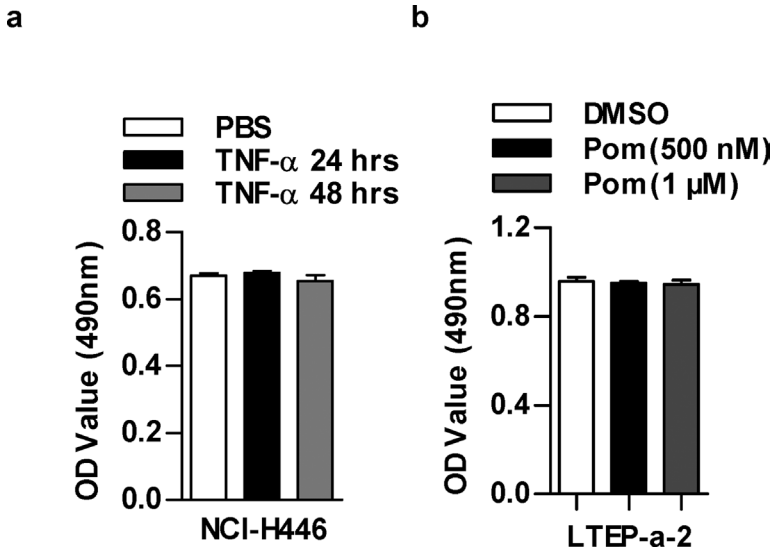
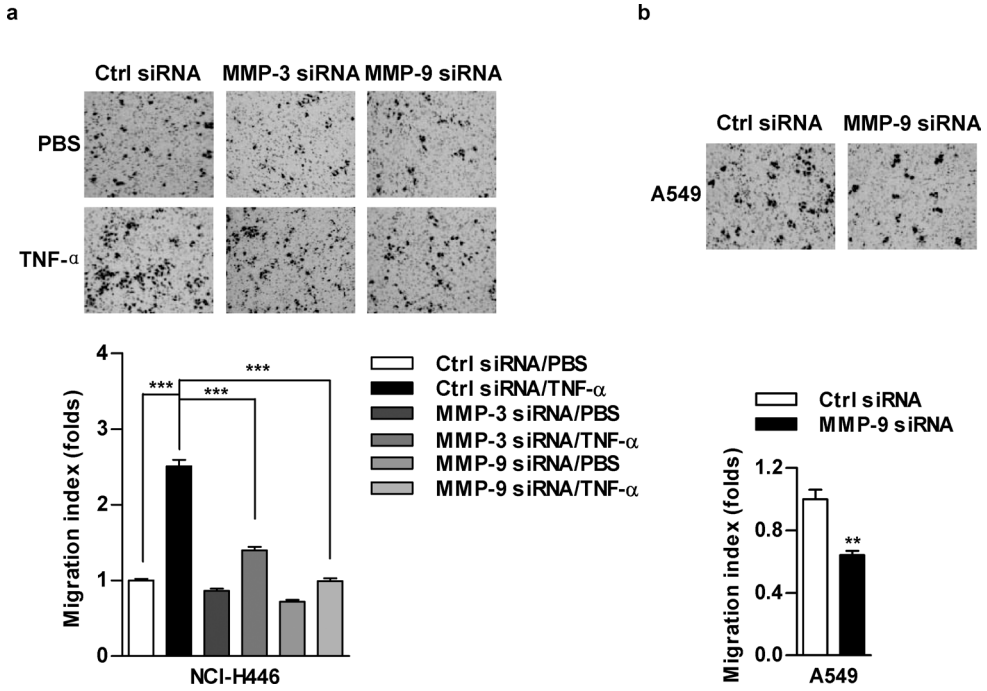
# Ataxia-telangiectasia mutated activation mediates tumor necrosis factor-alpha induced MMP-13 up-regulation and metastasis in lung cancer cells

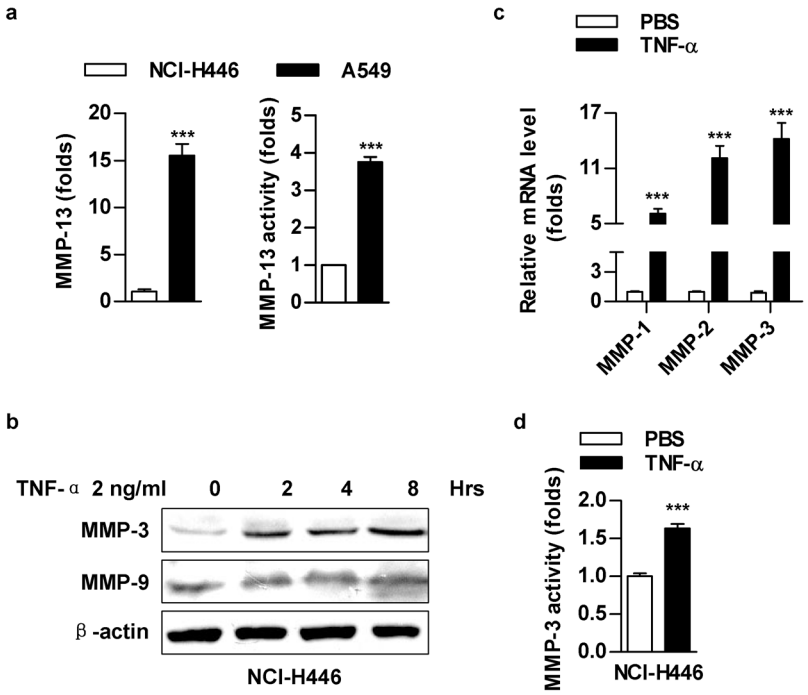
## SUPPLEMENTARY FIGURES AND TABLE



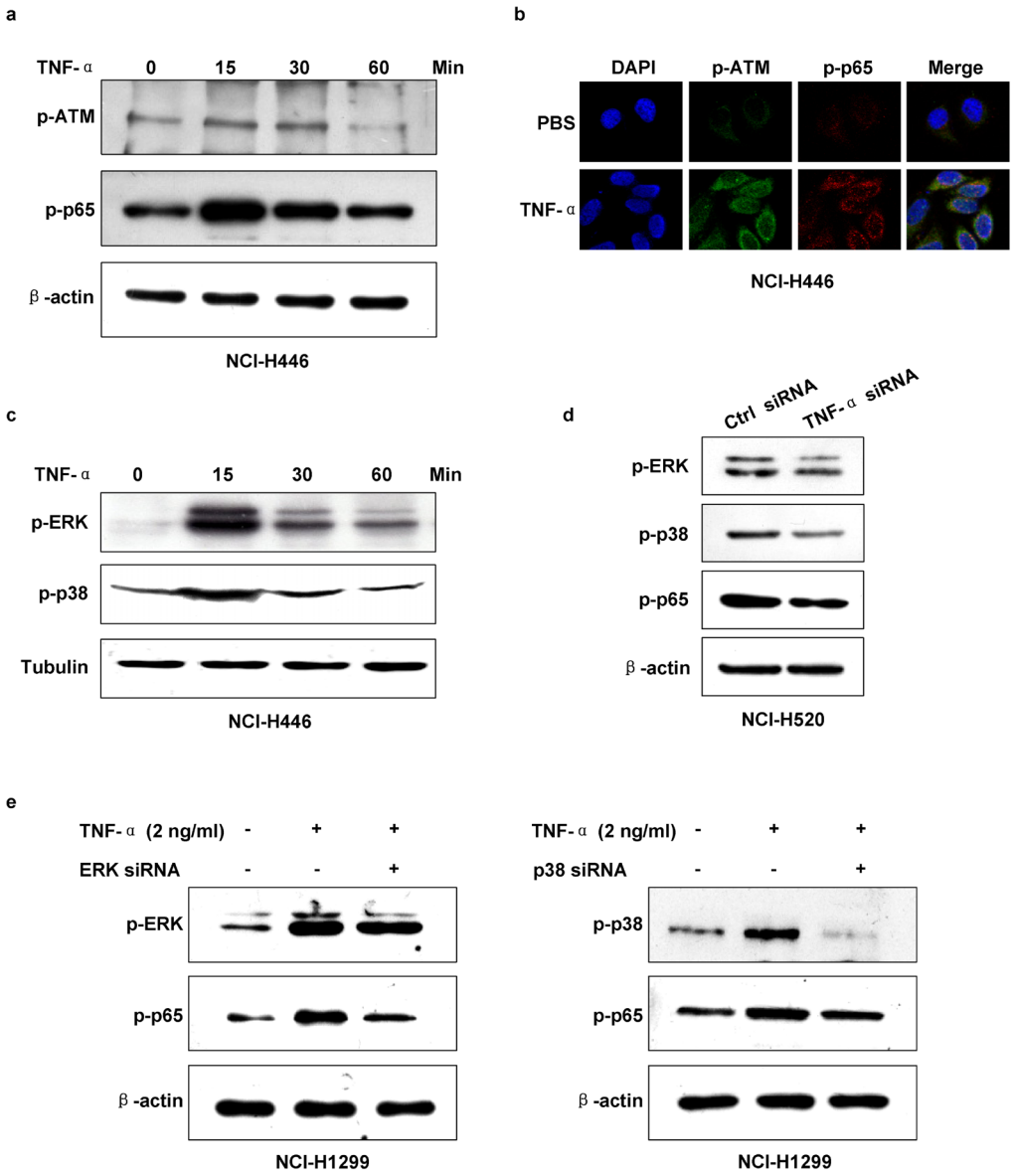

**Supplementary Figure S1: The effects of TNF-α and pomalidomide on lung cancer cell viabilities.** NCI-H446 **a.** or LTEP-a-2 **b.** cells were treated with TNF-α (2 ng/ml) (a) or TNF-α inhibitor Pom (b), respectively, for indicated period or concentration and cell viabilities were determined by MTT assay. Data are presented as the mean±SEM, n=3. One-way ANOVA with post Newman-Keuls test. Hours and pomalidomide was shown as Hrs or Pom for limited space in the figure.



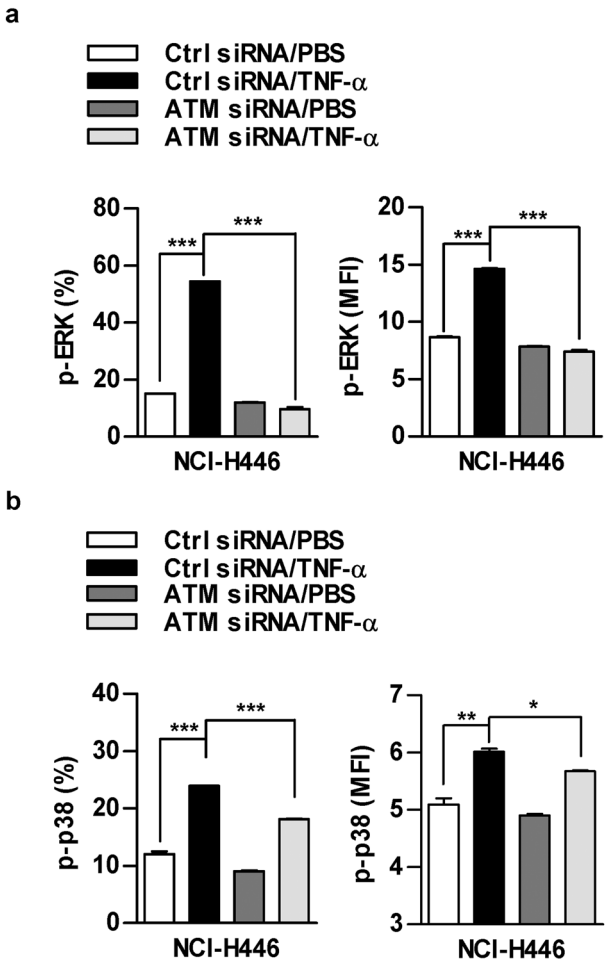

**Supplementary Figure S2: MMP-3 and MMP-9 are involved in TNF-α increasing lung cancer cell migration. a.** MMP-3 or MMP-9 deficient NCI-H446 and control cells were treated with TNF-α (2 ng/ml) and the effect of TNF-α on cell migration was determined by Transwell migration assay. **b.** A549 cells were conferred MMP-9 siRNA transfection and the cell migration was determined by Transwell migration assay. Data are presented as the mean±SEM, n=3. **p<0.01; ***p<0.001, Student *t* test or One-way ANOVA with post Newman-Keuls test. One representative from three experiments is shown.



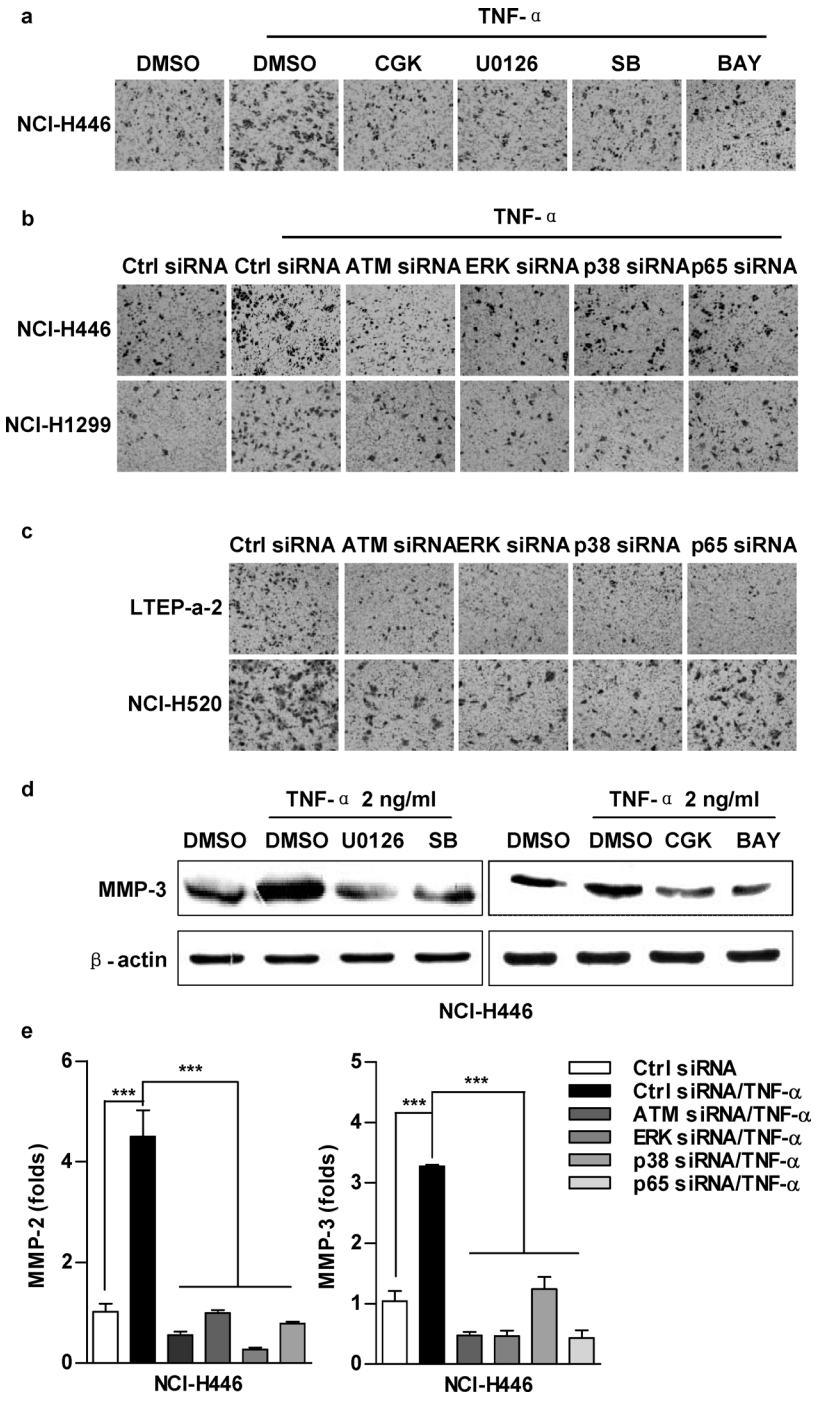

**Supplementary Figure S3: TNF-α increases MMPs expression and activity in lung cancer cells. a.** The cellular RNA and supernatant of NCI-H446 and A549 cells were prepared. The expression (left) and the activity (right) of MMP-13 was determined by RT-qPCR (left) or ELISA (right). **b-d.** NCI-H446 cells were treated with TNF-α (2 ng/ml) or control PBS. The expression (b-d) and activity (d) of indicated MMPs were determined by western blot (b), RT-qPCR (c) or ELISA (d), respectively. Data are presented as the mean±SEM, n=3. $^{***}p<0.001$, Student t test. One representative from three experiments is shown. The immunoblots were cropped to improve the clarity and conciseness of the presentation.



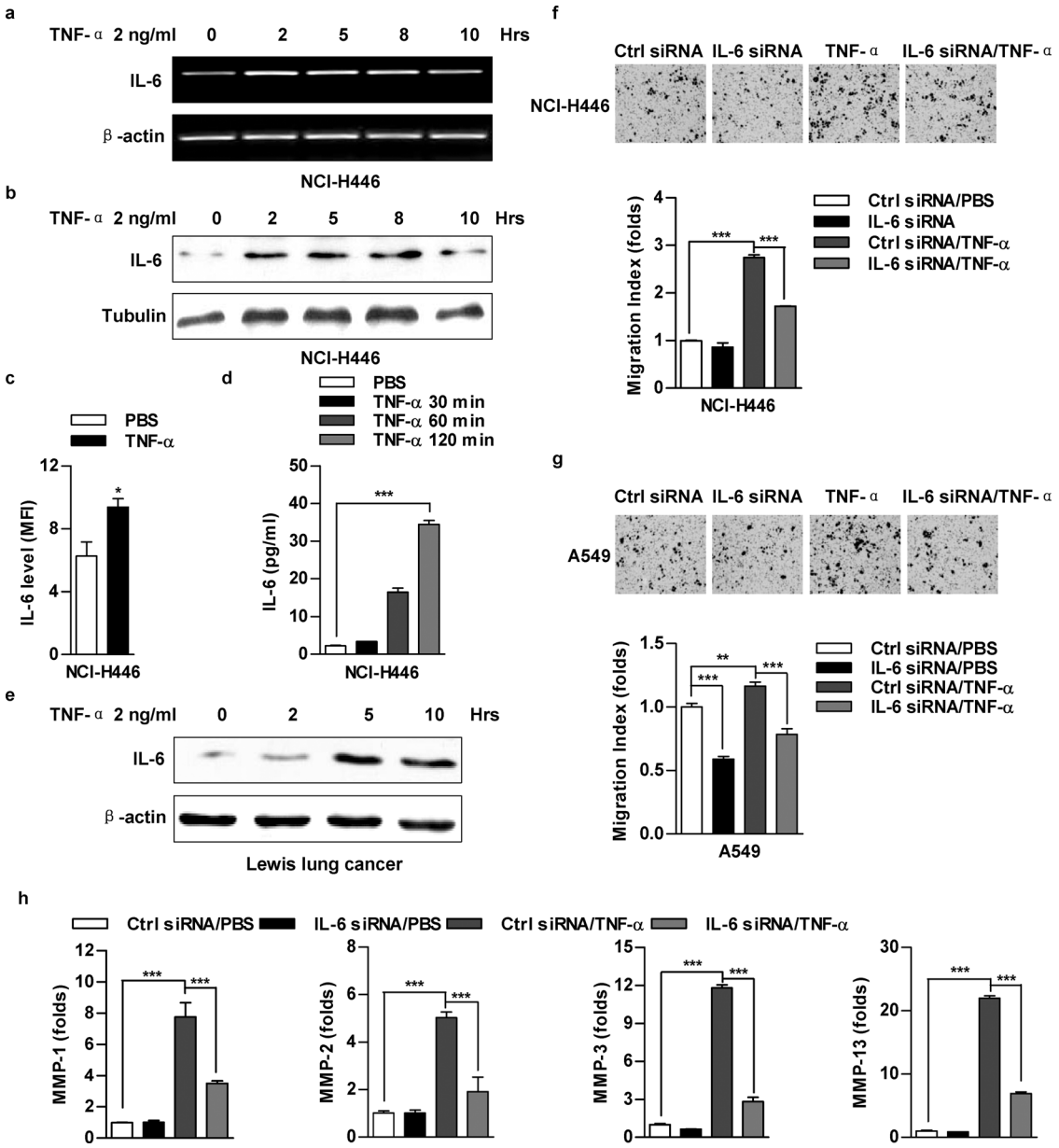

**Supplementary Figure S4: TNF-α activates ATM-ERK/p38- p65 pathway.** NCI-H446 cells **a-c.** were stimulated with TNF-α (2 ng/ml) for indicated periods while NCI-H1299 cells **d-e.** were conferred with indicated siRNA transfection, and the effect of TNF-α on related kinases were determined by western blot (a, c-e) and immunofluorescence observation (b). The immunoblots were cropped to improve the clarity and conciseness of the presentation.



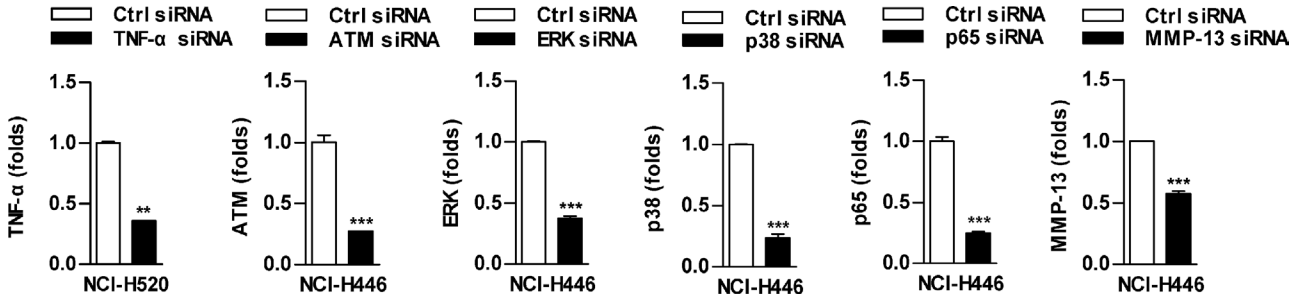

**Supplementary Figure S5: The deficiency of ATM decreases TNF-α-induced phosphorylation of ERK and p38.** NCI-H446 cells were conferred ATM siRNA transfection and further stimulated with TNF-α (2 ng/ml). The effects of TNF-α on ERK **a.**/ p38 **b.** activation in ATM deficient NCI-H446 cells was determined by flow cytometric analyses. Data are presented as the mean±SEM, n=3. *p<0.1; **p<0.01; ***p<0.001, One-way ANOVA with post Newman-Keuls test. One representative from three experiments is shown.




**Supplementary Figure S6: TNF-α up-regulate cell migrationg abilities and MMP-2/MMP-3 expression via ATM-ERK/p38-NF-κB pathway. a-c.** The migration images of Fig 5a-5c were shown, respectively. **d-e.** NCI-H446 cells were pretreated with indicated kinase inhibitors (d) or siRNA transfection (e) prior to TNF-α (2 ng/ml) stimulation. The effect of kinase inhibition on indicated MMPs expression was determined by western blot (d) and RT-qPCR (e), respectively. Data are presented as the mean±SEM, n=3. ***p<0.001, One-way ANOVA with post Newman-Keuls test. One representative from three experiments is shown. CGK733, SB203580 and BAY11-7082 were shown as CGK, SB and BAY, respectively, for limited space in the figure. The immunoblots were cropped to improve the clarity and conciseness of the presentation.




**Supplementary Figure S7: IL-6 is involved in TNF-α-increased MMPs expression and thereby augmented migration ability of lung cancer cells. a-e.** NCI-H446 (a-d) or Lewis lung cancer cells (e) cells were treated with TNF-α (2 ng/ml) for indicated periods and the expression of IL-6 was detected by RT-PCR (a), Western blot (b, e), flow cytometry (c) or ELISA assay (d). **f-h.** IL-6 deficient and control NCI-H446 (f, h) or A549 cells (g) were treated with TNF-α (2 ng/ml), and the effect of IL-6 deficiency on TNF-α-induced cell migration (f-g) and MMPs expression (h) were determined by Transwell migration assay (f-g) and RT-qPCR (h), respectively. Data are presented as the mean±SEM, n=3. $^{**}p<0.01$; $^{***}p<0.001$, Student t test or one-way ANOVA with post Newman-Keuls test. One representative from three experiments is shown. The immunoblots were cropped to improve the clarity and conciseness of the presentation.




**Supplementary Figure S8: siRNA transfection efficiently decreased relative gene expression in lung cancer cells.** NCI-H520 or NCI-H446 cells were conferred indicated siRNA transfection and the expressions of indicated gene were determined by RT-qPCR. Data are presented as the mean±SEM, n=3. **p<0.01; ***p<0.001, Student *t* test. One representative from three experiments is shown.

**Supplementary Table S1: Real-time PCR primers and small interference RNA sequences**

| Genes | F/R | Sequence |
|---|---|---|
| β-actin | F | 5'-TCAAGATCATTGCTCCTCCTG-3' |
| β-actin | R | 5'-CTGCTTGCTGATCCACATCTG-3' |
| Human TNF-α | F | 5'-CAGCCTCTTCTCCTTCCTGAT-3' |
| Human TNF-α | R | 5'-GCCAGAGGGCTGATTAGAGA-3' |
| MMP-13 | F | 5'-TTGTTGCTGCGCATGAGTTCG-3' |
| MMP-13 | R | 5'-GGGTGCTCATATGCAGCATCA-3' |
| Mouse TNF-α | F | 5'-ATGAGCACAGAA AGCATGATC-3 |
| Mouse TNF-α | R | 5'-TACAGGCTTGTCACTCGAATT-3' |
| MMP-1 | F | 5'-GGAGGGGATGCTCATTTTGATG-3 |
| MMP-1 | R | 5'-TAGGGAAGCCAAAGGAGCTGT-3' |
| MMP-2 | F | 5'-CCCCAAAACGGACAAAGAG-3' |
| MMP-2 | R | 5'-CTTCAGCACAAACAGGTTGC-3' |
| MMP-3 | F | 5'-CCTGCTTTGTCCTTTGATGC-3' |
| MMP-3 | R | 5'-TGAGTCAATCCCTGGAAAGTC-3' |