

## Supplementary Materials

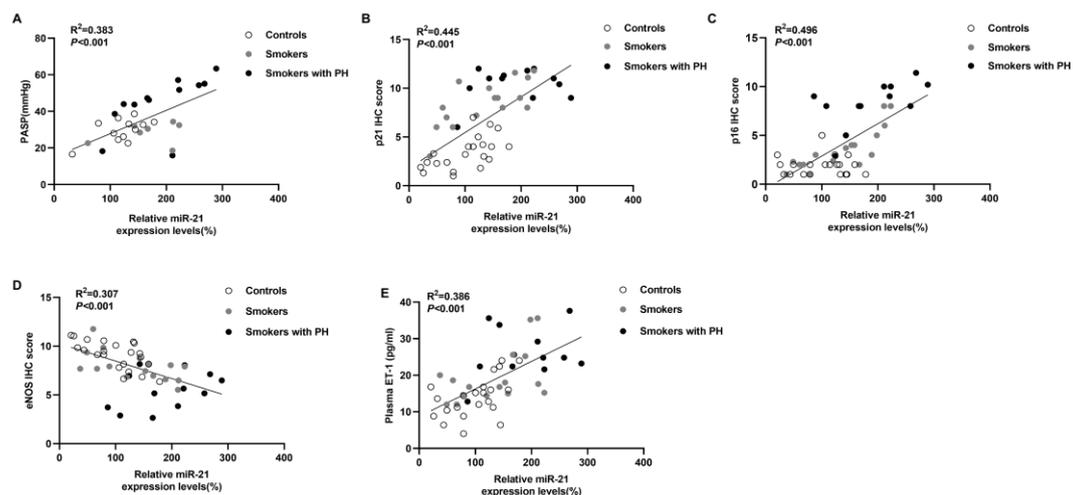
# miR-21-Mediated Endothelial Senescence and Dysfunction Are Involved in Cigarette Smoke-Induced Pulmonary Hypertension through Activation of PI3K/AKT/mTOR Signaling

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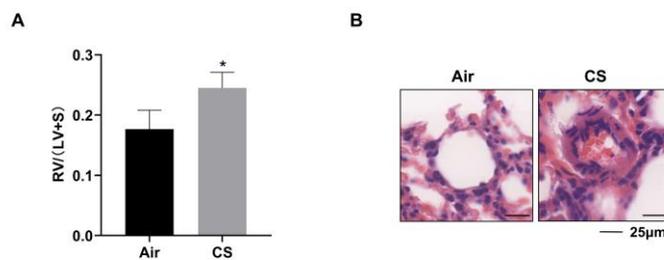
## Contents of supplementary information

### Supplementary figures.



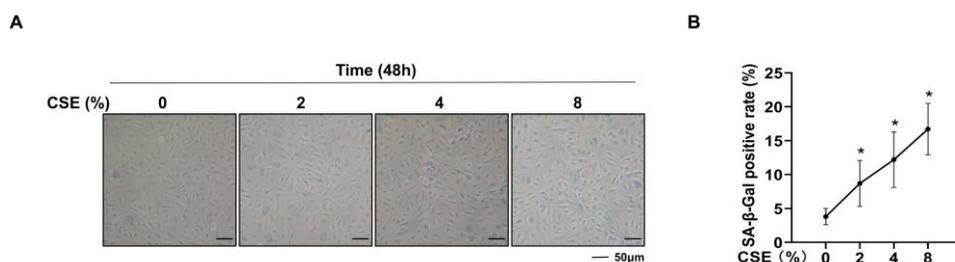
**Figure S1.** The correlation between miR-21 levels and PASP, markers of endothelial dysfunction and markers of cell senescence in lung tissues from patients

(A) miR-21 levels in lung tissues positively correlate with pulmonary artery systolic pressure (PASP). Only 31 patients (12 controls, 7 smokers, and 12 smokers with PH) were utilized, since their tricuspid regurgitation velocity (TRV) was measurable, and PASP could be calculated. (B, C) miR-21 levels positively correlate with IHC scores of p21- and p16-positive endothelial cells (ECs) in lung tissues from patients. (D) Upregulation of miR-21 levels correlates with decreased eNOS expression in lung tissues, and (E) raised plasma ET-1 levels of patients, related to miR-21 expression.



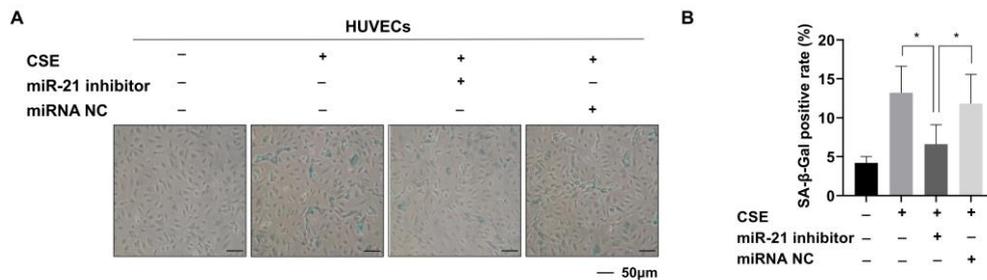
**Figure S2. CS exposure induces right-heart hypertrophy and pulmonary vascular proliferation in mice.**

(A) The degree of RV hypertrophy, given as the ratio of RV to the left ventricular plus septum (LV+S) mass was calculated for mice. (B) H&E staining of small pulmonary vessels (diameter: 0-70 μm) from mice (bars: 25 μm). \*  $p < 0.05$  compared with mice exposed to air.



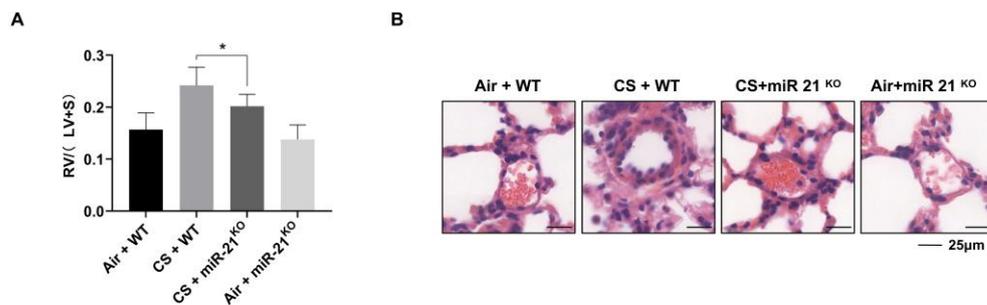
**Figure S3. CSE promotes the senescence of HUVECs.**

(A) Representative SA- $\beta$ -gal staining images of cells (blue-stained positive cells) and (B) the percentage of positive cells (n = 3, Scale bars: 50  $\mu$ m). \*  $p < 0.05$  different from HUVECs not treated with CSE.



**Figure S4. Downregulation of miR-21 alleviated the senescence of HUVECs induced by CSE.**

(A) Representative SA- $\beta$ -gal staining images of cells (blue-stained positive cells) by using the miR-21 inhibitor. (B) the percentages of positive cells (n = 3, Scale bars: 50  $\mu$ m). \*  $p < 0.05$  different from HUVECs treated with 8% CSE and miR-21 inhibitor.



**Figure S5. Knockout of miR-21 alleviates right-heart hypertrophy and pulmonary vascular proliferation induced by CS exposure in mice.**

(A) The ratios of RV and the left ventricular plus septum (LV+S) mass were calculated for mice. (B) H&E staining of small pulmonary vessels (diameter: 0-70  $\mu$ m) from mice (bars: 25  $\mu$ m). \*  $p < 0.05$  compared with WT mice exposed to CS.