**Supplementary Methods**

Public data query and mining

Gene and microRNA expression profiles by RNA-seq for cohorts of 428 cases stomach adenocarcinoma (STAD), 527 cases brain lower grade glioma (LGG), 496 cases kidney renal clear cell carcinoma (KIRC), 177 cases pancreatic adenocarcinoma (PAAD) that have clinical outcome data available were extracted from The Cancer Genome Atlas (TCGA). All expression values were converted into $\log_2$(TPM+1). MicroRNA expression datasets GSE23739, GSE26595, GSE28700, GSE93415 and gene expression dataset of Asian Cancer Research Group (ACRG; GSE66229) were extracted from the GEO database. We analyzed the relationship between microRNAs and the prognosis of cancer patients using Kaplan–Meier method. The cutoff point of each microRNA expression was determined by X-Tile software (Rimm Lab, USA).

Bioinformatics analysis

The target genes of miR-135b were predicted by TargetScan V7.2 (http://www.targetscan.org). And, we further performed gene ontology (GO) enrichment analysis, which was a useful method for annotating the target genes of microRNA with biological characteristics. miR-135b and its targets interaction network were visualized by Cytoscape 3.7.2 (https://www.cytoscape.org/), which could provide information on the molecular mechanism underlying biological process. The gene expression levels of the network were analyzed using Peking University Cancer Hospital Gastric Cancer Transcriptome Dataset (PUCH dataset; DOI: 10.21147/j.issn.1000-9604.2019.05.07). All data analyses were performed in the R programming and JAVA environment.

Immunohistochemistry (IHC) staining

Tissues from GC patients and xenograft tumors were fixed in 4% paraformaldehyde and embedded in paraffin blocks. The tissues were embedded into paraffin as blocks for storage and 5-μm-thick sections were cut into glass slides. Sections were incubated with primary antibody at 4°C overnight and then incubated with a secondary antibody at room temperature for 1 hour. After washing the slides, chromogen was applied and images were obtained with the digital pathology scaner (Aperio CS2, Leica Biosystems, USA). The primary Ki-67 antibody (#ZM-0166, ZSGB-BIO, China) and CAMK2D antibody (1:200, #15443-1-AP, Proteintech, China) were used.

Functional assay *in vivo*

Female BALB/c nude mice (Five-weeks old) were purchased from Beijing Vital River Laboratory Animal Technology Co. Ltd. Subcutaneous xenograft tumor model was used to estimate the effects of miR-135b on tumorigenicity *in vivo*. $2\times10^6$ BGC823 cells were transfected with miR-135b mimics or cotransfected with miRNA mimics and either CAMK2D overexpression plasmid or empty vector. After 24 hours, these cells were resuspended in PBS with 50% Matrigel and engrafted subcutaneously into each flank of nude mice. Antagomir-135b and antagomir-NC were obtained from RiboBio (China), tail vein injection three times, 100nM per mouse. The tumors observed in mice were measured every 3 days for three weeks. The tumor volume was calculated according to the formula: $\text{length}\times\text{width}^2/2$. At the end of experiment, the mice were sacrificed and the

tumors were collected. The dissected tissues were formalin-fixed and paffin-embedded for hematoxylin-eosin (H&E) staining, and Ki-67 IHC staining.

For experimental tumor metastatic model, BGC823 cells ($5\times10^6$ cells in a 100 μL volume per mouse) were injected into the tail vein of BALB/c nude mice. After 1 week, these mice were randomly divided into two groups: antagomir-135b and antagomir-NC. Mice were treated either antagomir-135b or antagomir-NC (tail vein injection 3 times, 100nM per mouse). Four weeks later, the mice were sacrificed and the lungs were excised from the body. Bouin's solution was injected from the main bronchi to fix the lung tissues. All animal experiments were conducted in accordance with the Institutional Animal Care and Use Committee guidelines at Peking University Beijing Cancer Hospital.

## Supplementary Data

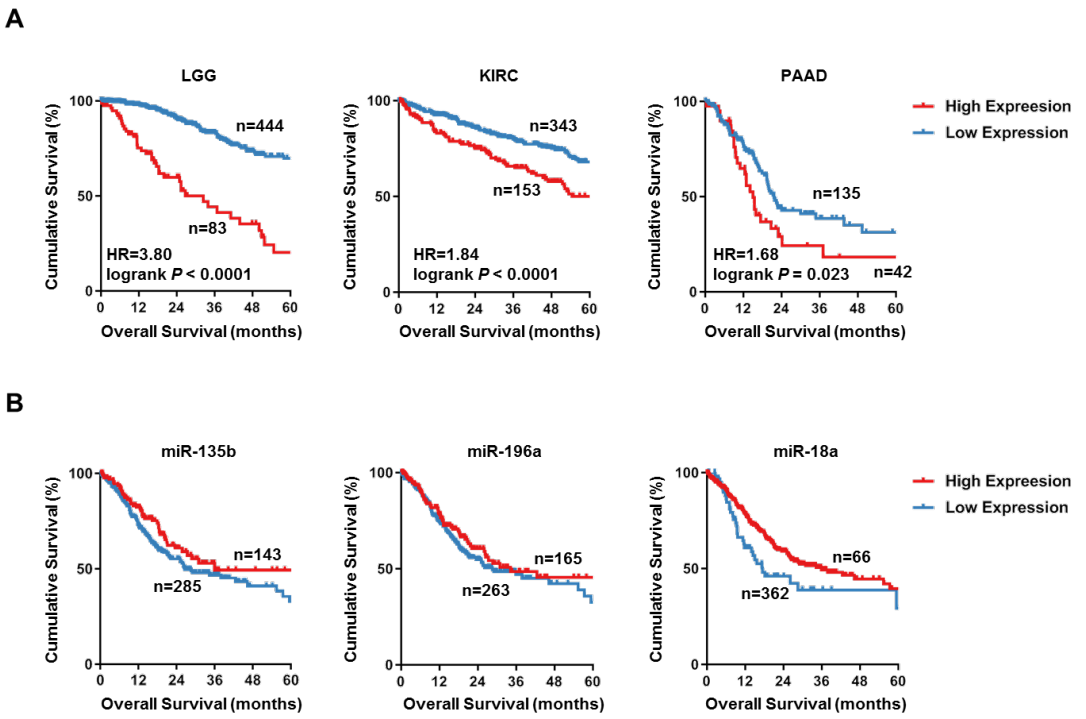


Figure S1. Survival analysis using TCGA data. (A) The high expression level of miR-135b was significantly associated with poor prognosis in patients with brain lower grade glioma (LGG), kidney renal clear cell carcinoma (KIRC), pancreatic adenocarcinoma (PAAD). (B) No prognostic significance in patients with gastric cancer was found among miR-135b, miR-196a and miR-18a. HR: hazard ratio.

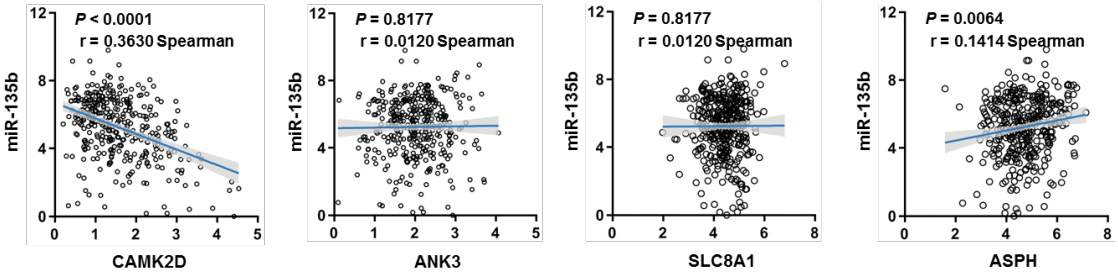


Figure S2. Correlation analysis of miR-135 and its predicted targets using TCGA data. The results showed that the expression levels between miR-135b and CAMK2D were significantly negatively correlated.

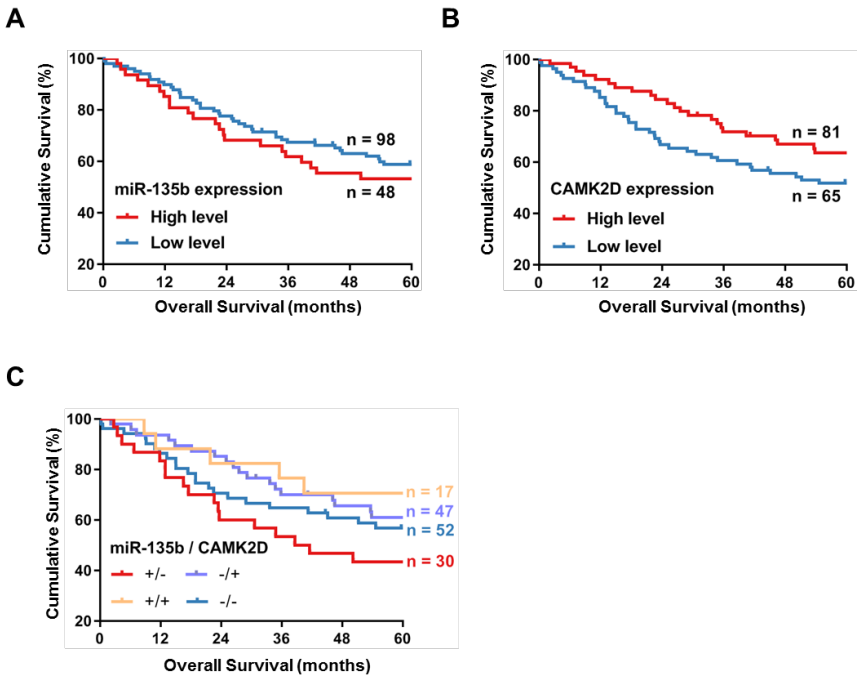


Figure S3. Survival analysis in GC patients based on expression levels of miR-135b and its target CAMK2D.

**Supplementary Tables**

Table S1. Pathological information of human gastric tissue samples for miRNA and mRNA qRT-PCR validation.

| Sample ID | Gender | Age | TNM | Stage | Lauren classification | Histological grade |
|---|---|---|---|---|---|---|
| 1 | M | 66 | T3N3M0 | IIIB | Diffuse | Poor |
| 2 | M | 51 | T3N3M0 | IIIB | Intestinal | Moderate |
| 3 | F | 47 | T3N2M0 | IIIA | Missing | Moderate |
| 4 | M | 59 | T3N3M0 | IIIB | Intestinal | Moderate |
| 5 | M | 53 | T2N2M0 | IIB | Mixed | Poor |
| 6 | M | 55 | T3N3M0 | IIIB | Diffuse | Missing |
| 7 | M | 56 | T3N2M0 | IIIA | Intestinal | Moderate |
| 8 | F | 74 | T3N0M0 | IIA | Diffuse | Poor |
| 9 | M | 58 | T3N0M0 | IIA | Intestinal | Moderate |
| 10 | M | 55 | T4N1M0 | III | Diffuse | Poor |
| 11 | M | 58 | T3N2M0 | IIIA | Intestinal | Moderate |
| 12 | M | 78 | T2N1M0 | IIA | Mixed | Moderate |
| 13 | F | 50 | T2N3M0 | IIIA | Intestinal | Moderate |
| 14 | M | 42 | T3N1M0 | IIB | Diffuse | Poor |
| 15 | F | 32 | T3N3M0 | IIIB | Diffuse | Poor |
| 16 | M | 68 | T3N3M0 | IIIB | Mixed | Moderate |
| 17 | M | 66 | T3N1M0 | IIB | Mixed | Poor |
| 18 | M | 69 | T3N3M0 | IIIB | Diffuse | Poor |
| 19 | F | 60 | T3N2M1 | IV | Intestinal | Poor |
| 20 | M | 59 | T2N2M0 | IIB | Mixed | Poor |
| 21 | M | 55 | T3N1M0 | IIB | Diffuse | Poor |
| 22 | F | 51 | T4N3M0 | III | Diffuse | Poor |
| 23 | M | 43 | T3N1M0 | IIB | Mixed | Moderate |
| 24 | F | 41 | T3N2M0 | IIIA | Intestinal | Moderate |
| 25 | F | 45 | T3N3M0 | IIIB | Diffuse | Poor |
| 26 | M | 49 | T2N0M0 | IIB | Mixed | Moderate |
| 27 | M | 75 | T3N0M0 | IIA | Intestinal | Moderate |
| 28 | M | 69 | T3N3M0 | IIIB | Diffuse | Poor |
| 29 | M | 75 | T3N1M0 | IIB | Mixed | Moderate |
| 30 | M | 75 | T3N0M0 | IIA | Intestinal | Moderate |
| 31 | M | 77 | T3N3M0 | IIIB | Intestinal | Moderate |
| 32 | M | 45 | T3N0M0 | IIA | Diffuse | Poor |
| 33 | M | 64 | T3N3M0 | IIIB | Diffuse | Missing |
| 34 | M | 55 | T1N0M0 | IA | Intestinal | Moderate |
| 35 | M | 44 | T3N1M0 | IIB | Intestinal | Moderate |

| | | | | | | |
|---|---|---|---|---|---|---|
| 36 | M | 60 | T3N1M0 | IIB | Intestinal | Moderate |
| 37 | M | 73 | T3N3M0 | IIIB | Mixed | Moderate |
| 38 | F | 53 | T2N0M0 | IB | Mixed | Moderate |
| 39 | F | 57 | T3N0M0 | IIA | Diffuse | Poor |
| 40 | M | 56 | T3N2M0 | IIIA | Diffuse | Poor |
| 41 | M | 38 | T3N3M0 | IIIB | Diffuse | Moderate |
| 42 | F | 56 | T3N0M0 | IIA | Diffuse | Poor |
| 43 | M | 62 | T2N0M0 | IB | Intestinal | Well |
| 44 | F | 48 | T2N0M0 | IB | Mixed | Moderate |
| 45 | F | 70 | T2N1M0 | IIA | Mixed | Poor |
| 46 | M | 54 | T3N0M0 | IIA | Intestinal | Moderate |
| 47 | M | 80 | T3N2M0 | IIIA | Diffuse | Poor |
| 48 | M | 65 | T3N1M0 | IIB | Mixed | Moderate |
| 49 | M | 39 | T3N0M0 | IIA | Diffuse | Missing |
| 50 | M | 59 | T3N3M0 | IIIB | Diffuse | Poor |
| 51 | F | 53 | T3N3M0 | IIIB | Mixed | Poor |
| 52 | F | 74 | T3N0M0 | IIA | Intestinal | Poor |
| 53 | M | 61 | T2N2M0 | IIB | Intestinal | Poor |
| 54 | M | 72 | T3N1M0 | IIB | Intestinal | Moderate |
| 55 | M | 49 | T3N1M0 | IIB | Intestinal | Moderate |
| 56 | F | 64 | T4N3M0 | III | Diffuse | Poor |
| 57 | F | 58 | T1N0M0 | IA | Diffuse | Poor |
| 58 | M | 71 | T2N1M0 | IIA | Intestinal | Poor |
| 59 | M | 70 | T2N1M0 | IIA | Intestinal | Missing |
| 60 | M | 60 | T2N1M0 | IIA | Intestinal | Moderate |
| 61 | M | 63 | T1N0M0 | IA | Mixed | Poor |
| 62 | M | 67 | T4N2M0 | III | Intestinal | Poor |
| 63 | M | 54 | T4N0M0 | IIB | Missing | Poor |
| 64 | M | 76 | T3N3M0 | IIIB | Intestinal | Moderate |
| 65 | M | 66 | T4N3M0 | III | Missing | Poor |
| 66 | M | 56 | T3N0M0 | IIA | Mixed | Moderate |
| 67 | F | 55 | T3N0M0 | IIA | Diffuse | Poor |
| 68 | M | 67 | T3N3M0 | IIIB | Mixed | Moderate |
| 69 | M | 52 | T3N3M0 | IIIB | Intestinal | Moderate |
| 70 | M | 41 | T3N0M0 | IIA | Mixed | Moderate |
| 71 | M | 36 | T3N0M0 | IIA | Diffuse | Poor |
| 72 | M | 60 | T4N2M0 | III | Diffuse | Poor |
| 73 | M | 51 | T3N3M0 | IIIB | Intestinal | Poor |
| 74 | F | 69 | T3N1M0 | IIB | Mixed | Moderate |
| 75 | M | 59 | T3N3M0 | IIIB | Intestinal | Well |
| 76 | M | 48 | T3N3M0 | IIIB | Diffuse | Poor |

| | | | | | | |
|---|---|---|---|---|---|---|
| 77 | M | 46 | T4N1M0 | III | Mixed | Poor |
| 78 | F | 41 | T3N2M0 | IIIA | Mixed | Moderate |
| 79 | M | 79 | T3N3M0 | IIIB | Intestinal | Moderate |
| 80 | M | 54 | T3N1M0 | IIB | Intestinal | Well |
| 81 | M | 52 | T3N1M0 | IIB | Intestinal | Moderate |
| 82 | F | 52 | T4N1M0 | III | Intestinal | Moderate |
| 83 | F | 64 | T3N3M0 | IIIB | Mixed | Moderate |
| 84 | M | 66 | T2N0M0 | IB | Intestinal | Moderate |
| 85 | M | 79 | T3N2M0 | IIB | Diffuse | Poor |
| 86 | M | 57 | T3N2M0 | IIB | Mixed | Poor |
| 87 | M | 56 | T2N0M0 | IB | Intestinal | Moderate |
| 88 | F | 57 | T3N0M0 | IIA | Intestinal | Moderate |
| 89 | M | 41 | T3N2M0 | IIIA | Diffuse | Poor |
| 90 | F | 56 | T1bN0M0 | IA | Mixed | Poor |
| 91 | M | 80 | T3N3M0 | IIIB | Intestinal | Moderate |
| 92 | M | 69 | T2N3M0 | IIIA | Intestinal | Moderate |
| 93 | M | 61 | T3N0M0 | IIA | Intestinal | Missing |
| 94 | M | 59 | T3N3M0 | IIIB | Intestinal | Poor |
| 95 | M | 45 | T1bN0M0 | IA | Diffuse | Poor |
| 96 | M | 58 | T2N3M0 | IIIA | Diffuse | Poor |
| 97 | M | 55 | T3N2M0 | IIIA | Diffuse | Poor |
| 98 | M | 62 | T3N2M0 | IIIA | Intestinal | Moderate |
| 99 | M | 51 | T2N0M0 | IB | Intestinal | Well |
| 100 | F | 68 | T4N2M0 | III | Intestinal | Moderate |
| 101 | M | 42 | T3N2M0 | IIIA | Intestinal | Moderate |
| 102 | F | 53 | T3N2M0 | IIIA | Diffuse | Poor |
| 103 | F | 34 | T2N0M0 | IB | Missing | Moderate |
| 104 | M | 49 | T3N3M0 | IIIB | Missing | Moderate |
| 105 | M | 71 | T3N0M0 | IIA | Missing | Moderate |
| 106 | M | 75 | T3N2M0 | IIIA | Missing | Moderate |
| 107 | M | 61 | T2N2M0 | IIB | Missing | Moderate |
| 108 | F | 58 | T3N2M0 | IIIA | Missing | Moderate |
| 109 | F | 67 | T2N2M0 | IIB | Intestinal | Moderate |
| 110 | M | 59 | T3N0M0 | IIA | Intestinal | Moderate |
| 111 | F | 52 | T3N1M0 | IIB | Intestinal | Moderate |
| 112 | M | 82 | T2N3M0 | IIIA | Diffuse | Poor |
| 113 | M | 74 | T3N1M0 | IIB | Intestinal | Moderate |
| 114 | F | 52 | T3N2M0 | IIIA | Intestinal | Moderate |
| 115 | M | 57 | T3N1M0 | IIB | Mixed | Poor |
| 116 | M | 62 | T3N3M0 | IIIB | Mixed | Poor |
| 117 | M | 69 | T3N0M0 | IIA | Intestinal | Moderate |

| | | | | | | |
|---|---|---|---|---|---|---|
| 118 | M | 62 | T3N3M0 | IIIB | Diffuse | Poor |
| 119 | F | 79 | T2N0M0 | IB | Intestinal | Poor |
| 120 | M | 58 | T3N3M0 | IIIB | Mixed | Poor |
| 121 | M | 36 | T2N2M0 | IIB | Diffuse | Poor |
| 122 | M | 46 | T3N3M0 | IIIB | Diffuse | Poor |
| 123 | M | 52 | T3N0M0 | IIA | Intestinal | Moderate |
| 124 | M | 60 | T4N2M0 | IIIB | Missing | Moderate |
| 125 | F | 71 | T3N2M0 | IIIA | Mixed | Moderate |
| 126 | F | 81 | T4N3M1 | IV | Mixed | Moderate |
| 127 | F | 46 | T3N2M0 | IIIA | Diffuse | Poor |
| 128 | M | 74 | T2N1M0 | IIA | Intestinal | Moderate |
| 129 | F | 62 | T2N3M0 | IIIA | Diffuse | Poor |
| 130 | M | 47 | T2N0M0 | IB | Diffuse | Poor |
| 131 | F | 71 | T2N0M0 | IB | Mixed | Poor |
| 132 | M | 69 | T3N0M0 | IIA | Diffuse | Poor |
| 133 | M | 57 | T3N2M0 | IIIA | Intestinal | Moderate |
| 134 | M | 63 | T3N3M0 | IIIB | Intestinal | Moderate |
| 135 | M | 57 | T3N3M0 | IIIB | Mixed | Moderate |
| 136 | M | 50 | T3N1M0 | IIB | Mixed | Moderate |
| 137 | M | 64 | T4N2M0 | III | Intestinal | Moderate |
| 138 | M | 71 | T2N0M0 | IB | Mixed | Poor |
| 139 | M | 33 | T3N2M0 | IIIA | Mixed | Poor |
| 140 | M | 69 | T3N0M0 | IIA | Intestinal | Moderate |
| 141 | M | 56 | T3N3M0 | IIIB | Intestinal | Moderate |
| 142 | M | 42 | T3N0M0 | IIA | Mixed | Moderate |
| 143 | M | 52 | T3N3M0 | IIIB | Mixed | Moderate |
| 144 | F | 64 | T3N3M0 | IIIB | Intestinal | Moderate |
| 145 | F | 60 | T3N1M0 | IIB | Missing | Moderate |
| 146 | M | 56 | T3N3M0 | IIIB | Missing | Moderate |

Table S2. Pathological information of blood samples for miRNA qRT-PCR validation.

| Sample ID | Gender | Age | Pathology/diagnosis |
|---|---|---|---|
| 1 | M | 68 | Adenocarcinoma |
| 2 | M | 52 | Adenocarcinoma |
| 3 | F | 33 | Adenocarcinoma |
| 4 | M | 71 | Adenocarcinoma |
| 5 | M | 54 | Adenocarcinoma |
| 6 | M | 68 | Adenocarcinoma |
| 7 | M | 57 | Adenocarcinoma |
| 8 | M | 64 | Adenocarcinoma |
| 9 | M | 57 | Adenocarcinoma |
| 10 | M | 64 | Adenocarcinoma |
| 11 | F | 57 | Adenocarcinoma |
| 12 | M | 65 | Adenocarcinoma |
| 13 | F | 67 | Adenocarcinoma |
| 14 | M | 53 | Adenocarcinoma |
| 15 | M | 69 | Adenocarcinoma |
| 16 | M | 50 | Hepatic metastases from GC |
| 17 | F | 74 | Adenocarcinoma |
| 18 | M | 71 | Adenocarcinoma |
| 19 | M | 72 | Adenocarcinoma |
| 20 | M | 69 | Suspected hepatoid adenocarcinoma |
| 21 | M | 48 | Adenocarcinoma |
| 22 | F | 45 | Adenocarcinoma |
| 23 | M | 58 | Adenocarcinoma |
| 24 | M | 37 | Normal |
| 25 | M | 44 | Normal |
| 26 | M | 56 | Normal |
| 27 | M | 61 | Normal |
| 28 | F | 65 | Normal |
| 29 | F | 60 | Normal |
| 30 | F | 55 | Normal |
| 31 | M | 47 | Normal |
| 32 | M | 48 | Normal |
| 33 | M | 67 | Normal |
| 34 | M | 68 | Normal |
| 35 | F | 70 | Normal |
| 36 | M | 66 | Normal |
| 37 | M | 54 | Normal |

| | | | |
|---|---|---|---|
| 38 | M | 55 | Normal |
| 39 | M | 49 | Normal |
| 40 | M | 60 | Normal |
| 41 | F | 41 | Normal |
| 42 | F | 43 | Normal |
| 43 | F | 51 | Normal |
| 44 | F | 47 | Normal |
| 45 | M | 62 | Normal |
| 46 | F | 54 | Normal |
| 47 | M | 53 | Normal |
| 48 | F | 49 | Normal |
| 49 | M | 53 | Normal |
| 50 | M | 51 | Normal |

Table S3. Primers list for miRNA and mRNA qRT-PCR validation.

| Primer | | Sequence (5'----------3') |
|---|---|---|
| miR-135b-5p | Forward | ACACTCCAGCTGGGTATGGCTTTCATTCCT |
| | Reverse | TGGTGTCGTGGAGTCG |
| miR-196a-5p | Forward | ACACTCCAGCTGGGTAGGTAGTTTCATGTT |
| | Reverse | TGGTGTCGTGGAGTCG |
| miR-18a-5p | Forward | ACACTCCAGCTGGGTAAGGTGCATCTAGTGC |
| | Reverse | TGGTGTCGTGGAGTCG |
| Let-7e | Forward | ACACTCCAGCTGGGTGAGGTAGTAGGTTGT |
| | Reverse | TGGTGTCGTGGAGTCG |
| U6 | Forward | CTCGCTTCGGCAGCACATATACT |
| | Reverse | ACGCTTCACGAATTTGCGTGTC |
| CAMK2D | Forward | AGGGCTTTCACTACTTGGTGT |
| | Reverse | AGCCAAAGTCTGCCAATTTCA |
| GAPDH | Forward | GACTCATGACCACAGTCCATGC |
| | Reverse | AGAGGCAGGGATGAT GTTCTG |