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 R_L 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 R_L (**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 R_L 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 R_L (**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 R_L (**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 R_L (**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 μm. Band density was expressed as fold change relative to corresponding β-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; $^{\dagger\dagger}P<0.01$, 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

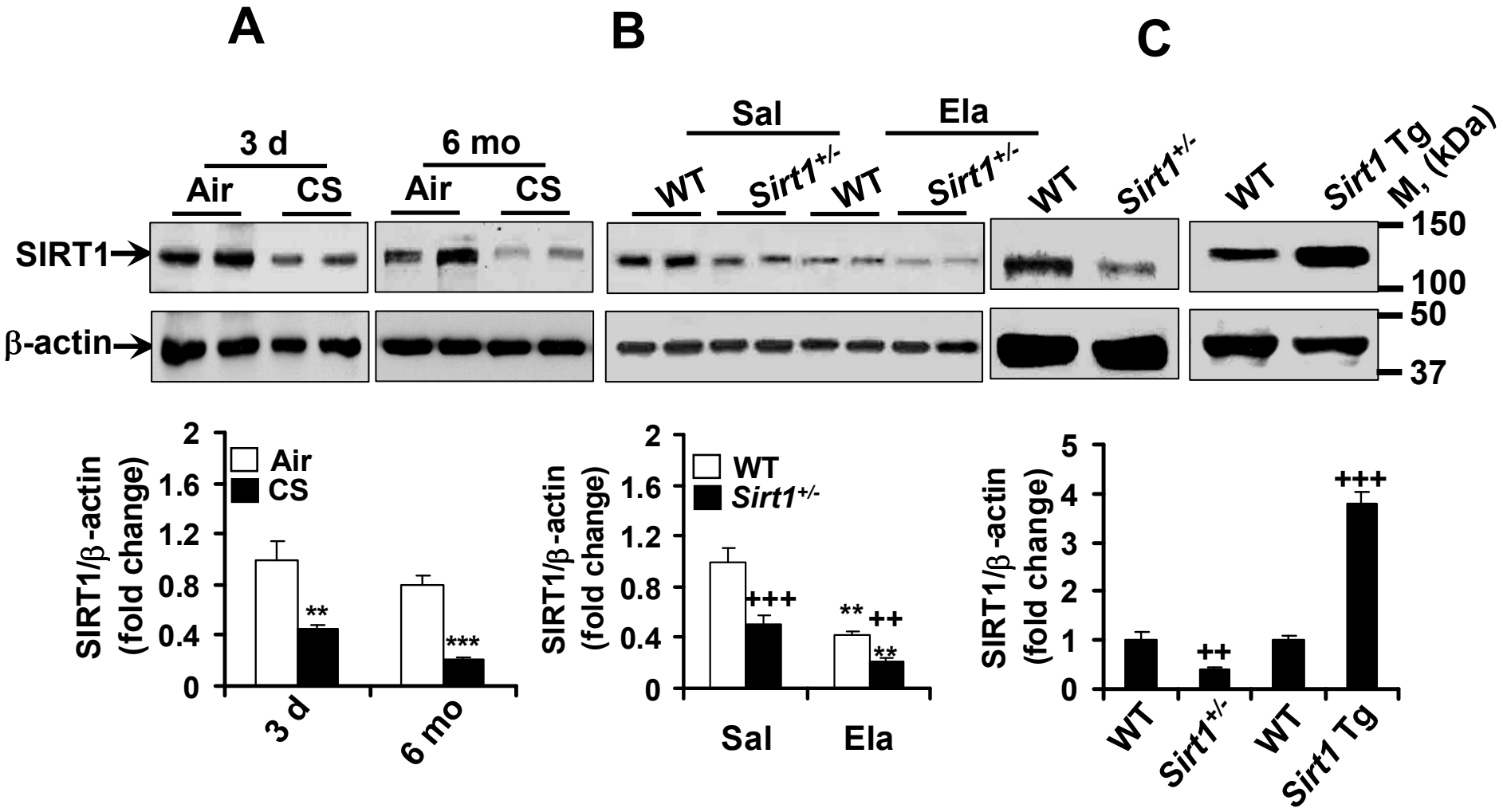
antibody or X-gal. Original magnification, ×400. Scale bar: 50 μm. Data are shown as mean ± SEM. $^{**}P<0.01$, $^{***}P<0.001$, significant compared with non-smokers.

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 (**B**) 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; $^{\dagger}P<0.05$, $^{\dagger\dagger}P<0.01$, $^{\dagger\dagger\dagger}P<0.001$, significant compared with corresponding vehicle-treated groups.

Supplemental Figure 1



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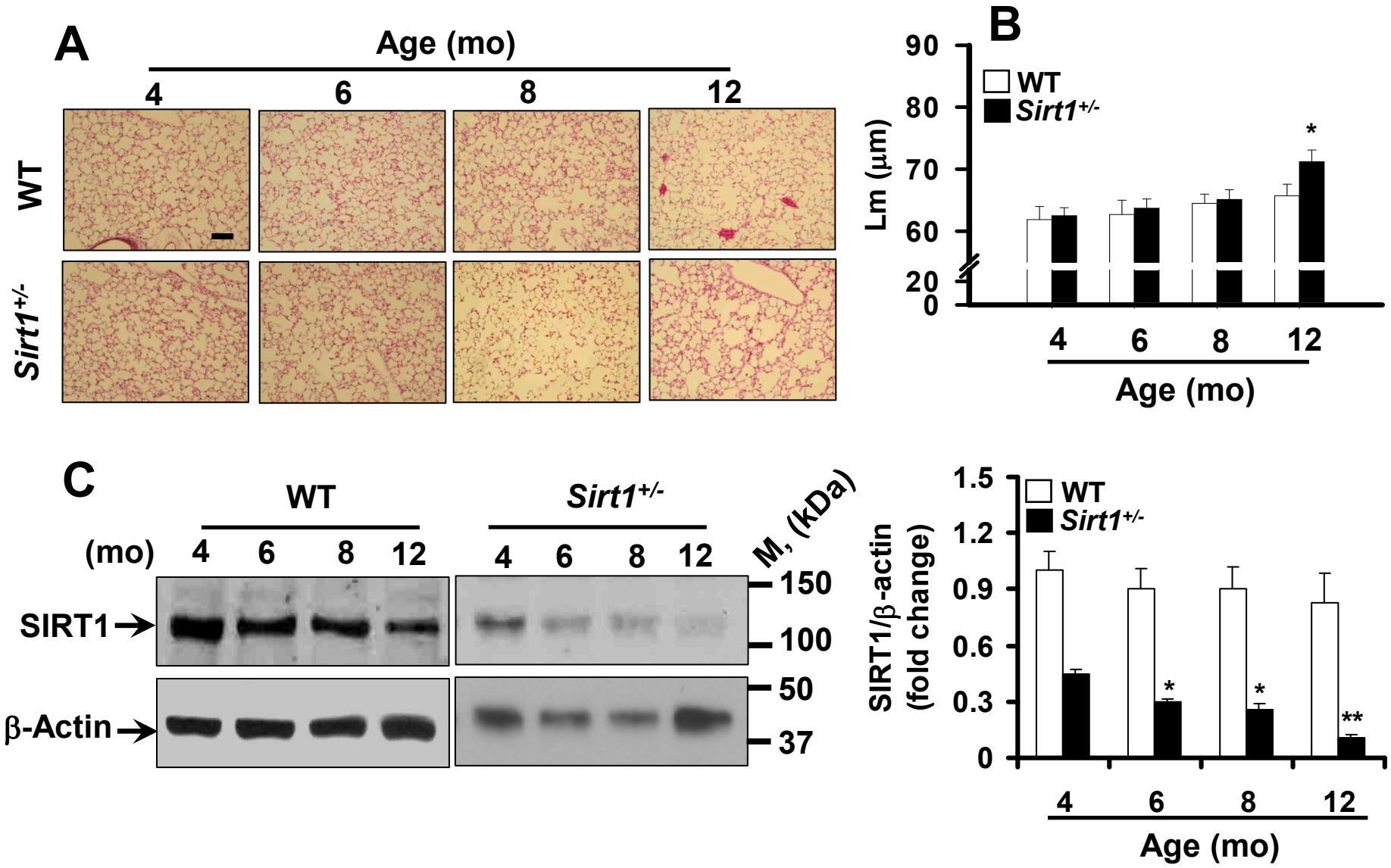

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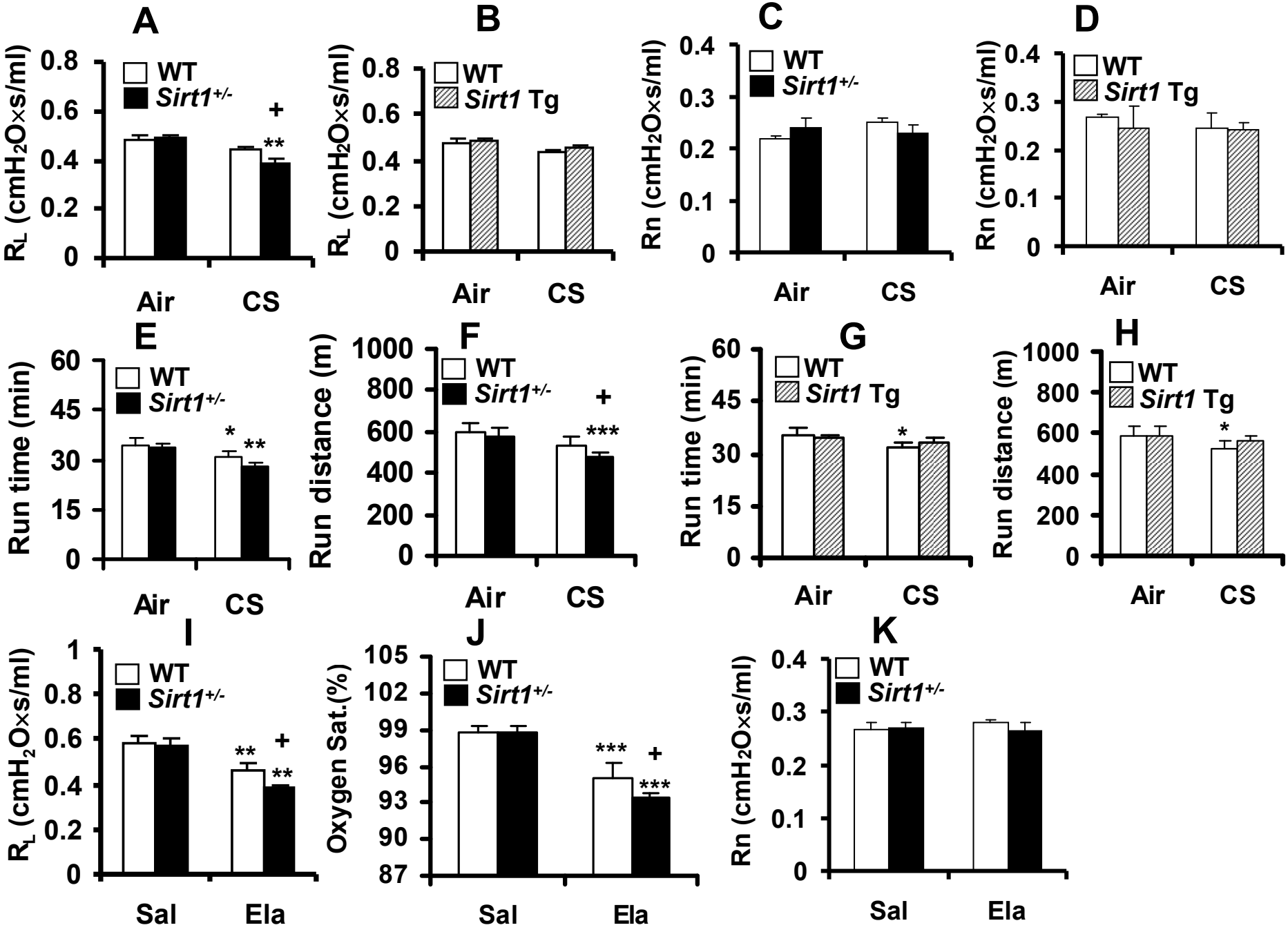

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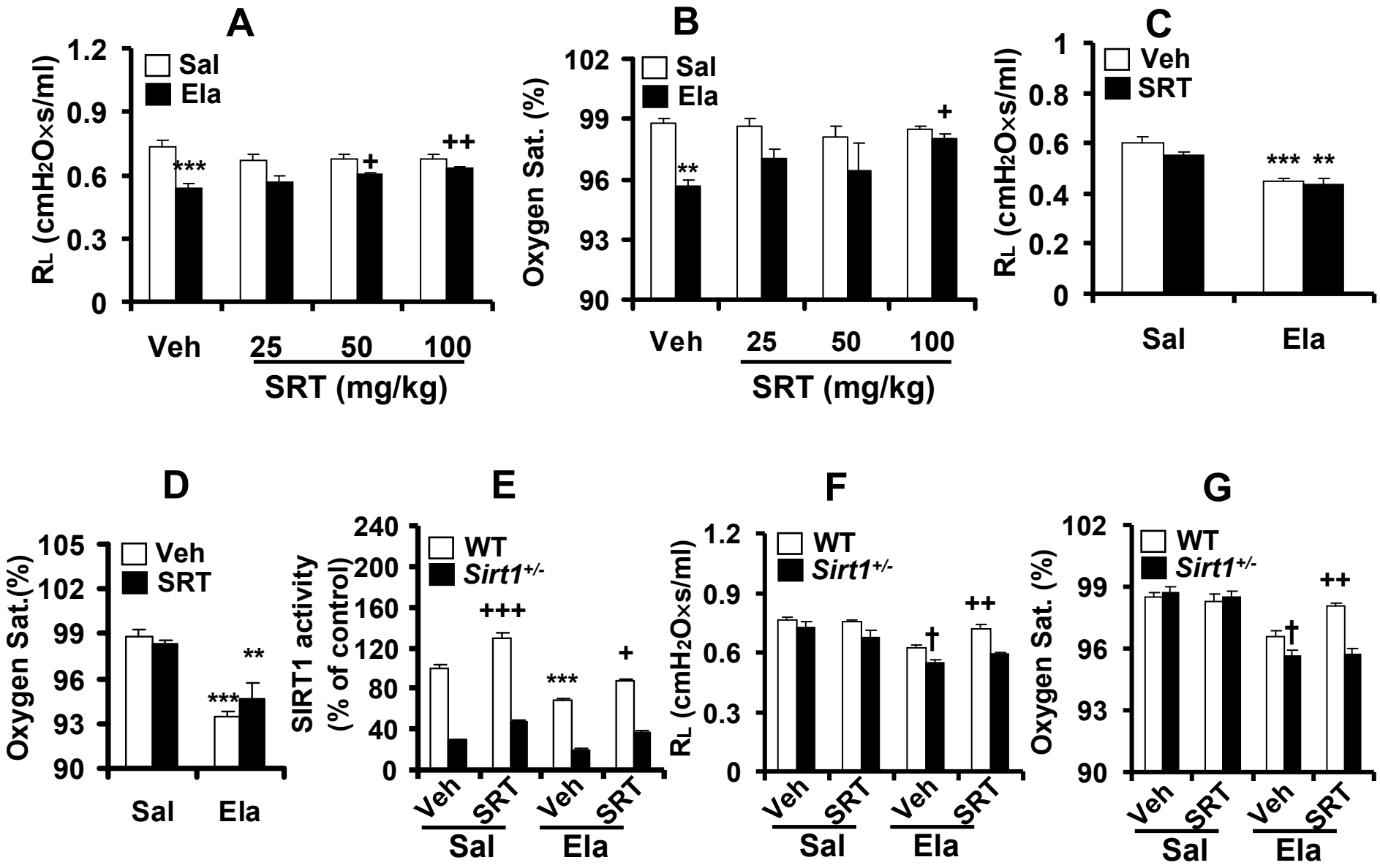

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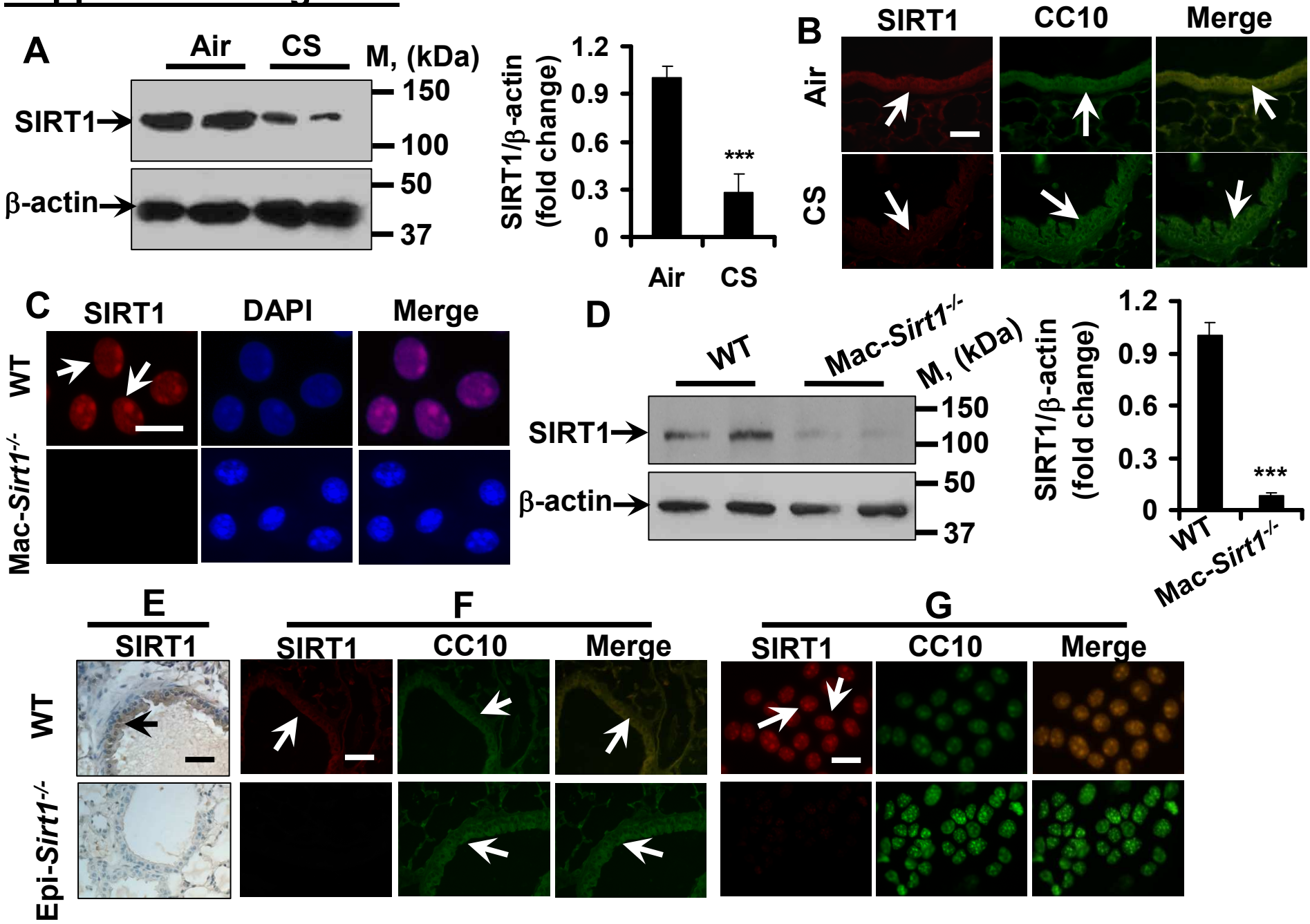

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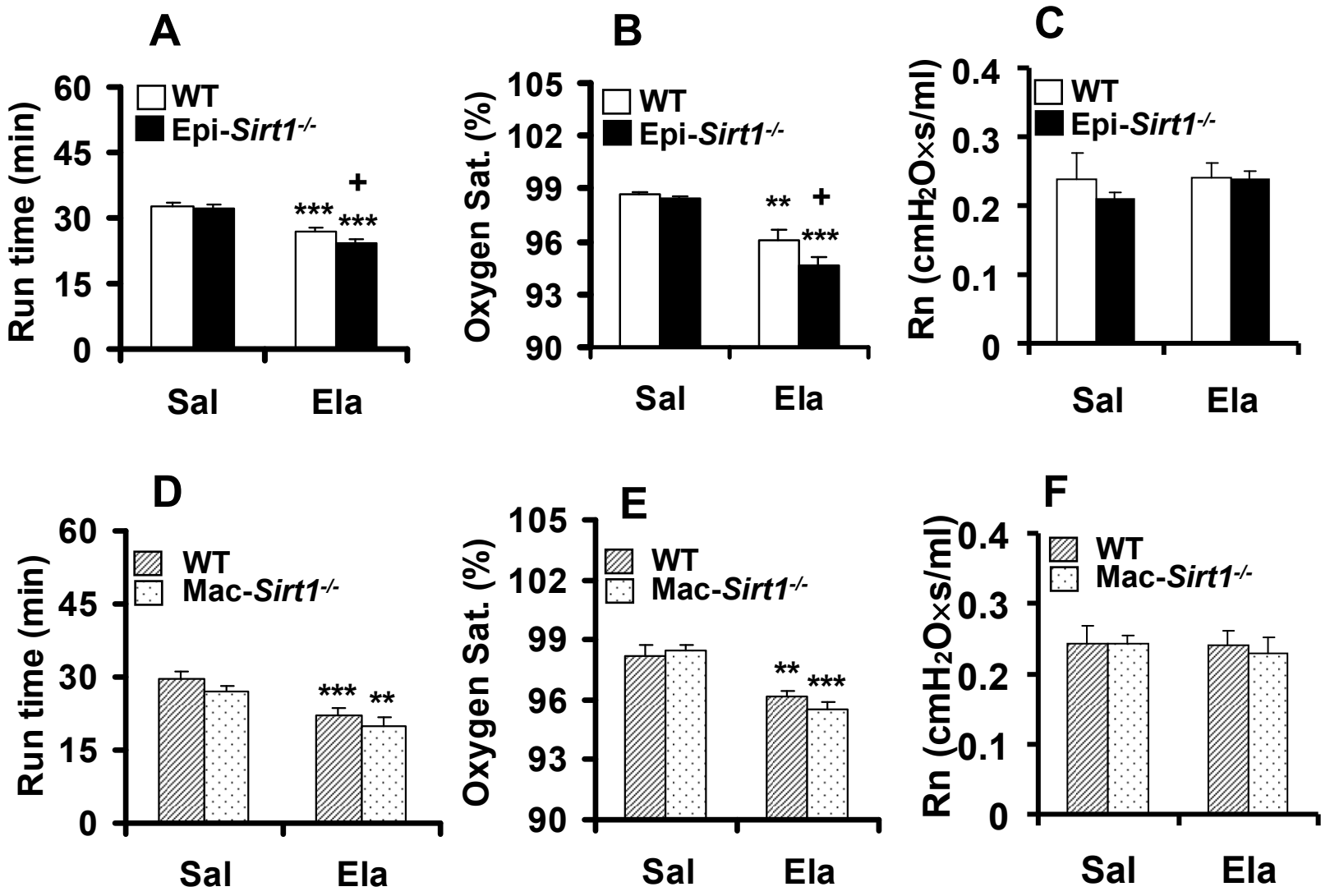

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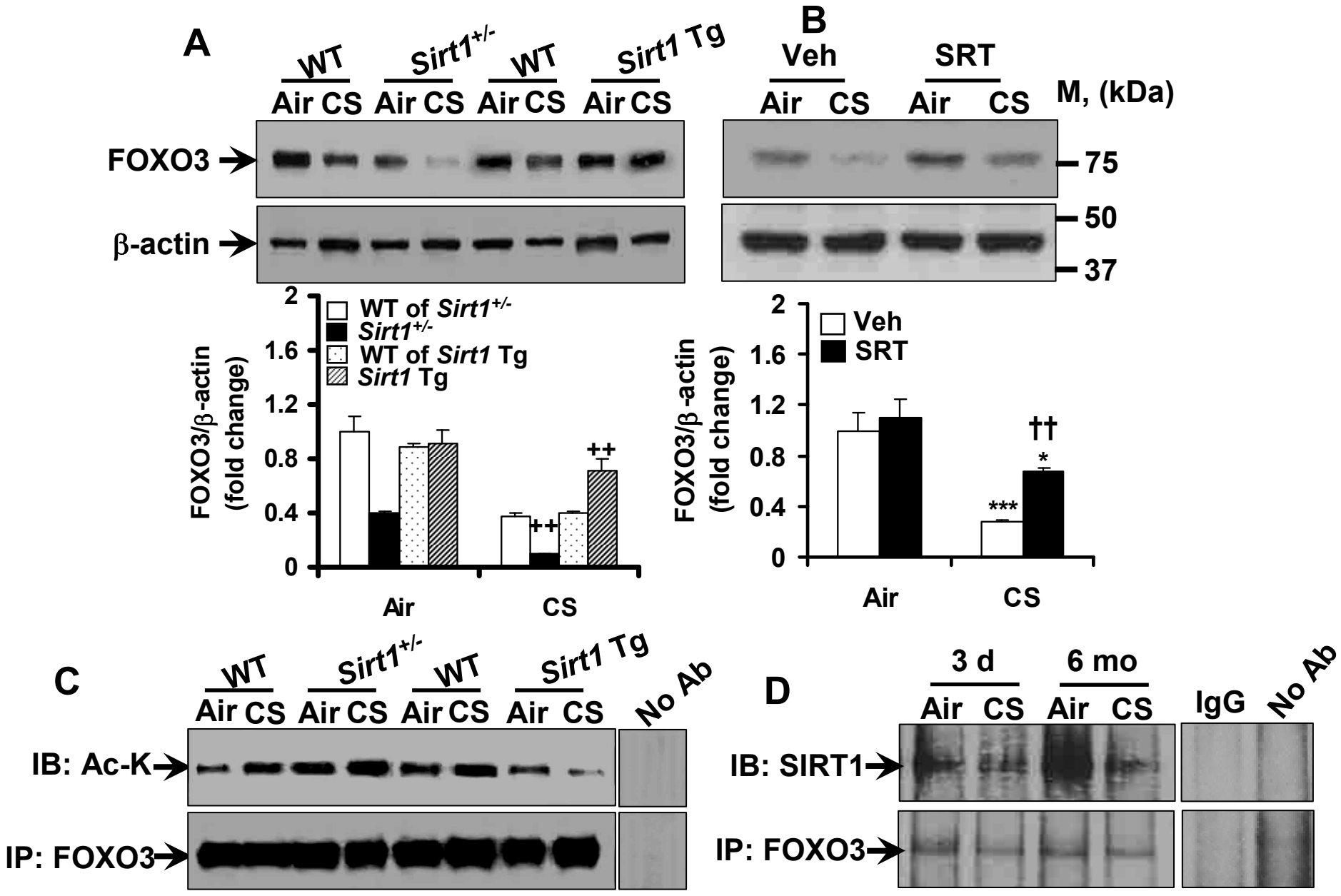

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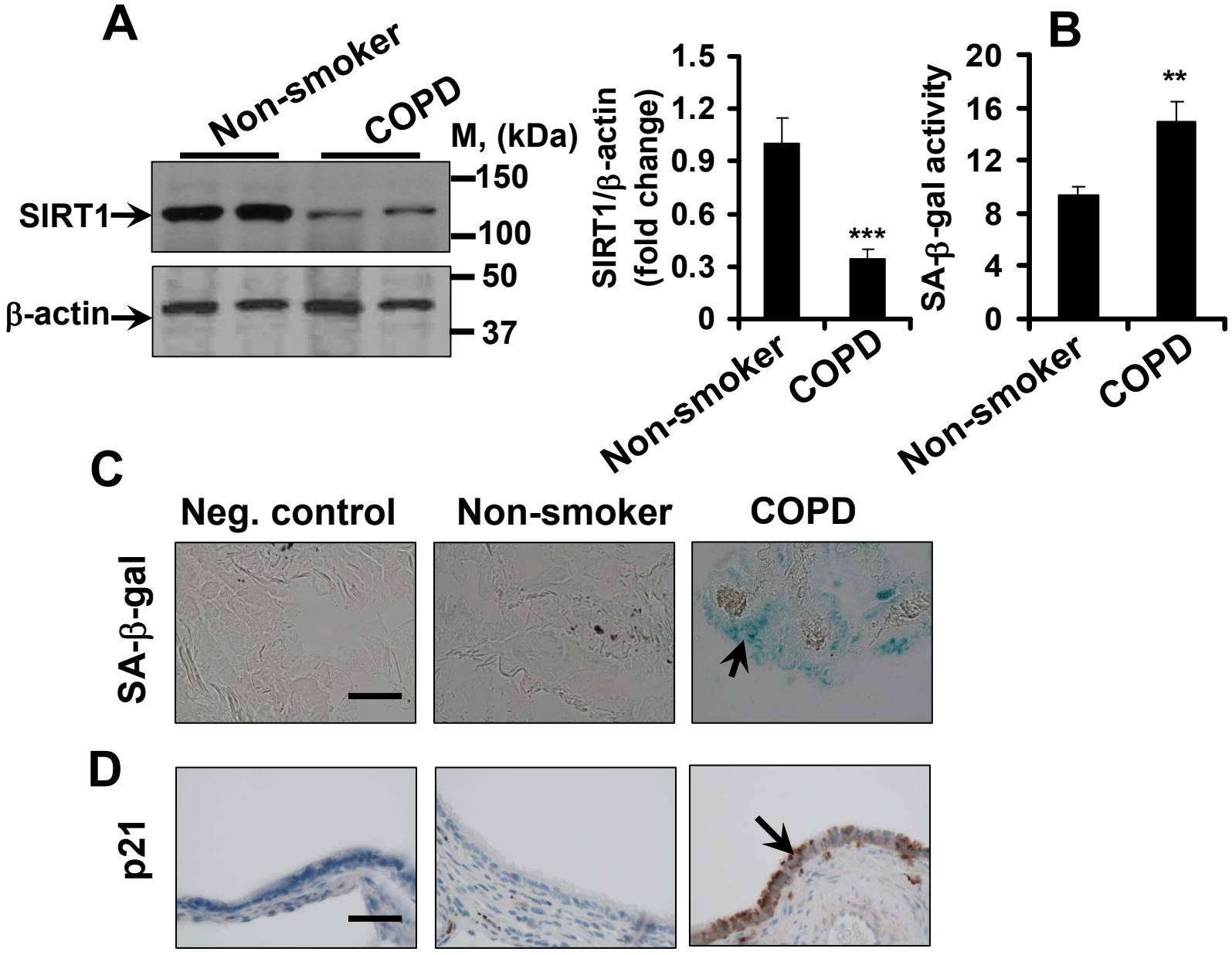

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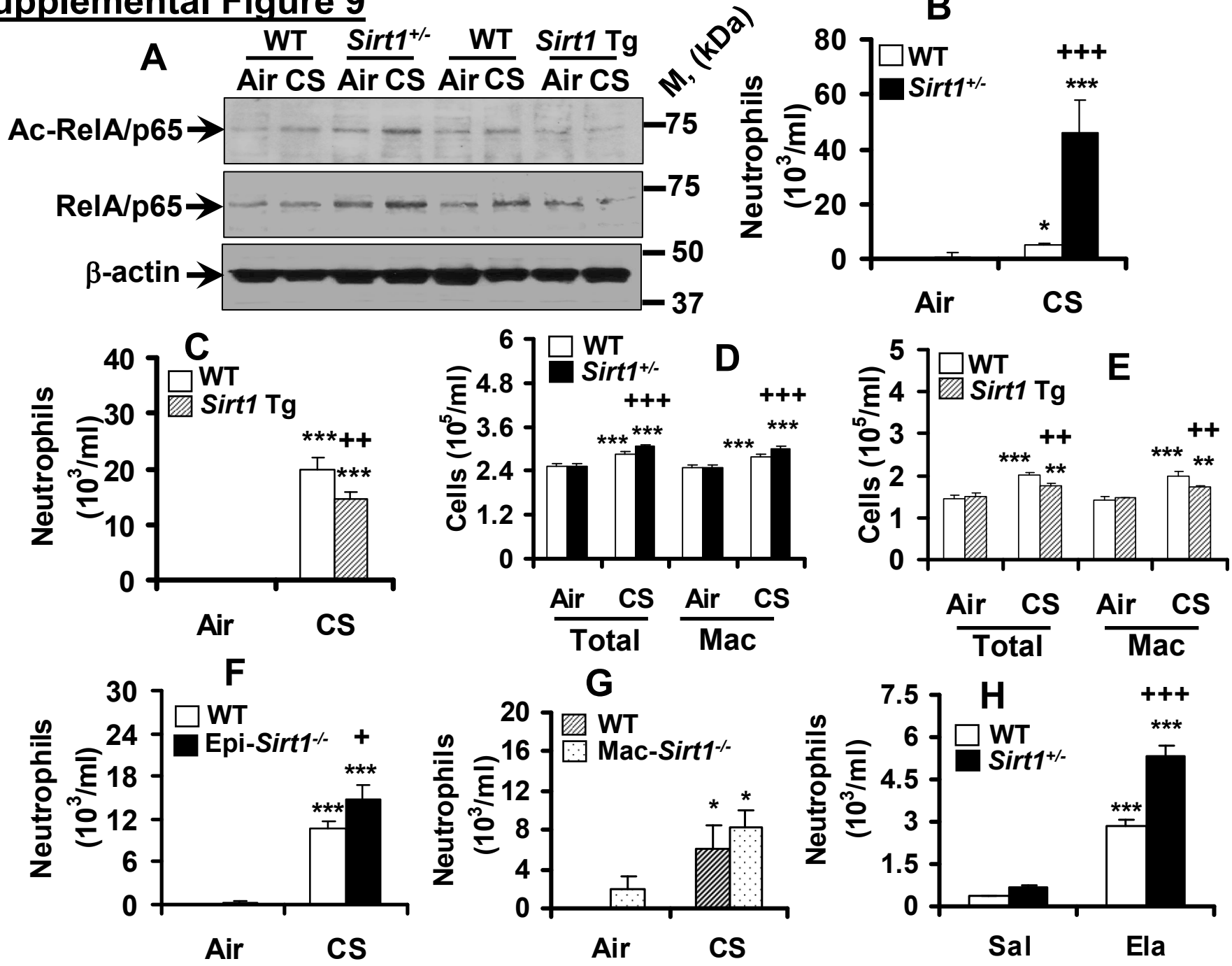

Supplemental Figure 10