SIRT1 protects against emphysema via FOXO3-mediated reduction of premature senescence in mice

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Supplemental Figure Legends

Supplemental Figure 1. Lung level of SIRT1 is decreased in mice exposed to CS and elastase, as well as in Sirt1+/− mice, whereas the level of SIRT1 is increased in Sirt1 Tg mice. The level of SIRT1 was determined by immunoblotting in mouse lung. (A and B) SIRT1 level was significantly reduced in WT mouse lung after 3 d and 6 mo CS exposures (A) as well as after elastase administration (B). (C) Lung SIRT1 protein level was significantly decreased in Sirt1+/− mice, but augmented in Sirt1 Tg mice as compared to their WT littermates. Sal: saline; Ela: elastase. Gel pictures shown are representative of at least 3 separate mice. Band density was expressed as fold change relative to corresponding β-actin. **P<0.01, ***P<0.001, significant compared with the corresponding air- or saline-exposed mice; ++P<0.01, +++P<0.001, significant compared with WT mice.

Supplemental Figure 2. Spontaneous airspace enlargement occurs in Sirt1+/− mice at the age of 1 yr, and an age-dependent decrease of SIRT1 can be seen in mouse lung. (A and B) No significant alteration in Lm of airspace was observed in Sirt1+/− mice until the age of 1 yr. (C) Lung SIRT1 level was decreased in an age-dependent manner. H&E-stained pictures represent three separate mice. Original magnification, ×100. Scale bar: 100 μm. Band density was expressed as fold change relative to corresponding β-actin. Data are shown as mean ± SEM (n=3 per group). The gel pictures shown are representative of at least 2 separate mice. *P<0.05, **P<0.01, significant compared with 4 mo old mice.

Supplemental Figure 3. SIRT1 prevents decline in lung function, impairment of exercise capacity, and reduction in arterial oxygen saturation in emphysematous mice. (A and B) R_L
was significantly decreased in *Sirt1*+/− mice (A), whereas no alteration of RL was seen in WT or *Sirt1* Tg (B) mice exposed to CS for 6 mo. (C and D) There was no significant change of Rn among *Sirt1*+/−, *Sirt1* Tg, and WT mice exposed to CS for 6 mo. (E-H) Deficiency of *Sirt1* decreased treadmill running time (E) and running distance (F), whereas no change in run time (G) or distance (H) was observed in *Sirt1* Tg mice exposed to CS for 6 mo. (I and J) Elastase injection significantly decreased RL (I) and arterial oxygen saturation (J) in WT mice, which were aggravated in *Sirt1*+/− mice. (K) No alteration of Rn was observed between *Sirt1*+/− mice and WT littermates after intratracheal elastase injection. Sal: saline; Ela: elastase. Data are shown as mean ± SEM (n=3 to 4 per group). *P<0.05, **P<0.01, ***P<0.001, significant compared with the corresponding air- or saline-exposed groups; †P<0.05, significant compared with the corresponding WT mice.

**Supplemental Figure 4. SRT1720 attenuates elastase-induced reduction in RL and arterial oxygen saturation associated with increased SIRT1 activity.** (A and B) Treatment with SRT1720 (50-100 mg/kg) before the development of emphysema attenuated elastase-induced reduction in RL (A) and arterial oxygen saturation (B) in WT mice. (C and D) SRT1720 administration (100 mg/kg) before the development of emphysema did not exhibit any effect on RL (C) or arterial oxygen saturation (D) in *Sirt1*+/− mice. (E) SIRT1 activity in lungs was significantly increased in WT, but not in *Sirt1*+/− mice, after SRT1720 (100 mg/kg) treatment before the development of emphysema. (F and G) SRT1720 administration (100 mg/kg) after the development of emphysema improved the reduced RL (F) and arterial oxygen saturation (G) in WT, but not in *Sirt1*+/− mice. Sal: saline; Ela: elastase; Veh: vehicle; SRT: SRT1720. Data are shown as mean ± SEM (n=3 to 4 per group). **P<0.01, ***P<0.001, significant compared with
corresponding saline-exposed groups; \( ^{+}P<0.05, ^{++}P<0.01, ^{+++}P<0.001 \), significant compared with corresponding Veh-treated mice; \( ^{\dagger}P<0.05 \), significant compared with WT mice.

**Supplemental Figure 5.** CS exposure decreases SIRT1 level in BAL cells and lung epithelial cells, as well as the confirmation of airway epithelium- and myeloid cell-specific deficiency of *Sirt1* in mice. (A and B) CS exposure for 6 mo significantly reduced the level of SIRT1 in BAL cells (A) and in lungs (B) of 129/SvJ mice. (C) There was no expression of SIRT1 in BAL cells (predominantly macrophages) from Mac-*Sirt1*\(^{-/-}\) mice as compared to WT control. (D) The level of SIRT1 was abolished in BAL cells from Mac-*Sirt1*\(^{-/-}\) mice. (E) SIRT1 expression was not seen in airway epithelium of Epi-*Sirt1*\(^{-/-}\) mice as compared to WT control. (F and G) The expression of SIRT1 in CC10-positive cells was not observed in lungs (F) and isolated Clara cells (G) from Epi-*Sirt1*\(^{-/-}\) mice. Scale bar: 50 \( \mu \)m. Band density was expressed as fold change relative to corresponding \( \beta \)-actin. Data are shown as mean ± SEM (n=3 per group). ***\( P<0.001 \), significant compared with air-exposed or WT mice.

**Supplemental Figure 6: Elastase-induced decrease in exercise capacity and arterial oxygen saturation are aggravated in mice deficient of *Sirt1* in airway epithelium, but not in myeloid cells.** (A) Elastase-induced reduction of treadmill run time was decreased in Epi-*Sirt1*\(^{-/-}\) mice as compared to WT littermates. (B) *Sirt1* deficiency in airway epithelium lowered the arterial oxygen saturation in response to intratracheal administration of elastase. (C) There was no change of Rn between Epi-*Sirt1*\(^{-/-}\) mice and WT littermates exposed to elastase (D and E) Elastase-induced a decrease in treadmill running time (D) and arterial oxygen saturation (E) were not altered between Mac-*Sirt1*\(^{-/-}\) mice and WT littermates in response to elastase administration. (F) No alteration in Rn was observed between Mac-*Sirt1*\(^{-/-}\) mice and WT
littermates exposed to elastase. Sal: saline; Ela: elastase. Data are shown as mean ± SEM (n=3 to 4 per group). **P<0.01, ***P<0.001, significant compared with corresponding saline-treated groups; †P<0.05, significant compared with corresponding WT littermates.

**Supplemental Figure 7. SIRT1 regulates CS-induced FOXO3 degradation and acetylation, and CS disrupts the interaction of SIRT1 with FOXO3 in mouse lung.** (A) CS exposure for 6 mo significantly reduced the level of FOXO3 in lungs of Sirt1+/− mice versus WT mice, which was attenuated by Sirt1 overexpression. (B) Administration of SRT1720 attenuated FOXO3 reduction in mouse lung exposed to CS for 3 d. (C) SIRT1 protected against FOXO3 acetylation in mouse lung in response to 6 mo of CS exposure. (D) CS disrupted SIRT1 interaction with FOXO3 in mouse lung at both 3 d and 6 mo exposures. Gel pictures shown are representative of at least 3 separate mice. Band density was expressed as fold change relative to corresponding β-actin. *P<0.05, ***P<0.001, significant compared with air-exposed mice; ‡‡P<0.01, significant compared with WT littermates; ††P<0.01, significant compared with Veh-treated mice.

**Supplemental Figure 8. SIRT1 level is decreased, whereas SA-β-gal activity and p21 expression are increased in lungs of patients with COPD.** (A) The level of SIRT1 was decreased in lungs of patients with COPD as compared to non-smokers. Band density was expressed as fold change relative to corresponding β-actin. (B and C) The activity of SA-β-gal was increased in lungs of patients with COPD as compared to non-smokers by both its quantitative assay (B) and staining (C). SA-β-gal activity was expressed as observed fluorescence of 4-MU after normalization to protein content (mg) in its quantitative assay. (D) The p21 expression was increased in lungs of patients with COPD when compared to non-smokers by an immunohistochemical staining. Negative control denotes the staining without p21
antibody or X-gal. Original magnification, ×400. Scale bar: 50 μm. Data are shown as mean ± SEM. **P<0.01, ***P<0.001, significant compared with non-smokers.

Supplemental Figure 9. SIRT1 decreases CS-induced RelA/p65 acetylation, and reduces CS- and elastase-mediated inflammatory cell influx in lungs. (A) SIRT1 protected against 6 mo of CS-induced increase in RelA/p65 and its acetylation (on K310) in lung nuclear proteins. (B and C) Three days of CS exposure increased neutrophil influx in BAL fluid of Sirt1+/− mice versus WT littermates, which was attenuated by Sirt1 overexpression (C). (D and E) The number of total cells and macrophages in BAL fluid were increased in Sirt1+/− mice (D) as compared to WT littermates in response to 6 mo of CS exposure, which was significantly attenuated in Sirt1 Tg mice (E). (F and G) The number of neutrophils in BAL fluid was increased in Epi-Sirt1+/− (D), but not in Mac-Sirt1+/− (E), mice as compared to their WT littermates exposed to CS for 3 d. (H) Deficiency of Sirt1 further increased neutrophil influx in BAL fluid after elastase intratracheal injection. The gel pictures shown are representative of at least 3 separate mice. Sal: saline; Ela: elastase; Mac: macrophages. Data are shown as mean ± SEM (n=3 to 4 per group). *P<0.05, **P<0.01, ***P<0.001, significant compared with the corresponding air- or saline-exposed groups; +P<0.05, ++P<0.01, +++P<0.001, significant compared with the corresponding WT littermates.

Supplemental Figure 10. Treatment with SRT1720 and IKK2 inhibitor decrease CS- and elastase-mediated neutrophil influx in BAL fluid. (A) Treatment with SRT1720 prior to CS exposure for 3 d attenuated neutrophil influx in BAL fluid of WT mice, but not in Sirt1+/− mice. (B) Neutrophil influx into BAL fluid was reduced by SRT1720 in WT mice, but not in Sirt1+/− mice, after elastase intratracheal injection. (C) Lung SIRT1 activity was significantly increased
in WT, but not in Sirt1⁺⁄⁻, mice after SRT1720 treatment in response to 3 d of CS exposure. (D) IKK2 inhibitor treatment prior to CS exposure for 3 d was more effective in attenuating the neutrophil influx in BAL fluid of Sirt1⁺⁄⁻ mice as compared to WT mice. (E) Sirt1⁺⁄⁻ mice were sensitive to IKK2 inhibition in reducing neutrophils in BAL fluid after elastase treatment. Sal: saline; Ela: elastase; Veh: vehicle; SRT: SRT1720; IKKi: IKK2 inhibitor. Data are shown as mean ± SEM (n=3 to 4 per group). ***P<0.001, significant compared with corresponding air- or saline-exposed groups; †P<0.05, ††P<0.01, †††P<0.001, significant compared with corresponding vehicle-treated groups.
Supplemental Figure 6

A
Run time (min)

WT
Epi-Sirt1−/−

Sal Ela

B
Oxygen Sat. (%)

WT
Epi-Sirt1−/−

Sal Ela

C
Rn (cmH$_2$O x s/ml)

WT
Epi-Sirt1−/−

Sal Ela

D
Run time (min)

WT
Mac-Sirt1−/−

Sal Ela

E
Oxygen Sat. (%)

WT
Mac-Sirt1−/−

Sal Ela

F
Rn (cmH$_2$O x s/ml)

WT
Mac-Sirt1−/−

Sal Ela
Supplemental Figure 8

(A) Western blot analysis showing SIRT1 and β-actin expression levels in non-smoker and COPD groups.

(B) Bar graph illustrating SIRT1/β-actin fold change and SA-β-gal activity levels.

(C) Immunohistochemical staining for SA-β-gal activity in neg. control, non-smoker, and COPD groups.

(D) Immunostaining for p21 expression in the indicated groups.
Supplemental Figure 9

A

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B

Neutrophils (10^3/ml)

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Cells (10^5/ml)

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H

Neutrophils (10^3/ml)

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Sal Ela