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## Supporting Information (SI Appendix)

# miR-196b-5p mediated downregulation of TSPAN12 and GATA6 promotes tumor progression in non-small cell lung cancer

Guang Liang [a, c, 1], Wei Meng [b,1], Xiangjie Huang [a,1], Wangyu Zhu [d], Changtian Yin [a], Canwei Wang [a, c], Matteo Fassan [e], Yun Yu [a], Masahisa Kudo [f], Sisi Xiao[a, g], Chengguang Zhao [a], Peng Zou [a], Yumin Wang [g], Xiaokun Li [a], Carlo M. Croce [f,2], Ri Cui [a, c, f, 2]

**Corresponding Authors**: Ri Cui (wzmucuiri@163.com) and Carlo M. Croce (Carlo.Croce@osumc.edu)

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**SI Tables**



**SI Materials and Methods**

**Plasmid construction, cell lines and regents**

The human pre-miRNA expression construct Lenti-miR-196b vector was purchased from System biosciences. Pre-miR miR-196b precursor and Pre-miR negative control were purchased from ThermoFisher. pLightSwtich empty, GATA6-3'UTR, and TSPAN12-3'UTR vectors were ordered from Active Motif. Mutations were generated by using the QuickChange II XL Site-Directed Mutagenesis Kit (Stratagene). The primers used in this study were shown in Table S1. siRNA negative control, siQKI-5, shRNA control (SHC001), shGATA6 (TRCN0000010938) and shTSPAN12 (TRCN0000127026) were purchased from Sigma. The cell lines used in this study were purchased from the American Type Culture Collection (ATCC). Human lung cancer cell lines H1299, U2020 and A549 were maintained in RPMI1640 medium containing 10% FBS and 100U/ml penicillin-streptomycin. 293T cells were cultured in

DMEM medium supplemented with 10% FBS and 100U/ml penicillin-streptomycin. Anti-QKI5 antibody (ab232502), anti-TSPAN12 antibody (ab93179), anti-CD31 antibody (ab28364) and anti-ki67 (ab16667) antibody were purchased from Abcam. Antibodies against GATA6 (D61E4), GAPDH (D16H11) and Cell Cycle Regulation Antibody Sampler Kit (9932T) were purchased from Cell signaling technology. Anti-vinculin antibody was ordered from Sigma-Aldrich.

**NanoString nCounter Assay and data analysis**

The NanoString nCounter Human v3 miRNA Expression Assay was used to perform the microRNA profiling analysis for RNAs extracted from QKI knockdown H1299 cells and control cells, following manufacturer's instructions (NanoString Technologies). The assay allows detecting and measuring the expression levels of up to 800 different microRNAs at the same time for each sample. Briefly, three µl of RNA were annealed with multiplexed DNA tags (miR-tag) and bridges target specific. Mature microRNAs were bond to specific miR-tags using a ligase enzyme and all the tags in excess were then removed through the enzyme clean-up step. The tagged microRNA product was then diluted (ratio 1:5) and 5 µl were combined with 20 µl of reported probes in hybridization buffer and 5 µl of capture probes. The overnight hybridization (16 to 20h) at 65°C allowed to complex the probes sequence specific with targets. Probe excess was then removed using two-step magnetic beads based purification on an automated fluidic handling system (nCounter Prep Station) and target/probe complexes were immobilized on the cartridge for data collection. The nCounter Digital Analyzer collected the data by taking images of immobilized fluorescent reporters in

the sample cartridge with a CCD camera through a microscope objective lens. For each cartridge, a high-density scan encompassing 600 fields of view was performed. Array values were scale normalized, using the sum of the control probe values to obtain a normalization factor for each profile.

**Virus infection and transfection**

The pre-miR196b expression construct and control vector were packaged with pPACKH1 Lentivector Packaging Plasmid mix (System Biosciences) in a 293T packaging cell line. The Transdux reagent (System Bioscience) was used for virus transduction, and infected cells were selected by fluorescence-activated cell sorting (FACS) analysis (FACSCalibur, BD Bioscience). Transfection of shRNAs against GATA6, TSPAN12 and control vector were carried out with Lipofectamine 3000 according to the manufacturer's instruction (Invitrogen) and transfected cells were selected by puromycin. siRNA against QKI-5 was transfected to cells by using Lipofectamine RNAiMAX according to the manufacturer's instruction (Invitrogen).

**Quantitative Real-time PCR**

Total RNAs were extracted using TRIzol Reagent (Invitrogen) according to the manufacturer's instruction. Expression of miRNA was quantified by qRT-PCR with TaqMan miRNA Reverse Transcription Kit and Taqman miRNA expression assay (Applied Biosystems). Small endogenous nucleolar U6 snRNA was used as control for normalization of miRNA. Taqman primiRNA assay for primiR-196b, TaqMan gene expression assays for GATA6 and TSPAN12 were purchased from Applied Biosystems to determine their expression. GAPDH was used as control for

normalization of primiRNA and mRNA expression. cDNAs were synthesized from 2μg total RNAs using High Capacity cDNA Reverse Transcription kit for RT-PCR (Thermo Fisher). All reactions were conducted in triplicates.

**Cell migration and proliferation assays**

*In vitro* cell migration was conducted using Boyden chambers (BD bioscience) that use 8-mm micropore membranes without Matrigel as previously described (1). The migration assay was carried out according to the manufacturer's instructions. *In vitro* cell growth rates were assessed by Cell Counting Kit8 (Dojindo) as described previously (2). Briefly, two thousand cells in 2% RPMI 1640 medium were added to each 48 well plate in quintuplex, and cultured at 37˚C for 4 days. The number of cells was quantified using a Cell Counting Kit8.

**Flow cytometry analysis**

For cell cycle analysis, the cells were fixed with 70% ethanol at -20°C overnight, washed with PBS and resuspended in PBS containing 50ug/ml propidium iodide, 100ug/ml RNase A, 0.05 % Triton X-100. The cells were incubated at 37°C for 40 min and analyzed by FACS Calibur Flow Cytometer (BD Biosciences).

**Western blot analysis**

The cells were lysed with RIPA buffer (25mM Tris-HCl (pH 7.6), 150mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS) supplemented with Protease/Phosphatase inhibitor Cocktail (Cell Signaling Technology), and separated on 4-20% Mini-protein TGX Gels (Bio-Rad). After SDS-PAGE, the proteins were electrotransferred to Immuno-Blot PVDF membrane (Bio-Rad). Then, Membranes

were blocked with 5% BSA in Tris-Buffered Saline with Tween 20 (TBST) buffer and incubated with primary antibody in 2% BSA in TBST, followed by incubation with appropriate horseradish peroxidase (HRP)-conjugated secondary antibody. Specific proteins were detected using the enhanced chemiluminescence system (GE Healthcare).

**TCGA dataset**

The TCGA miRNA-seq, RNA-seq data and Infinium Human DNA Methylation 450k beadchip with clinical information was downloaded on 31 July, 2013. Only log-2 transformed level 3 data were used for analysis. For analysis of the TCGA data set, Welch *t* test was conducted to determine if miR-196b-5p expression is different between patients with and without cancer to account for unequal variances. For the correlation analysis between miR-196b-5p expression and target gene expression or promoter methylation status, Pearson correlation coefficients were calculated.

**Luciferase Reporter Assay**

To determine if *miR-196b* directly targets the 3'UTRs of *GATA6* and *TSPAN12*, $5\times10^4$ 293T cells were seeded in 24-well plates overnight, then transfected with miR-196b mimic (Thermo Scientific) plus empty 3'UTR vector or 3'UTR vectors containing WT or mut-3'UTR. After 36 h, the cells were lysed and assayed using Dual Luciferase Assay (Promega) according to the manufacturer's instructions.

**Demethylation by 5-aza-CdR**

The H1299 and A549 cells were seeded 10cm tissue culture dishes and cultured with or without 7.5μM 5-aza-CdR for 3 days. The medium containing agent was replaced

every 24 h. RNAs were isolated and quantitative real-time PCR was carried out to evaluate the restoration of miR-196b-5p and primiR-196b expression after 5-aza-CdR treatment.

**Target analysis**

Bioinformatics analysis was performed by using these specific programs: Targetscan (http://www.targetscan.org/), Pictar (http://pictar.mdc-berlin.de/), miRWalk (http://mirwalk.umm.uni-heidelberg.de/) and RNAhybrid (http://www.bibiserv.techfak.uni-bielefeld.de/).

**Animal study**

All animal experimental procedures complied with the Wenzhou Medical University's Policy on the Care and Use of Laboratory Animals. Six-week-old, athymic BALB/c nu/nu female mice (19-23g) were purchased from Vital River Laboratories (Beijing, China). Stable miR-196b overexpressing H1299 cells (H1299/miR196b), GATA6 knockdown H1299 cells (H1299/shGATA6) or TSPAN12 knockdown H1299 cells (H1299/shTSPAN12) were harvested by trypsin, washed with PBS, and resuspended in Matrigel/RPMI medium (1:1). Each five million H1299/miR-196b, H1299/shGATA6 or H1299/shTSPAN12 and corresponding control cells were subcutaneously injected into the flank of nude mice, respectively. Injections were conducted in two flanks of each mouse. Tumor volumes were calculated from the length (a) and width (b) by using the following formula: volume ($mm^3$) = $ab^2/2$.

**Immunohistochemical staining**

Histological sections were fixed in 10% formalin, processed and embedded in paraffin. 5-micrometre-thick sections were placed on positively charged slides. Tissue sections were stained using routine immunohistochemical techniques and incubated with primarily antibodies against TSPAN12 (1:200), CD31 (1:200) or Ki-67 (1:100) overnight. Conjugated secondary antibodies and diaminobenzidine (DAB) were used for detection. Staining was absent when isotype-matched immunoglobulin was used as the control. For analyzing NSCLC tissue samples, according to the percentage of positive cells in the mean average of five fields, immunohistochemical reactivity for TSPAN12 was scored as follows: 0–5% (−), 5%–25 (1+), 25%–50% (2+), 50%–100% (3+). The TSPAN12 expression graded as 3+ was defined as strong, 2+ was defined as moderate and 1+ was defined as weak, respectively.

**Statistical analysis**

Statistical analyses were performed with the R program (version 3.0.2). Data are represented as means with standard deviation (SD) and statistical significance was determined with unpaired Student *t* tests unless indicated otherwise. *P* values less than 0.05 were considered statistically significant. Pearson correlation analysis was conducted to determine correlation between expression of *miR-196b* and its target genes.

**References**

1. Cui R*, et al.* (2015) MicroRNA-224 promotes tumor progression in nonsmall cell lung cancer. *Proceedings of the National Academy of Sciences of the United States of America* 112(31):E4288-4297.
2. Cui R*, et al.* (2009) Osteopontin is involved in the formation of malignant pleural effusion in lung cancer. *Lung Cancer* 63(3):368-374.

## SI Figures

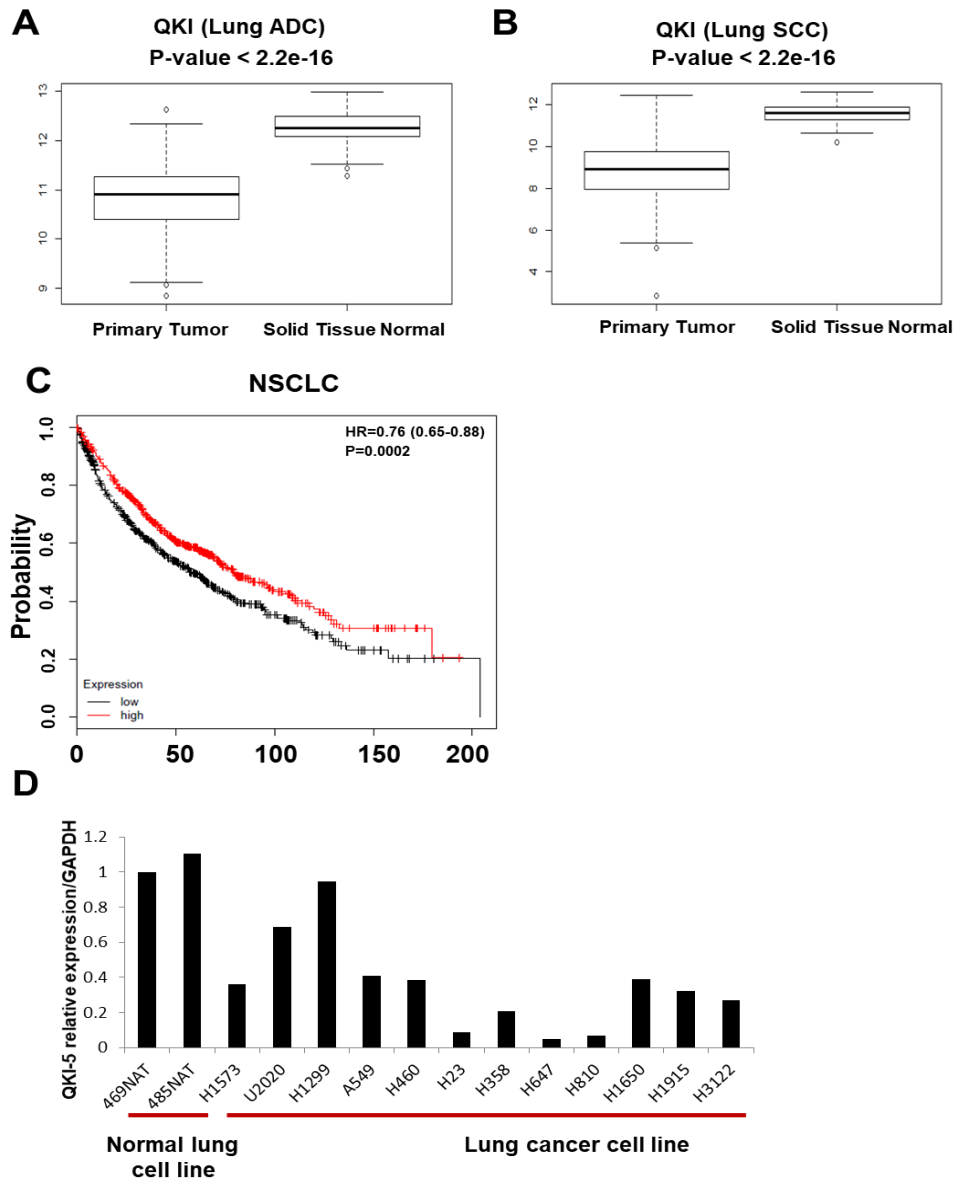



**Fig. S1. *QKI-5* plays tumor suppressive functions in NSCLC.** The *QKI* expression data were obtained from TCGA RNA-seq data containing a total of 334 lung ADC patients with 57 matched normal adjacent tissue (NAT) samples (**A**), and 349 lung SCC patients with 51 matched NAT samples (**B**). (**C**) Kaplan Meier Survival analysis of *QKI* for 3021 available NSCLC patients in the Kaplan Meier Plotter (http://kmplot.com/analysis/) database. (**D**) The *QKI-5* expression in different lung cancer cell lines and normal lung cell lines. *QKI-5* expressions were normalized by *GAPDH*.

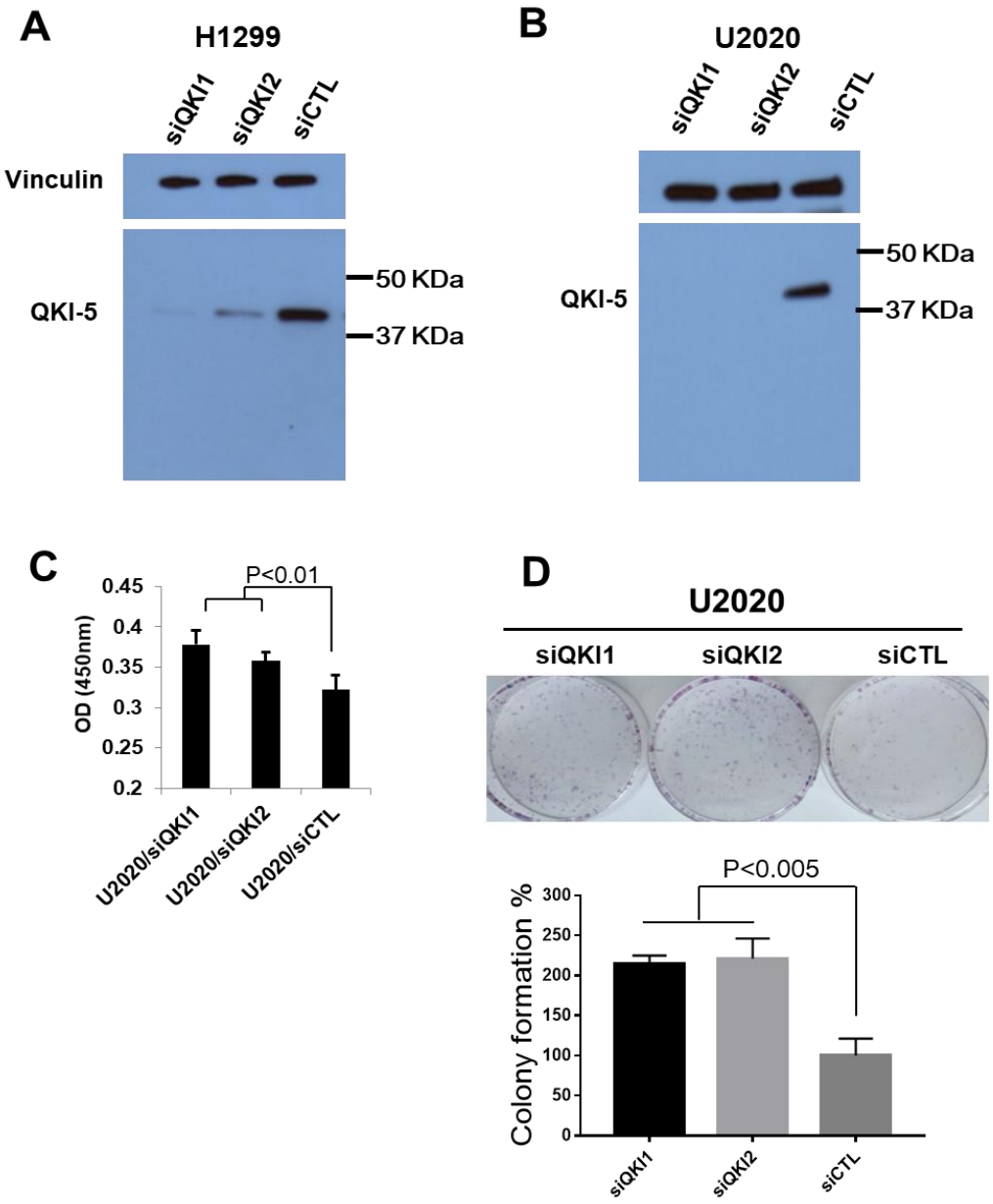



**Fig. S2. QKI-5 is involved in lung cancer cell proliferation and colony formation.** (**A-B**) Western blot analysis to examine QKI-5 protein expressions in H1299 (**A**) or U2020 (**B**) cells transfected with QKI-5 siRNA or control siRNA. (**C-D**) Cell proliferation assay **(C)** and colony formation assay **(D)** for QKI-5 knockdown U2020 cells. The cell growth rates were measured by cell counting kit 8. The values present mean ± S.D. as determined by quintuplet assays. Colony forming areas were measured by Image J software. The average values were derived from three random areas.

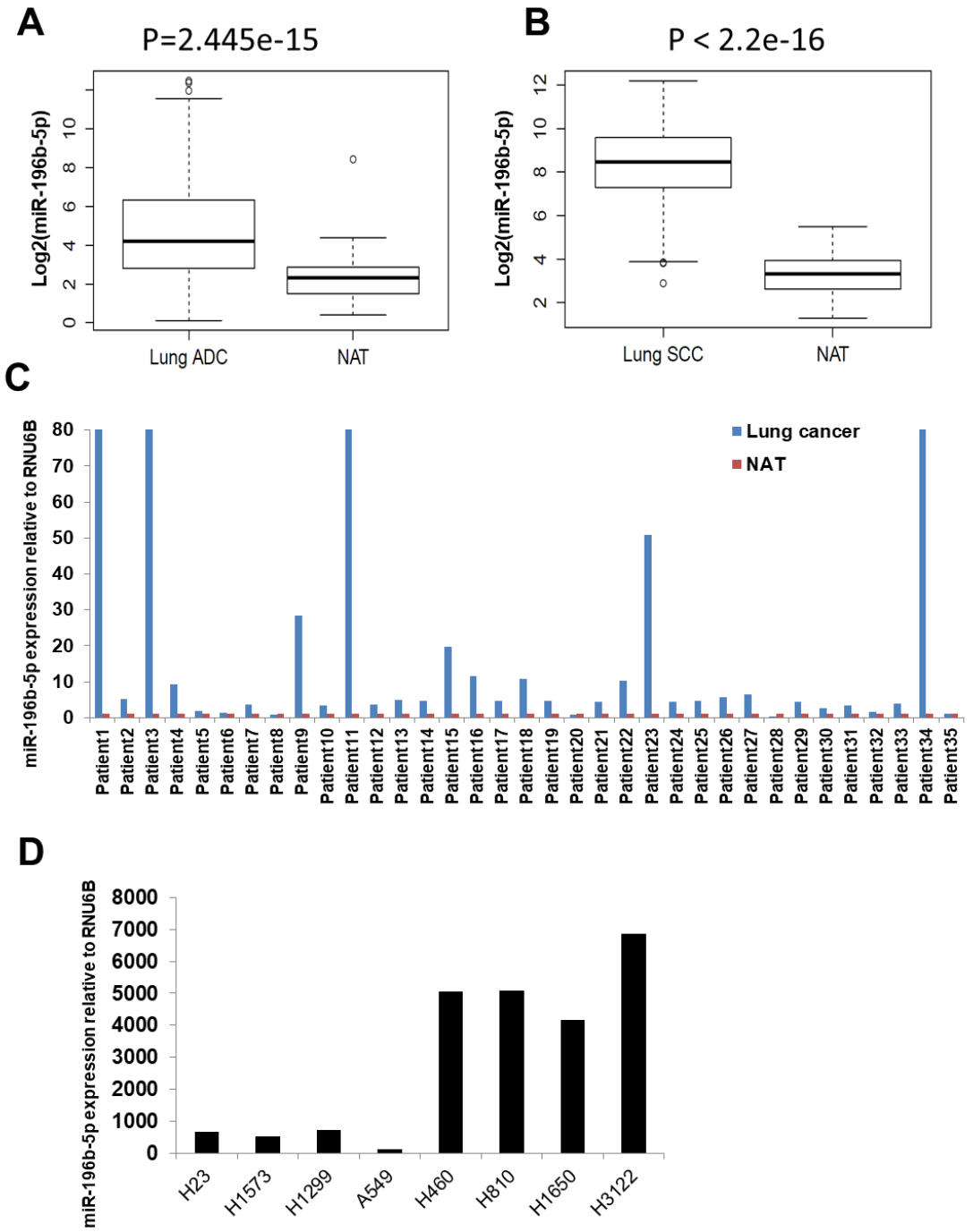



**Fig. S3. miR-196b-5p expression in NSCLC tissues and lung cancer cell lines.** (**A**-**B**) The miR-196b-5p expression data for lung ADC (**A**) and lung SCC (**B**) were acquired from TCGA miR-seq dataset. Of the evaluable 468 primary lung ADC patients, 46 patients had matched normal adjacent tissues. Of the evaluable 198 primary lung SCC patients, 40 patients had matched normal adjacent tissues. (**C**) Comparison of expression level of miR-196b-5p in cancerous and adjacent normal

tissues in 35 NSCLC patients. The RNA samples were extracted from cancerous and adjacent normal tissues of formalin-fixed, paraffin-embedded 35 NSCLC samples. The RNAs were subject to qRT-PCR with a miR-196b-5p probe and the expression was normalized by *U6B*. (**D**) The miR-196b-5p expression was evaluated in different lung cancer cell lines. miR-196b-5p expression was normalized by *RNU6B*.

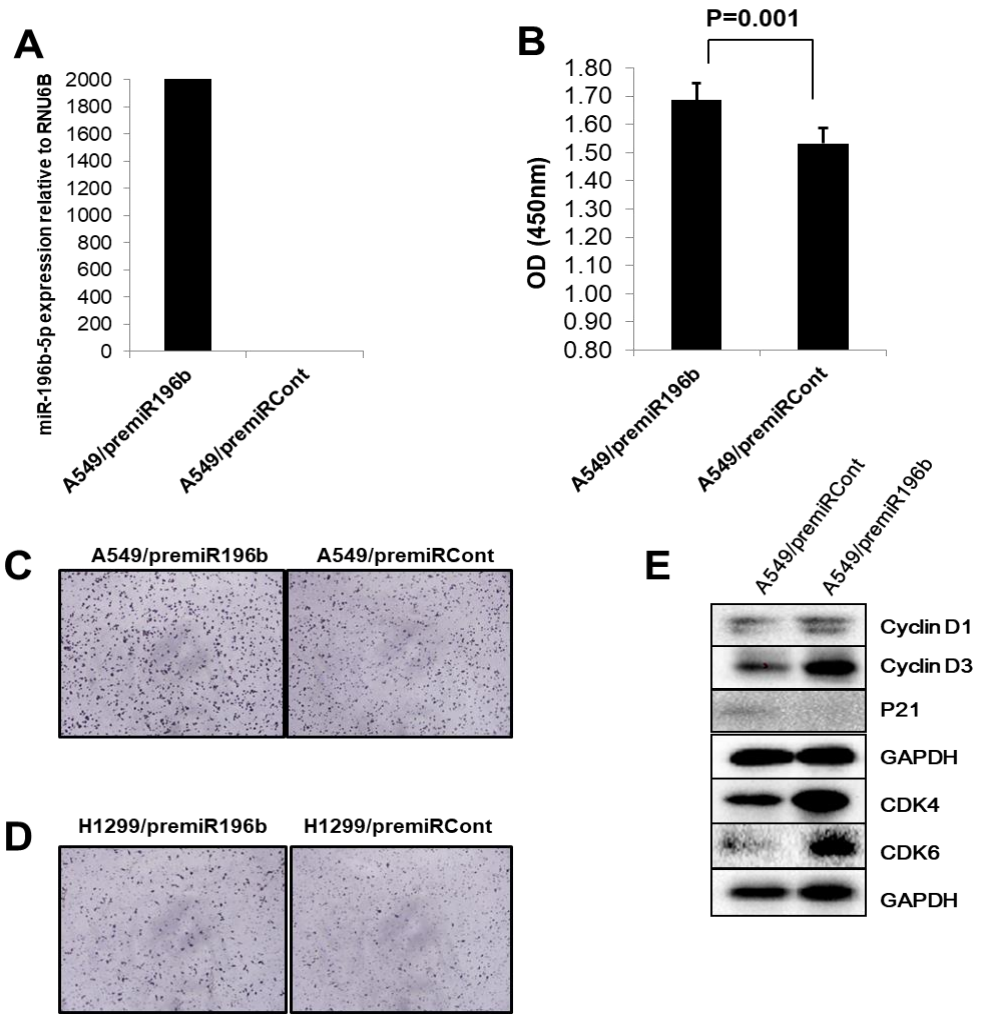
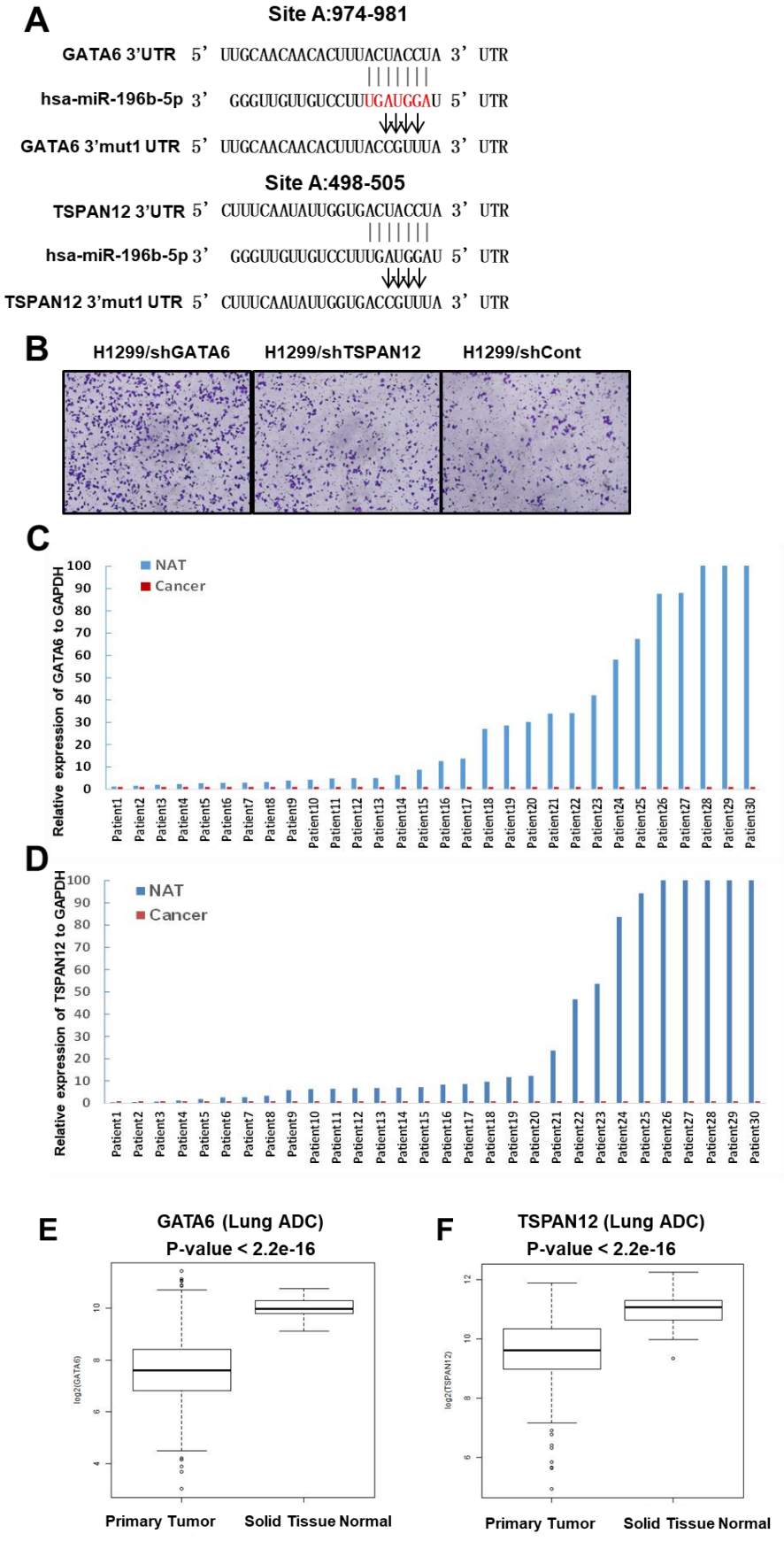



**Fig. S4. miR-196b-5p promotes lung cancer cell proliferation and migration.**

**(A)** qRT-PCR examines miR-196b-5p expression in miR-196b overexpressing A549 cells and control cells. (**B**) Cell proliferation assay for miR-196b-5p overexpressing A549 cells and control cells. The cell growth rates were measured by cell counting kit 8. The values present mean ± S.D. as determined by quintuplet assays. (**C**) Cell migration assay for miR-196b-5p overexpressing A549 cells and control cells using transwell membranes. Representative pictures of migration chambers are shown (×40 magnification). (**D**) Representative pictures of migration chambers for miR-196b-5p overexpressing H1299 cells and control cells are shown (×40 magnification). (**E**) Western blot analysis of cell cycle G1 phase related proteins (Cyclin D1, Cyclin D3, CDK4, CDK6 and P21) in miR-196b-5p overexpressing A549 cells and control cells.


A
Site A:974-981
GATA6 3'UTR 5' UUGCAACAACACUUUACUACCUA 3' UTR
hsa-miR-196b-5p 3' GGGUUGUUGUCCUUUGAUGGAU 5' UTR
GATA6 3'mut1 UTR 5' UUGCAACAACACUUUACCGUUUA 3' UTR
Site A:498-505
TSPAN12 3'UTR 5' CUUUCAAUAUUGGUGACUACCUA 3' UTR
hsa-miR-196b-5p 3' GGGUUGUUGUCCUUUGAUGGAU 5' UTR
TSPAN12 3'mut1 UTR 5' CUUUCAAUAUUGGUGACCGUUUA 3' UTR
B
H1299/shGATA6
H1299/shTSPAN12
H1299/shCont
C
Relative expression of GATA6 to GAPDH
NAT
Cancer
Patient1 Patient2 Patient3 Patient4 Patient5 Patient6 Patient7 Patient8 Patient9 Patient10 Patient11 Patient12 Patient13 Patient14 Patient15 Patient16 Patient17 Patient18 Patient19 Patient20 Patient21 Patient22 Patient23 Patient24 Patient25 Patient26 Patient27 Patient28 Patient29 Patient30
D
Relative expression of TSPAN12 to GAPDH
NAT
Cancer
Patient1 Patient2 Patient3 Patient4 Patient5 Patient6 Patient7 Patient8 Patient9 Patient10 Patient11 Patient12 Patient13 Patient14 Patient15 Patient16 Patient17 Patient18 Patient19 Patient20 Patient21 Patient22 Patient23 Patient24 Patient25 Patient26 Patient27 Patient28 Patient29 Patient30
E
GATA6 (Lung ADC)
P-value < 2.2e-16
log2(GATA6)
Primary Tumor
Solid Tissue Normal
F
TSPAN12 (Lung ADC)
P-value < 2.2e-16
log2(TSPAN12)
Primary Tumor
Solid Tissue Normal

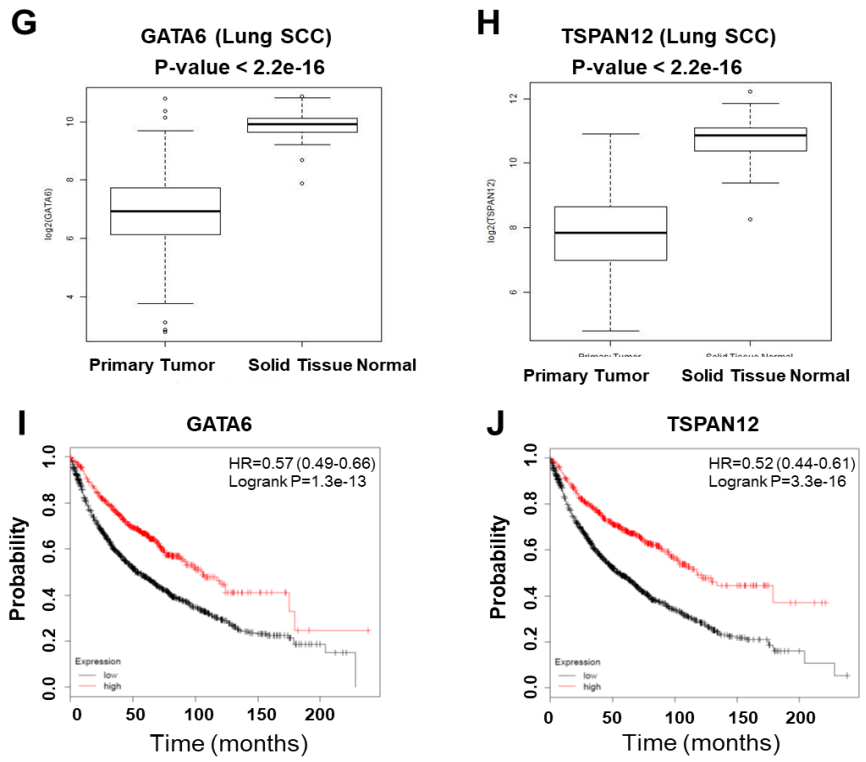



**Fig. S5. GATA6 and TSPAN12 play tumor suppressive functions in NSCLC.**

**(A)** Schematic diagram showing matched sequences between the seed sequence of *miR-196b* and 3'UTRs of *GATA6* and *TSPAN12*. The arrows indicate the mutagenesis nucleotides. (**B**) Cell migration assay for H1299/shGATA6, H1299/shTSPAN12 and control cells using transwell membranes. Representative pictures of migration chambers are shown (×40 magnification). (**C**-**D**) Comparison of expression level of *GATA6* (**C**) and *TSPAN12* (**D**) in 60 paired NSCLC tissues and their matched NATs. The RNA samples were extracted from 30 NSCLC tissues and 30 corresponding NATs. The RNAs were subject to qRT-PCR with a Taqman *GATA6* and *TSPAN12* probes and their expressions were normalized by *GAPDH*. (**E**-**H**) The *GATA6* and *TSPAN12* expression data were obtained from TCGA RNA-seq dataset. *GATA6* (**E**) and *TSPAN12* (**F**) expressions were analyzed in 334 lung ADC patients with 57 matched NAT samples. *GATA6* and *TSPAN12* expressions in 349 lung SCC patients with 51 matched NAT samples were shown in (**G**) and (**H**), respectively. (**I** -**J**) Kaplan Meier Survival analysis of *GATA6* (**I**) and *TSPAN12* (**J**) for 3021 available NSCLC patients in the Kaplan Meier Plotter (http://kmplot.com/analysis/) database.

**A**

| Methylation Probes (CpG51) (Chr:position) | Log2 (has-miR-196b-5p) | |
|---|---|---|
| | Pearson correlation coefficients | P-value |
| cg18181034 (chr7:27208884) | 0.03 | 0.57 |
| cg04298953 (chr7:27208886) | -0.027 | 0.68 |
| cg16447012 (chr7:27209130) | -0.024 | 0.72 |
| cg18311537 (chr7:27209195) | -0.13 | 0.05 |
| cg15912800 (chr7:27209197) | -0.24 | 0.0003 |
| cg26259537 (chr7:27209246) | -0.01 | 0.78 |
| cg05250768 (chr7:27209269) | 0.104 | 0.12 |
| cg01024168 (chr7:27209280) | 0.108 | 0.11 |
| cg14780416 (chr7:27209337) | -0.09 | 0.16 |
| cg05027336 (chr7:27209349) | -0.05 | 0.43 |
| cg08964780 (chr7:27209462) | -0.016 | 0.8 |

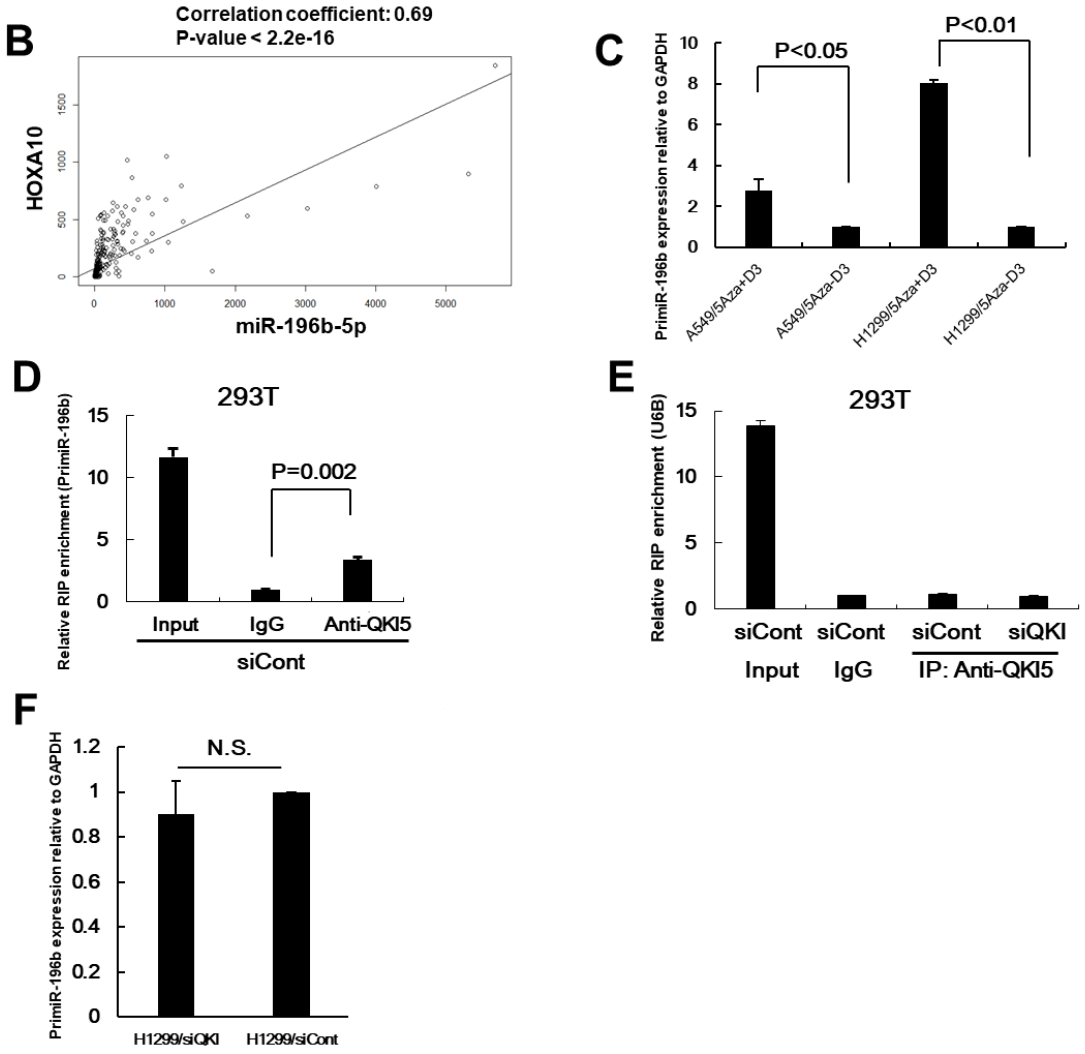



**Fig. S6. miR-196b-5p promoter region's methylation and QKI-5 modulate miR-196b-5p expression.** (**A**) Correlation between miR-196b-5p expression from TCGA dataset and methylation probes in the promoter region of miR-196b (CpG51) from TCGA Illumina Infinium Human DNA Methylation 450k beadchip in NSCLC. (**B**) The normalized expression values of miR-196b-5p and *HOXA10* were extracted from

TCGA dataset for Pearson correlation analysis. (**C**) qRT-PCR to measure priprimiR-196b expression in lung cancer cell lines, A549 and H1299 cells after treatment with 7.5μM 5-aza-CdR for 3days. The values present mean ± S.D. as determined triplicated assays. (**D**-**E**) Lysates from QKI-5 knockdown 293T cells or control cells were subjected to RIP analysis. The cell extracts were subjected to IP with immunoglobulin G or anti-QKI5 antibody. Pull-down RNA was analyzed by qRT-PCR using specific primer for primiR-196b (**D**) or U6B (**E**). Data are presented as mean ± SD as determined by triple assays. (**F**) RNAs were extracted from QKI-5 knockdown 293T cells or control cells treated with 20 ug/ml α-amanitin for 9 hours and then subjected to qRT-PCR with a primiR-196b probe. GAPDH probe was used for normalization. Data are presented as mean ± SD as determined by triple assays.

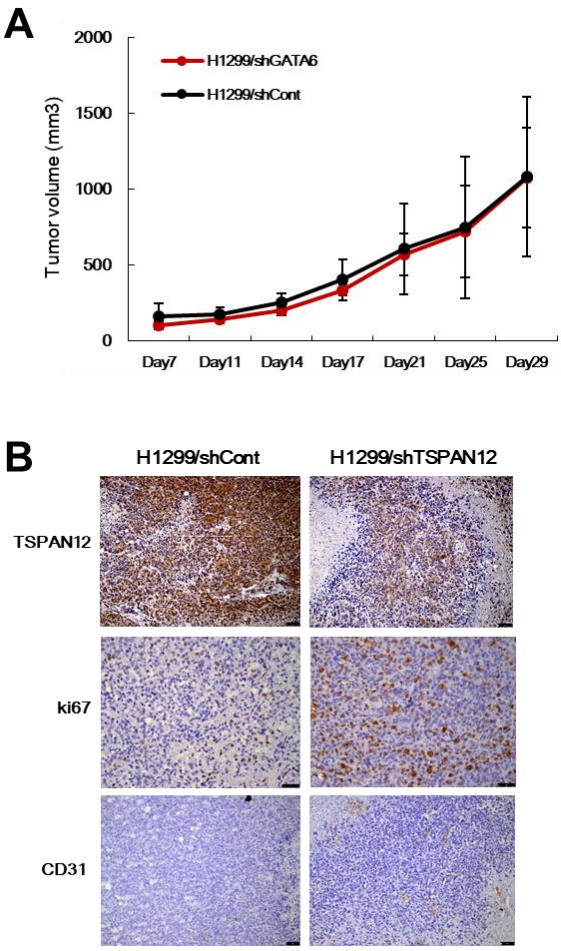



**Fig. S7. Effects of GATA6 or TSPAN12 on tumor growth in mouse model.**

(**A**) Tumor growth in nude mice subcutaneously injected into flanks with H1299/shGATA6 or H1299/shCont cells. Data are presented as mean ± SD (n=6 per group). (**B**) Representative sections for TSPAN12 positive cells (x200), ki-67 positive cells (x200) and CD31 positive cells (x100) were depicted from tumors derived from H1299/shTSPAN12 or H1299/shCont cells.

# SI Table

| MicroRNA | Gene | miRanda | miRDB | miRWalk | RNAhybrid | PICTAR5 | RNA22 | Targetscan | SUM |
|---|---|---|---|---|---|---|---|---|---|
| hsa-miR-196b-5p | GATA6 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 7 |
| hsa-miR-196b-5p | HOXC8 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 7 |
| hsa-miR-196b-5p | SORCS1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 6 |
| hsa-miR-196b-5p | TOX3 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 6 |
| hsa-miR-196b-5p | HOXB6 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 6 |
| hsa-miR-196b-5p | TRERF1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 6 |
| hsa-miR-196b-5p | CCDC47 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 6 |
| hsa-miR-196b-5p | CDKN1B | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 5 |
| hsa-miR-196b-5p | ARFGEF1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 5 |
| hsa-miR-196b-5p | IGF2BP3 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 5 |
| hsa-miR-196b-5p | RXFP2 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 5 |
| hsa-miR-196b-5p | COL1A2 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 5 |
| hsa-miR-196b-5p | COL3A1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 5 |
| hsa-miR-196b-5p | C2orf60 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 5 |
| hsa-miR-196b-5p | TMEM161B | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 5 |
| hsa-miR-196b-5p | EPHA7 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 5 |
| hsa-miR-196b-5p | TSPAN12 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 5 |
| hsa-miR-196b-5p | NAALADL2 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 5 |
| hsa-miR-196b-5p | DPY19L4 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 5 |
| hsa-miR-196b-5p | CPSF1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 5 |
| hsa-miR-196b-5p | SCHIP1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 5 |
| hsa-miR-196b-5p | ANXA1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 5 |
| hsa-miR-196b-5p | HOXA9 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 5 |
| hsa-miR-196b-5p | HOXB7 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 5 |
| hsa-miR-196b-5p | FAS | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 5 |
| hsa-miR-196b-5p | AQP4 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 5 |
| hsa-miR-196b-5p | ZC3H6 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 5 |
| hsa-miR-196b-5p | LAMA4 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 5 |
| hsa-miR-196b-5p | NFKBIA | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 5 |
| hsa-miR-196b-5p | PBX3 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 5 |
| hsa-miR-196b-5p | MRPS7 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 5 |
| hsa-miR-196b-5p | LRP1B | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 5 |
| hsa-miR-196b-5p | BIRC6 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 5 |
| hsa-miR-196b-5p | IFT80 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 5 |
| hsa-miR-196b-5p | RBM26 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 5 |
| hsa-miR-196b-5p | VSNL1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 5 |
| hsa-miR-196b-5p | ZNF24 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 5 |
| hsa-miR-196b-5p | HMGA2 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 5 |
| hsa-miR-196b-5p | USP32 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 5 |
| hsa-miR-196b-5p | MAP4K3 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 5 |
| hsa-miR-196b-5p | CASK | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 5 |
| hsa-miR-196b-5p | SMC3 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 5 |
| hsa-miR-196b-5p | CLEC2B | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 5 |

**Table S1.** Predicted miR-196b-5p targets from miRWalk (http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/). At least 5 prediction sites predicted targets were shown.

| Target genes | Correlation coefficient | P-value |
|---|---|---|
| GATA6 | -0.56 | 1.66E-25 |
| LAMA4 | -0.51 | 1.93E-20 |
| TSPAN12 | -0.51 | 2.23E-20 |
| AQP4 | -0.50 | 2.32E-19 |
| NFKBIA | -0.48 | 1.13E-17 |
| FAS | -0.33 | 5.96E-09 |
| TMEM161B | -0.32 | 3.90E-08 |
| PBX3 | -0.27 | 3.20E-06 |
| ZC3H6 | -0.27 | 4.50E-06 |
| RXFP2 | -0.63 | 5.17E-06 |
| TOX3 | -0.22 | 1.68E-04 |
| CLEC2B | -0.21 | 3.60E-04 |

**Table S2.** Correlation between miR-196b-5p and its targets expression. Target genes expression from TCGA RNA-seq data and miR-196b-5p expression from miR-seq data were used to examine correlation between miR-196b-5p and its target genes in lung SCC dataset (n = 289).

| Names of primers | Primer sequences |
|---|---|
| gata6-3UTR-mut1-S | agagtatttacaaatgctatccgttaaacggtaaagtgttgttgcaatttttccagcacatattcc |
| gata6-3UTR-mut1-AS | ggaatatgtgctggaaaaattgcaacaacactttaccgtttaacggatagcatttgtaaatactct |
| tspan-3UTR-mut1-S1 | agtggtaagaatattttagtaaccagcaaaaatcacatttaaacggtcaccaatattgaaagttttctgatatat |
| tspan-3UTR-mut1-AS1 | atatatcagaaaactttcaatattggtgaccgtttaaatgtgatttttgctggttactaaaatattcttaccact |

**Table S3.** Primers used for 3'UTR mutagenesis.