

Supplementary Materials: miR-29a Participated in Nacre Formation and Immune Response by Targeting Y2R in *Pinctada martensii*

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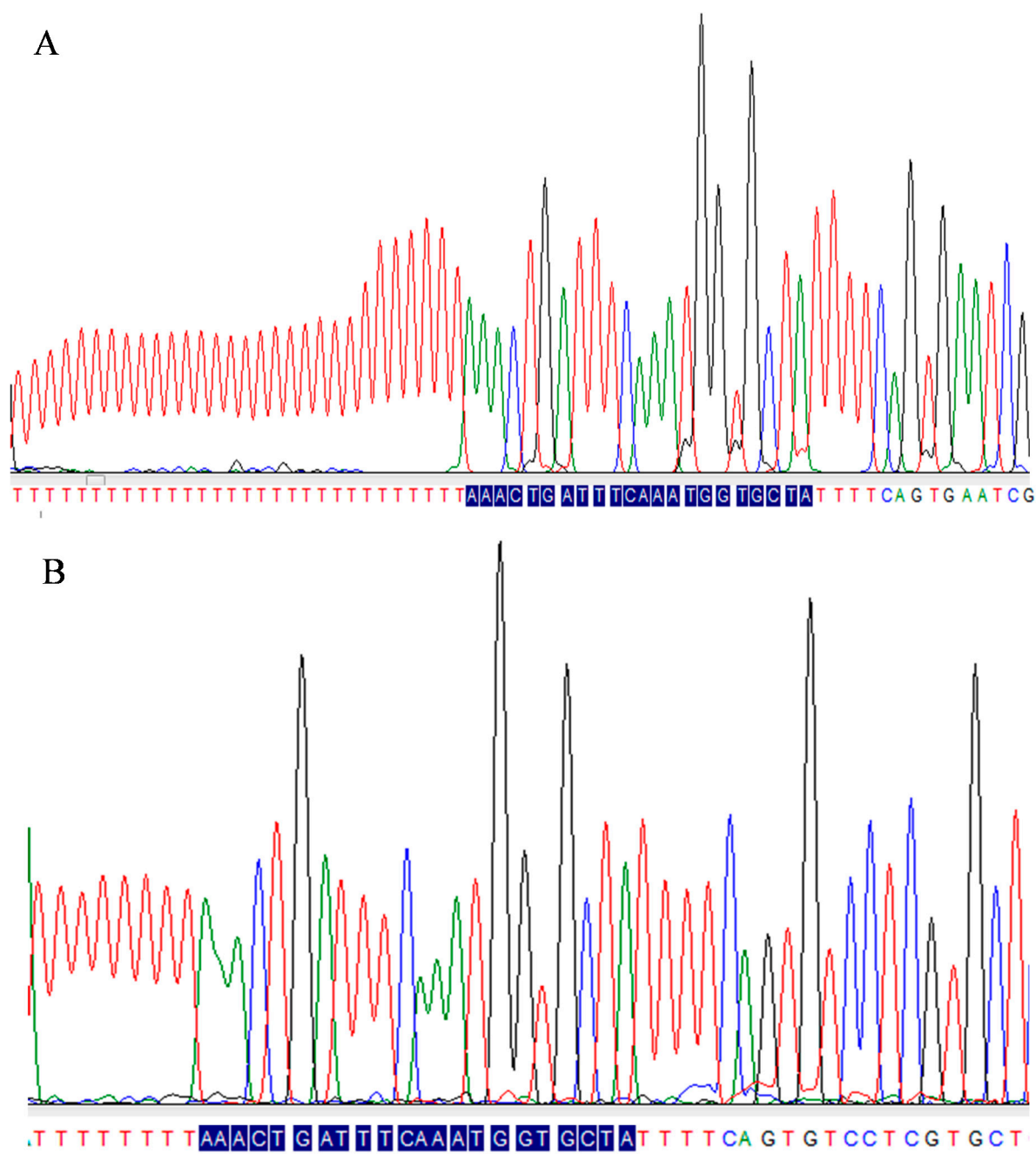


Figure S1. Sequence verification of the mature pm-miR-29a by miR-RACE. The result of 3' miR-RACE (A) and 5' miR-RACE (B), highlighted parts represented the mature sequence of pm-miR-29a. The different colors means different bases, red is T, green is A, blue is C and blank is G. The curves means the reliability of sequencing.

<p>target: SOCS-2 (cytokine-induced suppressor of cytokine signaling 2)</p> <p>miRNA: miR-29a</p> <p>mfe: -18.2 kcal/mol</p> <p>position 310</p> <p>target 5' U UA GU UC U 3'</p> <p>UUG GUU A GUGGUGU</p> <p>GAC UAA U UACCAUGA</p> <p>miRNA 3' UUU GU U 5'</p>	<p>target: FGF18(fibroblast growth factor18)</p> <p>miRNA: miR-29a</p> <p>mfe: -24.0 kcal/mol</p> <p>position 871</p> <p>target 5' U AAG U 3'</p> <p>AUUG UUUCAA GGUGCUA</p> <p>UGAC AAAGUUU CCACGAU</p> <p>miRNA 3' UU U A 5'</p>
<p>target: toll-like receptor 3</p> <p>miRNA: miR-29a</p> <p>mfe: 14.3 kcal/mol</p> <p>position 159</p> <p>target 5' A UGUUUGUUUC AA A 3'</p> <p>AAAUUGGUU UCAAG GGU</p> <p>UUUGACUAA AGUUU CCA</p> <p>miRNA 3' A CGAU 5'</p>	<p>target : TNF-alpha factor</p> <p>miRNA: miR-29a</p> <p>mfe: -18.6 kcal/mol</p> <p>position 181</p> <p>target 5' A AA UA U 3'</p> <p>UGA UCA GGUGCU</p> <p>ACU AGU CCACGA</p> <p>miRNA 3' UUUG AA UUA U 5'</p>
<p>target: N19 (Nacre protein)</p> <p>miRNA: miR-29a</p> <p>mfe: -18.6 kcal/mol</p> <p>position 285</p> <p>target 5' A GGAAAA U AG A 3'</p> <p>UGA UUCA AUGG GCUG</p> <p>ACU AAGU UACC CGAU</p> <p>miRNA 3' UUUG A U A 5'</p>	<p>target: dermatopontin</p> <p>miRNA : miR-29a</p> <p>mfe: -11.8 kcal/mol</p> <p>position 103</p> <p>target 5' U AA C 3'</p> <p>ACU AUUUA GUGU</p> <p>UGA UAAAGU CACG</p> <p>miRNA 3' UU C UUAC AU 5'</p>
<p>target: alkaline phosphatase</p> <p>miRNA: miR-29a</p> <p>mfe: -15.8 kcal/mol</p> <p>position 119</p> <p>target 5' C CCG A 3'</p> <p>AAUUG UUA GGUGC</p> <p>UUGAC AGU CCACG</p> <p>miRNA 3' U UAA UUA AU 5'</p>	<p>target:IL-17(interleukin 17)</p> <p>miRNA : miR-29a</p> <p>mfe: -13.9 kcal/mol</p> <p>position 37</p> <p>target 5' U UG UUA C C 3'</p> <p>GGCUG CAGAU GU GUUG</p> <p>UUGAC GUUUA CA CGAU</p> <p>miRNA 3' U UAAA C 5'</p>
<p>target: IRF-2 (interferon regulatory factor 2)</p> <p>miRNA : miR-29a</p> <p>mfe: -18.4 kcal/mol</p> <p>position 122</p> <p>target 5' U GUU CAAUUGA A 3'</p> <p>GACUGAU GAUGG GC</p> <p>UUGACUA UUACC CG</p> <p>miRNA 3' U AAGU A AU 5'</p>	<p>target: SK (protein serine kinase)</p> <p>miRNA: miR-29a</p> <p>mfe: -20.1 kcal/mol</p> <p>position 71</p> <p>target 5' A UUAUAG U U 3'</p> <p>UG UCAA GUGGUGCU</p> <p>AC AGU UACCACGA</p> <p>miRNA 3' UUUG UAA U 5'</p>
<p>target: allograft inflammatory factor-1</p> <p>miRNA: miR-29a</p> <p>mfe: -15.8 kcal/mol</p> <p>position 308</p> <p>target 5' C CAGGCAACA CU A 3'</p> <p>AAACUGA UUUUAAA UGUUA</p> <p>UUUGACU AAAGUUU ACGAU</p> <p>miRNA 3' ACC 5'</p>	<p>target: PFTy (tyrosinase-like protein)</p> <p>miRNA: miR-29a</p> <p>mfe: -17.6 kcal/mol</p> <p>position 157</p> <p>target 5' U CAAGA U AG C 3'</p> <p>CUG GUUUC GUGG GCUA</p> <p>GAC UAAAG UACC CGAU</p> <p>miRNA 3' UUU UU A 5'</p>
<p>target: amorphous calcium carbonate binding protein 1</p> <p>miRNA: miR-29a</p> <p>mfe: -14.2 kcal/mol</p> <p>position 119</p> <p>target 5' A A CAAG A 3'</p> <p>UUCAAA GG UGUUA</p> <p>AAGUUU CC ACGAU</p> <p>miRNA 3' UUUGACUA A 5'</p>	<p>target:BMP-7(bone morphogenetic protein 7)</p> <p>miRNA: miR-29a</p> <p>mfe: -18.9 kcal/mol</p> <p>position 1099</p> <p>target 5' U UU UCUUAUAUGUGCAA U 3'</p> <p>UUGAUUUU AUG GUGCUA</p> <p>GACUAAAG UAC CACGAU</p> <p>miRNA 3' UUU UU 5'</p>
<p>target: Y2R(neuropeptide Y receptor type 2)</p> <p>miRNA: miR-29a</p> <p>mfe: -20.6 kcal/mol</p> <p>position 365</p> <p>target 5' A AAUUG C G 3'</p> <p>GAACUGAU CAG AUGGU CUA</p> <p>UUUGACUA GUU UACCA GAU</p> <p>miRNA 3' AA C 5'</p>	<p>target: tissue inhibitor of matrix metalloproteinase</p> <p>miRNA: miR-29a</p> <p>mfe: -12.4 kcal/mol</p> <p>position 124</p> <p>target 5' A GCCAAG A 3'</p> <p>AAAU UUCA UGGU</p> <p>UUUG AAGU ACCA</p> <p>miRNA 3' ACUA UU CGAU 5'</p>

Figure S2. Target prediction by RNAhybrid. RNAhybrid software was used to predict the target relationship between pm-miR-29a and the 3' UTR of known genes which was related to the immune response or biomineralization in *P. martensii*.

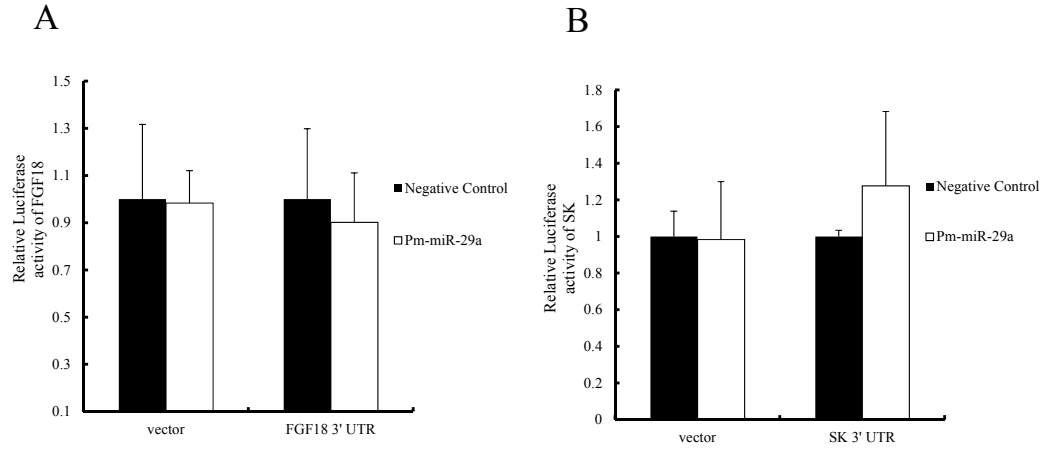


Figure S3. The relative luciferase activity of the reporter containing the 3' UTR of *FGF18* and serine kinase (*SK*) gene. The luciferase activity of the reporter containing the 3' UTR of *FGF18* (A) and *SK* (B) gene were not affected by pm-miR-29a mimics.

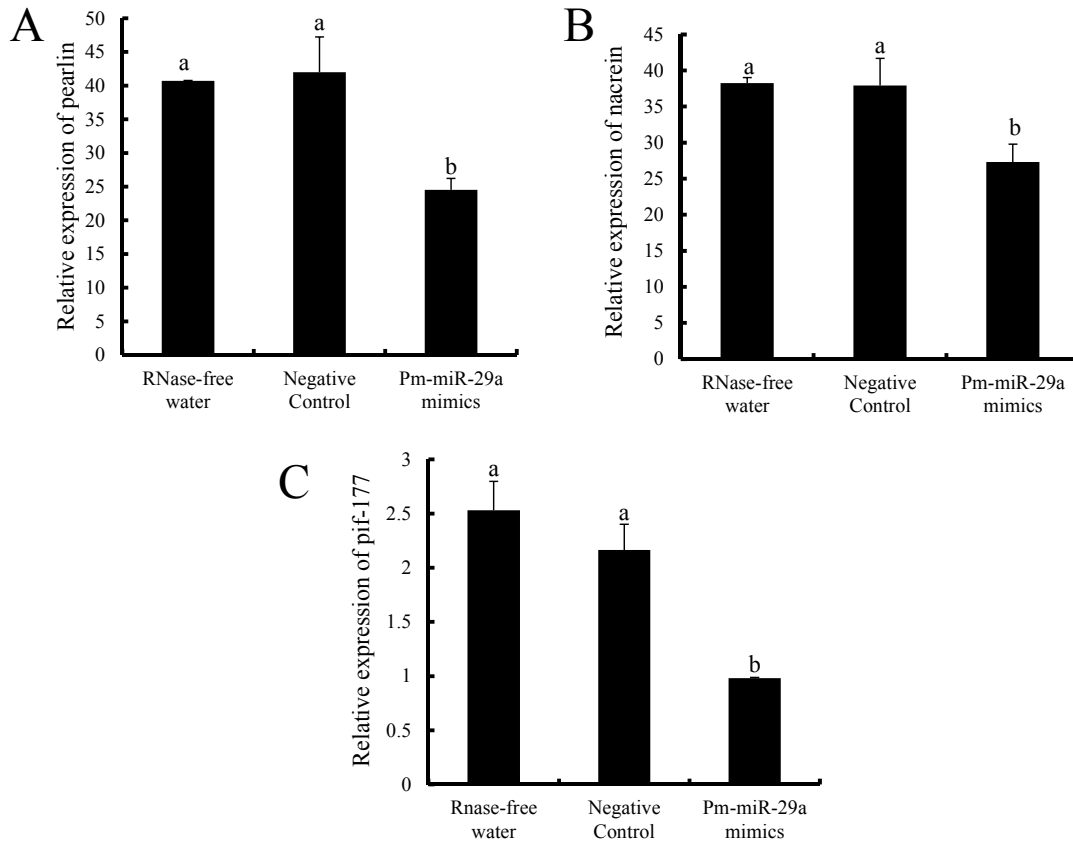


Figure S4. Expression of nacre formation-related genes after over-expression of pm-miR-29a. qRT-PCR was applied to detect the expression of nacre formation-related genes including *pearlin*, *nacrein* and *pif-177* in the mantle tissues of five individuals after pm-miR-29a mimics injection. Results showed that the expression of *pearlin* (A), *nacrein* (B), *pif-177* (C) were significantly down-regulated after over-expression of pm-miR-29a. Different letters mean a significant difference ($p < 0.05$). Error bars correspond to mean \pm SD.