

Supplementary materials

Supplementary tables

Table S1. Clinical characteristics of study subjects

| | Never smokers | Smokers | Smokers with COPD/emphysema |
|------------------------------|---------------|----------------|--------------------------------|
| n | 20 | 20 | 20 |
| Age (years) | 64 ± 8.5 | 67 ± 10.7 | 69 ± 9.3 |
| Gender (male/female) | 20/0 | 20/0 | 20/0 |
| BMI (kg/m ²) | 23 ± 3 | 26 ± 4 | 24 ± 4 |
| Smoking history (pack-years) | 0 | 27 ± 12 | 36 ± 18 [#] |
| FEV1/FVC (%) | 86.49 ± 8.12 | 81.56 ± 6.00** | 60.41 ± 5.33*** [#] |

***p* < 0.05, compared with the non-smoker group, [#]*p* < 0.05 and ^{##}*p* < 0.01 compared with the smokers group.

Table S2. Sequences of primers used

| Gene Name | Sequences (5'-3') |
|----------------------|---|
| <i>miR-125a-5p F</i> | <i>GGGTCCCTGAGACCCTTTAAC</i> |
| <i>miR-125a-5p R</i> | <i>CAGTGCGTGTCGTGGAGT</i> |
| <i>miR-34a-5p F</i> | <i>GGGTGGCAGTGTCTTAGCT</i> |
| <i>miR-34a-5p R</i> | <i>CAGTGCGTGTCGTGGAGT</i> |
| <i>miR-20a-5p F</i> | <i>GGGTAAAGTGCTTATAGTGC</i> |
| <i>miR-20a-5p R</i> | <i>CAGTGCGTGTCGTGGAGT</i> |
| <i>U6 F</i> | <i>CGCTTCGGCAGCACATATACTAAAATTGGAAC</i> |

| | |
|-------------------------|-------------------------------------|
| <i>U6 R</i> | <i>GCTTCACGAATTTGCGTGTCATCCTTGC</i> |
| <i>SIRT1 promoter F</i> | <i>TCATTCTGACTGTGATGACGA</i> |
| <i>SIRT1 promoter R</i> | <i>CTGCCACAGTGTCATATCCAA</i> |
| <i>TGF-β1 F</i> | <i>ACTGGAGTTGTACGGCAGTG</i> |
| <i>TGF-β1 R</i> | <i>GGGGCTGATCCCGTTGATTT</i> |
| <i>Snail F</i> | <i>ATGGAGTGCCTTTGTACCCG</i> |
| <i>Snail R</i> | <i>CAGTAACCACCCTGCTGAGG</i> |
| <i>p21 F</i> | <i>TAAGGACGTCCCACCTTGCC</i> |
| <i>p21 R</i> | <i>CTGAGGATCACCCCCAGGTA</i> |
| <i>GAPDH F</i> | <i>CCCTTAAGAGGGATGCTGCC</i> |
| <i>GAPDH R</i> | <i>ACTGTGCCGTTGAATTTGCC</i> |

Supplementary Figures and figure legends

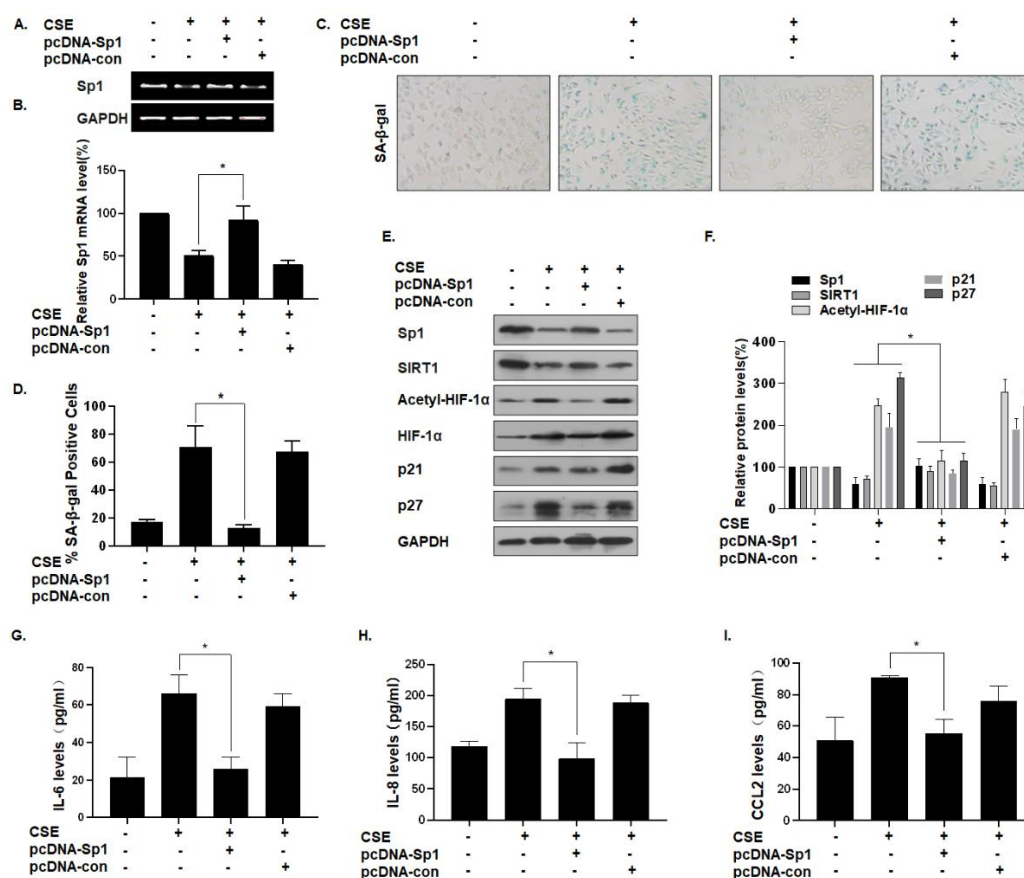


Figure S1. Sp1 is involved in CSE-mediated MLE-12 cell senescence and secretion of SASP factors.

Densities of bands were quantified by Image J software. GAPDH levels, measured in parallel, served as controls. MLE-12 cells were exposed to 0 or 8% CSE for 48 h after cells were transfected with an pcDNA-Sp1 or an pcDNA-con for 24 h. (A and B) The levels of Sp1 in MLE-12 cells were measured by RT-PCR (n = 3) and qRT-PCR (n = 3). (C) Photographs of senescence-associated β-galactosidase (SA-β-gal) staining and (D) the percentage of SA-β-gal positive cells (n = 3). (E) Western blots were performed, and (F) the relative protein levels of Sp1, SIRT1, acetyl-HIF-1α, HIF-1α, p21, and p27 in MLE-12 cells were determined (n = 3). The levels of IL-6 (G), IL-8 (H) and CCL2 (I) in MLE-12 cells were assessed by ELISA (n = 3). Values were

expressed as means \pm SD. * $p < 0.05$ compared with individual CSE treatment groups.