNAs was the same as that of NGS. It is known that *S. frugiperda* and AcMNPV are very close to *B. mori* and BmNPV at the genomic levels. Wu *et al.* (2016a) showed that 38 silkworm miRNAs were differentially expressed after BmNPV infection. The differentially expressed miRNAs of silkworm and *S. frugiperda* were different. The expression level of miR-278-3p was down-regulated following AcMNPV-EGFP infection by RT-qPCR. Moreover, Wu *et al.* (2016b)

reported that the expression level of *B. mori* miR-278-3p was down-regulated after BmCPV infection, and miR-278-3p affected the replication of BmCPV by regulating the expression of the IBP2 gene.

The expression of key genes in the miRNA pathway was measured by RT-qPCR. The role of exportin-5 is to transport pre-miRNA from the nucleus to the cytoplasm, and pre-miRNA is cleaved by Dicer1. Ago1 is the key component of RISC. Upon AcMNPV-EGFP infection, the transcriptional levels of *Exportin-5*, *Dicer1*, and *Ago1* were increased, especially at 48–72 h p.i. There was no significant change in the transcriptional level of *Ran*. The transcriptional levels of these key genes are similar to previous studies (Karamipour *et al.*, 2019; Rahimpour *et al.*, 2019). These results showed the miRNA pathway in Sf9 cells was more active during AcMNPV infection. MiRNA pathway played important roles in the Sf9-AcMNPV interaction.

Host-encoded miRNAs not only regulate the cellular processes but also play important roles in defending against pathogen infection (Hussain and Asgari, 2014; Asgari, 2015; Shrivastava *et al.*, 2022). Among the many differentially expressed miRNAs, which one or more play key roles in insect-virus interaction? Five miRNAs (miR-11-3p, miR-2a-3p, miR-34-5p, miR-92a-3p, and miR-278-3p) were selected, which are conversed, highly expressed in Sf9 cells and are sensitive to AcMNPV-EGFP infection. Among them, miR-11-3p and miR-92a-3p were up-regulated, and miR-2a-3p, miR-34-5p, and miR-278-3p were down-regulated during AcMNPV-EGFP infection. These miRNAs play regulated roles in insect growth and the immune process. miR-11-3p was found to be involved in DNA damage-induced apoptosis. The pro-apoptotic genes reaper (rpr) and head involution defective (hid) are direct targets of miR-11 in *Drosophila* (Truscott *et al.*, 2011). miR-2a regulates chitin biosynthesis through phoacetylglucosamine mutase (PAGM) in *Nilaparvata lugens* (Chen *et al.*, 2013). miR-92a suppresses *Drosophila* olfactory memory consolidation by constraining the expression of kinesin heavy chain 73 (khc73) (Guven-Ozkan *et al.*, 2020). miR-278-3p encoded by *S. frugiperda*, and miR-278 encoded by *B. mori* have the same sequence. Down-regulation of miR-278 in BmCPV-infected silkworms could promote the IBP2 mRNA level,
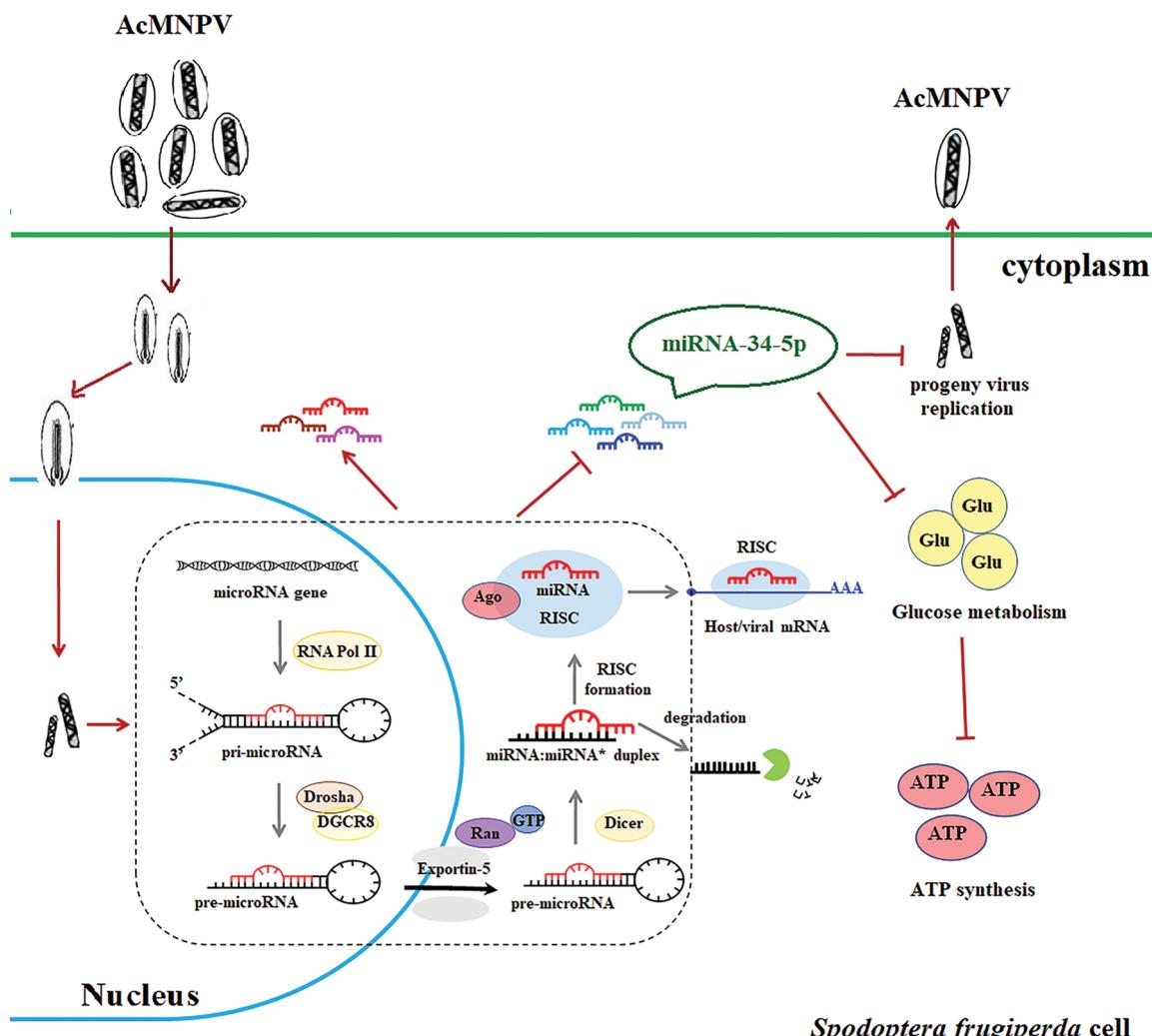
FIGURE 5. Schematic diagram of the response of the miRNA pathway in *S. frugiperda* cells upon AcMNPV infection. The diagram shows the key role of the miRNA pathway in response to baculovirus infection. By increasing the expression of some core genes of the miRNAs biogenesis pathway, the miRNA pathway in *S. frugiperda* cells is activated after AcMNPV infection. It allows the expression of miRNA encoded by *S. frugiperda* to be up- or down-regulated. Among many differentially expressed miRNAs, miR-34-5p has attracted our attention because of its regulatory effect on viral replication. miR-34-5p inhibits the infectivity of progeny virus and affects the energy metabolism of host cells.

which may play a positive role in BmCPV replication (Wu *et al.*, 2016b). We found that overexpression and inhibition of miR-11-3p, miR-2a-3p, miR-92a-3p, and miR-278-3p did not affect the BVs titer of AcMNPV-EGFP. However, miR-34-5p inhibits the infectivity of progeny virus of AcMNPV-EGFP. MiR-34-5p is highly conserved among multiple species, such as arthropods, nematodes, and mammals (Lau *et al.*, 2001; Cheng *et al.*, 2014). In arthropods and Nematoda, miR-34-5p is involved in aging, stress response, neurodevelopment, and insect immunity and development (Lai *et al.*, 2016; Guo *et al.*, 2017; Ye *et al.*, 2019; Wang *et al.*, 2022). In *B. mori*, miR-34-5p can regulate larval growth and wing formation by targeting *BmE74* and *BmCPG4* and directly regulating ecdysone signal and stratum corneum protein (Liu *et al.*, 2020). miR-34-5p can mediate the cross-regulation among JH, 20E, and IIS pathways to regulate the wing polymorphism of brown planthopper (Ye *et al.*, 2019). miR-34-5p can regulate the innate immune pathway of *Drosophila* by inhibiting the expression of two target genes, *Dlg1* and *Eip75B* (Xiong *et al.*, 2016). A recent study found that miR-34-5p targeted the *ecdysone receptor* (*EcR*) to regulate the growth and development of lepidopteran pests (Li *et al.*, 2022). In this study, we first discovered that miR-34-5p could regulate the proliferation of baculovirus progeny virus.

Virus infection has significant impacts on host physiology, establishing optimal conditions for successful propagation (Lin *et al.*, 2020). Viruses rely on host cell metabolism to provide the necessary energy and biosynthetic precursors for successful viral replication (Feng *et al.*, 2021). We commonly think that the modulation of host physiology is for virus multiplication, but on the flip side, it can also be an aspect of host resistance. Virus growth relies heavily on host resources, and the distribution and transfer of energy in hosts are important factors that affect viral replication. MiRNA may be a key factor in regulating host metabolism. Of the five miRNAs that we selected, miR-11-3p, miR-2a-3p, and miR-34-5p affect the glucose consumption, ATP production, and lactic acid accumulation in the host cells. Virus proliferation and energy metabolism of the host may be reciprocal causation and form a complex relationship of virus-host interaction.

Conclusions

The study exhibits the differential expression of miRNAs in *S. frugiperda* cells regulated by AcMNPV. The expression of some core genes in miRNA biogenesis pathways was significantly increased after AcMNPV-EGFP infection. It's not hard to deduce that the miRNA biogenesis pathway in Sf9 cells is activated to resist AcMNPV infection. MiR-34-5p inhibits the infectivity of progeny virus. MiR-11-3p, miR-2a-3p, and miR-34-5p affected the energy metabolism of host cells (Fig. 5). In general, the study provides evidence for a possible mechanism of insect-virus interaction. That is, the miRNA biogenesis pathway in hosts plays an extremely significant role in antiviral immunity. In future work, we will focus on the targets of miRNAs in *S. frugiperda* and

provide more ideas and evidence for host-virus interaction mechanism research.

Availability of Data and Materials: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Supplementary Materials: The supplementary material is available online at DOI 10.32604/biocell.2023.026182.

Author Contributions: Conceptualization, methodology, software, validation, formal analysis, investigation, Q.J.; resources, data curation, Q.J. and Y.F.; writing—original draft preparation, Q.J.; writing—review and editing, Y.F.; supervision, Y.F.; project administration, Y.F.; funding acquisition, Y.F. All authors have read and agreed to the published version of the manuscript.

Ethics Approval: Not applicable.

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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(a)

(b)

(c)

(d)
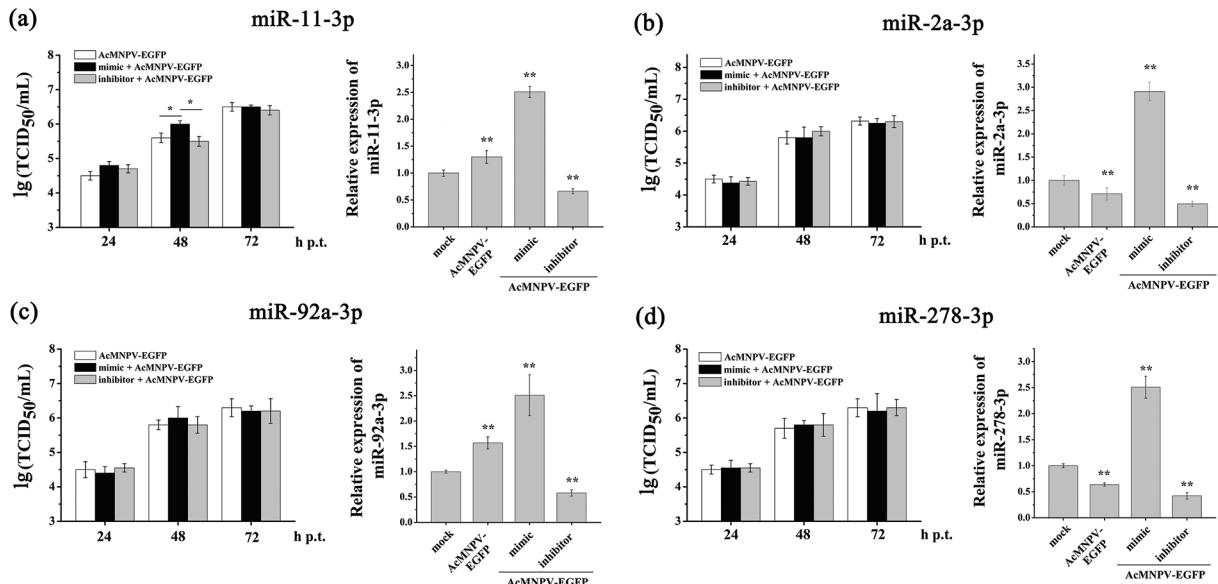
FIGURE S1. $TCID_{50}$ analysis of the effects of four pairs of mimic and inhibitor on the BV titer. They were miR-11-3p (a), miR-92a-3p (b), miR-92a-3p (c), and miR-278-3p (d), respectively. Sf9 cells were infected with AcMNPV-EGFP (5 MOI) for 8 h after transfection with the mimic and inhibitor, respectively. The expression of miRNA was analyzed by qPCR after transfection with mimic and inhibitor. The asterisks indicated that the *t*-test showed a signifcant difference relative to the control (*$P < 0.05$; **$P < 0.01$).
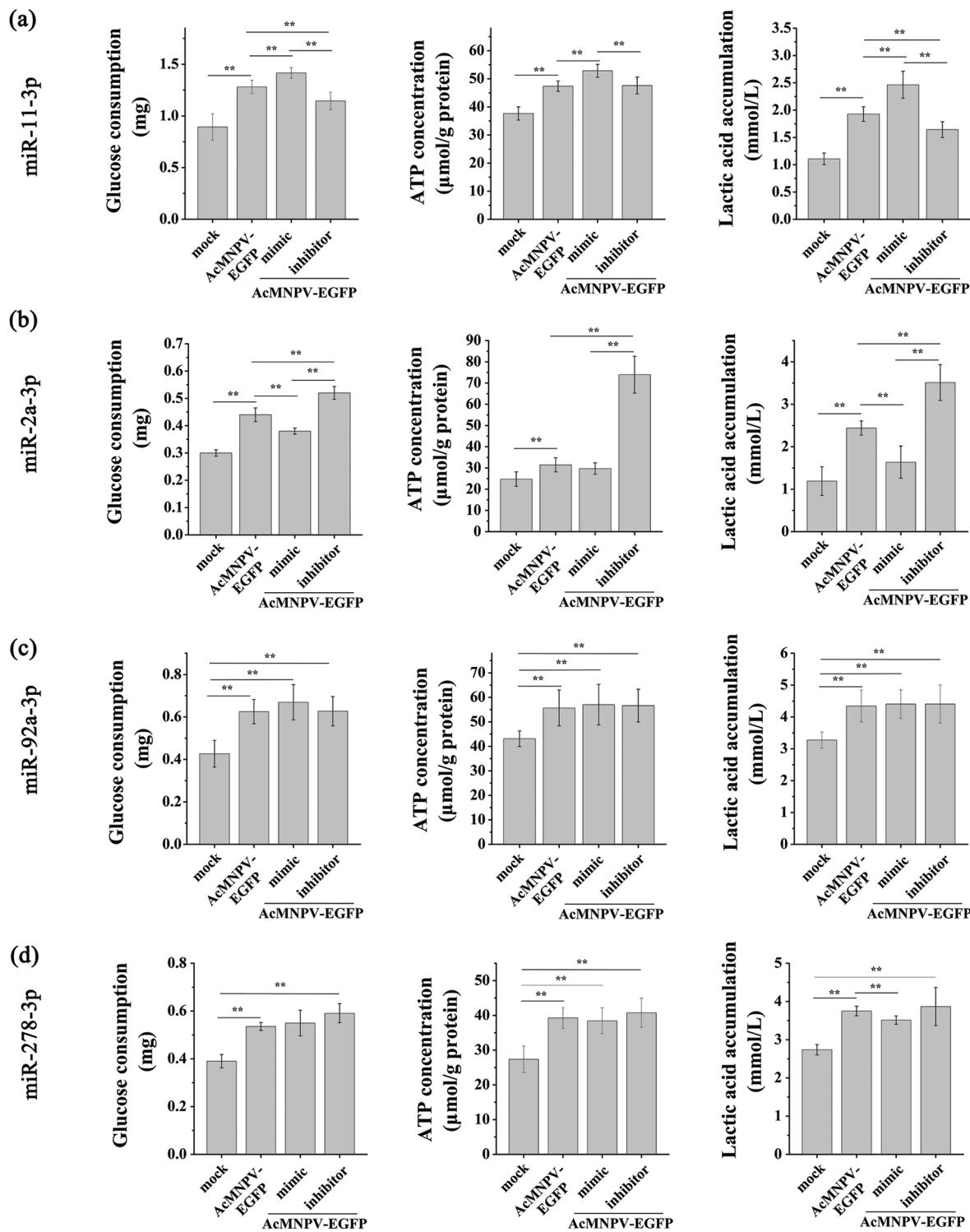
FIGURE S2. Effects of miRNAs on energy metabolism of host cells. The effect of the four pairs of mimic and inhibitor on the glucose consumption, ATP content, and lactic acid accumulation of host cells. They were miR-11-3p (a), miR-2a-3p (b), miR-92a-3p (c), and miR-278-3p (d), respectively. Sf9 cells were infected with AcMNPV-EGFP (5 MOI) for 8 h after transfection with the mimic and inhibitor, respectively. The asterisks indicated that the t-test showed a signifcant difference relative to the control (**$P < 0.01$).

SUPPLEMENTARY TABLE 1

Sequence of primers for RT-qPCR and mimics and inhibitors

Primers	Sequences (5′→3′)
qPCR forward primers of miRNAs by Tailing Reaction	
miR-10461a-5p 5' FP	CGAGGACTGGACTAGTGTCA
miR-275-3p 5' FP	TCAGGTACCTGAAGTAGCGCGC
miR-10453-5p 5' FP	ATGAATACGCGTATCAATGACA
miR-10484-3p 5' FP	CTGGCGGGCTTTCTGCCCT
miR-2765-5p 5' FP	TGGTAACTCCACCACCGTTGGC
miR-263b-5p 5' FP	CTTGGCACTGGGAGAATTCACAG
miR-10499b-5p 5' FP	CAAGGGTTTGAAACTCCATACA
miR-745-5p 5' FP	CGGCTCATCGTATGGCAGTTTGC
miR-79-5p 5' FP	CTTTGGCGATTTAGCTCCGTGA
miR-252-5p 5' FP	CTAAGTACTAGTGCCGCAGGAG
miR-92a-3p 5' FP	TATTGCACCAGTCCCGGCCTAT
miR-92b 5' FP	AATTGCACCAATCCCGGCCTGC
bantam-3p 5' FP	TGAGATCATTGTGAAAGCTGAT
miR-2a-3p 5' FP	TCACAGCCAGCTTTGATGAGCA
miR-11-3p 5' FP	CATCACAGTCAGAGTTCTAGCT
miR-34-5p 5' FP	TGGCAGTGTGGTTAGCTGGTTGT
miR-278-3p 5' FP	TCGGTGGGATCTTCGTCCGTTT
Primers of miR-34-5p by Stem-loop Method	
miR-34-5p stem-loop	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCAC TGGATACGACACAACCAG
miR-34-5p F	GGCTGGCAGTGTGGTTAG
universal primer	GCAGGGTCCGAGGTATTC
5S rRNA R	GCCAACGTCCATACCATGTT
5S rRNA F	GTGGTGTTCCCAGGCGGTCA
mimics and inhibitors	
miR-11-3p mimic	CAUCACAGUCAGAGUUCUAGCU
miR-11-3p inhibitor	AGCTAGAACTCTGACTGTGATG
miR-2a-3p mimic	UCACAGCCAGCUUUGAUGAGCA
miR-2a-3p inhibitor	TGCTCATCAAAGCTGGCTGTGA
miR-34-5p mimic	UGGCAGUGUGGUUAGCUGGUUGU
miR-34-5p inhibitor	ACAACCAGCTAACCACACTGCCA
miR-92a-3p mimic	UAUUGCACCAGUCCCGGCCUAU
miR-92a-3p inhibitor	ATAGGCCGGGACTGGTGCAATA
miR-278-3p mimic	UCGGUGGGAUCUUCGUCCGUUU
miR-278-3p inhibitor	AAACGGACGAAGATCCCACCGA
mimic NC	UUGUACUACACAAAAGUACUG
inhibitor NC	CAGUACUUUUGUGUAGUACAA
qPCR primers for protein genes	
Sf-*Dcr1* F	GATCTACCAGCACACGGAGG
Sf-*Dcr1* R	GCTTCTTACCCCTTCTGCGT
Sf-*Ago1* F	TTCGCCACAGACCCTAAGC
Sf-*Ago1* R	GGAATATCACCGGCTCGTT
Sf-*Exp5* F	GCCGGTCTAGCTCAACAACT
Sf-*Exp5* R	GTATGAGCGAGCGTGAGTGA
Sf-*Ran* F	AAAGCCCTTCCTGTGGTTAG
Sf-*Ran* R	GCAGTGTCTTGGGCTTCTT
β-actin F	AAGGCTAACCGTGAGAAGATGAC
β-actin R	GATTGGGACAGTGTGGGAGAC

SUPPLEMENTARY TABLE 2

Details of known miRNA in all libraries

Mature miRNA	Total reads	Accession number	Mature miRNA sequence (5′→3′)	Length
Bantam-3p	328692	MIMAT0007907	UGAGAUCAUUGUGAAAGCUGAU	22
Bantam-5p	469	MIMAT0015278	UGGUUUUCAUAAUGAUUUGACAGA	24
miR-92b	21849	MIMAT0009155	AAUUGCACCAAUCCCGGCCUGC	22
miR-745-5p	18812	MIMAT0013625	CGGCUCAUCGUAUGGCAGUUUGC	23
miR-927-5p	5042	MIMAT0009157	UUUAGAAUUCCUACGCUUUACC	22
miR-278-3p	4692	MIMAT0009164	UCGGUGGGAUCUUCGUCCGUUU	22
miR-79-5p	701	MIMAT0015275	CUUUGGCGAUUUAGCUCCGUGA	22
miR-79-3p	635	MIMAT0045672	AUAAAGCUAGAUUACCAAAGC	21
miR-306a-5p	873	MIMAT0012629	UCAGGUACUAGGUGACUCUGAGU	23
miR-283-5p	968	MIMAT0004426	AAAUAUCAGCUGGUAAUUCU	20
miR-275-3p	724	MIMAT0004201	UCAGGUACCUGAAGUAGCGCGC	22
miR-263a-5p	385	MIMAT0014289	AAUGGCACUGGAAGAAUUCACGG	23
miR-929-5p	81	MIMAT0009158	AAAUUGACUCUAGUAGGGAGU	21
miR-252-5p	42	MIMAT0010124	CUAAGUACUAGUGCCGCAGGAG	22
miR-137	105	MIMAT0005507	UAUUGCUUGAGAAUACACGUAG	22
miR-71-3p	2914	MIMAT0015243	UCUCACUACCUUGUCUUUCAUG	22
miR-305-5p	51	MIMAT0004207	AUUGUACUUCAUCAGGUGCUCUGG	24
miR-11-3p	315814	MIMAT0041881	CAUCACAGUCAGAGUUCUAGCU	22
miR-2b-3p	71155	MIMAT0041877	UAUCACAGCCAGCUUUGAUGAGC	23
miR-2a-3p	71131	MIMAT0041871	UCACAGCCAGCUUUGAUGAGCA	22
miR-10482-5p	29437	MIMAT0041857	UCGGUGAAUGCCAACGGAUUU	21
miR-2755-3p	28202	MIMAT0041957	CACCCUGUCAGACCAUACUUGUU	23
miR-2c-3p	27142	MIMAT0041764	UAUCACAGCCAGCUUUGUUGACU	23
miR-10458-3p	21616	MIMAT0041783	CCUAAAACAUGUAUCCGGCUCA	22
miR-10483-5p	20473	MIMAT0041859	CCUGUAAUUGUUCGUUCCCUU	21
miR-92a-3p	17452	MIMAT0041865	UAUUGCACCAGUCCCGGCCUAU	22
miR-317-3p	13277	MIMAT0041885	UGAACACAGCUGGUGGUAUCU	21
miR-279b-3p	6442	MIMAT0041968	UGACUAGAUUAUCACUUAUCCU	22
miR-13b-3p	4376	MIMAT0041893	UAUCACAGCCAUUUUUGACGAGUU	24
miR-34-5p	2298	MIMAT0041878	UGGCAGUGUGGUUAGCUGGUUGU	23
miR-10503-5p	1764	MIMAT0041944	CAUUGGAUCGUUCGAUUCCC	20
miR-10509-3p	1364	MIMAT0041962	CCCCAUUUUACUGUACGUGACA	22
miR-10483-2-3p	890	MIMAT0041861	AGGGGCCGAACAAUUCCAGA	20
miR-10458-5p	745	MIMAT0041782	CGCCGGGAUACGUGUUUACCG	21
miR-11-5p	613	MIMAT0041880	CUAGCACUUUGGCUGUGACCU	21
miR-10510-5p	509	MIMAT0041969	CAGAUUGCAGACUUGGCUUC	20
miR-10454d-5p	482	MIMAT0041808	UGUCAGAAUGGUCGAUCAUU	20
miR-10483-3p	463	MIMAT0041860	AGGGGCUGAACAAUUCCAGA	20
miR-2b-5p	450	MIMAT0041876	CUCACAAAGUGGCUGUAAUGUG	22
miR-2765-5p	448	MIMAT0041902	UGGUAACUCCACCACCGUUGGC	22
miR-10505-3p	439	MIMAT0041951	UAGGGUUAGAAACUUGGCUC	20
miR-285-3p	412	MIMAT0041966	UAGCACCAUUCGAAUUCAGUGC	22
miR-10454e-5p	340	MIMAT0041810	UGUCACAAUGGUCGAUCACU	20
miR-10488-3p	312	MIMAT0041889	UGCUGUCGGAAUAAACAUUGAUAA	24
miR-10-5p	313	MIMAT0041933	UACCCUGUAGAUCCGAAUUUGU	22

(Continued)

Supplementary Table 2 (continued).

Mature miRNA	Total reads	Accession number	Mature miRNA sequence (5′→3′)	Length
miR-2a-5p	283	MIMAT0041870	GCAUCAAAGUCGGCUUGUCAU	21
miR-10456-5p	282	MIMAT0041778	UAACGUCAUAAAUAUCCUGC	20
miR-263b-5p	271	MIMAT0041982	CUUGGCACUGGGAGAAUUCACAG	23
miR-10501-3p	247	MIMAT0041939	UUUGGCAUGGAGGUAGGUCU	20
miR-10454g-5p	227	MIMAT0041830	AAUUGUGUCAGAAUCGUCGA	20
miR-13a-3p	217	MIMAT0041907	UAUCACAGCCACUUUGAUGUGGU	23
miR-2c-5p	203	MIMAT0041763	UCAGCAAAGUGGUUGUGUCUUA	22
miR-317-5p	179	MIMAT0041884	GGGUGCCACGCUGUGCUCUCU	21
miR-10466-5p	168	MIMAT0041802	UAUAAUUAUCAGCACCGUCCA	21
miR-10504-3p	160	MIMAT0041947	UCAAAUCUCUGUGUAUGAUUGG	22
miR-281-5p	140	MIMAT0041898	AAGAGAGCUAUCCGUCGACAGU	22
miR-10507-5p	122	MIMAT0041954	UGCAAGUUUAUUCUGUAGUGGUCU	24
miR-10502-5p	117	MIMAT0041942	GGGCAGAAAGCCCGCCAGGCUG	22
miR-10501-5p	97	MIMAT0041938	ACCACUUCCAAUGGUCGUAU	20
miR-10490-5p	87	MIMAT0041900	UUCCAACGAAUGCAAGACCG	20
miR-13a-5p	83	MIMAT0041906	CUGUCAAAGCGGCGGUGAAAUG	22
miR-281-3p	78	MIMAT0041899	CUGUCAUGGAGUUGCUCUCUU	21
miR-375-5p	65	MIMAT0041882	ACCCGAGCGGAUUGAGCAAACU	22
miR-10497-5p	65	MIMAT0041927	CACACCAAACGGUGUCUGGUC	21
miR-10484-3p	61	MIMAT0041869	CUGGCGGGCUUUCUGCCCU	19
miR-34-3p	61	MIMAT0041879	CAGCCAGUAACGACACUGCCUC	22
miR-10510-3p	61	MIMAT0041970	UUGAAGUUGGGACGGCGACUU	21
miR-10506-3p	58	MIMAT0041953	UUGGACGAUGUAGUUGUGAAAC	22
miR-10499b-3p	46	MIMAT0041972	CAUGGAGUUUCAAACUCUUGAA	22
miR-10462-3p	38	MIMAT0041795	AUGGGUCCCGACUGCUUAAAU	21
miR-10508a-5p	34	MIMAT0041958	AAACUCGAGUCGUCAGUAGC	20
miR-10499b-5p	34	MIMAT0041971	CAAGGGUUUGAAACUCCAUACA	22
miR-10499b-2-5p	34	MIMAT0041973	AAGGGUUUGAAACUCCAUACAU	22
miR-10461a-5p	31	MIMAT0041788	CGAGGACUGGACUAGUGUCA	20
miR-92a-5p	28	MIMAT0041864	GGGCGGUGACUGGCGCUAUAU	21
miR-10489-3p	21	MIMAT0041897	CGAUGUCCAAGUCGAUCUAUU	21
miR-2765-3p	21	MIMAT0041903	CCAACGGGGGCAGAGUUCCUAUU	23
miR-375-3p	20	MIMAT0041883	AGUUUGUUCGCCCCGGCUCGA	21
miR-10474-5p	19	MIMAT0041832	CGCGGAAUUAGAUUGUGAUC	20
miR-13b-5p	19	MIMAT0041892	UCGUAAAAAUGGUUGUGCCAUGU	23
miR-10454d-3p	18	MIMAT0041809	AGUGAUCGGCCAUUCUGACA	20
miR-10454e-3p	18	MIMAT0041811	AGUGAUCGGCCAUUCUGACA	20
miR-10499a-3p	17	MIMAT0041932	CAUGGAGUUUCAAACUCUUGGA	22
miR-263b-3p	17	MIMAT0041983	CGUGAAUUUCCUGAUGCCUUAG	22
miR-10502-3p	16	MIMAT0041943	CUGGUCGGACUUUCUGCCCCCU	22
miR-10507-3p	15	MIMAT0041955	GAACCACAGCAGUUUGAGCGGACC	24
miR-2755-5p	14	MIMAT0041956	CAAGGUGGCCUAGCAGCGUGUU	22
miR-10453-5p	11	MIMAT0041771	AUGAAUACGCGUAUCAAUGACA	22
miR-10497-3p	11	MIMAT0041928	UGACCAGACUCGUUUUGGUUG	21
miR-10465-3p	10	MIMAT0041801	CUGACUUGUAUUCUCGCUGCCC	22
miR-10468-3p	10	MIMAT0041807	UGCACUUGGCGAAGUUGCCUG	21

SUPPLEMENTARY TABLE 3

Novel miRNAs in all libraries

Provisional ID	miRDep 2 score	Total Read Count	Mature Read Count	Consensus Mature Sequence (5'→3')	Consensus Star Sequence (5'→3')	Consensus Precursor Sequence (5'→3')
sfr-novel-11	882.1	1729	1698	CCCCUUGUUACAAACCUCACCU	UGUGCGUUGUGGCAAAGGAGAU	UGUGCGUUGUGGCAAAGGAGAUCUGCUCCAGUCGGAUCAUCCCUUGUUACAAACCUCACCU
sfr-novel-12	771.6	1507	1504	UUUGGCACUUGAUCUAUUUCUAA	UGAAAUAGACAAGGGUCACU	UGAAAUAGACAAGGGUCACUAUGUAGAUUUGGCACUUGAUCUAUUUCUAA
sfr-novel-17	510.1	998	756	AAAAAAUUGAGAAAUACCAGAA	CUGGUAUUUCUCAAUUUUUUUU	CUGGUAUUUCUCAAUUUUUUUUAUUAAGAAUAAAAAAUUGAGAAAUACCAGAA
sfr-novel-23	209.7	419	331	CCUGGGAAACAAUCCGGAGUU	CCUCGGAUUCCUUCGCAAUC	CCUCGGAUUCCUUCGCAAUCCUGCAAUCCUGCAAUCACACGGCUCCUGGGAAACAAUCCGGAGUU
sfr-novel-24	164.9	322	239	AUUUCGACUUUCUCAGUAACG	UUACUGAGAGUUUCGAAAAUU	UUACUGAGAGUUUCGAAAAUUGAGGGAGCUGCCAUUUCGCAUUUCGACUUUCUCAGUAACG
sfr-novel-26	154.4	301	300	ACCGUGAGACCGAGACUUUAU	AGAGCUCCUUUCUACAGUAUC	ACCCUGUAGACCGAGACUUUAUAUUGGUUACGACGUGCAUAUAGAGCUCCUUUCUACAGGUAUC
sfr-novel-27	147.4	299	216	UUUCUUGUAUUAAUGUAGGUAGACU	UAUCCUACACUGAUUGAGAAAU	UAUCCUACACUGAUUGAGAAAUAUUUGCCUUUAAAAUAUUGCUUUAUAAUGUAGGUAGGUAGACU
sfr-novel-28	92.7	181	60	AAAGGAGGACUUACGAGGUUA	ACCCUGUAGAUCCGAACUUAA	ACCCUGUAGAUCCGAACUUAAACCUGUAGAUCCGAACUUUAACAUGUAAACUCACAAUAAUAGCACCCUGGC
sfr-novel-29	74.3	145	144	UACACAAUAAUAGCACCCUGGC	AGGGUGCAAUUAUUGUGGGGCU	AGGGUGCAAUUAUUGUGGGGCUGUUGCACAAAAACUCACAAUAAUAGCACCCUGGC
sfr-novel-32	70.2	135	132	UCACUAUGUGACCGUUUCGACU	GUCGAAACGGUCACUAUGUGGC	GUCGAAACGGUCACUAUGUGGCGUGUUUAGCUGAUACAGUCACUAUGUGACCGUUUCGACU
sfr-novel-34	65	125	124	UGAUUUAAUCGAUGAAUAGACU	UCUAUUCAUCGAUUAAAUCAU	UGAUUUAAUCGAUGAAUAGACUCUAUUCAUCGAUUAAAUCAU
sfr-novel-36	58.3	105	98	GUGGGGCAAAUUGCGAAAGCU	UUUGCCAAUUUGCCGCACCC	UUUCGCAAUUUGCGCACCCGUAAGUGAGCCUGGGGCAAAUUGCGAAAGCU
sfr-novel-37	37.9	76	50	UCUCCAACUGCUUAACUGUAU	AACAGUCAUCCGUUACGUAU	UCUCCAACUGCUUAACUGUAUCGUAUCGAUAUCGAUACUAACAGUCAUCCGUUACGUAU
sfr-novel-39	33.5	66	59	UUCCUGUUGACGCCAAUUUUUUU	AAAAUUCGGCUCUAUAGGUAGA	UUCCUGUUGACGCCAAUUUUUUUUAAUUUGCAUAAAAUUCGGCUCUAUAGGUAGA
sfr-novel-40	32.4	65	62	AAUCUUUCAUUAUCGUGCCGGU	CGACAUGACAGUAAAGAUGAA	AAUCUUUCAUUAUCGUGCCGGUGAAAUAAUAUUACCGACAGUAAAGAUGAA
sfr-novel-42	29	56	55	UUGGGUCUCUGAAGCCACAGUAU	CUUUGGUUCCACAGGCCA	UUGGGUCUCUGAAGCCACAGUAUAUGUGGCUCUAGCCUCUGAAAACUUUGGUUCCACAGGCCA
sfr-novel-43	15.4	40	18	AUGGCUUGAAACUUGUAGAGU	UCGGGUCGACGACGCAAAAACGG	UCGGGUCGACGACGCAAAAACGGUGUAUAUGUGGCGUCAAUGAUGAUGAGCCUCUAGCAUCAUGGCUUGAAACUUGUAGAGU
sfr-novel-45	15.1	27	26	AACAUCGCAGUUCGGUACACU	UGUACCGAACGAUGUUGACUG	UGUACCGAACGAUGUUGACUGUUCGUACGACGAGUCAGUCAUCAACAUCGCAGUUCGGUACACU
sfr-novel-46	12.2	28	20	AUUCUUUCCUUACCACUUUUGU	AAACGGUGAGAAAAGACGC	AUUCUUUCCUUACCACUUUUGUGUUUCGAGUAUAACAAAGUGGUAGGAAAGACGC
sfr-novel-47	10.9	20	14	UUCCUCAUGGCGAAAACCUCGUA	UGAGGUCGUCGACAUGUGGAGU	UUCCUCAUGGCGAAAACCUCGUAGUGUGUCGACAUGUCAUGACAGUAAAACUGAGGUCGUCGACAUGUGGAGU
sfr-novel-48	9.6	18	11	CCCGUCUUCUGUUCCUAGUGU	ACAAGGAUAGAAGGGAGUGAU	CCCGUCUUCUGUUCCUAGUGUGCCUAUAAAUUACAAGGAUAUAGAAGGGAGUGAU
sfr-novel-49	9.1	15	5	CGCGCUACUUCCAGGUACCUGACU	CCCGGCGCCGGAGUAGCGCG	CGCGCUACUUCCAGGUACCUGAGCACGAUCAGCAGCCCGGCGCCGGAGUAGCGCG
sfr-novel-55	8.5	16	11	UUCGAUCCACCGUGCUCAUGGCG	CCAGAGGACUGUGUAUUGUGAACAU	UUCGAUCCACCGUGCUCAUGGCGCUUUGCACCAGGCGUACUGGCACUCCAGAGGACUGUGUAUUGUGAACAU
sfr-novel-57	8.3	14	13	AGUUUUCUUUCCAUUCCCACU	UGGGGGAAAGAAAAAACAUA	AGUUUUCUUUCCAUUCCCACUCCCCGCCCCCACUCGGGGGGAAAGAAAAAACAUA
sfr-novel-58	8.1	15	5	GAGUGUAGGACAGAAAUUUAU	AAAAUACUGUGCCUAUAUUACGA	GAGUGUAGGACAGAAAUUUAUAUUUAUUUUGCCCUGCCGACCGGUUGCAUAUUAAAAUACUGUGCCUAUAUUACGA
sfr-novel-64	7.1	21	20	UUUUCGUUGUGUACUUCUUGCGCC	GCAGAGUGGACGGAUGUGUGAGA	UUUCGUUGUGUACUUCUUGCGCCGCGACCGCGUGAUGCAGAGUGGACGGAUGUGUGAGA
sfr-novel-66	5.9	12	11	UACCCUGACGGGCCAUCCUUUUA	AAAGUAUGCUAAAGGGAUGC	UACCCUGACGGGCCAUCCUUUUAUUGAUUAUUUGGGGUAUCAAAAGUAUGCUAAAGGGAUGC
sfr-novel-70	5.5	54	54	UACCCUGUAAAUCCGGGACCUAUAG	CUGGGUCCAGGUAUACAGGGAAAC	CUGGGUCCAGGUAUACAGGGAAACGGUCAUUUUAUUGUCCCGGGUUUCUUAGUGGGUCCUUAGCCUAUAG
sfr-novel-80	5.1	385	385	AAUGGCACUGGAAGAAUUCACGG	GUGGUCUCUAGUGGCAUCAC	AAUGGCACUGGAAGAAUUCACGGGUUUAGCGGUUUACCGUGUUCCCAAUUCCUGAUGGGUCUUAGUGGCAUCAC
sfr-novel-79	5.1	13	13	AAAUUCCGAAAUAGAGGGGGUUU	ACCUCUAUGCCGAAUGUAA	AAAUUCCGAAAUAGAGGGGGUUUGCAAAUGUAUAAAACCCCUCUUUAUUAGGUUCCCUUCAUGUCCGAAUGUAA
sfr-novel-78	5.1	12	12	UUGGUCCCUUCAACCAGCUGU	AGCUGGUUGACUUCGGGUCAAAU	AGCUGGUUGACUUCGGGUCAAAUAGCUGGUUGCAAAUGUAAUUGGAUAUCCUUCAACCUAGUACCGGAUGGC
sfr-novel-81	5	18	18	CAAAUUGGCUCAUGUAGAUCU	AUGCCAUGUACCGAAUGGC	CAAAUUGGCUCAUGUAGAUCUAGGUUCAAAUGUAGAUCUCCAUGUACCGAAUGGC
sfr-novel-84	4.8	24	24	UCUUUGGUUAUCUACGCUGUAGA	AUAAUGACUAGGUACCGAGGUUA	UCUUUGGUUAUCUACGCUGUAGAUAGCUAGGUUGAUUGGAUGAUGUAGAGGUGUGGAGGUAGUA
sfr-novel-83	4.8	12	5	AUUCUUUAUUGUGGCCACUUUUGU	CAAUAUGACAAAGGAAGAUAGA	AUCUUUAUUGUGGCCACUUUUGUUGUGAAGUGAAUUGACAAAGGAAGAUAGA
sfr-novel-85	4.6	11	11	AAAGCUCGUGCUACUACAGGUGAU	AGCCUGUAGUUCGGAGCUAUUUG	AGCCUGUAGUUCGGAGCUAUUUGGGCUCGAGCAAACAAUUUGCAUCCUAUACGGUGUGCUACACGGUAU
sfr-novel-88	4.1	11	9	UUGGUCCCGUAACCAAACU	ACUUUGGUACCACGGACCAAG	UGGUCCCUGUAACCAAAUAGUUUACAUCCCAUACGUGUCGUACCACGGACCAAG
sfr-novel-89	4	11	11	AAAGCUCGUUCCCUACAGGUAAU	AGCCUGUAGUUCGGAGCUAUUUG	AGCCUGUAGUUCGGAGCUAUUUGGGCUCGAGCAAACAAUAAUUCAAAAGCUGCGUUCCCUACAGGUAUU
sfr-novel-95	3	11	11	CCCUUUCGUGUUUAUUAACAA	AUAAUAAAAAUACGAAGAGAUCA	CCCUUUCGUGUUUAUUAACAAAUAAUAAAAAUACGAAGAGAUCA
		67811	67811			

(Continued)

Supplementary Table 3 (continued).

Provisional ID	miRDeep2 score	Total Read Count	Mature Read Count	Consensus Mature Sequence (5'→3')	Consensus Star Sequence (5'→3')	Consensus Precursor Sequence (5'→3')
sfr-novel-96	2.5	38	38	AGCACCGAUCGCGCUGCGC	GAGAGGCGUUCGGCGGCUCU	GAGAGGCGUUCGGCGGCUCUGAUAGGUUGGUCCAUUCUCCGCGGCCAGUCAGAGCACCGAUCGCGGCUGUCGC
sfr-novel-98	2.2	36	36	UUUAGGCAUUGGAAUUAUUG	AUAAUUCCAAUGCCUAAAAC	AUAAUUCCAAUGCCUAAAACUGUGUUGUUUAGGCAUUGGAAUUAUUG
sfr-novel-97	2.2	19	18	UAUACCACUACUUGGCAGCGAGG	CCGAUAAGUGGUAUAGAAUGCC	UAUACCACUACUUGGCAGCGAGGCACUCGCCGAAGACUUGCCGAUAGUGGUAUAGAAUGCC
sfr-novel-101	2.2	19	16	CCCUUUCGUGUUUAUUAACA	AAAACACGAAGAGGUCAGAAGU	CCCUUUCGUGUUUAUUAACAAAAAUAAAAUAAACACGAAGAGGUCAGAAGU
sfr-novel-99	2.2	12	12	UUCUAUGCUCGGAUUCUGUGAC	CACAGAAUCCGAGCAUAGAAUA	CACAGAAUCCGAGCAUAGAAUAUAGGUUACCUAUUCCUCGGAUUCUGUGAC
sfr-novel-104	2.1	139	139	UACAGGAUACAGUAUACUUGC	AAGUAUACUGUAUCGUGUAUA	UACAGGAUACAGUAUACUUGCGAAGUCCAGUGCAAGUAUACUGUAUCGUGUAUA
sfr-novel-103	2.1	34	34	CCGCCGCUGUGUCGUGUGCU	CACCAGCGCACGCUGCGCGU	CACCAGCGCACGCUGCGCGUGGCGCCGCCGCCGCUGUGUCGUGUGCU
sfr-novel-102	2.1	19	19	UUUUUGACAUCGGUCGUAAAAAGGA	CUUUUUACCGAUGUCAAAAACA	UUUUUGACAUCGGUCGUAAAAAGGAGGAGCAAUUGUCUUUUUACCGAUGUCAAAAACA
sfr-novel-107	2.1	19	19	UUUUUGACAUCGGUCGUAAAAAGGA	CUUUUUACCGAUGUCAAAAACA	UUUUUGACAUCGGUCGUAAAAAGGAGGAAUUGUCUCCUUUUUACCGAUGUCAAAAACA
sfr-novel-105	2.1	18	18	CUAUUAGGUAAAUGUAGGCAUU	UGCCUACAUUACCUAAUAGUA	UGCCUACAUUACCUAAUAGUAGAUAUAUAUAGAUAUUACCAUCUAUUAGGUAAAUGUAGGCAUU
sfr-novel-106	2.1	12	12	AGCCGGUCGGGUCUGAUU	UGAGGCGCGAACGCGCUCU	AGCCGGUCGGGUCUGAUUGGCCGUGAGCAUUAAACUAACCAAUGAGCGGCCGAACGCGCUCU
sfr-novel-110	2	405	405	AUGGGCUAGCUGAACGAUCA	UCCGCUUGAGCUAGCGCAUUC	AUGGGCUAGCUGAACGAUCAAUCCGUUGAGCUAGCGCAUUC
sfr-novel-115	2	405	405	AUGGGCUAGCUGAACGAUCA	UCCGCUUGAGCUAGCGCAUUC	AUGGGCUAGCUGAACGAUCAAUCCGUUGAGCUAGCGCAUUC
sfr-novel-114	2	149	149	UACACGAUACAGUAUACUUGC	AAGUAUACUGUAUCGUGUAUG	UACACGAUACAGUAUACUUGCUGGACUUCCGAAGUAUACUGUAUCGUGUAUG
sfr-novel-108	2	102	102	AUUGGAACCGAGAUUGUACAAUCU	AUUAUACAAUCUCGGUUCCAAUUA	AUUGGAACCGAGAUUGUACAAUCUCAAUCAAUUACAACUCGGUUCCAAUUA
sfr-novel-109	2	25	24	UCGUCUACGUAGAUGGGUACC	CAUUAUAAUCGUAGACAGA	CAUUAUAAUCGUAGACAGAGAGAGAUAUAUAUGUCUACGUAGAUGGGUACC
sfr-novel-111	2	18	18	CUAUUAGGUAAAUGUAGGCAUU	UGCCUACAUUACCUAAUAGUA	UGCCUACAUUACCUAAUAGUAGAUAUUAUAUGUAAAUGUAGGCAUU
sfr-novel-117	1.9	14	14	CGUCGCAGGCACUGAGACUAGU	UGAAAGUUCAGUGCCCGCGACGCG	UGAAAGUUCAGUGCCCGCGACGCGUAUCGCGACGGUCGCAGGCACUGAGACUAGU
sfr-novel-118	1.9	14	14	UAUAGAACUUCAGCAAGUAGC	UACUGUCGAAGUUCUAUAGA	UACUGUCGAAGUUCUAUAGAGAAAACUCUAUAGAACUUCAGCAAGUAGC
sfr-novel-116	1.9	12	12	AGCCCGUCGGCGAACGGCCGC	UCAGACGGCGAACGGCGCGC	AGCCCGUCGGCGAACGGCCGCGAGAUCAAUCAACCUACAACGGCGCAGCCCGUCGGCGAACGGCGCGC
sfr-novel-121	1.8	26	13	ACCAGCACGGCCACCAGCGC	CUGGUCGUAGUGUGCUUCGUGG	CUGGUCGUAGUGUGCUUGGGUCCGAGUACCGCCGUACCGCAGGCGCAGGUCGCACCGCACCAGCACGGCCACCAGCGGCCACCAGCGC
sfr-novel-120	1.8	20	20	CGAGGUCAGCGGUUGCUCUAUGGU	CAUAGAGCAACCGGGCCUCGUA	CAUAGAGCAACCGGGCCUCGUAUAGAGAUACUCCGAGGUCAGCGGUUGCUCUAUGGU
sfr-novel-119	1.8	13	13	UAUAGAACUUCAGCAAGUAGC	UACUGUCGAAGUUCUAUAGA	UACUGUCGAAGUUCUAUAGAUGAUAGAACUUCUAUAGAACUUCAGCAAGUAGC
sfr-novel-122	1.8	11	11	UACCCAAUUCAGCUUGUACCC	GUAUGAGCUGAAUUGGGUGAU	UACCCAAUUCAGCUUGUACCCUCGUAUAUACAGAGUCGUAUGAGCUGAAUUGGGUGAU
sfr-novel-125	1.7	86	86	UGAGUCGUGAUUUUGUCUAGA	CAGACAAAAUCACAGACACUA	CAGACAAAAUCACAGACACUAUAAGUGUAUGUAUGGUACACAGAGUCGUGAUUUUGUCUAGA
sfr-novel-123	1.7	35	35	AGAGAGGAACUGCCAGCGCCC	GCGUUGUUAUUUUGUCGUCCGC	AGAGAGGAACUGCCAGCGCCCGCCUCCGGUGGGCUUCGGUUGUUAUUUUGUCGUCCGC
sfr-novel-127	1.6	938	923	UAAAAUGGCAAACAAAGACU	ACUCUUUAUUUGCCACUUUAGU	ACUCUUUAUUUGCCACUUUAGUGUGACAUAAUAUUCUAAAUGGCAAACAAAGACU
sfr-novel-128	1.6	198	198	UCAUCCAAGCGCGGGCUUUCU	AGAGUCCGGCAUUGGAGGACU	AGAGUCCGGCAUUGGAGGACUGCGUCCUCCAAUGCUCCAAGCGCGGGCUUUCU
sfr-novel-130	1.6	11	11	CAAGCUCAGUCAGUAAACAGU	UGUUUACUGCUGAGCUUGGA	UGUUUACUGCUGAGCUUGGAGCUCGGUGACUCAGCUCAGUAAACAGU
sfr-novel-131	1.5	48	48	CGGAAUAAAAUUUUGCAUAAA	UAUGCAAAUUUUAUUCCGUA	UAUGCAAAUUUUAUUCCGUACUACUAAGGUACGGAAUAAAAUUUUGCAUAAA
sfr-novel-132	1.5	41	41	ACCAACUUCGAAAUUAUUCUGU	AGAAUAAUUUCGAAGUUGGUCG	AGAAUAAUUUCGAAGUUGGUCGGUCGAUUAUAGAGGUGCAACUUCGAAAUUAUUCUGU
sfr-novel-135	1.5	21	21	UCGGGGAAUGCUGGUCACGGGGU	CAGUGAGCAGUAAUCCGGGGCA	CAGUGAGCAGUAAUCCGGGGCACGACGUCGCUCAGUGGAAUGCUGGUCACGGGGU
sfr-novel-138	1.5	18	18	GUUUCGAUGGUGUAUACCAUG	UGGUUAACAAUGUCAAAUCAU	UGGUUAACAAUGUCAAAUCAUACAUGAGAUGUUUCGAUGGUGUAUACCAUG
sfr-novel-136	1.5	17	17	UGAGAAUUCAUGAUGAUGAAG	UCUCCAUCAUGAAUUUUUAGU	UGAGAAUUCAUGAUGAUGAAGAAGGAGUCUCCAUCAUGAAUUUUUAGU
sfr-novel-142	1.4	3077	3077	CGGCGAUGAAAUCUGUCGGUA	CAGCGGGUUUCAUCCCCGGCU	CAGCGGGUUUCAUCCCCGGCUGCUCUGCGAGGUCGGCGAUGAAAUCUGUCGGUA
sfr-novel-144	1.4	172	172	UCCAAGCUACGAUCUGAAUCUAGC	CAGCGGGCGAUAGUCGGGGGCG	CAGCGGGCGAUAGUCGGGGGCGGCUCAGUCACGCUUGAUUCCAAGCUACGAUCUGAAUCUAGC
sfr-novel-141	1.4	52	52	ACUACCCUUAUGUGAUGACU	CCAUCACCCUUAUGUGAUGUGA	CCAUCACCCUUAUGUGAUGUGACACUGCUUCUACUAGGUGAUGACU
sfr-novel-143	1.4	19	19	UUUCACAUUGGAACUGGCGGAG	CGCCAAAAUUCAAUGUGAUAUG	CGCCAAAAUUCAAUGUGAUAUGUUUCACAUUGGAACACGGCGGAG
sfr-novel-147	1.3	60	60	CGGAAUAAAAUUUUGCAUAAA	UAUGCAAAUUUUAUUCCGUA	UAUGCAAAUUUUAUUCCGUACUACUAGGUACGGAAUAAAAUUUUGCAUAAA
sfr-novel-146	1.3	24	24	AAGUGACCAGGGGAUUUCAUGU	AUGAAACCCGGCCAUUACC	AAGUGACCAGGGGAUUUCAUGUAUGUGUCGCACCACGGGAAGUUGCACCACGGGCCAUUACC

(Continued)

Supplementary Table 3 (continued).

Provisional ID	miRDeep2 score	Total Read Count	Mature Read Count	Consensus Mature Sequence (5'→3')	Consensus Star Sequence (5'→3')	Consensus Precursor Sequence (5'→3')
sfr-novel-145	1.3	11	11	UGUUUGGCGCUUUGAUUGGCAG	ACCAAUCAGAGCGCCGAAUGAG	UGUUUGGCGCUUUGAUUGGCAGGCGGUGAAAUAACCACCAAUCAGAGCGCCGAAUGAG
sfr-novel-148	1.1	2285	2284	CACCUAUUAUUUGUUGUUUCGC	ACAAAUAAUAAGGAGGUACGCU	ACAAAUAAUAAGGAGGUACGCUCCAGACGUCACCUAUUAUUUGUUGUUUCGC
sfr-novel-153	1.1	1794	1794	UUAGGGAACCGAAGAAACCACGU	UGGAUUCUCGUUCCCAAAGA	UUAGGGAACCGAAGAAACCACGUCAAAUCUCGUUCCCAAAGA
sfr-novel-149	1.1	11	11	UCCCCAAUAGGAUUGCACAAAAU	UUUGUGCUAUCCUAUUGGUUAAU	UCCCCAAUAGGAUUGCACAAAAUCUACGCUCCUCUUUAGUGGACAUAUUUGUGCUAUCCUAUUGGUUAAU
sfr-novel-150	1.1	11	11	UUGCAUCGGCUGCUAUUGUGUC	GACAAUAGUCCGGCCGCCGCCGUA	GACAAUAGUCCGGCCGCCGCCGUAUUGCGACGAGCUGUUAUUGCUCUAUUGUGUC
sfr-novel-154	1.1	11	11	UCCCCAAUAGGAUUGCACAAAAU	UUUGUGCUAUCCUAUUGGUUAAU	UCCCCAAUAGGAUUGCACAAAAUCUACGCUCCUCUUUAGUGGACAUAUUUGUGCUAUCCUAUUGGUUAAU
sfr-novel-155	1	1583	1582	CCCUUUCGUGUUUUAUUAA	AAAUACGAAGAGGCCAGUAAG	CCCUUUCGUGUUUUAUUAAAAAAUACGAAGAGGCCAGUAAG
sfr-novel-157	1	20	20	UUUUUUCUGAACACUUUUGAACU	UUCAAAGUCGUCGGAGAAAAUA	UUUUUUCUGAACACUUUUGAACUCCUAUUAGUCAAAGUCGUCGGAGAAAAUA
sfr-novel-158	1	20	20	UUUUUUCUGAACACUUUUGAACU	UUCAAAGUCGUCGGAGAAAAUA	UUUUUUCUGAACACUUUUGAACUCCUAUUAGUCAAAGUCGUCGGAGAAAAUA
sfr-novel-163	0.9	250	214	CAAAAGGACAAUAAGAGAUA	CUCUUUUAUUGCCACUUUGGU	CUCUUUUAUUGCCACUUUGGUGAAACAAUAUUAACCAAAAGGACAAUAAGAGAUA
sfr-novel-162	0.9	38	38	CUGGCGGGCUUUCUGCAA	GUGGAUUCUCCGACCAGC	GUGGAUUCUCCGACCAGCGCAGGCGAGGUGAUCCUGCCUGGCGGGCUUUCUGCAA
sfr-novel-161	0.9	37	37	CCCUGUUAAAAAUUACUCAAU	ACAGGAAUUUUUAGCAGGGUUA	ACAGGAAUUUUUAGCAGGGUUAACAGGUAAUACCUGUUAAAAAUUACUCAAU
sfr-novel-166	0.8	16	16	AGGGCAUGUAGGGUUCGCCACA	UGGCAACCCCAUGCCACCA	AGGGCAUGUAGGGUUCGCCACAAGGUUGGAGUAUUAUUUUUGGCAACCCCAUGCCACCA
sfr-novel-167	0.7	1588	1588	CCCUUUUCGUGUUUUAUUAA	AAUAAGAUACGAAGAGGCCA	CCCUUUUCGUGUUUUAUUAAAAAAUAAUAUAAGGAAUACGAAGAGGCCA
sfr-novel-168	0.7	64	64	GGGAACGUCGUCGAUUUGAC	CACUUCCGCCCACUUCCGCUC	GGGAACGUCGUCGAUUUGACGUUUGCGAUUUGUGCAACUGUCAAAUUCAAUUGACGAGGAUAAGACAAGGCGUCGACUUCCGCCCACUUCCGCUC
sfr-novel-169	0.7	11	11	UGGGGAUCGACCUAUCCAUG	AGUAUAGAUAGUCCAAUCCCGAC	AGUAUAGAUAGUCCAAUCCCGACGAGGAUGACGACCAUCCCGACUCAGGUCAAAAUGUGGGGAUCGACCUAUCCAUG
sfr-novel-170	0.6	27	22	UGUGUCAAAAUUCUACCUCUGG	CAAGAGAUAGUAUUUUGACAUA	CAAGAGAUAGUAUUUUGACAUAAGCUCCAUCACAAAAUGUGUCAAAAUUCUACCUCUGG
sfr-novel-171	0.6	27	22	UGUGUCAAAAUUCUACCUCUGG	CAAGAGAUAGUAUUUUGACAUA	CAAGAGAUAGUAUUUUGACAUAAGCUCCAUCACAAAAUGUGUCAAAAUUCUACCUCUGG
sfr-novel-173	0.5	27	27	CCAAUUAGAGUGAAGAACGCGC	CCGUCUGCCCUCGAUUUAGUC	CCGUCUGCCCUCGAUUUAGUCGGUCAAUAACCAACCAAUUAGAGUGAAGAACGCGC
sfr-novel-172	0.5	12	12	GAGAACGCAGGUUGAUUGAGU	UGGUACCCGUGUGGUCCUAG	GAGAACACGCAGGUUGAUUGAGUGUGUAUAUAAAAAUAAUAAAAUAACGAAGAGGUCA
sfr-novel-174	0.4	1631	1631	CCCUUUCGUGUUUUAUUAA	AAUAAAAUACGAAGAGGUCA	CCCUUUCGUGUUUUAUUAAUAAAAAUAAUAAAAUACGAAGAGGUCA
sfr-novel-175	0.4	459	459	CUUUUCGUGUGUUUUAUUAAAC	UAAUAACUUUCCACCACGAUAAAGGC	CUUUUCGUGUGUUUUAUUAAACAGGUGAAUGACAGGUAAUAACUUUCCACCACGAUAAAGGC
sfr-novel-176	0.4	78	78	CUCAGUAUUACUUUGGACCCACC	UCAGUCCAAAGUAAUACAGCGUA	UCAGUCCAAAGUAAUACAGCGUACCUGGACCCAGCUGCUACUCAGUAUUACUUUGGACCCACC
sfr-novel-177	0.2	12	5	AUUCUUUAUUUGCCACUUUGGU	CAAUAUGACAAUGAAGGAGAUGA	AUUCUUUAUUUGCCACUUUGGUGAAGGUGAAAAAUAUAUUUCCAAUAUGACAAUGAAGGAGAUGA
sfr-novel-178	0	96651	96650	CAAUAUACGAGAUACACAUUGA	UAUAUUUCGUAUGUAUGUCAAAGA	CAAUAUACGAGAUACACAUUGAGAAAAAUAUAUUUGUAUAUUUCGUAUGUAUGUCAAAGA
sfr-novel-179	0	13	12	UUACAUUUGAUGUGGUUGUUCUGCAAA	UGCAGAAGUGCCAUGAGGUU	UGCAGAAGUGCCAUGAGGUUGCGUAAUAGUAGCGGUUGUUUAAGCUUCUUUACAUUUGAUGUGGUUGUUCUGCAAA