Supplementary information

Tanshinone IIA sulfonate protects against cigarette smoke-induced COPD and down-regulation of CFTR in mice

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Running title: TIIA inhibits cigarette smoke-induced COPD

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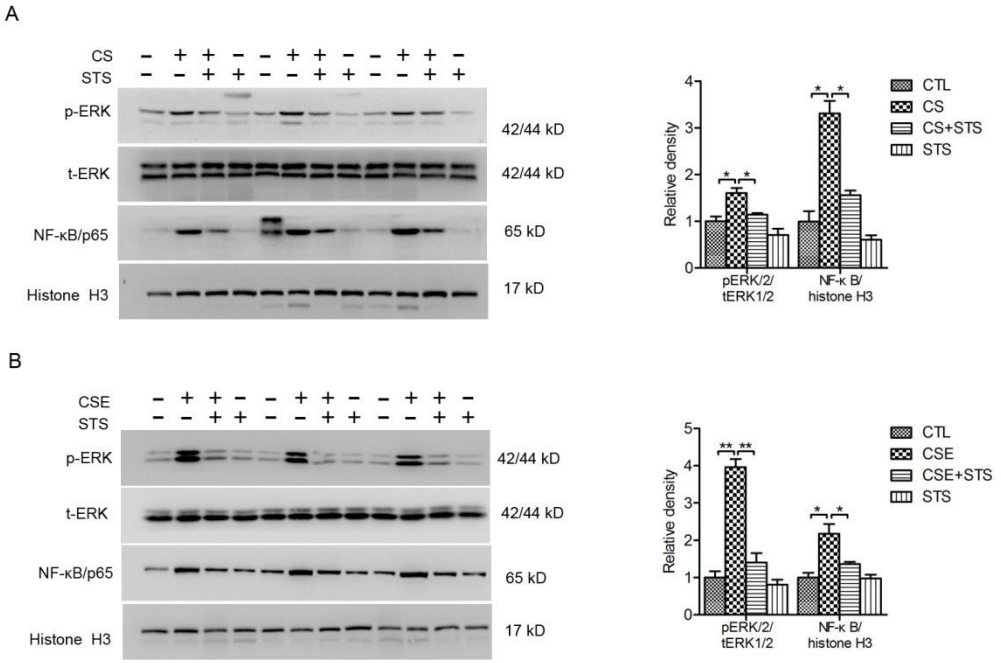
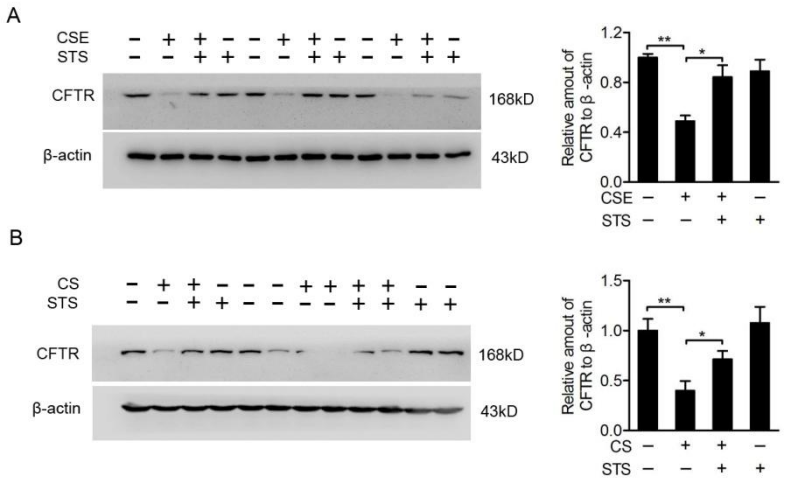
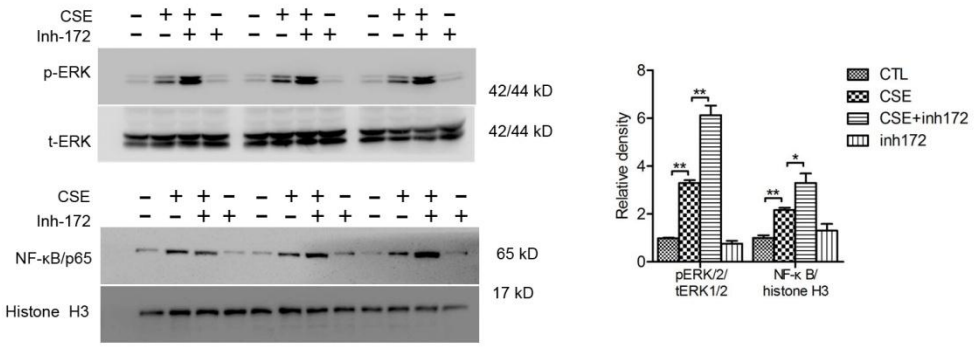


Supplementary figure 1. STS reduces CS-induced activation of ERK/NF-κB pathway in 16HBE cells and mouse lungs. (A) Levels of phosphorylated ERK1/2 in cytosol protein and levels of NF-κB in nuclear protein from lung tissue of mice exposed to CS with or without STS treatment**.** *P< 0.05 and **P< 0.01, n=3. CSE, cigarette smoke extract; CS, cigarette smoke. (B) 16HBE cells were treated with 2% CSE for 1 h with or without 2 h of STS pretreatment. Levels of ERK1/2 and phosphorylated ERK1/2 in cytosol protein and levels of NF-κB with histone H3 as loading control in nuclear protein**.** *P< 0.05 and **P< 0.01. CFTR, cystic fibrosis transmembrane conductance regulator; CSE, cigarette smoke extract; CS, cigarette smoke; STS, Sodium tanshinone IIA sulfonate.



Supplementary figure 2. STS protects against CS-induced reduction of CFTR in 16HBE cells and mouse lungs. 16HBE cells were treated with 2 % CSE for 48 h with 2 h of STS (10 μg/ml) pre-incubation. (A) The level of CFTR in total cell extract was determined with β-actin as internal control (n=4 per group). (B) Western blotting tested the impact of STS on CFTR expression in lungs from CTL, CS, CS plus STS (CS+STS) and STS-treated mice (n=6 mice per group). *P< 0.05 and **P< 0.01. CFTR, cystic fibrosis transmembrane conductance regulator; CSE, cigarette smoke extract; CS, cigarette smoke; STS, Sodium tanshinone IIA sulfonate.



Supplementary figure 3. CFTR inhibition increases CSE-induced activation in ERK/NF-κB pathway. 16HBE cells were treated with 2% CSE for 1 h with 2 h of STS (10 μg/ml) pre-incubation. In order to inhibit CFTR function, cells were treated with CFTR specific inhibitor CFTR-Inh172 (10 μM) 1 h prior to STS treatment. The levels of phosphorylated ERK1/2 in cytosol protein were determined with ERK1/2 as internal control (n=3). The level of p65/NF-κB in nuclear extraction was determined with histone H3 as internal control (n=6). *P< 0.05 and **P< 0.01, CFTR, cystic fibrosis transmembrane conductance regulator; CSE, cigarette smoke extract; STS, Sodium tanshinone IIA sulfonate.