Supplementary Fig.S1 Schematic diagram of *Gigantidasplatifrons* gill tissue.

(A) Overview of *G.platifrons* specimens collected during the R/V Kexue 2017 cruise. The tissues of fresh collected mussels remained intact after sampling, indicating the fitness of samples. Mussel samples acclimated at atmosphere were subsequently used for either a methane-oxidizing bacteria (MOB) or *Vibrio*bacteria challenge.

(B) Transverse section of gill tissue were then conducted and subjected to 4’, 6-diamidino-2-phenylindole (DAPI) staining. As demonstrated, filamentous gill tissue was consisted by monolayer epithelial cells while symbionts were only harbored in bacteriocytes (marked as “bc”) instead of ciliated cells (marked as “cc”).

(C) With magnification, it was further demonstrated that both symbionts (marked as “en” with black triangles) and the nucleus (marked as “n” with white triangles) of host cells could be visualized by DAPI staining. Moreover, symbionts were found located exclusively in the lateral region of the bacteriocytes (marked as “bc”). In contrast, the cell nucleuswas found to be located in the basal region, close to the basal membrane (marked as “bm” with orange triangles). Lumens containing haemocytes (marked as “hae” with green triangles) could also be observed inside gill filaments.

(D) The ultrastructure of *G. platifrons*bacteriocytes (marked as “bc”) were further displayed with transmission electron microscopy. As observed, endosymbotic MOBs (marked as “en” with black triangles) were distributed exclusively in the lateral regions of bacteriocytes, while lysosomes (marked as “ls”) were located in the central and basal regions. Endoplasmic reticulum (marked as “er”) could also be found closely adjacent to the nucleus (marked as “n”). Granularhaemocytes (marked as “g”) were found inside the gill lumen separated from bacteriocytes by the basal membrane (marked as “bm”).

(E) The majority of endosymbionts were found to be engulfed separately by vesicles (black triangles) inside bacteriocytes,althoughsome could be engulfed by lysosomes (white triangles) for digestion.

Supplementary Fig.S2 Bacteria used in the study were visualized by scanning electron microscopy.

(A) Symbiotic methane-oxidizing bacteria (MOB) collected from fresh retrieved *G.platifrons* mussels by centrifugation and successive filtration were subjected to scanning electron microscopy for visualization. The majority of symbiotic MOBs remained intact after collection, with a similar size (~2 µm, rod shape) as reported previously.

(B) *Vibrio alginolyticus*was cultured overnight and also visualized by scanning electron microscopy. It was observed that*V.alginolyticus*retained its classic rod shape, with a length less than 2 µm.

Supplementary Fig.S3 Illustration of miRNAs identified in *G. platifrons*.

(A) precursors, mature sequences (red) and star sequences of gpl-miR-544a.

(B) precursors, mature sequences (red) and star sequences of gpl-novel-9.

Supplementary Fig.S4Heat map and hierarchical clustering of differentially expressed miRNAs.

Expression levels of differentially expressed miRNAs in all groups were subjected to an expression clustering analysis and illustrated by pheatmap package. The legend represents relative expressional alternations between groups where orange indicated increased expression and blue indicated decreased expression.

Supplementary Fig.S5Gene ontology (GO) distribution of differentially expressed genes (DEGs).

The GO distributions of DEGs in EN12 group in comparison with CT12 group (A), EN24 group in comparison with CT24 group (B), VA12 group in comparison with CT12 group (C), and VA24 group in comparison with CT24 group (D) were analyzed by Blast2GO and visualized by WEGO. Consequently, GO of all DEGs could be clustered into three classes as cellular component, molecular function and biological process. Noticeable, multiple immune related processes, including the response to stress, cell death, and immune responses, could be found in molecular function and biological process of almost all groups.

Supplementary Fig.S6 GO distribution of target genes of differentially expressed miRNAs.

The GO distributions of target genes of differentially expressed miRNAs in EN12 group in comparison with CT12 group (A), EN24 group in comparison with CT24 group (B), VA12 group in comparison with CT12 group (C), and VA24 group in comparison with CT24 group (D) were analyzed by Blast2GO and visualized by WEGO.

Supplementary Fig.S7Heat map and hierarchical clustering of differentially expressed pattern recognition receptors (PRRs).

Expression levels of differentially expressed PRRs in all groups were subjected to an expression clustering analysis and illustrated by pheatmap package. The legend represents relative expressional alternations between groups where orange indicated increased expression and blue indicated decreased expression. It was found that the expression pattern of PRRs could branch distinctly between challenges, with PRRs in the EN12 and EN24 groups branching first before joining with the PRRs in the VA12 and VA24 groups.

Supplementary Table 1.Overview of transcriptome and miRNA sequencing.

Supplementary Table 2. Expression level of *G.platifrons* genes given as a fragments per kilobase of transcript per million (FPKM) value.

Supplementary Table 3.List of all differentially expressed genes (DEGs).

Supplementary Table 4.List of all miRNAs identified in*G.platifrons*.

Supplementary Table 5.Expression level of all miRNAs in*G.platifrons* given as TPM value.

Supplementary Table 6.List of all targets of DE miRNAs identified in*G.platifrons*.

Supplementary Table 7.Differentially expressed pattern recognition receptors (PRRs) with annotations.

Supplementary Table 8.Differentially expressed immune effectors, with annotations.