Supplemental Information

miR-409 Inhibits Human Non-Small-Cell Lung Cancer Progression by Directly Targeting SPIN1

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Figure S1

Figure S1. Screening for potential SPIN1-targeting miRNAs. Immunoblot analysis of the A549 cells transfected with empty vector, miR-23-3p, miR-409, miR-431-5p, or miR-489. Histograms on the right of the immunoblot graphs show corresponding expression levels of miRNAs by qRT-PCR. *p < 0.05 and **p < 0.01.
Figure S2. miR-409 regulates SPIN1 expression in a posttranscriptional way. Analysis of A549, H460 cells transfected with scramble or miR-409 mimics. The corresponding miRNA expression was determined using qRT-PCR. Each bar represents the mean of at least three independent experiments performed in triplicate ± standard deviation. *p < 0.05 and **p < 0.01.
Figure S3. miR-409 regulates the activation of PI3K/AKT pathway. (A) Immunofluorescent staining were conducted in A549 cells transfected with miR-409. (B) CREB1 expression levels were assessed by qRT-qPCR. (C) BCL2 expression levels were assessed by qRT-qPCR. (D) Immunofluorescent staining were conducted in A549 cells transfected with anti-miR-409. (E) CREB1 expression levels were assessed by qRT-qPCR. (F) BCL2 expression levels were assessed by qRT-qPCR. *p < 0.05, **p < 0.01. (G) Immunoblot analysis of the A549 transfected with miR-409. (G) Immunoblot analysis of the A549 transfected with anti-miR-409.
Figure S4. SPIN1 is upregulated in NSCLC patients. SPIN1 expression was analyzed in human lung cancer tissues and adjacent tissues from 3 representative donors by Western blotting.
### Supplementary Table 1 Clinicopathological characteristics of NSCLC patients

| Characteristics | n (%)     |
|-----------------|-----------|
| **Age**         |           |
| ≥ 60            | 47 (55.3%)|
| < 60            | 38 (44.7%)|
| **Gender**      |           |
| Male            | 62 (72.9%)|
| Female          | 23 (27.1%)|
| **Tumor size (cm)** |     |
| > 3             | 34 (40.0%)|
| ≤ 3             | 51 (60.0%)|
| **TNM Stage**   |           |
| I               | 43 (50.6%)|
| II              | 31 (36.5%)|
| III             | 11 (12.9%)|
| **Pleural invasion** |   |
| NO              | 68 (80.0%)|
| Yes             | 17 (20.0%)|
| **Metastasis**  |           |
| NO              | 51 (60.0%)|
| Yes             | 34 (40.0%)|
**Supplementary Table 2** Primer sequences of Oligonucleotides

| Name | Forward (5’→3’) | Reverse (5’→3’) |
|------|-----------------|-----------------|
| **Primer sequences for real-time quantitative RT-PCR** | | |
| SPIN1 | GTTCTGGACCAGGTGCCCTGTAAA | CTGTCTCAAAACATATGTCCACTG |
| β-actin | ATCACCATTTGCAATGAGCG | TTGAAGGTAGTTTCGATGGAT |
| **Primers sequences for PCR** | | |
| SPIN1 3'UTR | ATGTCATCACAAACTCTGCC | TTTACAGTGAGGATTTGCACATAAA |
| SPIN1 3'UTR Mut | CTTGTCCAGGAAATTACCAGGA | TCCGTGTAATTCCTGGAACAAG |
| SPIN1 | ATGAAGACCCCATTCGGAAAGAC | CTAAGGATGTTCACCAAAATCGTA |