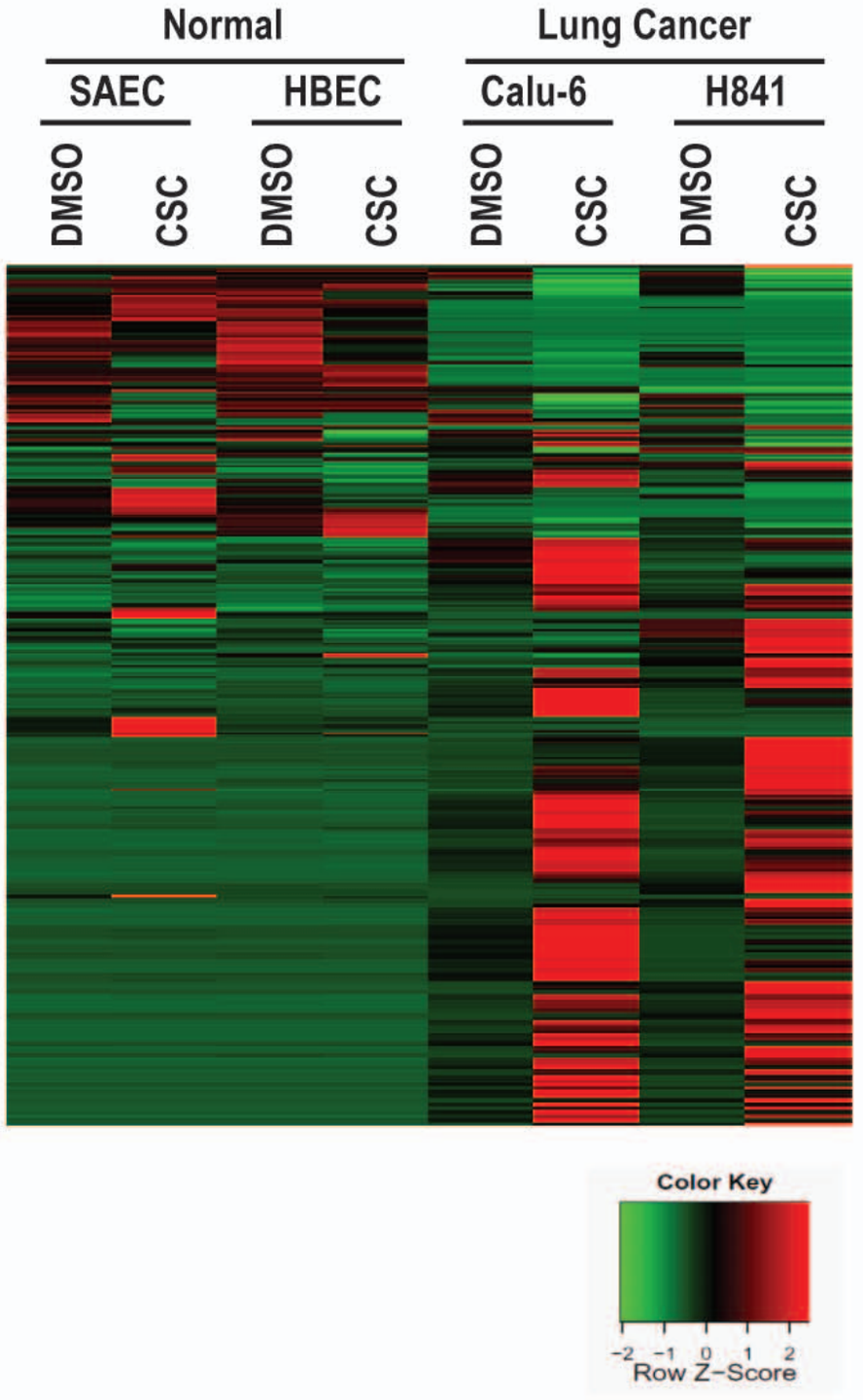
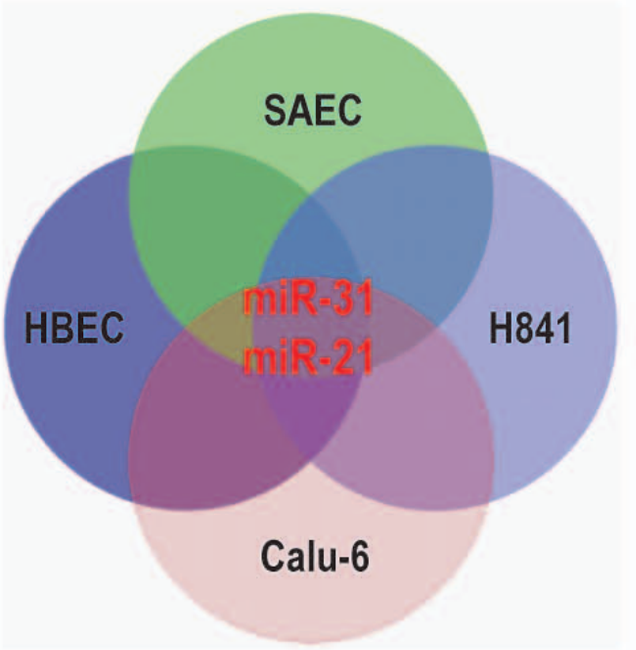
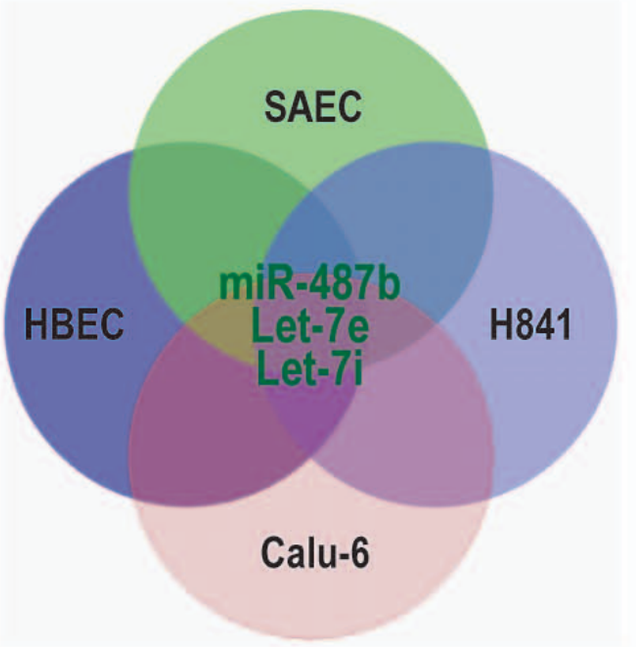
S1



Up-regulated miRNA in lung cancer cells

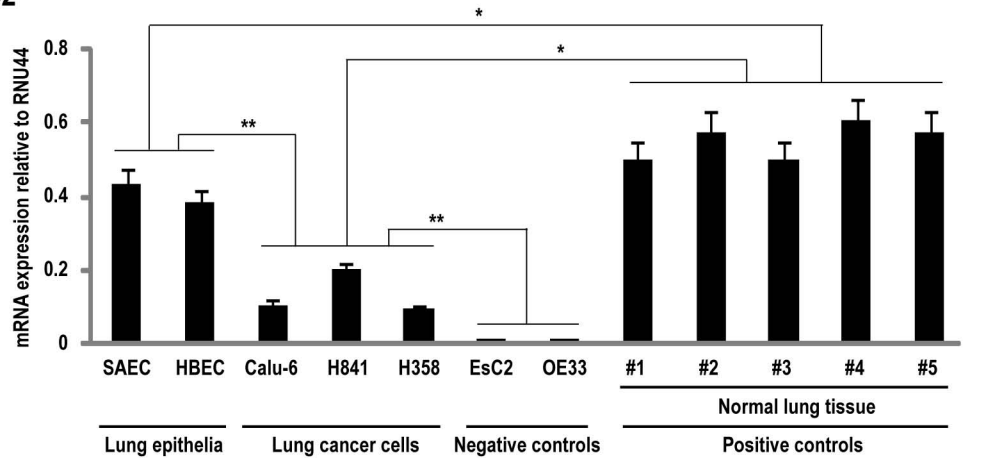


Down-regulated miRNA in lung cancer cells



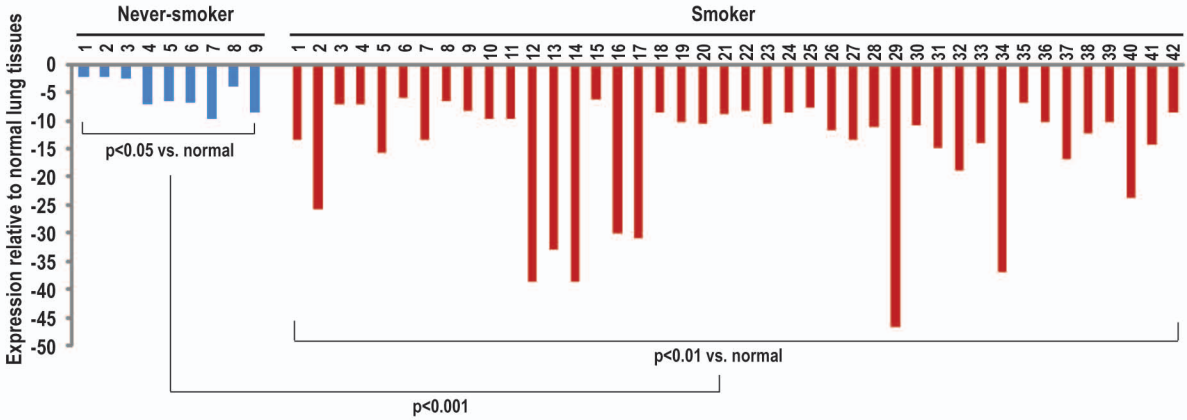
Supplementary Figure 1. Summary of microRNA array analysis of CSC-mediated effects in cultured human normal respiratory epithelia and lung cancer cells. The expression of all 376 miRNAs from our microRNA array analysis in SAEC, HBEC, Calu-6, and H841 cells with or without CSC exposure are showed in this heatmap. miRNAs that were consistently up-regulated or down-regulated following CSC exposure in all four cell lines are depicted in Venn diagrams.

S2

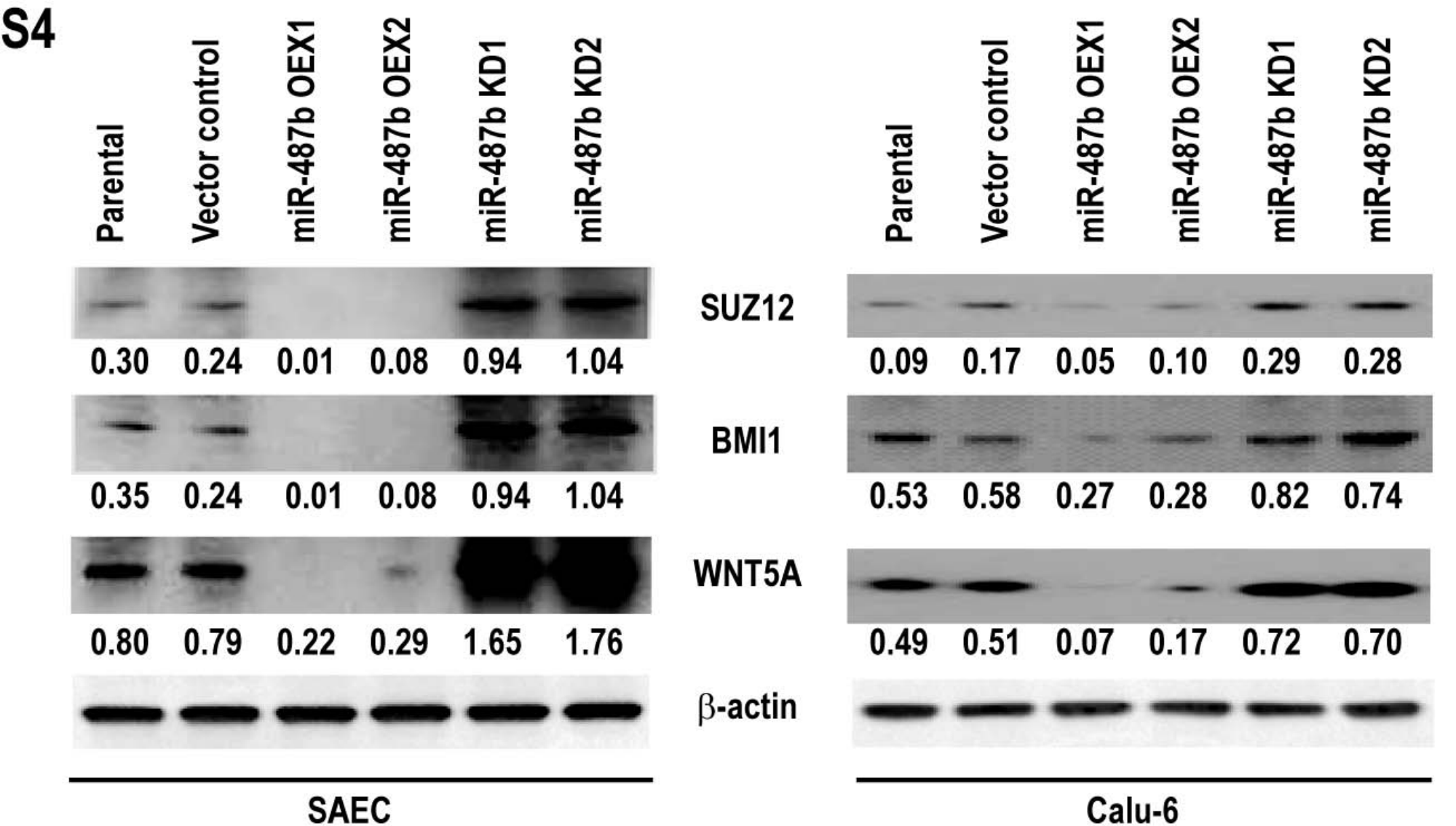


Supplementary Figure 2. qRT-PCR analysis of endogenous miR-487b expression levels normalized with control miRNA (RNU44) in SAEC, HBEC, Calu-6, H841, and H358 cells. Five normal lung tissues served as positive controls. EsC2 and OE33 (esophageal cancer lines) with no miR-487b genomic copy number (supplementary figure 11A) served as negative controls. (*p<0.05; **p<0.01)

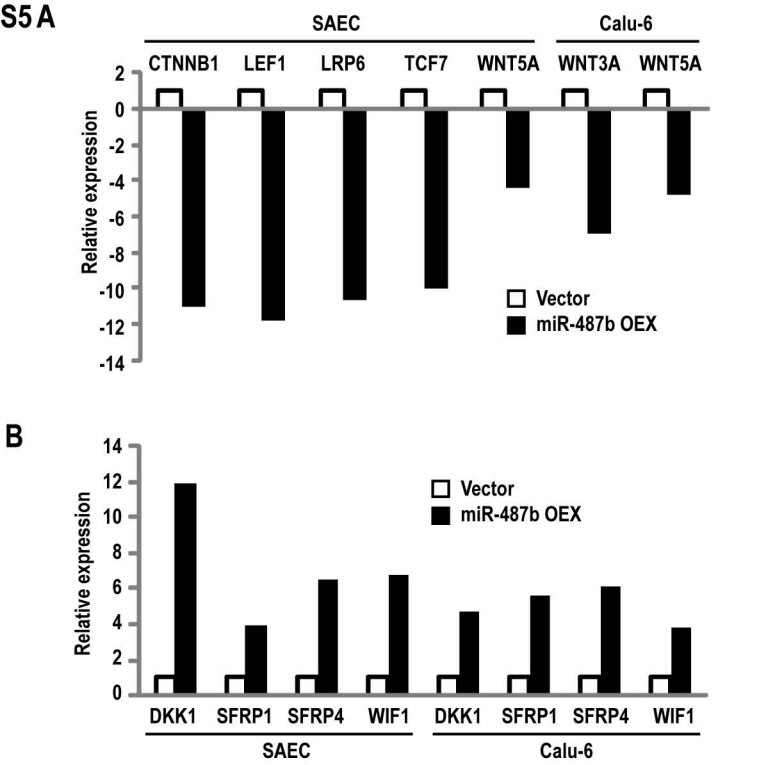
S3



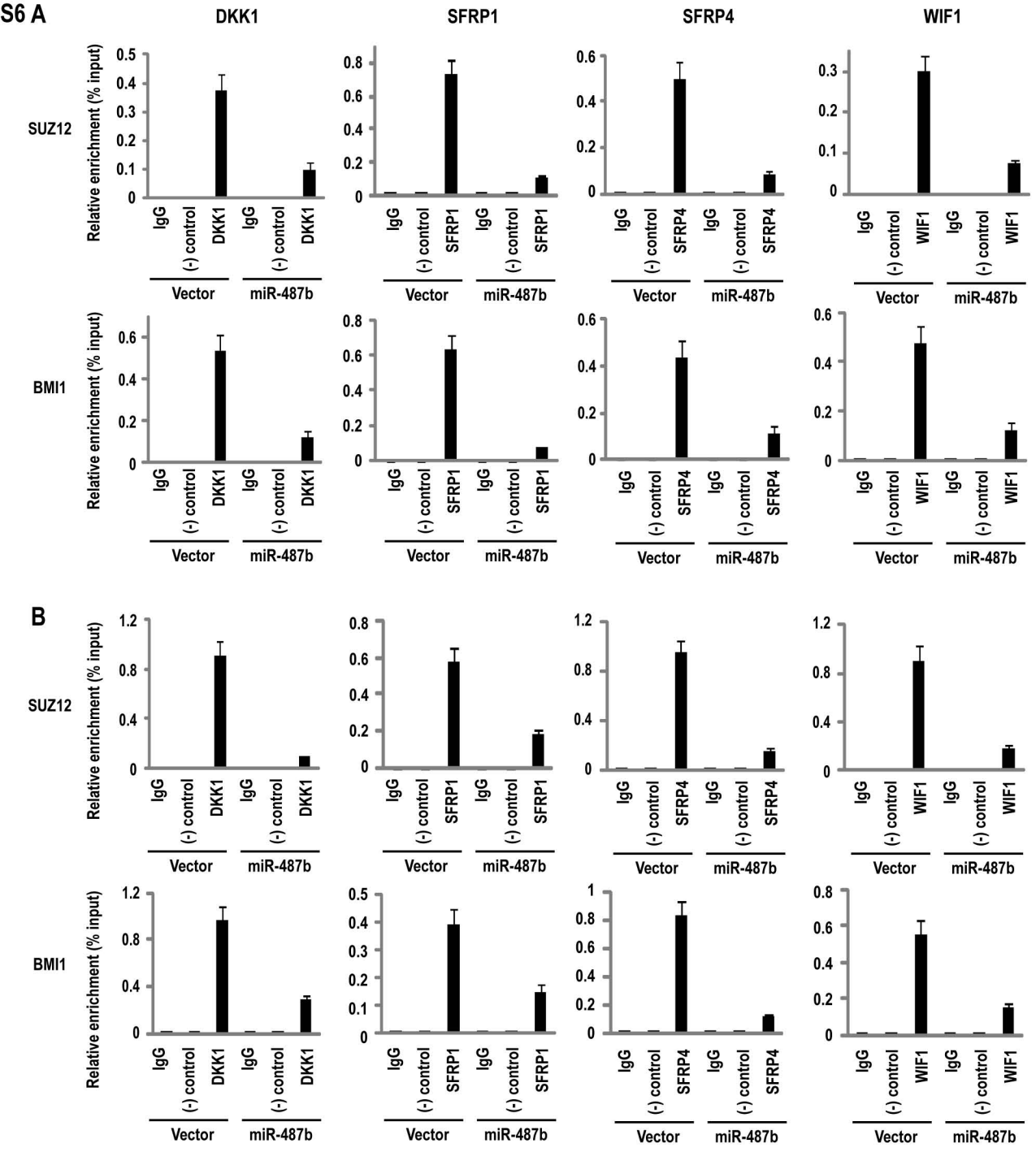
Supplementary Figure 3. qRT-PCR analysis of miR-487b expression in human lung cancers relative to paired adjacent normal lung tissues (n=51). miR-487b levels in tumors were significantly lower than corresponding adjacent normal lung tissues. Furthermore, miR-487b levels were significantly more repressed in lung cancers from smokers/former smokers compared to those from never-smokers.



Supplementary Figure 4. Densitometry of Figure 2F, confirming that over-expression of miR-487b decreases, whereas knock-down of miR-487b increases SUZ12, BMI1 and WNT5A protein levels in SAEC and Calu-6 cells.

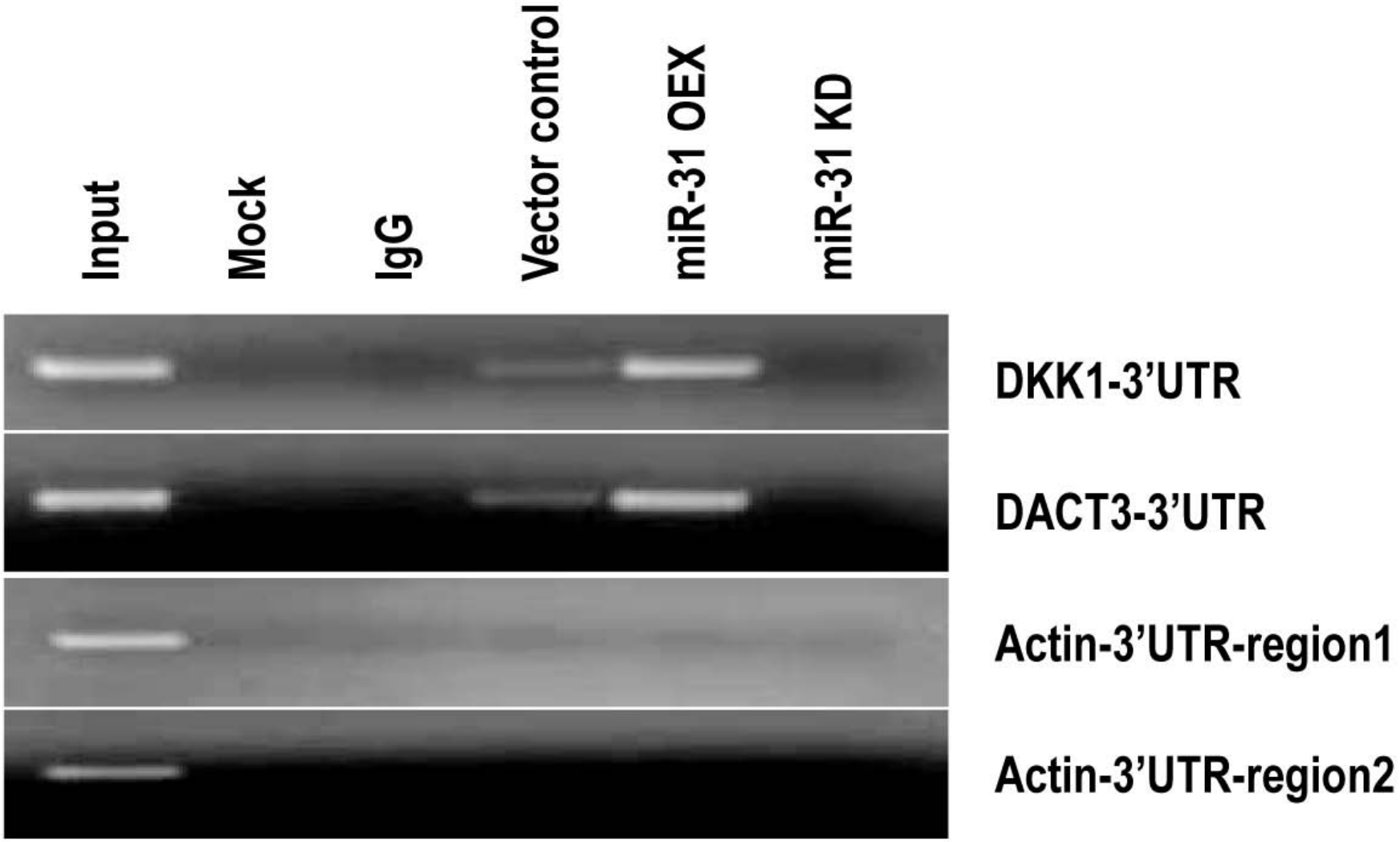


Supplementary Figure 5. Effects of miR-487b on Wnt signaling in SEAC and Calu-6 cells.
A) Results of focused Wnt PCR array demonstrating that constitutive expression of miR-487b (miR-487b OEX) down-regulates a variety of genes encoding agonists of canonical and non-canonical Wnt signaling.
B) Results of focused Wnt PCR array demonstrating that constitutive expression of miR-487b coincides with up-regulation of a variety of Wnt antagonists aberrantly silenced in lung cancer cells.

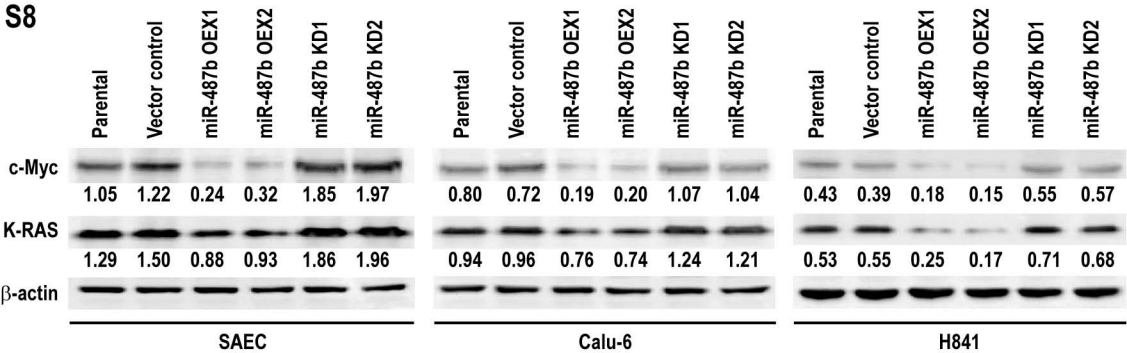


Supplementary Figure 6. miR-487b-mediated epigenetic regulation of Wnt antagonists.
A/B) Quantitative ChIP analysis of SUZ12 and BMI1 levels within promoter regions of DKK1, SFRP1, SFRP4, and WIF1 in SAEC (A) or Calu-6 (B) cells. Over-expression of miR-487b decreases occupancy of repressive polycomb proteins in promoter regions of DKK1, SFRP1, SFRP4, and WIF1.

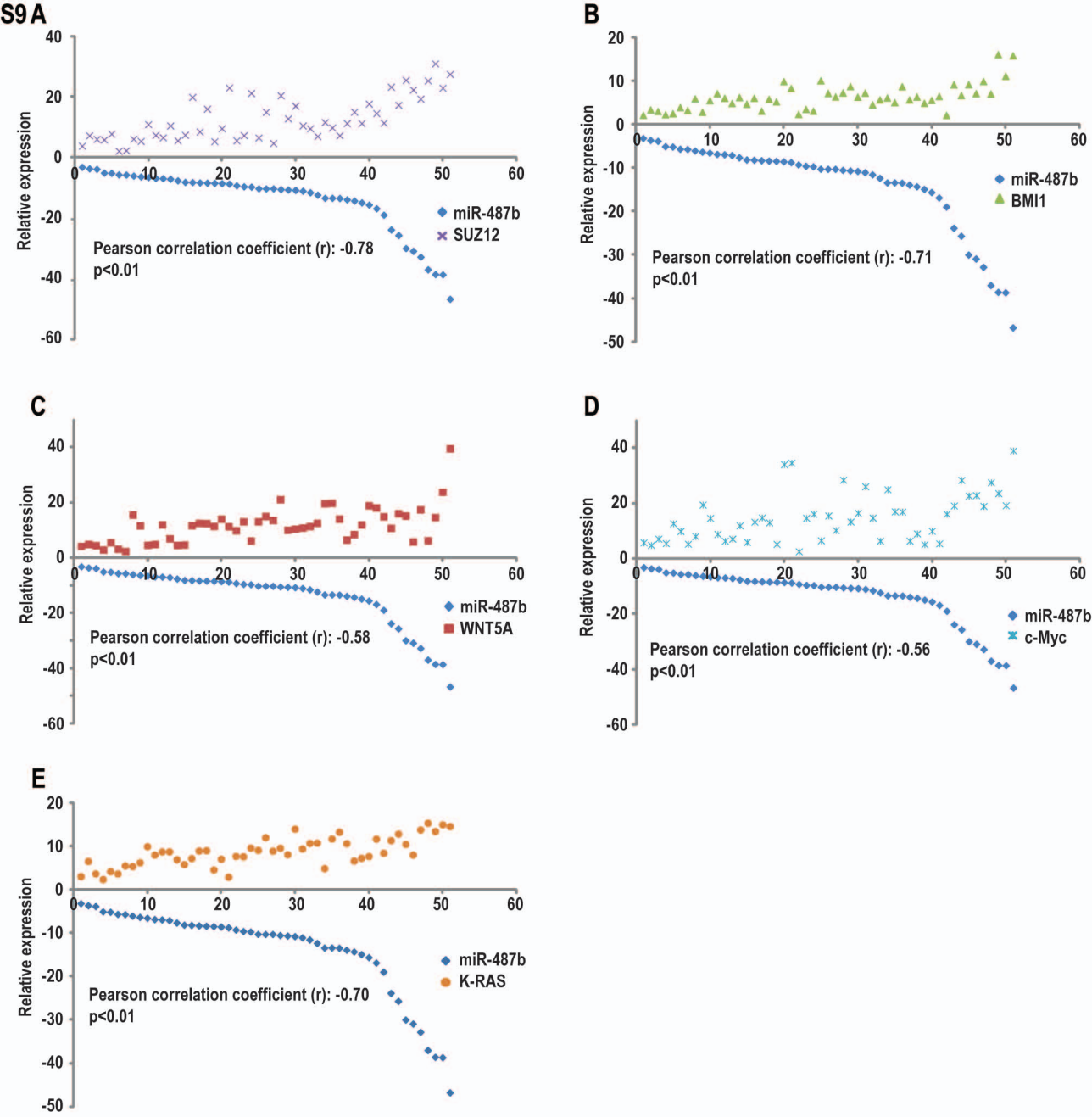
S7



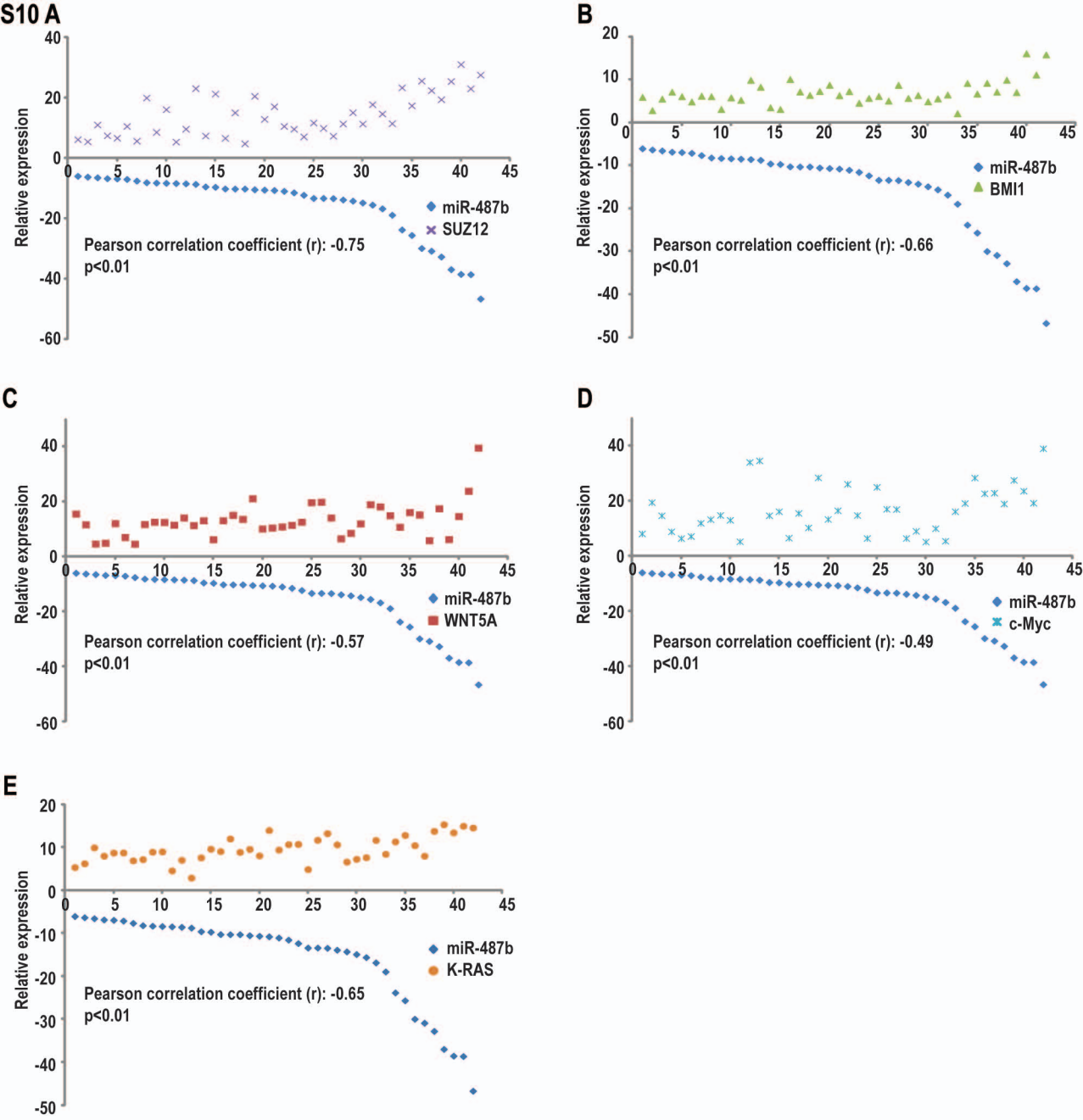
Supplementary Figure 7. Ago-CLIP analysis of miR-31 interactions with DKK1 and DACT3 in SAEC (see Xi et al, PLoS ONE, 2010). Over-expression of miR-31 significantly increased precipitation of DKK1 and DACT3, indicating direct interaction of miR-31 with the respective 3' UTRs.



Supplementary Figure 8. Densitometry of Figure 4E.

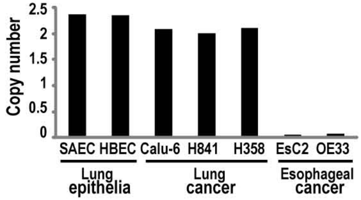


Supplementary Figure 9. Correlation between miR-487b and (A) SUZ12, (B) BMI1, (C) WNT5A, (D) c-Myc, and (E) K-RAS expression assessed by qRT-PCR techniques in 51 lung cancers including 9 from never smokers.

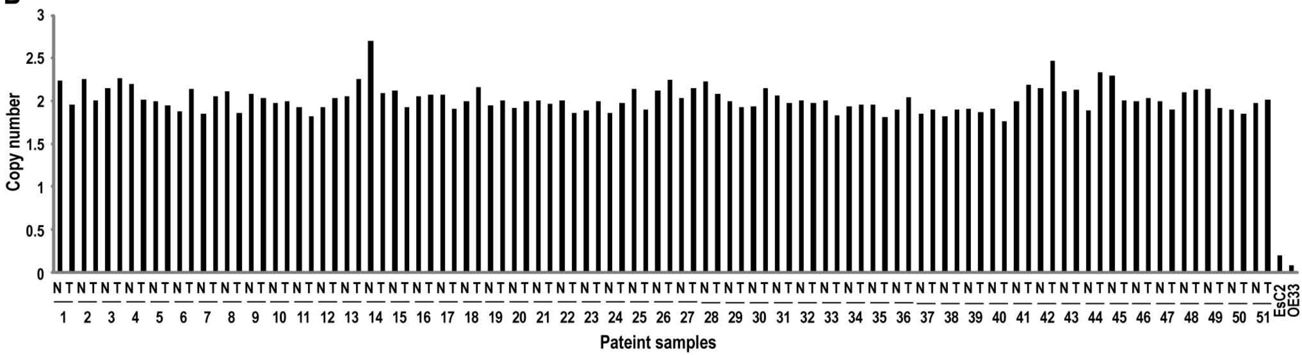


Supplementary Figure 10. Correlation patterns and corresponding correlation coefficient values between miR487b and (A) SUZ12, (B) BMI1, (C) WNT5A, (D) c-Myc, and (E) K-RAS in lung cancers from smokers. (n=42)

S11 A



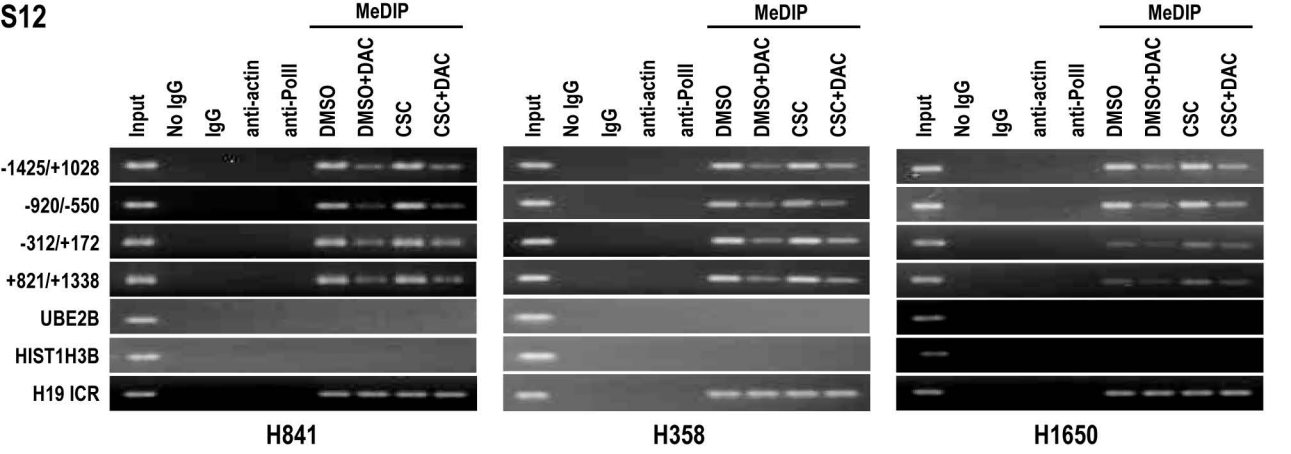
B



C

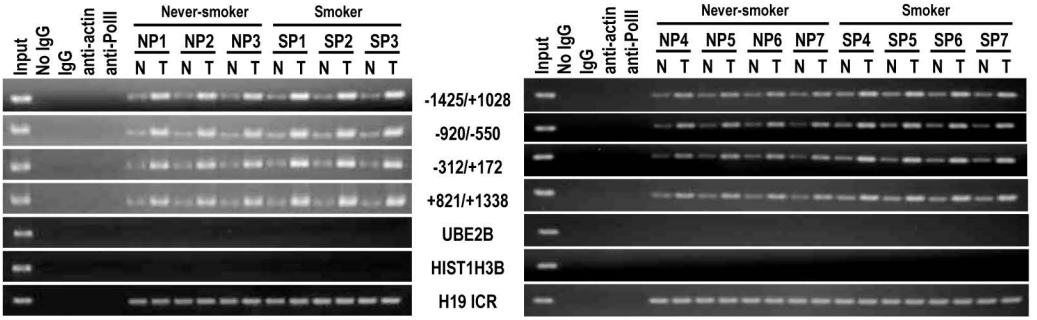
mir-487b RNA	UUGGUACUUGGAGAGUGGUUAUCCCUGUCCUGUUCGUUUUGCUCAUGUCGAAUCGUACAGGGUCAUCCACUUUUUCAGUAUCAA
miR-487b genome	TTGGTACTTGGAGAGTGGTTATCCCTGTCCTGTTCGTTTTGCTCATGTCGAATCGTACAGGGTCATCCACTTTTTCAGTATCAA
SAEC	TTGGTACTTGGAGAGTGGTTATCCCTGTCCTGTTCGTTTTGCTCATGTCGAATCGTACAGGGTCATCCACTTTTTCAGTATCAA
HBEC	TTGGTACTTGGAGAGTGGTTATCCCTGTCCTGTTCGTTTTGCTCATGTCGAATCGTACAGGGTCATCCACTTTTTCAGTATCAA
Calu-6	TTGGTACTTGGAGAGTGGTTATCCCTGTCCTGTTCGTTTTGCTCATGTCGAATCGTACAGGGTCATCCACTTTTTCAGTATCAA
H841	TTGGTACTTGGAGAGTGGTTATCCCTGTCCTGTTCGTTTTGCTCATGTCGAATCGTACAGGGTCATCCACTTTTTCAGTATCAA
H358	TTGGTACTTGGAGAGTGGTTATCCCTGTCCTGTTCGTTTTGCTCATGTCGAATCGTACAGGGTCATCCACTTTTTCAGTATCAA
P1	TTGGTACTTGGAGAGTGGTTATCCCTGTCCTGTTCGTTTTGCTCATGTCGAATCGTACAGGGTCATCCACTTTTTCAGTATCAA
⋮	⋮ ⋮ ⋮
P51	TTGGTACTTGGAGAGTGGTTATCCCTGTCCTGTTCGTTTTGCTCATGTCGAATCGTACAGGGTCATCCACTTTTTCAGTATCAA

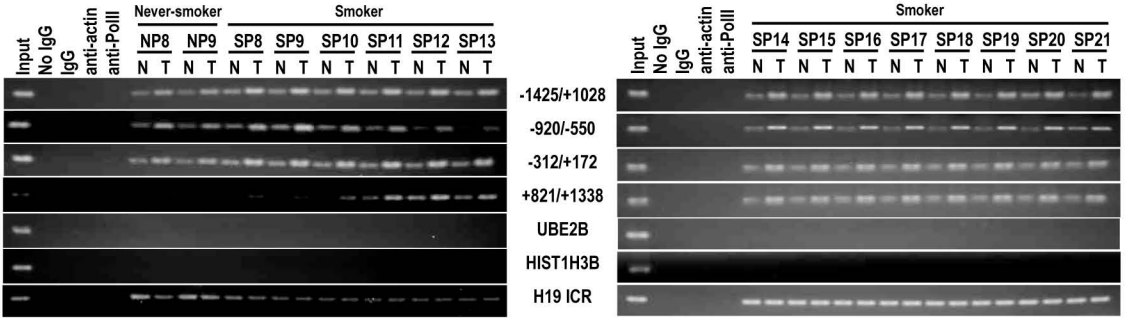
Supplementary Figure 11. Evaluation of genomic profiles around miR-487b genomic locus in cultured cells and human respiratory tissues.
A/B) Quantitative PCR analysis demonstrating no genomic copy loss of miR-487b in cultured normal respiratory epithelia and lung cancer cells (A), or 51 human lung cancers relative to paired adjacent normal lung tissues (B). Genomic copy number examination of chromosome 14q32.31 locus (transcription site of miR-487b) reveals no allele loss or deletion in SAEC, HBEC, Calu-6, H841, and H358. EsC2 and OE33 (esophageal cancer lines) served as negative control with no copy number detectable.
C) Direct genomic DNA sequencing demonstrating no sequence alteration around miR-487b genomic locus in normal respiratory epithelia, lung cancer cells, and 51 human lung cancers paired with adjacent normal lung tissues.

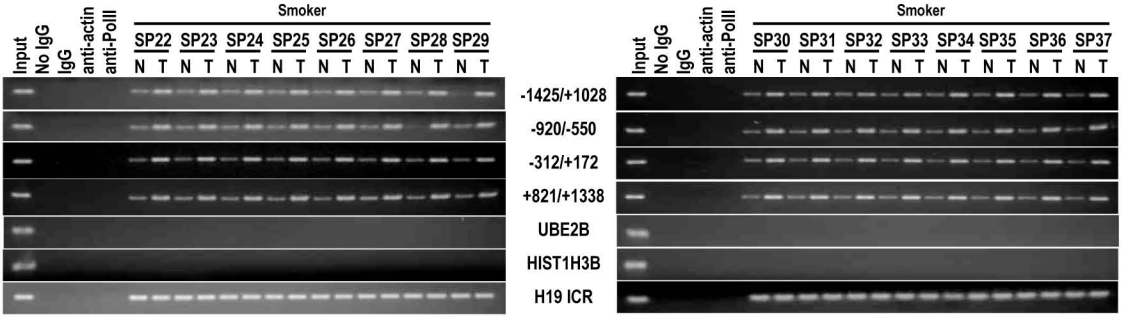


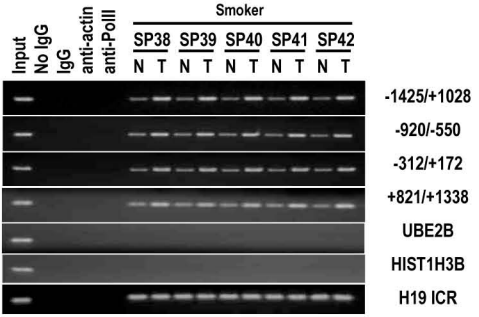
Supplementary Figure 12. MeDIP analysis of DNA methylation profiles within the miR-487b genomic locus in H841, H358, and H1650 cells treated with DAC or CSC. DAC significantly decreased methylation near miR-487b, and partially abrogated CSC-mediated CpG methylation of these regions in these cells.

S13

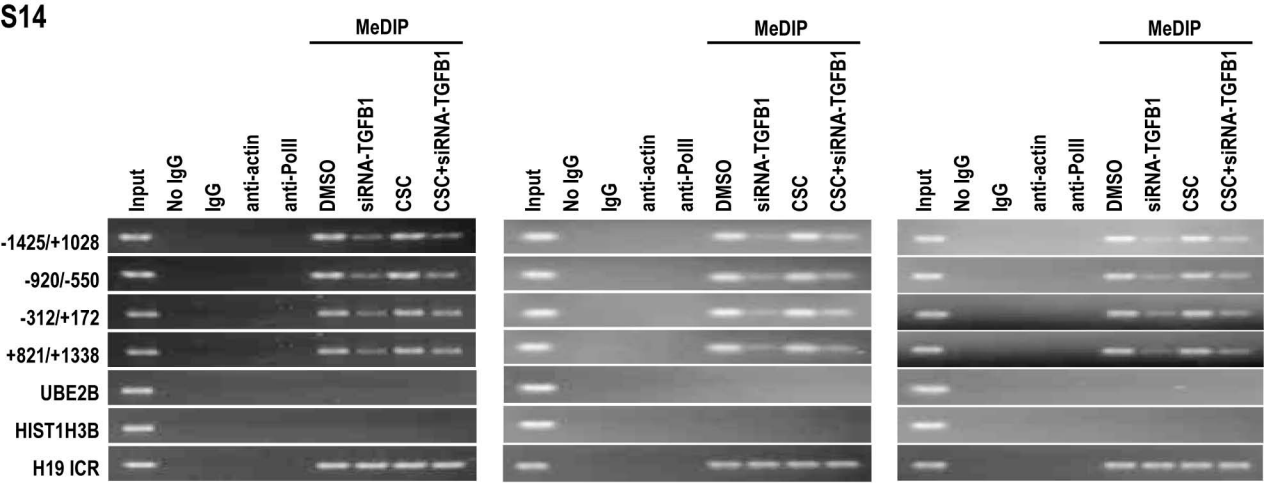








Supplementary Figure 13. MeDIP analysis of DNA methylation profiles within the miR-487b genomic locus in human lung cancers relative to paired adjacent normal lung tissues. CpG methylation levels near miR-487b in tumors were higher than corresponding adjacent normal lung tissues. Furthermore, methylation levels in these regions were higher in lung cancers from smokers compared to those from never-smokers.



Supplementary Figure 14. MeDIP analysis demonstrating that knock-down of TGFB1 markedly decreases DNA methylation levels in untreated H841, H358, and H1650 cells, and diminishes CSC-mediated increases in DNA methylation around the miR-487b genomic locus in these cells.

Supplementary Methods

Quantitative RT-PCR. For mRNA, total RNA was prepared using TRIzol reagent (Invitrogen) and genomic DNA was eliminated with TURBO DNA-free Kit (Ambion). One μg of total RNA was reverse transcribed using iScript reverse transcriptase (Bio-Rad). Omission of reverse transcriptase served as a negative control. cDNA was amplified using Platinum PCR SuperMix (Invitrogen). PCR was performed as follows: 5 min at 94^{o}C, 35 cycles of 60 s at 94^{o}C, 60 s at 57-60^{o}C, and 60 s at 72^{o}C, followed 5 min at 72^{o}C. Real-time quantitative RT-PCR analysis was done as described (10) using WNT5A, BMI1, SUZ12, SFRP1, SFRP4, WIF1, c-Myc, K-RAS, TCF-1, TGFB1, and β-actin primers from Applied Biosystems or Integrated DNA Technologies. For miR-487b and miR-665 expression analysis, these miRs were isolated with the RT2 qPCR-Grade miRNA Isolation kit (Qiagen), and amplified using real-time RT-PCR techniques and using specific primers for the qRT-PCR miRNA Detection Kit (ABI).

siRNA and shRNA knockdown. Calu-6 and H841 cells were transiently transfected with siRNAs targeting TGFB1, TCF-1(Santa Cruz Biotechnology), c-Myc, K-RAS, or sham siRNA sequences (Dharmacon) using Lipofectamine 2000 (Invitrogen). Target gene knockdown was confirmed by qRT-PCR and western blot techniques.

RT-PCR arrays. Wnt signaling qRT-PCR super-arrays (PAH-043A) were obtained from SABiosciences. One μg of total RNA was used for reverse transcription and the entire cDNA reaction was diluted and distributed amongst the 96 wells of the super-array plate. The reactions were performed with RT² SYBR Green / ROX PCR Master Mix (SA Biosciences). Results were analyzed using software provided by the vendor (http://www.sabiosciences.com/pcr/arrayanalysis.php).

MicroRNA PCR array analysis. Human RT2 miRNA PCR Arrays (MAH-3100E-12) were obtained from SA Biosciences. Two hundred ng of isolated miRNA was used for reverse transcription and the entire first strand cDNA was diluted and distributed amongst the 384 wells of the super-array plate. The reactions were performed with RT² SYBR Green / ROX PCR Master Mix (SABiosciences). Results were analyzed using software provided by the vendor (http://www.sabiosciences.com/pcr/arrayanalysis.php).

Western blot analysis. Protein extracts were generated as previously described (61). Samples were separated on NuPAGE 4-12% Bis-Tris gels (Invitrogen) and blotted onto Immobilon P membrane (Millipore), and proteins detected using enhanced chemiluminescence detection reagents (Amersham). Antibodies used for western analysis were rabbit anti-SUZ12, rabbit anti-BMI1, rabbit anti-WNT5A, mouse anti-K-RAS, rabbit anti-c-Myc (Abcam), and β-actin antibody (Santa Cruz Biotechnology.)

In Vitro Invasion. Cell invasion was evaluated *in vitro* using extracellular matrix protein (ECM)-coated semipermeable modified Boyden chambers (Millipore). Briefly, cells were plated at a density of 5×104 cells/well in the chamber or insert. Both the insert and the holding well were subjected to the same medium composition with the exception of serum. The insert contained no serum, whereas the lower well contained 10% FBS that served as a chemo-attractant. Chambers were treated with reagents depending on the experiment. After 48 h of treatment at 37°C in a 5% CO2 incubator, the cells in the insert were removed by wiping gently with a cotton swab. Cells on the reverse side of the insert were stained and quantified according to the manufacturer's instructions.

In vitro apoptosis assays. Calu-6, H841, and H358 cells with over-expression or depletion of miR-487b and control cells (vector control) were detached by trypsinization, counted and pelleted (1000 rpm for 5 min). Cell pellets were washed once with PBS (pH 7.4) and resuspended in 100 µl Annexin V binding buffer (10 mM HEPES, pH 7.4, 140 mM NaCl, 2.5 mM $CaCl_2$). Cells (5 x10^5) were transferred to a 12 x75 mm tube and 5 µl of Annexin V-Cy3 (Abcam) was added per tube and allowed to incubate at room temperature for 15 min in the dark. Then the stained cell suspension was dropped on the slides and covered with coverslips. The membrane of apoptosis cells are stained a bright orange color when analyzed with fluorescence microscope. The ratio (percentage) of apoptotic to total cells (apoptotic plus nonapoptotic cells) was calculated for each high-power field (HPF). For each treatment, 5–10 high-power fields of view were quantitated on each section.

Senescence-associated β-galactosidase staining. Cells (5×10^4) were plated in Lab-Tek II chamber slides (LAB-TEK, Naperville, IL) for 48 hours, followed by evaluation for senescence-activated β-galactosidase (SA-βGal) expression using the Senescence-βGal Staining Kit (Cell Signaling Technology) according to manufacturer's instructions. The ratio (percentage) of senescent to total cells (senescent plus nonsenescent cells) was calculated for each high-power field (HPF). For each treatment, 20 high-power fields of view were quantitated on each section.

Cell cycle analysis. Trypsinized cells were collected and fixed 70% ethanol for 30 min, centrifuged at 2,000 rpm (800 × g) for 10 min, and washed in ice-cold PBS. Cell pellets were resuspended in 100µl PBS with 100 units of RNase (Sigma-Aldrich Chemical Co.), and incubated at 37°C for 20 min, followed by dilution with 0.5 ml PBS containing 50 µg/ml propidium iodide, and incubation at 4^{o}C in the dark for 30 min, and then analyzed using a FACSCalibur flow cytometer (Becton Dickinson).

TaqMan Gene Copy Number Assays. Normal respiratory epithelia, lung cancer cells, and cancer specimens from patients were assayed for copy number of the 14q32.31 region using two TaqMan Gene Copy Number Assays. Primers and probes were designed using Applied Biosystems proprietary software. Each assay was run as a duplex TaqMan real-time PCR reaction, utilizing a FAM dye-based assay targeted to 14q32.31 (PN4400291, ABI), and a VIC dye-based assay for the reference gene, TERT (PN 4401633, ABI) or RNase P (PN 4401631, ABI). Each PCR assay was performed in quadruplicate, and comprised 10 ng gDNA, 1xTaqMan probe/primer mix in 1xTaqMan Universal Master Mix in a 10 μl reaction amplified using an Applied Biosystems 7900HT SDS instrument for 2 mins at 50^{o}C, 10 mins at 95°C, followed by 40 cycles of 15 secs at 95°C and 60 secs at 60°C. Real-time data were collected by the SDS 2.3 software. The method involves relative quantification of the test sequence versus a reference gene known to be two copies for diploid genome. Relative quantity was determined by the ΔΔCt [(FAM Ct- VIC Ct) sample - (FAM Ct – VIC Ct) calibrator] method, where a reference sample or calibrator known to have two copies of the test sequence is used as the basis for comparative results. Gene copy number was analyzed with the CopyCaller™ Software (PN4400042, ABI).

Mutation screening of miR-487b genomic locus. miR-487b genomic site (-200bp and +200bp) was amplified with specific primers (Supplementary Table 1) from human lung cancer lines and lung cancer tissues followed by purification. DNA fragments were subcloned into Topclone sequencing vectors (Invitrogen) for DNA sequencing, and further identification of sequence variations or mutation.

Supplementary Table 1: Primer sequences

ChIP primers

Name	Position	Sequence
DKK1-F	P(-0.45/-0.05kb)	5'-CCA AGT TCC CAG AGT TCC TG-3'
DKK1-R	P(-0.45/-0.05kb)	5'-TTG GGA GGG AGA CAA CAA AG-3'
SFRP1-F	P(-0.5/-0.1kb)	5'-GCA AGC CAA TGC GAG TTA AT-3'
SFRP1-R	P(-0.5/-0.1kb)	5'-GGC TCA ACA CCC CTT AAA AA-3'
SFRP4-F	P(-0.4/-0.05kb)	5'-TCT AAG GCA GAG GGA GCA AA-3'
SFRP4-R	P(-0.4/-0.05kb)	5'-CTG CTT TCC CCC TAA AGT CC-3'
WIF1-F	P(-0.3/+0.1kb)	5'-GAG TGA TGT CCC AGG GGT CT-3'
WIF1-R	P(-0.3/+0.1kb)	5'-GGG CAA ATA GAG CGA GAA CA-3'
miR-487b-F	P(-0.4/0.0 kb)	5'-GCA CCT GGA TGA CTT TAG GG-3'
miR-487b-R	P(-0.4/0.0 kb)	5'-TGG GTG GAC TTG GTA CGT CT-3'

MEDIP primers

Name	Position	Sequence
miR-487b-F	P(-312/+172)	5'-ATG CAC GAT GTG TGT GGT CT-3'
miR-487b-R	P(-312/+172)	5'-TGG GTG GAC TTG GTA CGT CT-3'
miR-487b-F	P(-920/-550)	5'-CAC ACT GGG AAG GAG ATG GT-3'
miR-487b-R	P(-920/-550)	5'-GTG CCC ATA GGT CAG CTC TC-3'
miR-487b-F	P(-1425/-1028	5'-ACG CCT GGG TAA TGA GTC AC-3'
miR-487b-R	P(-1425/-1028	5'-TCT CAC AGA GCC GTT GAC AC-3'
miR-487b-F	P(+821/+1338)	5'-TGT GCC CTA ACC TCA GAA CC-3'
miR-487b-R	P(+821/+1338)	5'-CTG GGG GTC AGT CAC ACT CT-3'
H19 ICR-F		5'-GAG CCG CAC CAG ATC TTC AG-3'
H19 ICR-R		5'-TTG GTG GAA CAC ACT GTG ATC A-3'
UBE2B-F		5'-CTC AGG GGT GGA TTG TTG AC-3'
UBE2B-R		5'-TGT GGA TTC AAA GAC CAC GA-3'
H3B-F		5'-CCC ACA CTT CTT ATG CGA CA-3'
H3B-R		5'-CTG TGC CTG GTT GCA GAT TA-3'

Nucleosome primers

Name	Position	Sequence
miR-487b-F1	P(-2.0/-1.8 kb)	5'-GGA GAG GAG GGA CCT TTT TG-3'
miR-487b-R1	P(-2.0/-1.8 kb)	5'-CCA AGA AGA GGC AGA TCA ATG-3'
miR-487b-F2	P(-2.0/-1.8 kb)	5'-CTT CCT GTT CCC AGC ATC A-3'
miR-487b-R2	P(-2.0/-1.8 kb)	5'-CCA CCA TCA CTG CTG TCT GT-3'
miR-487b-F1	P(-1.8/-1.6 kb)	5'-TCC TGG AGG TGG TAT TGA CC-3'
miR-487b-R1	P(-1.8/-1.6 kb)	5'-TGA ACA CCC AAG CAT CAA AA-3'
miR-487b-F2	P(-1.8/-1.6 kb)	5'-GGC TTC CTG GAG GTG GTA TT-3'

miR-487b-R2	P(-1.8/-1.6 kb)	5'-TGA ACA CCC AAG CAT CAA AA-3'
miR-487b-F1	P(-1.6/-1.4 kb)	5'-GTG CAT GTG GGA GGT CAG A-3'
miR-487b-R1	P(-1.6/-1.4 kb)	5'-GCC ATC CTA GGT GTG AGC AG-3'
miR-487b-F2	P(-1.6/-1.4 kb)	5'-CTT CTG CCT CGA TGT CTG TG-3'
miR-487b-R2	P(-1.6/-1.4 kb)	5'-GAA CAA GAG TCC CAT TCT CAG G-3'
miR-487b-F1	P(-1.4/-1.2 kb)	5'-ACA TTC GCA GCA TCC TCA G-3'
miR-487b-R1	P(-1.4/-1.2 kb)	5'-GTC CAG GTG GCA GGT TAT GT-3'
miR-487b-F2	P(-1.4/-1.2 kb)	5'-AAG CCA GCT TCA GGA AGG AC-3'
miR-487b-R2	P(-1.4/-1.2 kb)	5'-CGT GGT GCT CAC CTC TCT TC-3'
miR-487b-F1	P(-1.2/-1.0 kb)	5'-AGA GAC CAC TGT GGG GAA TG-3'
miR-487b-R1	P(-1.2/-1.0 kb)	5'-AGA AAG AGC ACA GGG TCA ACA-3'
miR-487b-F2	P(-1.2/-1.0 kb)	5'-TTC TTA GCA TGG CTT CAT GG-3'
miR-487b-R2	P(-1.2/-1.0 kb)	5'-TCT CAC AGA GCC GTT GAC AC-3'
miR-487b-F1	P(-1.0/-0.8 kb)	5'-CAG GGC CTT TGC TTT GTC TA-3'
miR-487b-R1	P(-1.0/-0.8 kb)	5'-GGC ACC TCT CCA GGA AGT TT-3'
miR-487b-F2	P(-1.0/-0.8 kb)	5'-GGC CTT TGC TTT GTC TAA GG-3'
miR-487b-R2	P(-1.0/-0.8 kb)	5'-GGC ACC TCT CCA GGA AGT TT-3'
miR-487b-F1	P(-0.8/-0.6 kb)	5'-GGA GTG ATG CCT GGA AAA GA-3'
miR-487b-R1	P(-0.8/-0.6 kb)	5'-GTG CCC ATA GGT CAG CTC TC-3'
miR-487b-F2	P(-0.8/-0.6 kb)	5'-AAT CTG GAG TGA TGC CTG GA-3'
miR-487b-R2	P(-0.8/-0.6 kb)	5'-GTC AGC TCT CCA TCC CAA AG-3'
miR-487b-F1	P(-0.6/-0.4 kb)	5'-ACA AGG GCA AGC TCT CTG TG-3'
miR-487b-R1	P(-0.6/-0.4 kb)	5'-ATG CAC ACA CAT ACC GCA TC-3'
miR-487b-F2	P(-0.6/-0.4 kb)	5'-TTA AAG CGA GGT TGC CCT TT-3'
miR-487b-R2	P(-0.6/-0.4 kb)	5'-GGG TCC AAG ACA AGG TTT GA-3'
miR-487b-F1	P(-0.4/-0.2 kb)	5'-GCA CCT GGA TGA CTT TAG GG-3'
miR-487b-R1	P(-0.4/-0.2 kb)	5'-AGA CCA CAC ACA TCG TGC AT-3'
miR-487b-F2	P(-0.4/-0.2 kb)	5'-TTT TGT GTG TGG CAA ATC GT-3'
miR-487b-R2	P(-0.4/-0.2 kb)	5'-GCC ACT GCC TTC CCT AAA GT-3'
miR-487b-F1	P(-0.2/0.0 kb)	5'-GCA TGT TCT GCT TGG GGT AT-3'
miR-487b-R1	P(-0.2/0.0 kb)	5'-TGG GTG GAC TTG GTA CGT CT-3'
miR-487b-F2	P(-0.2/0.0 kb)	5'-TTA CTC GGT CCG TGA GTG TG-3'
miR-487b-R2	P(-0.2/0.0 kb)	5'-CCT GGG GAT GAA TTA GAT GC-3'
miR-487b-F1	P(0.0/+0.1 kb)	5'-TGT CGA ATC GTA CAG GGT CA-3'
miR-487b-R1	P(0.0/+0.1 kb)	5'-GAG GTG GGA TCC AAA CAC AG-3'
miR-487b-F2	P(0.0/+0.1 kb)	5'-GTG TTT GGA TCC CAC CTC TG-3'
miR-487b-R2	P(0.0/+0.1 kb)	5'-ATG TGA GAC AAT GGG CAC AG-3'
miR-487b-F1	P(+0.1/+0.3 kb)	5'-CAT TGG CGT CCA TTT CTT G-3'
miR-487b-R1	P(+0.1/+0.3 kb)	5'-GGC TAC CAC CTA CGA AGT GC-3’
miR-487b-F2	P(+0.1/+0.3 kb)	5'-GCT GGG GCT GAA CGA GTT A-3'
miR-487b-R2	P(+0.1/+0.3 kb)	5'-GTC CTG GCT ACC ACC TAC GA-3'
miR-487b-F1	P(+0.3/+0.5 kb)	5'-CTT CAT CGG GTA TGG AAT GG-3'
miR-487b-R1	P(+0.3/+0.5 kb)	5'-TTA GCT GCC AAG CAT CAA GA-3'

miR-487b-F2	P(+0.3/+0.5 kb)	5'-GTC CTT CAT CGG GTA TGG AA-3'
miR-487b-R2	P(+0.3/+0.5 kb)	5'-TTA GCT GCC AAG CAT CAA GA-3'
miR-487b-F1	P(+0.5/+0.7 kb)	5'-TCT TGA CCT CGG CCA CTA TC-3'
miR-487b-R1	P(+0.5/+0.7 kb)	5'-GGG AAG CAA GAT GCC ATA GA-3'
miR-487b-F2	P(+0.5/+0.7 kb)	5'-GAA GGA GTC TTG AGC GAG GA-3'
miR-487b-R2	P(+0.5/+0.7 kb)	5'-AAT GCA TGC ACC AAG TTT GA-3'
miR-487b-F1	P(+0.7/+0.9 kb)	5'-CTT GAG CCA AAG ACC TGG AG-3'
miR-487b-R1	P(+0.7/+0.9 kb)	5'-AAA GCG AAC ACA CCA AGG AT-3'
miR-487b-F2	P(+0.7/+0.9 kb)	5'-AAC CTT GAG CCA AAG ACC TG-3'
miR-487b-R2	P(+0.7/+0.9 kb)	5'-AAA GCG AAC ACA CCA AGG AT-3'
miR-487b-F1	P(+0.9/+1.1 kb)	5'-TGT GCC CTA ACC TCA GAA CC-3'
miR-487b-R1	P(+0.9/+1.1 kb)	5'-GCC AAT GCA AAT CCC TGA TA-3'
miR-487b-F2	P(+0.9/+1.1 kb)	5'-GTT TGT GGT GGG GAC ACT TG-3'
miR-487b-R2	P(+0.9/+1.1 kb)	5'-TCC CTG ATA GCA CCA AGG AG-3'
miR-487b-F1	P(+1.1/+1.3 kb)	5'-TGC TTC TTG GAG GCA CTT CT-3'
miR-487b-R1	P(+1.1/+1.3 kb)	5'-CTG GGG GTC AGT CAC ACT CT-3'
miR-487b-F2	P(+1.1/+1.3 kb)	5'-CAA ACC TTA GGG ACC GAT CA-3'
miR-487b-R2	P(+1.1/+1.3 kb)	5'-ATT GAG AGT CAA GCC CAT GC-3'
miR-487b-F1	P(+1.3/+1.5 kb)	5'-TGA TGA TTA ATA TCG GAC AAC CA-3'
miR-487b-R1	P(+1.3/+1.5 kb)	5'-ACC AAT GTC CCC TCA CTG AA-3'
miR-487b-F2	P(+1.3/+1.5 kb)	5'-CAG CTC TTC TTG ATG GCA CA-3'
miR-487b-R2	P(+1.3/+1.5 kb)	5'-AAA ACA ATG GTT GTC CGA TAT T-3'
miR-487b-F1	P(+1.5/+1.7 kb)	5'-AGA ATG CAA GCC ACC AGT GT-3'
miR-487b-R1	P(+1.5/+1.7 kb)	5'-ATA TGG CAC CAA GCC CCT A-3'
miR-487b-F2	P(+1.5/+1.7 kb)	5'-CAG AAT GCA AGC CAC CAG T-3'
miR-487b-R2	P(+1.5/+1.7 kb)	5'-ATA TGG CAC CAA GCC CCT A-3'
miR-487b-F1	P(+1.7/+2.0 kb)	5'-AGC TGC CTG TTG GGT ATT TG-3'
miR-487b-R1	P(+1.7/+2.0 kb)	5'-GTC TAA GAT GGG GTG GTT CC-3'
miR-487b-F2	P(+1.7/+2.0 kb)	5'-CAC TTC CTG TAG GGA ATG CAA-3'
miR-487b-R2	P(+1.7/+2.0 kb)	5'-GCT TAG GAG CTG CCA AAA AG-3'

Bisulfite primers

Name	Position	Sequence
miR-487b-F	P(-312/+172)	5'-AAG GTA GTG GTT TTT GGG ATA TTT T-3'
miR-487b-R	P(-312/+172)	5'-ATA AAA TCC TTA CCC AAA ATT AAA C-3'
miR-487b-F	P(-920/-550)	5'-ATT TTT AGG AAA ATT TTT TGG AGA G-5'
miR-487b-R	P(-920/-550)	5'-AAA CAA ATT CCT CAC TAT CAT TCA C-3'
miR-487b-F	P(-1425/-1028	5'-TTG TTT TGT GTT TGT GGA GAT ATT T-3'
miR-487b-R	P(-1425/-1028	5'-TAT CAT TCT CCA TAC CAA CCC TTA C-3'
miR-487b-F	P(+821/+1338)	5'-AAT TTT GAG TTA AAG ATT TGG AGG T-3'
miR-487b-R	P(+821/+1338)	5'-CAC ACA TTA AAA ATC AAA CCC ATA C-3'

Primers for amplification of 3'-UTRs of target genes

WNT5A-F	3'UTR(3700-4317)	5'-AAG CTT GAG ACC GGT ACT AGC TAA CTC CA-3'
WNT5A-R	3'UTR(3700-4317)	5'-ACT AGT ATC TCA TTT CTA GCC CAG CA-3'
WNT5A-F	3'UTR(2000-2750)	5'-AAG CTT TTA GGC AGG TTG GCT TTC AT-3'
WNT5A-R	3'UTR(2000-2750)	5'-ACT AGT GAG AAT TCC CCT TTT GTT CCA-3'
SUZ12-F	3'UTR(1250-1933)	5'-AAG CTT TTC ATT CCA CCA CCA TCA GA-3'
SUZ12-R	3'UTR(1250-1933)	5'-ACT AGT TGT ACC ATT CAA ATG CTT TAT CAT C-3'
BMI1-F	3'UTR(1-750)	5'-AAG CTT CGT CCA ATT TGC TTT CTT TTG-3'
BMI1-R	3'UTR(1-750)	5'-ACT AGT CCC GCT TTT AGG CAT ACA GA-3'

miR-487b 3'UTR mutation primers

Name	Position	Sequence
WNT5A-3'UTR-mutation-F	3'UTR(3700-4317)	5'-CCA TTT TTA TAT GCA GTG TGC TGG GCT AGA AAT G-3'
WNT5A-3'UTR-mutation-R	3'UTR(3700-4317)	5'-CAT TTC TAG CCC AGC ACA CTG CAT ATA AAA ATG G-3'
WNT5A-3'UTR-mutation-F	3'UTR(2000-2750)	5'-TAT CTT CTA GCC TTT ATT AAT GTA CAT ATT TCT GTC-3'
WNT5A-3'UTR-mutation-R	3'UTR(2000-2750)	5'-GAC AGA AAT ATG TAC ATT AAT AAA GGC TAG AAG ATA-3'
SUZ12-3'UTR-mutation-F	3'UTR(1250-1933)	5'-AAC AGA TTT TGA AGA AAT GAT AAA GCA TTT GAA T-3'
SUZ12-3'UTR-mutation-R	3'UTR(1250-1933)	5'-ATT CAA ATG CTT TAT CAT TTC TTC AAA ATC TGT T-3'
BMI1-3'UTR-mutation-F	3'UTR(1-750)	5'-GCC ATG TCA CTG TGA ATA TCT TGC ATA TTT AGC CAT-3'
BMI1-3'UTR-mutation-F	3'UTR(1-750)	5'-ATG GCT AAA TAT GCA AGA TAT TCA CAG TGA CAT GGC-3'

miR-487b CLIP primers

Name	Position	Sequence
WNT5A-F	3'UTR(2300-2450bp)	5'-GAG GAA AAT TGC ATC TTA GAC CAT-3'
WNT5A-R	3'UTR(2300-2450bp)	5'-ATC TTT ATC TCA TTT CTA GCC CAG CA-3'
WNT5A-F	3'UTR(4200-4350bp)	5'-TGT ACA TAT TTC TGT CTT GCG TGA-3'
WNT5A-R	3'UTR(4200-4350bp)	5'-GGC ATA AGC CTT TCG ATG TT-3'
SUZ12-F	3'UTR(1850-1950bp)	5'-AAA GTG CTT TTC TAT ATG TAC CCT TGA-3'
SUZ12-R	3'UTR(1850-1950bp)	5'-TGT ACC ATT CAA ATG CTT TAT CA-3'
BMI1-F	3'UTR(450-550bp)	5'-GCA TTC TAT GTA GCC ATG TCA CTG-3'
BMI1-R	3'UTR(450-550bp)	5'-AAA TCA AAC AGG AAT CAA AAT GG-3'
c-Myc-F	3'UTR(1700-1800bp)	5'- TCT GAC ACC CAT GAC TCC AC -3'
c- Myc -R	3'UTR(1700-1800bp)	5'- AGG CAG TTT GGG GGA AGG -3'
K-RAS-F	3'UTR(2750-2850bp)	5'- AAC ACG ATG CGT ATT TTA GTT TTG -3'
K-RAS-R	3'UTR(2750-2850bp)	5'- AGC TTG ATC GAA GAG TTT CAG TG -3'

Supplementary Table 2: Antibodies

Name	Vendor	Location	Catalogue #
TCF-1	Millipore	Billerica, MA	17-604
SUZ12	Abcam Inc.	Cambridge, MA	ab12073
BMI1	Abcam Inc.	Cambridge, MA	ab38295
H2AZ	Abcam Inc.	Cambridge, MA	ab4174
WNT5A	Abcam Inc.	Cambridge, MA	ab72583
K-RAS	Abcam Inc.	Cambridge, MA	ab55391
c-Myc	Abcam Inc.	Cambridge, MA	ab32072
β-actin	Santa Cruz Biotechnology, Inc.	Santa Cruz, CA	sc-1616
AGO proteins	Millipore	Billerica, MA	04-085
elF2C	Santa Cruz Biotechnology, Inc.	Santa Cruz, CA	sc-32877