*SUPPLEMENTAL INFORMATION*

*Wang et al.*

## 1. Supplemental Material and Methods

**Plasmid construction.** For human miRNA overexpressing plasmids, primers were designed based on the genomic sequences from the miRBase (microrna.sanger.ac.uk), with the forward primers (Table S2) carrying a *HindIII* site and the reverse primers (Table S2) carrying a *XbaI* site respectively. Pre-miRNA gene fragments were individually PCR-amplified using genomic DNA prepared from human GC cell BGC823 with Pyrobest DNA Polymerase (TaKaRa). The resultant DNA fragments were subcloned into pFlag-CMV2, using the *HindIII* and *XbaI* sites. The insertion sequences of the resultant plasmids were confirmed by sequencing. To construct lentiviral vectors overexpressing miR-15a-3p or miR-16-1-3p, human *miR-15a* or *miR-16-1* gene DNA fragment was PCR amplified from human BGC823 cell genomic DNA with the primers listed in Table S2. The PCR-amplified fragments were inserted to a lentiviral vector pLV-EF1α-MCS-IRES-Puro (pLV-ctrl) to generate pLV-miR-15a or pLV-miR-16-1. To construct lentiviral vector overexpressing *Twist1*, human *Twist1* gene DNA fragment was PCR amplified from pHA-Twist1 plasmid with the primers listed in Table S2. The PCR-amplified fragment was inserted to a lentiviral vector pLV-EF1α-MCS-IRES-Puro (pLV-ctrl) to generate pLV-Twist1.Viral vector pLV-miR-15a, pLV-miR-16-1, pLV-Twist1 or pLV-ctrl as well as three lentivirus packaging plasmids (pMDL, pVSVG and pREV) were co-transfected into HEK293T cells. pLV-EF1α-MCS-IRES-Puro, pMDL, pVSVG and pREV were kind gifts from Prof. Jiahuai Han from Xiamen University. Media containing lentiviruses (LV-miR-15a, LV-miR-16-1, LV-Twist1 and LV-ctrl) were collected every 24 h for 3 times and the lentiviruses were purified by ultra-speed centrifugation.

The resulting plasmids are shown in Figure S4 in the Supplemental information section. hGH-pA, poly-A for human growth hormone gene; Luc, luciferase; SV40-pA, SV40 poly-A.

## 2. Supplemental figure and table legends

**Figure S1 Overexpression of miR-15a-3p and miR-16-1-3p significantly reduced MMP2 and MMP9 activity.** BGC823 cells were transfected with miRNA expression

plasmids or control plasmid as indicated. Cell medium was harvested for Gel Zymography analysis 24 h after the transfection.

**Figure S2 miR-15a-3p, miR-16-1-3p and *Twist1* mRNA expression in human GC clinical samples.** (**A**) miR-15a-3p expression was significantly down-regulated in human GC tissues. (**B**) miR-16-1-3p expression was significantly down-regulated in human GC tissues. (**C**) Twist1 mRNA expression was significantly up-regulated in human GC tissues. Abbreviations: T, GC tissue; N, adjacent noncancerous gastric tissue; 18S rRNA and U6 mRNA were calibrated for qPCR analysis.

**Figure S3 Twist1 mRNA expression in clinical tumor samples as reported in microarray studies [1-6].** Data were downloaded from the Oncomine database (www.oncomine.org) and used for the plot without any further processing. T, tumor tissue; N, adjacent noncancerous tissue; NS, not significant; **, $p < 0.01$; ***, $p < 0.001$, all compared to the normal controls. (**A)** dot plots showing *Twist1* mRNA expression in clinical GC samples as studied by D'Errico M [1]. (**B)** dot plots showing *Twist1* mRNA expression in clinical lung cancer samples as studied by Hou J [2]. (**C)** dot plots showing *Twist1* mRNA expression in clinical pancreas cancer samples as studied by Badea L [3]. (**D)** dot plots showing *Twist1* mRNA expression in clinical prostate cancer samples as studied by Varambally S [4]. (**E)** dot plots showing *Twist1* mRNA expression in clinical colon cancer samples as studied by Kaiser S [5]. (**F)** dot plots showing *Twist1* mRNA expression in clinical brain cancer samples as studied by Murat A [6]. (**G)** dot plots showing *Twist1* mRNA expression in clinical ovarian cancer samples as studied by The Cancer Genome Atlas.

**Figure S4 Schematic graphs for the plasmids used in the current study.** See the Materials and Methods section for more details.

**Table S1** A list of chemically-synthesized and HPLC-purified miRNA mimics and miRNA inhibitor purchased from Genepharm (Shanghai, China).

**Table S2** Plasmids and the primers used for their construction. Lic, ligation independent cloning.

**Table S3** A list of the primers used for qPCR analyses of mRNAs and miRNAs. LNA, locked nucleic acid.

**Table S4** A list of the antibodies used for immunoblots (IB) in current study.

**Figure S1**

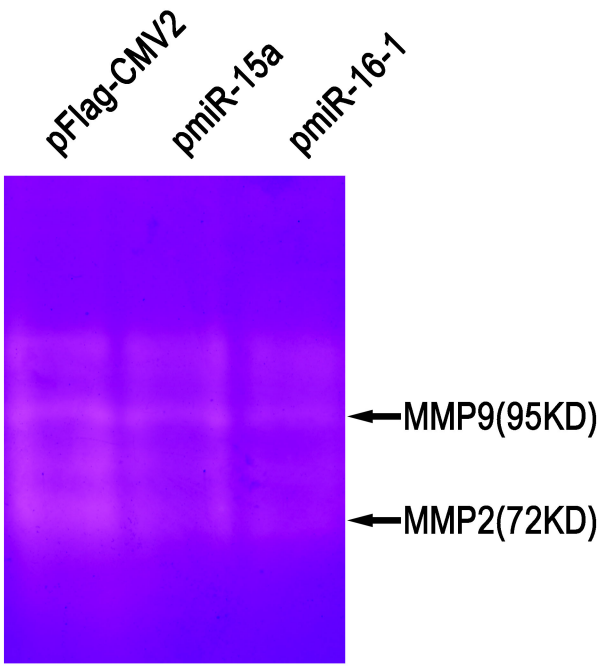
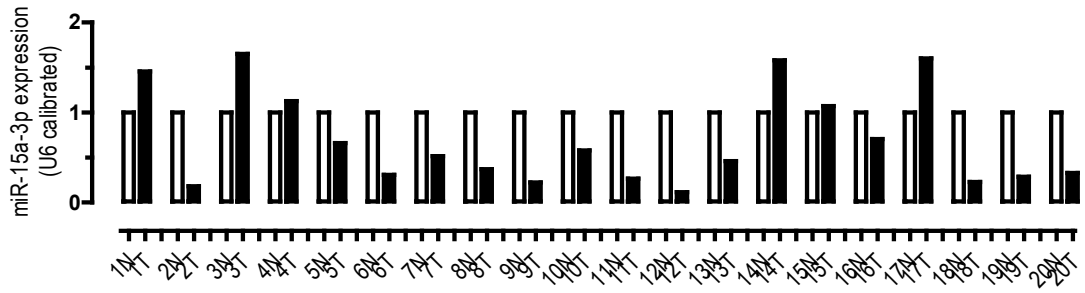
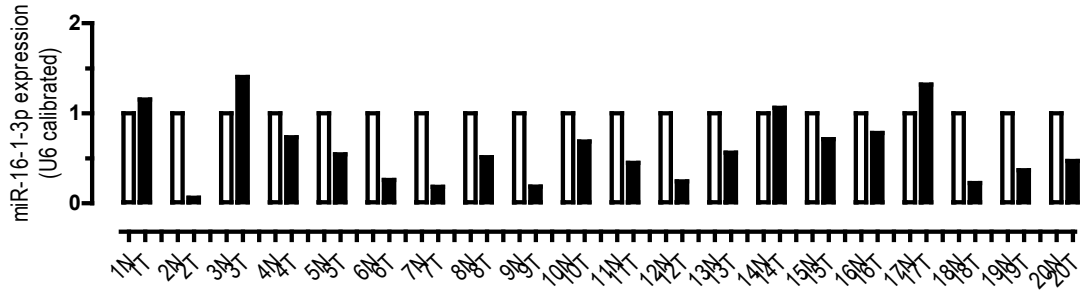


**Figure S2**

**A**




**B**




**C**

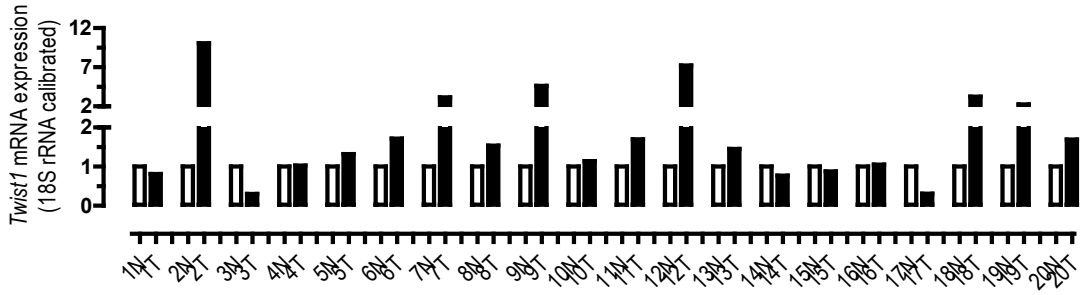


**Figure S3**

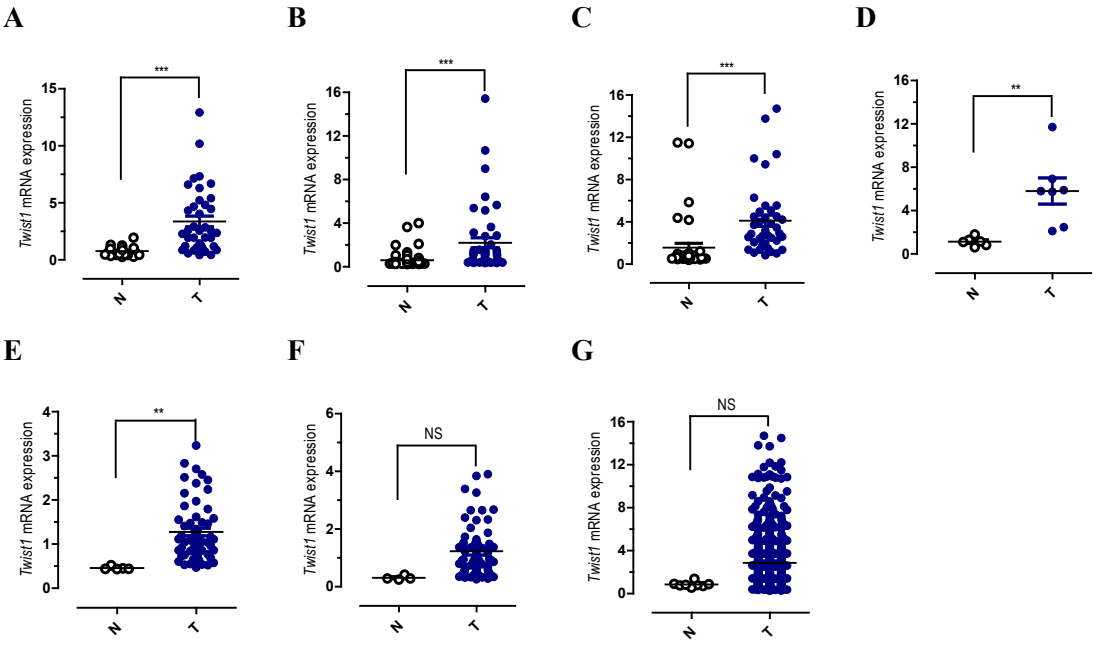


**Figure S4**

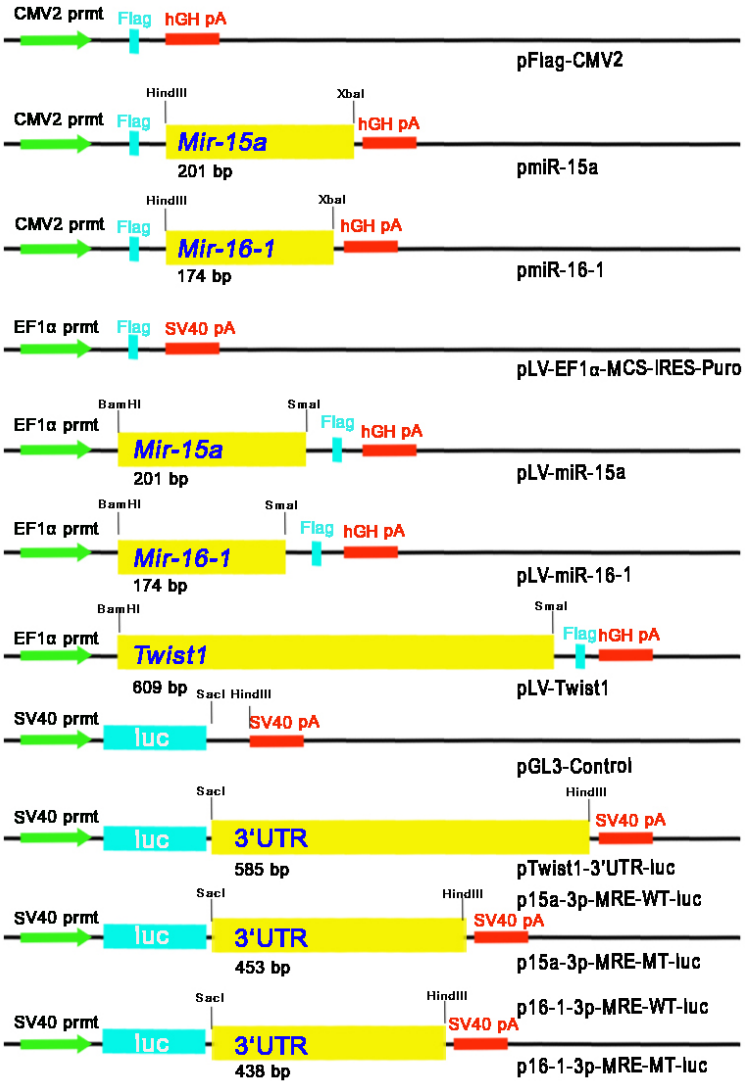

CMV2 prmt
Flag
hGH pA
pFlag-CMV2
HindIII
XbaI
Mir-15a
201 bp
pmiR-15a
Mir-16-1
174 bp
pmiR-16-1
EF1α prmt
SV40 pA
pLV-EF1α-MCS-IRES-Puro
BamHI
SmaI
pLV-miR-15a
pLV-miR-16-1
Twist1
609 bp
pLV-Twist1
SacI
luc
pGL3-Control
3'UTR
585 bp
pTwist1-3'UTR-luc
453 bp
p15a-3p-MRE-WT-luc
p15a-3p-MRE-MT-luc
438 bp
p16-1-3p-MRE-WT-luc
p16-1-3p-MRE-MT-luc

**Table S1**

| Name | Sequence (5' →3') | Supplier |
|---|---|---|
| mimics-ctrl | UUCUCCGAACGUGUCACGUTT<br>ACGUGACACGUUCGGAGAATT | GenePharma, Shanghai |
| miR-15a-3p mimics | CAGGCCAUAUUGUGCUGCCUCA<br>AGGCAGCACAAUAUGGCCUGUU | GenePharma, Shanghai |
| miR-16-1-3p mimics | CCAGUAUUAACUGUGCUGCUGA<br>AGCAGCACAGUUAAUACUGGUU | GenePharma, Shanghai |
| inhibitor-ctrl | CAGUACUUUUGUGUAGUACAA | GenePharma, Shanghai |
| miR-15a-3p inhibitor | UGAGGCAGCACAAUAUGGCCUG | GenePharma, Shanghai |
| miR-16-1-3p inhibitor | UCAGCAGCACAGUUAAUACUGG | GenePharma, Shanghai |

**Table S2**

| Plasmid | Backbone vector | Template | Primer sequence (from5'→3') | Gene ID | Insert size (bp) |
|---|---|---|---|---|---|
| pmiR-15a | pFlag-CMV2 | BGC823 genomic DNA | Forward: GAC*AAGCTT*TAGGCGCGAATGTGTGTTTAA<br>Reverse: GAC*TCTAGA*TATTTACGTGCTGCTAAGGCA | MI0000069 (miRBase) | 201 |
| pmiR-16-1 | pFlag-CMV2 | BGC823 genomic DNA | Forward: GAC*AAGCTT*AGGATCTGATCTTCTGAAGAA<br>Reverse: GAC*TCTAGA*CATTAAAACACAACTGTAGAG | MI0000070 (miRBase) | 174 |
| pTwist1-3'UTR-luc | pGL3-control | BGC823 cDNA | Forward: ATA*GAGCTC*CCTAGATGTCATTGTTTCCAG<br>Reverse: CGC*AAGCTT*GACACCGGATCTATTTGCATT | NM_000474.3 | 585 |
| p15a-3p-MRE-WT-luc | pGL3-control | BGC823 cDNA | Forward: ATA*GAGCTC*CCTAGATGTCATTGTTTCCAG<br>Reverse: CGC*AAGCTT*TGCAGGCCAGTTTGATCCCAG | NM_000474.3 | 453 |
| p15a-3p-MRE-MT-luc | pGL3-control | BGC823 cDNA | Forward: ATA*GAGCTC*CCTAGATGTCATTGTTTCCAG<br>Reverse: CGC*AAGCTT*TGCACGGCAGTTTGATCCCAG | NM_000474.3 | 453 |
| p16-1-3p-MRE-WT-luc | pGL3-control | BGC823 cDNA | Forward: ATA*GAGCTC*CCTAGATGTCATTGTTTCCAG<br>Reverse: CGC*AAGCTT*TCCCAGTATTTTTATTTCTAA | NM_000474.3 | 438 |
| p16-1-3p-MRE-MT-luc | pGL3-control | BGC823 cDNA | Forward: ATA*GAGCTC*CCTAGATGTCATTGTTTCCAG | NM_00047 | 438 |

| | | | | | |
|---|---|---|---|---|---|
| | | | Reverse: CGC*AAGCTT*TCCCTGAAATTTTATTTCTAA | 4.3 | |
| pLV-miR-15a | pLV-EF1α-MCS-IRES-Puro | BGC823 genomic DNA | Forward (Lic): *AGAGAATTCGGATCC*TAGGCGCGAATGTGTGTTTAA Reverse (Lic): *CCATGGCTCGAGCCC*TATTTACGTGCTGCTAAGGCA | MI0000069 (miRBase) | 201 |
| pLV-miR-16-1 | pLV-EF1α-MCS-IRES-Puro | BGC823 genomic DNA | Forward (Lic): *AGAGAATTCGGATCC*AGGATCTGATCTTCTGAAGA Reverse (Lic): *CCATGGCTCGAGCCC*CATTAAAACACAACTGTAGAG | MI0000070 (miRBase) | 174 |
| pLV-Twist1 | pLV-EF1α-MCS-IRES-Puro | pHA-Twist1 | Forward (Lic): *AGAGAATTCGGATCC*ATGATGCAGGACGTGTCCAGC Reverse (Lic): *CTTCCATGGCTCGAG*CTAGTGGGACGCGGACATGGA | NM_000474 | 609 |

**Table S3**

| Gene | Gene ID | Primer sequence (5' → 3') | Amplicon (bp) |
|---|---|---|---|
| miR-15a-3p | MIMAT0004488 | Reverse transcription:<br>GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACTTGAGGC<br>Forward: GGGGCAGGCCATATTGTG (LNA)<br>Reverse: TGCGTGTCGTGGAGTC | 64 |
| miR-16-1-3p | MIMAT0004489 | Reverse transcription:<br>GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACTTCAGCA<br>Forward：GGGGCCAGTATTAACTGT (LNA)<br>Reverse：TGCGTGTCGTGGAGTC | 64 |
| U6 | NR_004394.1 | Reverse transcription: CGCTTCACGAATTTGCGTGTCAT<br>Forward: GCTTCGGCAGCACATATACTAAAAT (LNA)<br>Reverse: CGCTTCACGAATTTGCGTGTCAT | 89 |
| Twist1 | NM_000474.3 | Forward: AAGCTGCAGCTATGTGGCTCACG<br>Reverse: AATCACTGTCCACGGGCCTGTCT | 317 |
| 18S rRNA | NR_003286.2 | Forward: CGACGACCCATTCGAACGTCT<br>Reverse: CTCTCCGGAATCGAACCCTGA | 102 |

**Table S4**

| Antibody | Cat# | Dilution/ Working concentration | Supplier |
|---|---|---|---|
| Goat anti-Mouse IgG | 31430 | 1:5000 | Pierce Biotechnology, Inc., Rockford, IL, USA |
| Goat anti-Rabbit IgG | 31360 | 1:5000 | Pierce |
| anti-α-tubulin | sc-5286 | 1:5000 | Santa Cruz Biotechnology, Inc., CA, USA |
| anti-β-actin | sc-47778 | 1:5000 | Santa Cruz |
| anti-TWIST1 | ab49254 | 1:3000 | Abcam, Cambridge, London, UK |
| anti-N-cadherin | ab18203 | 1:3000 | Abcam |
| anti-Fibronectin | ab6328 | 1:2000 | Abcam |
| anti-α-SMA | ab66050 | 1:3000 | Abcam |

Reference List

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3. Badea L, Herlea V, Dima SO, Dumitrascu T, Popescu I. Combined gene expression analysis of whole-tissue and microdissected pancreatic ductal adenocarcinoma identifies genes specifically overexpressed in tumor epithelia. Hepatogastroenterology. 2008; 55: 2016-27.

4. Varambally S, Yu J, Laxman B, Rhodes DR, Mehra R, Tomlins SA, et al. Integrative genomic and proteomic analysis of prostate cancer reveals signatures of metastatic progression. Cancer Cell. 2005; 8: 393-406.

5. Kaiser S, Park YK, Franklin JL, Halberg RB, Yu M, Jessen WJ, et al. Transcriptional recapitulation and subversion of embryonic colon development by mouse colon tumor models and human colon cancer. Genome Biol. 2007; 8: R131.

6. Murat A, Migliavacca E, Gorlia T, Lambiv WL, Shay T, Hamou MF, et al. Stem cell-related "self-renewal" signature and high epidermal growth factor receptor expression associated with resistance to concomitant chemoradiotherapy in glioblastoma. J Clin Oncol. 2008; 26: 3015-24.