## Supporting Information (SI Appendix)

# MicroRNA-224 promotes tumor progression in non-small cell lung cancer

Ri Cui[#], Wei Meng[#], Hui-Lung Sun, Taewan Kim, Zhenqing Ye, Matteo Fassan, Young-Jun Jeon, Bin Li, Caterina Vicentini, Yong Peng, Tae Jin Lee, Zhenghua Luo, Lan Liu, Dongyuan Xu, Esmerina Tili, Victor X. Jin, Justin Middleton, Arnab Chakravarti, Tim Lautenschlaeger*, Carlo M. Croce*

region of miR-224 from TCGA Illumina Infinium Human DNA Methylation 450k beadchip in NSCLC

## SI Materials and Methods

### Genome-wide miRNA sequencing and data analysis

As described previously (1), small RNAs were purified from fresh frozen tissue samples (6 with N0 stage and 4 with N1+ stage lung ADC) and ligated with 5' and 3' adapter mix using RNA ligase. Ligated products were amplified by PCR using SOLID Small RNA Expression Kit (Applied Biosystems) and applied for Applied Biosystems SOLID next generation high-throughput sequencing system for data acquisition. Expression analysis of miRNA sequencing data was conducted using the R/Bioconductor package EdgeR as described previously (1).

### Plasmid construction, cell lines and regents

The human pre-miRNA expression construct Lenti-miR-224 vector and human miRZip-224 anti-miR-224 miRNA construct were purchased from System biosciences. pLightSwtich empty, TNFAIP1-3'UTR, and SMAD4-3'UTR vectors were ordered from Active Motif. Mutations were generated by using the QuickChange II XL Site-Directed Mutagenesis Kit (Stratagene). To generate SMAD4 expressing constructs, the ORFs were amplified by PCR and cloned into pCDH-CMV-MCS-EF1-Neo vector at NheI and NotI restriction enzyme sites (System biosciences). The primers used in this study were shown in Table S2. shRNA control (SHC001) and shTNFAIP1 (TRCN0000156906) were purchased from Sigma. siRNA against c-Jun and control siRNA were purchased from Dharmacon. The cell lines used in this study were purchased from the American Type Culture Collection (ATCC). Human lung cancer cell lines H1299, H1573, A549 and H460 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. The Phorbol 12-myristate 13-acetate (PMA) and 5-Aza-deoxycytidine were purchased from Cayman chemicals and Sigma-Aldrich, respectively. Antibodies against Histon H3 (D1H2), cleaved PARP1 (Asp214) and SMAD4 were purchased from Cell signaling technology. Anti-vinculin antibody and anti-TNFAIP1 antibody were ordered from Sigma-Aldrich and Abcam, respectively.

**Virus infection and transfection**

The pre-miR224 expression construct, miRZip-224 anti-miR-224 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 SMAD4 expressing vector and shRNA against TNFAIP1 were carried out with Lipofectamine LTX according to the manufacturer's instruction (Invitrogen) and transfected cells were selected by neomycin or puromycin, respectively. siRNA against C-jun 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 (Applied Biosystems). Small endogenous nucleolar U6 snRNA was used as control for normalization of miRNA. TaqMan gene expression assays for TNFAIP1 and SMAD4 were purchased from Applied Biosystems to determine their expression. β2M was used as control for normalization of mRNA expression. cDNAs were synthesized from 3μg total RNAs

using SuperScript First-Strand Synthesis System for RT-PCR (Invitrogen). All reactions were conducted in triplicates.

**Cell migration, invasion and proliferation assays**

*In vitro* cell migration and invasion assays were conducted using Boyden chambers (BD bioscience) that use 8-mm micropore membranes without Matrigel (for migration assay) or with Matrigel (for invasion assay) as previously described (1). Both assays were 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). The cytosol and nuclear proteins were extracted by using NE-PER Nuclear and Cytoplasmic extraction Regeants (Thermo). After SDS-PAGE, the proteins were electrotransferred to Immun-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 3% 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).

**In situ hybridization of miR224**

*In situ* hybridization (ISH) was performed as previously descrived (3). The *miR-224* probe was tagged with 5' digoxigenin and LNA modified (Exiqon). The probe–target complex was detected using an antidigoxigenin-alkaline phosphates conjugate and nitro-blue tetrazolium and 5-bromo-4-chloro-3′-indolyphosphate as the chromogen. Cases were classified according to cytoplasmic *miR-224* intensity as: negative= negative or faint expression in most cells; low expression= low expression in most cells or moderate expression in <50% of the cells; high expression= moderate to strong expression in most cells.

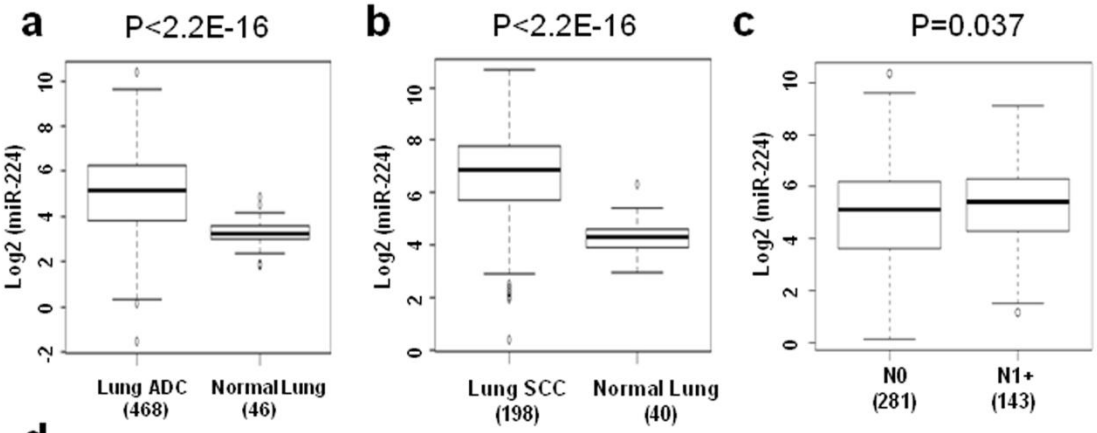
**Isolation of cells from normal lung tissues from lung cancer patients**

Normal lung tissues were obtained distal to tumor sites when the surgical specimen was large enough to include both tumor and normal lung margin from two lung cancer patients. The cell isolation was processed within 2 hours after surgical resection as previously described (4) with some modification. Briefly, the tissues were minced with scissors until ~1mm$^2$ or smaller and digested with 1×collagenase (Stemcell Technologies) in HBSS at 37˚C for 4 hours with shaking. Following incubation, using 18 gauge needle to further dissociate undigested pieces of tissues. The tissue slurry was filtered with 10μm filters and overlay onto the 40%-70% percoll gradient. After centrifugation at 2500 rpm for 20min, collect cells from the 40%-70% percoll interface. The cells were maintained with RPMI1640 medium containing 20% FBS and 100U/ml penicillin-streptomycin. We isolated two cell lines from normal lung tissues and designated them as 469NAT and 485 NAT, respectively.

## SI References

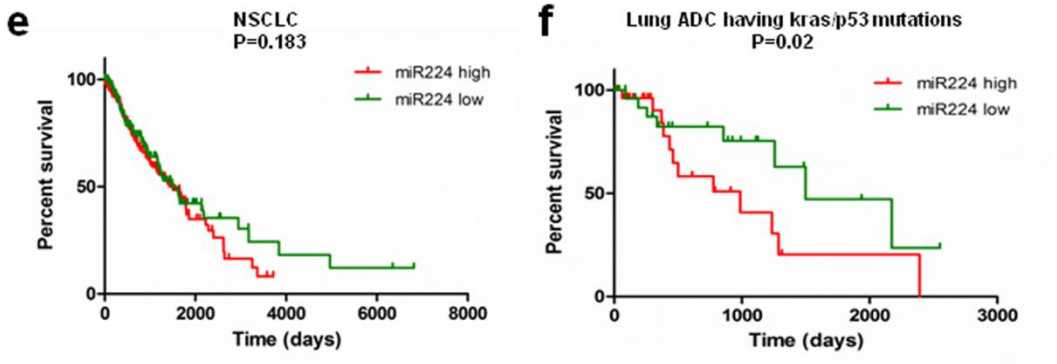

1. Meng W, *et al.* (2013) MicroRNA-31 predicts the presence of lymph node metastases and survival in patients with lung adenocarcinoma. *Clin Cancer Res* 19(19):5423-5433.
2. Cui R, *et al.* (2009) Osteopontin is involved in the formation of malignant pleural effusion in lung cancer. *Lung Cancer* 63(3):368-374.
3. Fassan M, *et al.* (2011) MicroRNA expression profiling in human Barrett's carcinogenesis. *Int J Cancer* 129(7):1661-1670.
4. Cook JA, *et al.* (1991) Cellular glutathione and thiol measurements from surgically resected human lung tumor and normal lung tissue. *Cancer Res* 51(16):4287-4294.

**SI Figures**




d

| | miR224 expression levels | | |
|---|---|---|---|
| | 0 | 1 | 2 |
| Normal (51) | 17 (33%) | 26 (51%) | 8 (16%) |
| Primary (51) | 1 (2%) | 18 (35%) | 32 (63%) |
| Primary - Normal (paired) | 20 (39%) | 22 (43%) | 9 (18%) |



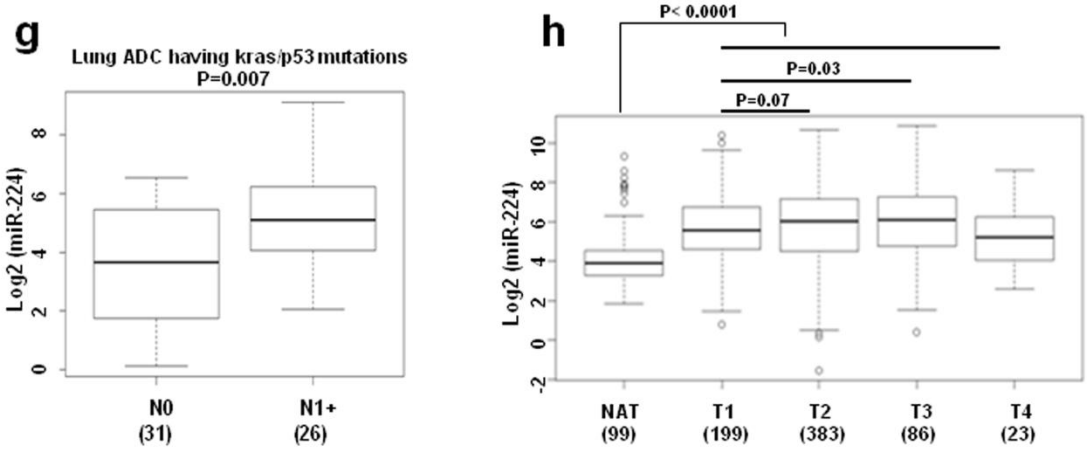



**Fig.S1. MiR-224 expression in NSCLC and its clinical implications.** The miR-224 expression data for lung ADC (a) and lung SCC (b) were acquired from TCGA miR-seq dataset. Of the evaluable 468 primary lung ADC patients, 46 patients had matched normal adjacent tissues. Of the evaluable 198 primary lung squamous carcinoma patients, 40 patients had matched normal adjacent tissues. (c) miR-224 expression in 281 N0 patients versus 143 N1+ patients from TCGA lung ADC dataset. (d) Summary of tissue micro array data for lung cancer and corresponding normal tissue. Paired analysis represents subtraction of NAT staining score from matched lung cancer tissue scores. (e) Kaplan Meier survival analysis for 642 available TCGA NSCLC patients. The p-value was calculated from Cox proportional hazards model. (f) Cox proportional hazards model on 59 lung ADC patients who have TP53 and/or KRAS mutations (mutation data were downloaded from www.cbioportal.org, Lung Adenocarcinoma TCGA Provisional). (g) miR-224 expression in 31 N0 patients versus 26 N1+ patients from lung ADC patients who have TP53 and/or KRAS mutations. (h) miR-224 expression according to T stage in the TCGA dataset.

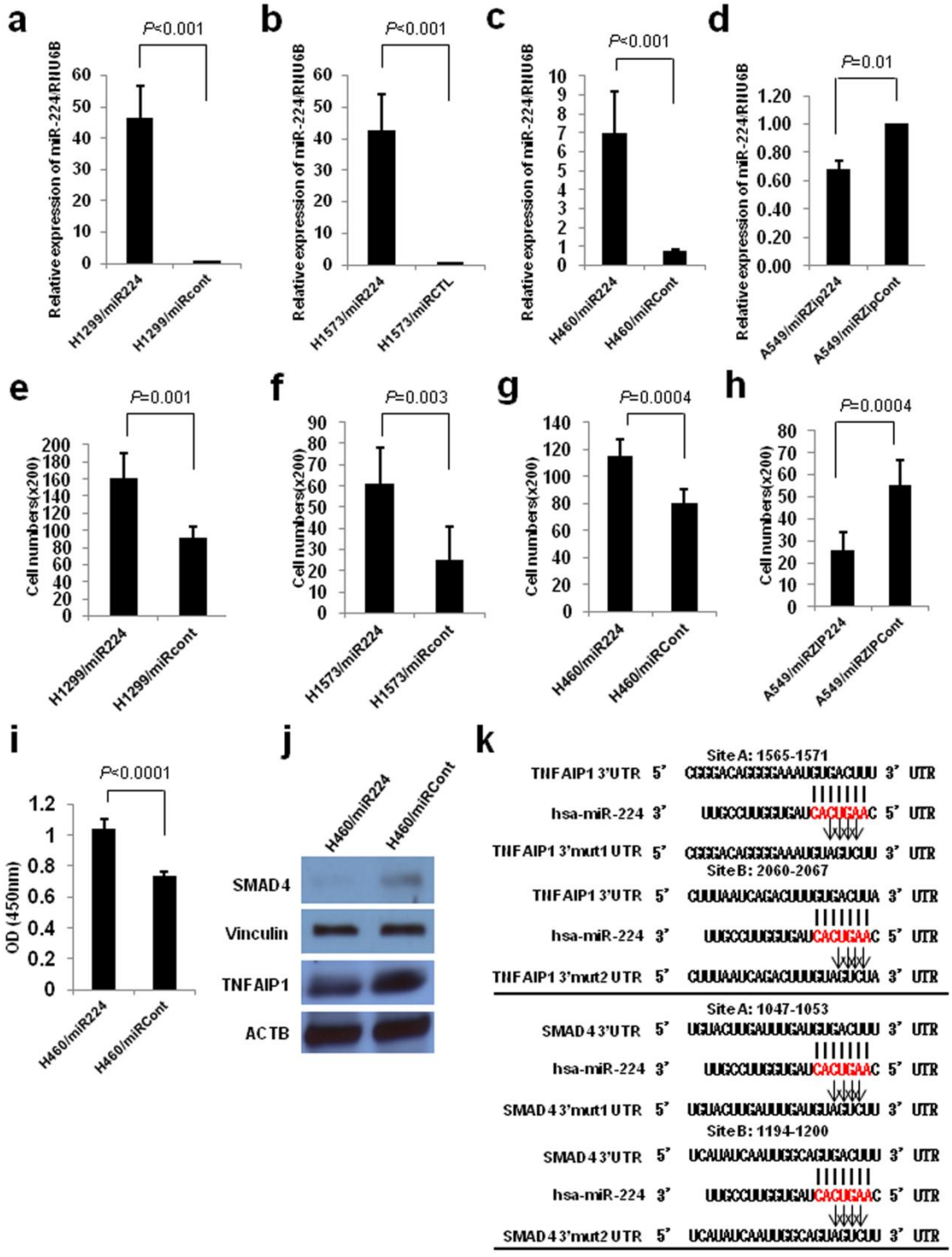

a
P<0.001
Relative expression of miR-224/RNU6B
H1299/miR224
H1299/miRcont
b
P<0.001
Relative expression of miR-224/RNU6B
H1573/miR224
H1573/miRCTL
c
P<0.001
Relative expression of miR-224/RNU6B
H460/miR224
H460/miRCont
d
P=0.01
Relative expression of miR-224/RNU6B
A549/miRZip224
A549/miRZipCont
e
P=0.001
Cell numbers(x200)
H1299/miR224
H1299/miRcont
f
P=0.003
Cell numbers(x200)
H1573/miR224
H1573/miRcont
g
P=0.0004
Cell numbers(x200)
H460/miR224
H460/miRCont
h
P=0.0004
Cell numbers(x200)
A549/miRZIP224
A549/miRZIPCont
i
P<0.0001
OD (450nm)
H460/miR224
H460/miRCont
j
H460/miR224
H460/miRCont
SMAD4
Vinculin
TNFAIP1
ACTB
k
Site A: 1565-1571
TNFAIP1 3'UTR 5' CGGGACAGGGGAAAUGUGACUUU 3' UTR
hsa-miR-224 3' UUGCCUUGGUGAUCACUGAAC 5' UTR
TNFAIP1 3'mut1 UTR 5' CGGGACAGGGGAAAUGUAGUCUU 3' UTR
Site B: 2060-2067
TNFAIP1 3'UTR 5' CUUUAAUCAGACUUUGUGACUUA 3' UTR
hsa-miR-224 3' UUGCCUUGGUGAUCACUGAAC 5' UTR
TNFAIP1 3'mut2 UTR 5' CUUUAAUCAGACUUUGUAGUCUA 3' UTR
Site A: 1047-1053
SMAD4 3'UTR 5' UGUACUUGAUUUGAUGUGACUUU 3' UTR
hsa-miR-224 3' UUGCCUUGGUGAUCACUGAAC 5' UTR
SMAD4 3'mut1 UTR 5' UGUACUUGAUUUGAUGUAGUCUU 3' UTR
Site B: 1194-1200
SMAD4 3'UTR 5' UCAUAUCAAUUGGCAGUGACUUU 3' UTR
hsa-miR-224 3' UUGCCUUGGUGAUCACUGAAC 5' UTR
SMAD4 3'mut2 UTR 5' UCAUAUCAAUUGGCAGUAGUCUU 3' UTR

**Fig. S2. MiR-224 is involved in lung cancer cell proliferation and migration.** (a-c) Expressions of *miR-224* following infection with Lenti-miR vector containing miR-224 precursor into H1299 (a), H1573 (b), and H460 (c) were confirmed by TaqMan real-time PCR. (d) Expression of *miR-224* following infection with miRZip-224 anti miR-224 construct was confirmed by qRT-PCR. (e-g) Migrated cell counts after overexpressing miR-224 in H1299 (e), H1573 (f), and H460 (g) cells. (h) Migrated cell counts after knockdown miR-224 in A549 cells. (i) Cell proliferation assay for miR-224 overexpressing H460 cells. (j) Western blot analyses to measure SMAD4 and TNFAIP1 protein levels in H460 cells infected with lentivirus expressing premiR-224 or control vector. (k) Schematic diagram showing matched sequences between the seed sequence of *miR-224* and 3'UTRs of TNFAIP1 and SMAD4. The arrows indicate the mutagenesis nucleotides.

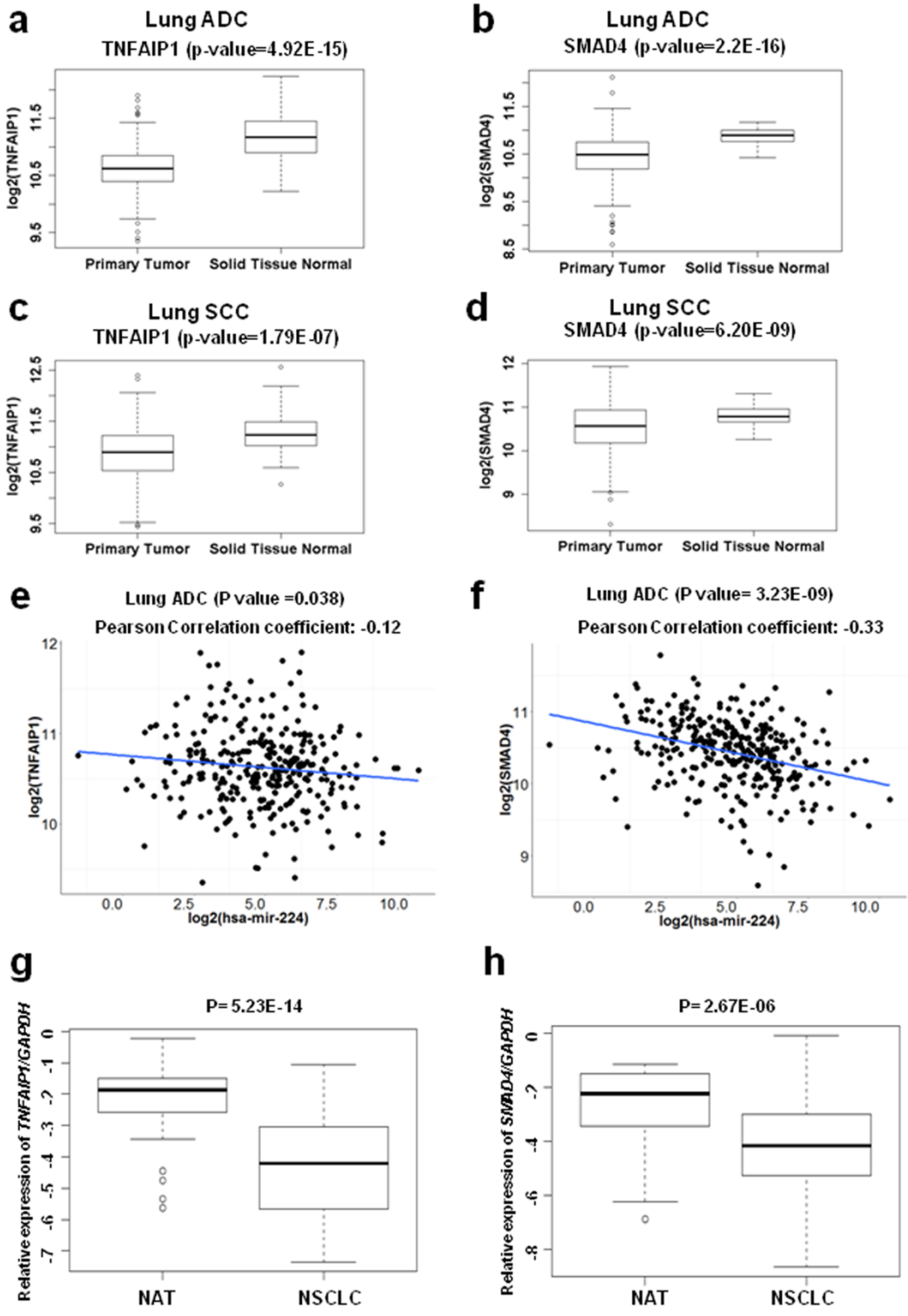

a
Lung ADC
TNFAIP1 (p-value=4.92E-15)
log2(TNFAIP1)
Primary Tumor
Solid Tissue Normal
b
Lung ADC
SMAD4 (p-value=2.2E-16)
log2(SMAD4)
Primary Tumor
Solid Tissue Normal
c
Lung SCC
TNFAIP1 (p-value=1.79E-07)
log2(TNFAIP1)
Primary Tumor
Solid Tissue Normal
d
Lung SCC
SMAD4 (p-value=6.20E-09)
log2(SMAD4)
Primary Tumor
Solid Tissue Normal
e
Lung ADC (P value =0.038)
Pearson Correlation coefficient: -0.12
log2(TNFAIP1)
log2(hsa-mir-224)
f
Lung ADC (P value= 3.23E-09)
Pearson Correlation coefficient: -0.33
log2(SMAD4)
log2(hsa-mir-224)
g
P= 5.23E-14
Relative expression of TNFAIP1/GAPDH
NAT
NSCLC
h
P= 2.67E-06
Relative expression of SMAD4/GAPDH
NAT
NSCLC

**Fig. S3. *TNFAIP1* and *SMAD4* expressions in NSCLC.** The *TNFAIP1* and *SMAD4* 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, and 349 lung SCC patients with 51 matched NAT samples. In lung ADC, TNFAIP1 (a) and SMAD4 (b) expression were dramatically reduced in primary tumor tissues. In lung SCC, a similar reduction of TNFAIP1 (c) and SMAD4 (d) expression levels were observed. (e and f) *SMAD4* and *TNFAIP1* expression from TCGA RNA-seq data and *miR-224* expression from miR-seq data were examined for correlation between miR-224 expression and its target genes in lung ADC dataset (n = 306). (g and h) *TNFAIP1* and *SMAD4* expressions in 63 NSCLC patients with 48 matched NATs from OSU cohort.

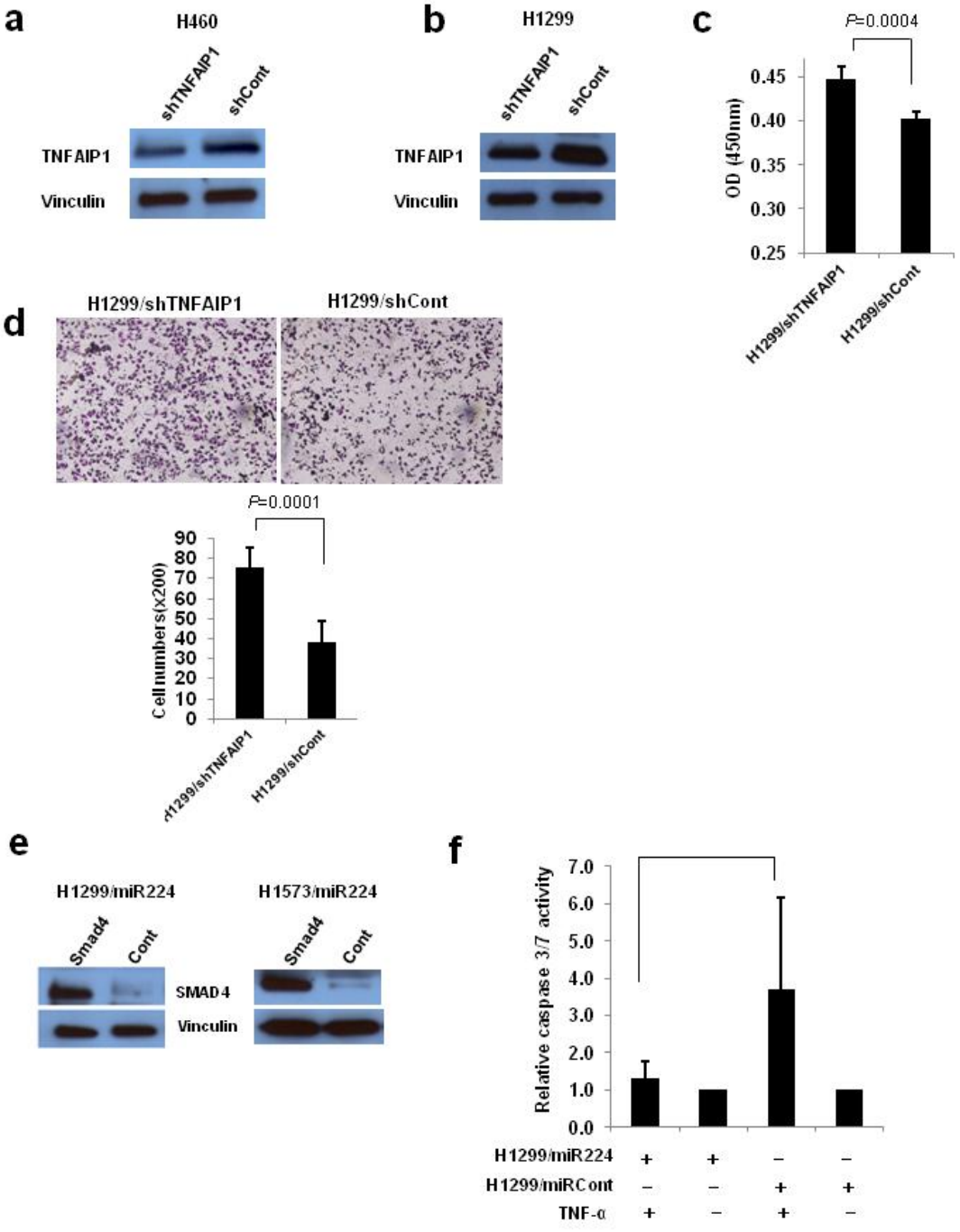
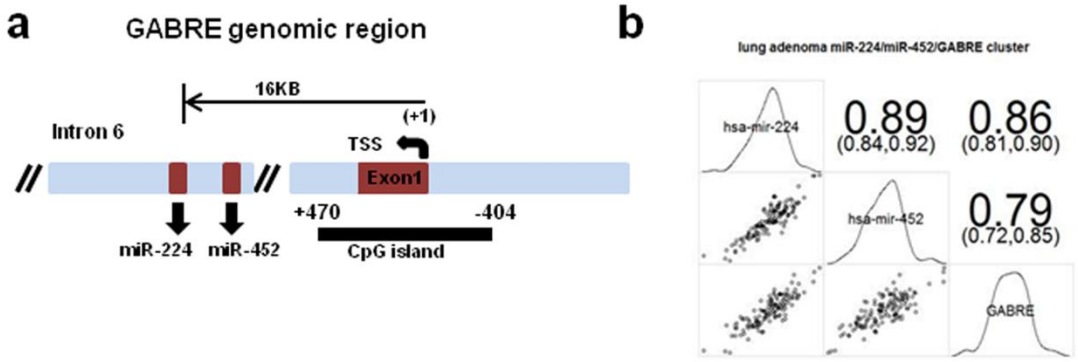
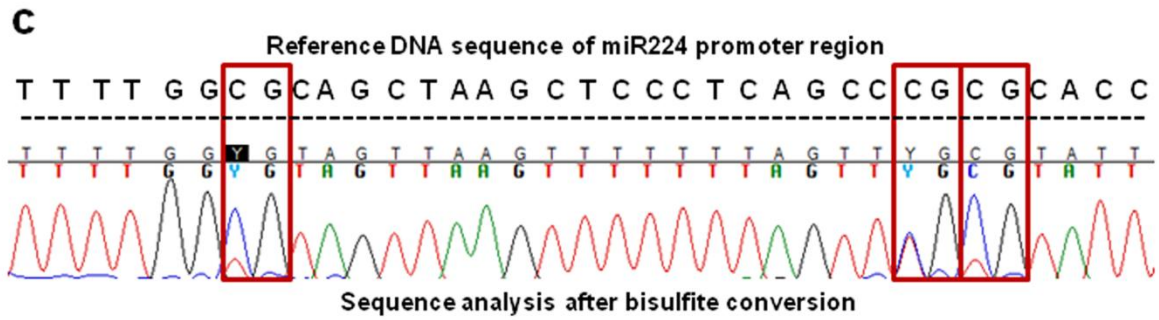
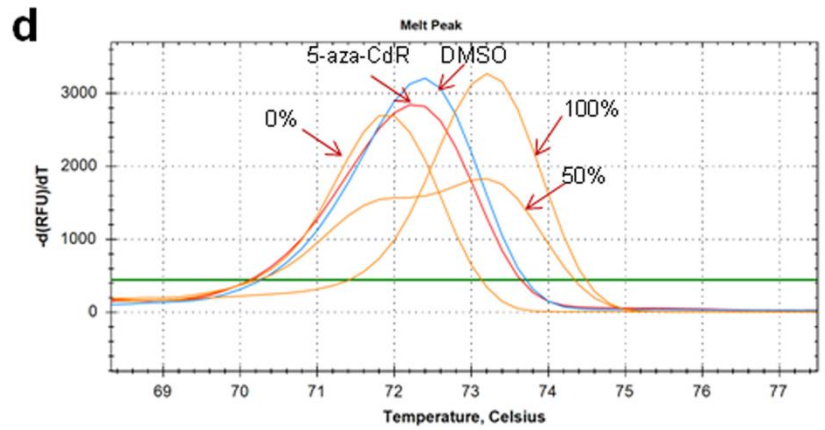

a
H460
shTNFAIP1
shCont
TNFAIP1
Vinculin
b
H1299
shTNFAIP1
shCont
TNFAIP1
Vinculin
c
P=0.0004
OD (450nm)
0.45
0.40
0.35
0.30
0.25
H1299/shTNFAIP1
H1299/shCont
d
H1299/shTNFAIP1
H1299/shCont
P=0.0001
Cell numbers(x200)
90
80
70
60
50
40
30
20
10
0
H1299/shTNFAIP1
H1299/shCont
e
H1299/miR224
H1573/miR224
Smad4
Cont
Smad4
Cont
SMAD4
Vinculin
f
Relative caspase 3/7 activity
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
H1299/miR224 + + – –
H1299/miRCont – – + +
TNF-α + – + –

**Fig. S4. TNFAIP1 and SMAD4 are targets of miR-224.** (a and b) Western blot analyses of TNFAIP1 knockdown H460 (a) and H1299 (b) lung cancer cells. (c) Cell proliferation assay for TNFAIP1 knockdown H1299 lung cancer cells. The cell growth rates were measured by cell counting kit 8. (d) Cell migration assay for TNFAIP1 knockdown H1299 cells using transwell membranes. The average counts were derived from six random microscopic fields. (e) Western blot analysis of miR-224 overexpressing cells transfected with SMAD4 (do not contain 3'UTR) or empty vector. (f) Caspase 3/7 assay after TNF-α treatment in miR-224 overexpressing H1299 cells.


a
GABRE genomic region
16KB
(+1)
Intron 6
TSS
Exon1
+470
-404
miR-224
miR-452
CpG island
b
lung adenoma miR-224/miR-452/GABRE cluster
hsa-mir-224
hsa-mir-452
GABRE
0.89
(0.84,0.92)
0.86
(0.81,0.90)
0.79
(0.72,0.85)


c
Reference DNA sequence of miR224 promoter region
T T T T G G C G C A G C T A A G C T C C C T C A G C C C G C G C A C C
Sequence analysis after bisulfite conversion


d
Melt Peak
5-aza-CdR
DMSO
0%
100%
50%
-d(RFU)/dT
3000
2000
1000
0
69
70
71
72
73
74
75
76
77
Temperature, Celsius

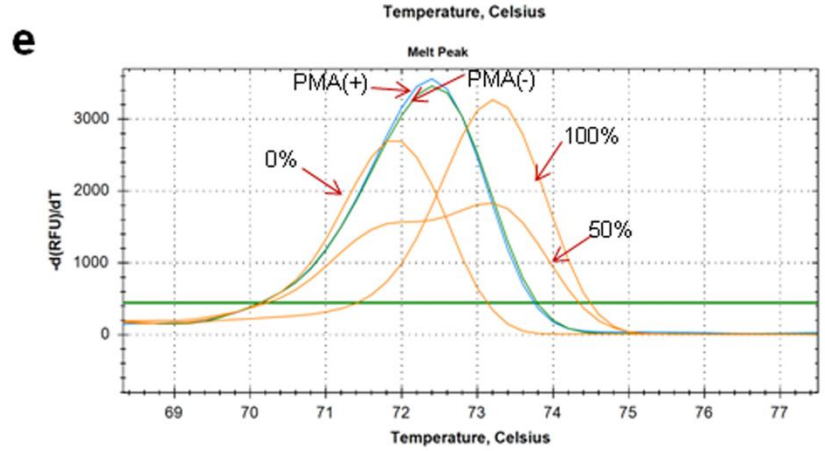

e
Melt Peak
PMA(+)
PMA(-)
0%
100%
50%
-d(RFU)/dT
3000
2000
1000
0
69
70
71
72
73
74
75
76
77
Temperature, Celsius

**Fig. S5. MiR-224 promoter methylation modulates miR-224 expression.** (a) Schematic structure of *miR224~miR452~GABRE* genomic locus (not to scale). *miR-224* and *miR452* are located in intron 6 of *GABRE*. TSS indicated putative transcription start site. Black box shows CpG island which expand exon1 of *GABRE*. (b) The normalized expression values of *miR-224*, *miR-452* and *GABRE* were extracted from TCGA dataset for Pearson correlation analysis between β-values of each other. (c) Bisulfate modified genomic DNA was amplified by miR224 promoter specific primers and subject to sequencing analysis. (d) MS-HRM analyses of 485NAT cells with and without 5-aza-CdR treatment. (e) MS-HRM analyses of 485NAT cells with and without PMA treatment.

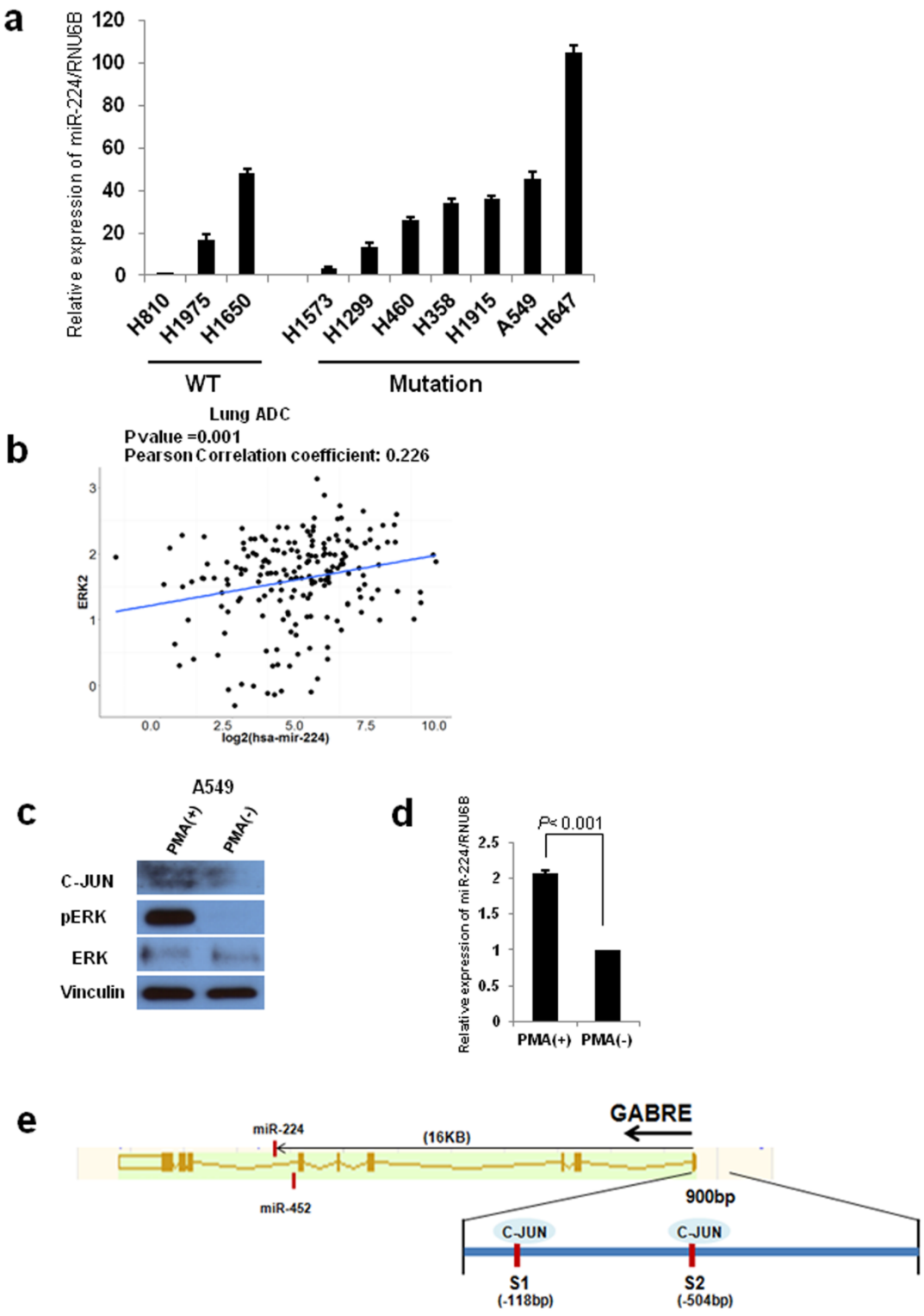

a
Relative expression of miR-224/RNU6B
120
100
80
60
40
20
0
H810
H1975
H1650
H1573
H1299
H460
H358
H1915
A549
H647
WT
Mutation
b
Lung ADC
Pvalue =0.001
Pearson Correlation coefficient: 0.226
ERK2
log2(hsa-mir-224)
c
A549
PMA(+)
PMA(-)
C-JUN
pERK
ERK
Vinculin
d
P< 0.001
Relative expression of miR-224/RNU6B
PMA(+)
PMA(-)
e
GABRE
miR-224
(16KB)
miR-452
900bp
C-JUN
C-JUN
S1
(-118bp)
S2
(-504bp)

**Fig. S6. ERK signaling modulates miR-224 expression.** (a) miR-224 expression in different lung cancer cell lines. The cell lines were divided two sub-groups, RAS wild type and RAS mutation groups. The RAS gene status was collected from http://cancer.sanger.ac.uk/cancergenome/projects/cell_lines/. (b) Total ERK2 expression from TCGA RPPA data and *miR-224* expression from miR-seq data were examined for correlation between miR-224 expression and ERK2 proteins in lung ADC dataset (n = 188). Western blot analysis (c) and qRT-PCR (d) for A549 cells with and without PMA treatment to measure miR-224 expression. (e) Schematic diagram of *miR224~miR452~GABRE* genomic locus. *MiR-224* is located in intron6 of *GABRE*. Two C-jun binding sites (red line) were located in -118 bp and -504 bp upstream of GABRE transcription start site.

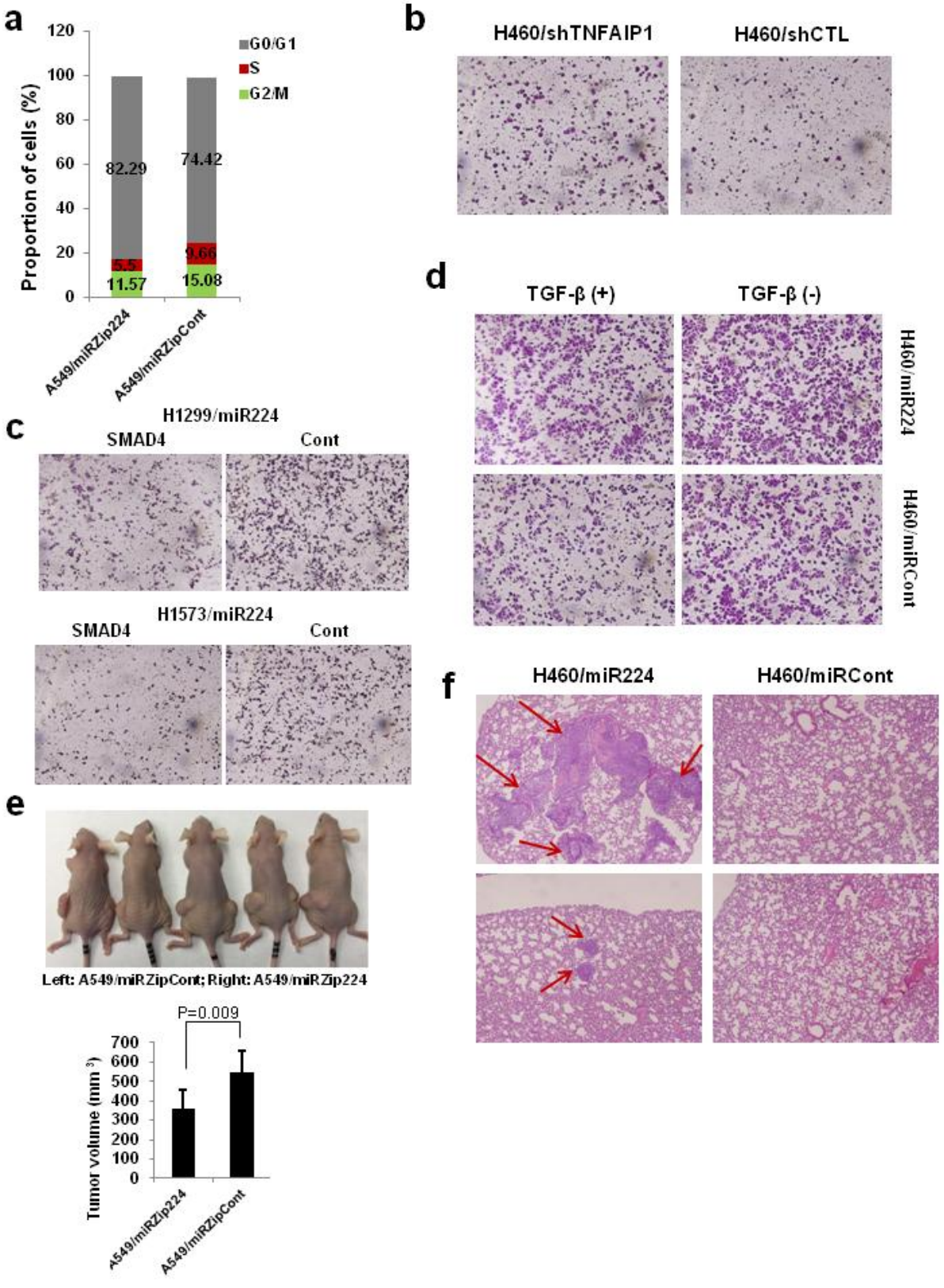

a
Proportion of cells (%)
120
100
80
60
40
20
0
G0/G1
S
G2/M
82.29
74.42
5.5
9.66
11.57
15.08
A549/miRZip224
A549/miRZipCont
b
H460/shTNFAIP1
H460/shCTL
c
H1299/miR224
SMAD4
Cont
H1573/miR224
SMAD4
Cont
d
TGF-β (+)
TGF-β (-)
H460/miR224
H460/miRCont
e
Left: A549/miRZipCont; Right: A549/miRZip224
P=0.009
Tumor volume (mm 3)
700
600
500
400
300
200
100
0
A549/miRZip224
A549/miRZipCont
f
H460/miR224
H460/miRCont

**Fig. S7. Representative pictures of cell migration assay and animal studies using miR-224 knockdown lung cancer cells.** (a) Proportion of cells in each cell-cycle phase in miR-224 knockdown A549 cells were determines by flow cytometry analysis. (b) Representative pictures of cell migration assay for TNFAIP1 knockdown H460 cells (× 100 magnification). (c) Representative pictures of cell migration assay for miR-224 overexpressing cells transfected with SMAD4 or empty vector. (× 100 magnification). (d) Representative pictures of cell migration assay for H460/miR224 and H460/miRCont cells treated with or without TGF-β. (× 100 magnification). (e) Tumor growth in nude mice s.c. injected into flanks with A549/miRZip224 or A549/miRZipCont after day 45. Data are presented as mean ± SD (n=6 per group). (f) Representative pictures of lung metastasis after s.c. injection of H460/miR-224 or H460/miRCont cells. Red arrow indicates tumor mass in lung in H460/miR-224 injected mice.

**SI Tables**

| miR ID | Fold change (N1+ vs N0) | P value |
|---|---|---|
| hsa-miR-371b-5p | 0.01 | 1.37E-05 |
| hsa-miR-371a-3p | 0.01 | 1.37E-05 |
| hsa-miR-373-3p | 0.04 | 2.35E-03 |
| hsa-miR-34b-5p | 0.06 | 4.99E-03 |
| hsa-miR-3975 | 47.65 | 1.82E-05 |
| hsa-miR-1269a | 28.69 | 2.15E-04 |
| hsa-miR-31-3p | 27.17 | 1.65E-04 |
| hsa-miR-224-5p | 21.07 | 3.46E-04 |
| hsa-miR-147b | 19.55 | 4.58E-04 |
| hsa-miR-31-5p | 17.07 | 7.38E-04 |
| hsa-miR-3977 | 13.49 | 1.72E-03 |
| hsa-miR-1269b | 12.60 | 2.07E-03 |
| mir-novel-59 | 12.41 | 2.79E-03 |
| hsa-miR-363-3p | 11.84 | 2.72E-03 |
| hsa-miR-452-3p | 8.70 | 7.86E-03 |
| hsa-miR-224-3p | 8.44 | 8.31E-03 |

**Table S1.** Identification of differentially expressed miRNAs between N0 and N1+ groups of patients.

| Primers for 3'UTR mutagenesis | |
|---|---|
| TNFAIP1_3UTR_mut1_s | catgacggcgggacaggggaaatgtagtcttctaattaggcattttatgttagtc |
| TNFAIP1_3UTR_mut1_as | gactaacataaaatgcctaattagaagactacatttcccctgtcccgccgtcatg |
| TNFAIP1_3UTR_mut2_s | acatgatcttagcttctttaatcagactttgtagtctaaaagtttgggggttttctttgaaagtttcc |
| TNFAIP1_3UTR_mut2_as | ggaaactttcaaagaaaaccccccaaacttttagactacaaagtctgattaaagaagctaagatcatgt |
| SMAD4_3UTR_mut1_s | gtttggatatttttgtacttgatttgatgtagtctttttttggtataatgtttaaatcatgtatg |
| SMAD4_3UTR_mut1_ns | catacatgatttaaacattataccaaaaaagactacatcaaatcaagtacaaaaatatccaaac |
| SMAD4_3UTR_mut2_s | ctttgccatcaatgatcatatcaattggcagtagtcttgtatagagaatttaagtagaaaagttgcag |
| SMAD4_3UTR_mut2_as | ctgcaacttttctacttaaattctctatacaagactactgccaattgatatgatcattgatggcaaag |
| primers for SMAD4 cloning | |
| SMAD4_nhe_F | CTAGCTAGCACCATGGACAATATGTCTATTACG |
| SMAD4_not_R | ATAGTTTAGCGGCCGCTCAGTCTAAAGGTT |

**Table S2.** Primers for 3' UTR mutagenesis and SMAD4 cloning

| Methylation Probes (Chr:position) | Log2 (has-miR-224) | |
|---|---|---|
| | Pearson correlation coefficients | P-value |
| cg27049053 (chr X: 151142517) | -0.36 | 1.29E-08 |
| cg12204574 (chr X: 151142773) | -0.19 | 2.68E-03 |
| cg04929599 (chr X: 151142863) | -0.15 | 2.09E-02 |
| cg08783090 (chr X: 151143126) | -0.24 | 1.31E-04 |
| cg18748981 (chr X: 151143213) | -0.06 | 3.56E-01 |
| cg25528646 (chr X: 151143303) | -0.13 | 4.43E-02 |
| cg07053880 (chr X: 151143516) | -0.17 | 8.95E-03 |
| cg01480550 (chr X: 151143527) | -0.17 | 8.52E-03 |
| cg10276549 (chr X: 151143686) | -0.23 | 3.34E-04 |

**Table S3.** miR-224 expression from TCGA dataset and methylation probes in the promoter region of miR-224 from TCGA Illumina Infinium Human DNA Methylation 450k beadchip in NSCLC.