

Overexpression of Fatty Acid Synthase Attenuates Bleomycin Induced Lung Fibrosis by restoring mitochondrial dysfunction in mice

Running title: Overexpression of fatty acid synthase reduces lung fibrosis

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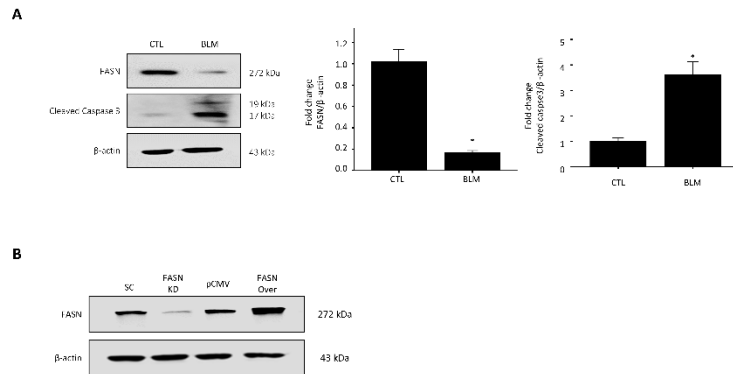
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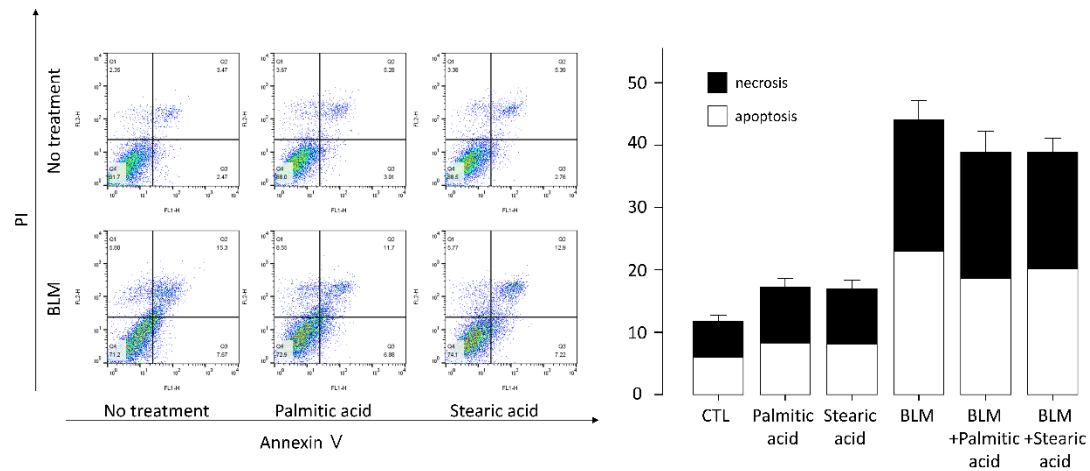
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Supplementary Figure 1.



(A) Immunoblotting analysis of FASN, cleaved forms of caspase-3 in control and BLM(10 μ g/ml)-treated AEC cells obtained from WT mice. The intensity of FASN and cleaved caspase-3 were quantified by densitometry, and the data were normalized to β -actin. The data are expressed as the mean $\pm$ SEM. Error bars represent mean $\pm$ SEM (n = 4, each group) *P < 0.05, vs. CTL. (B) Immunoblotting analysis of FASN expression in stable A549 cells (FASN_Over), FASN knockdown (FASN_KD), and their respective control vector-transfected (pCMV) and scramble control shRNA-transfected (SC) cells. Cropped images are displayed, uncropped blots are displayed in Supplementary Figure. 5.

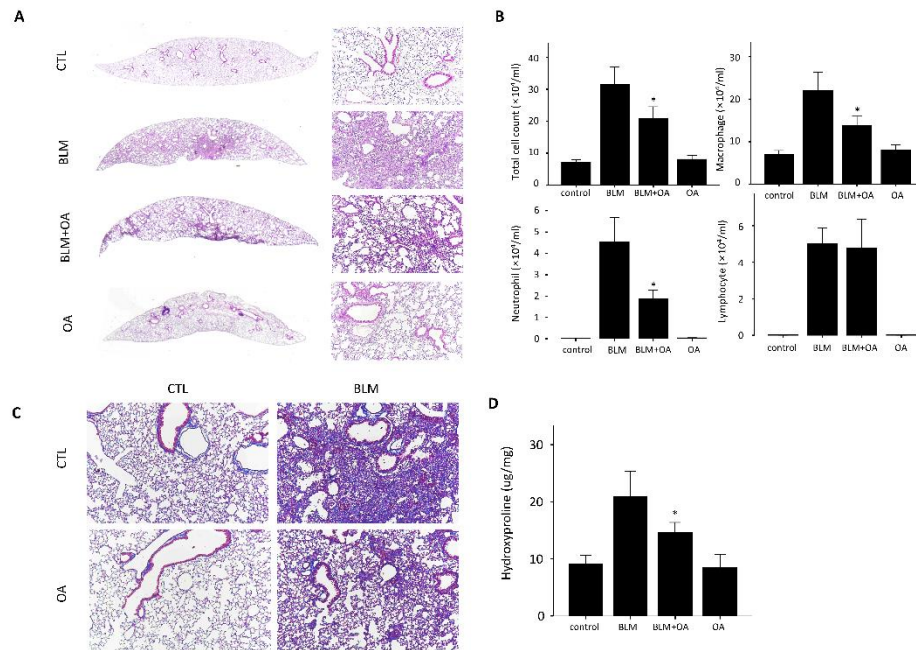
Supplementary Figure 2.



Treatment with Palmitic acid (100 μ M) and stearic acid (100 μ M) did not have a beneficial effect on AECs from BLM (10 μ g/ml)-induced apoptotic cell death. Apoptosis was defined by Annexin V+/both PI+and PI-staining. Necrosis was defined by PI+/both Annexin V+and Annexin V-staining. The data are expressed as the mean $\pm$ standard error of the mean (n = 4).

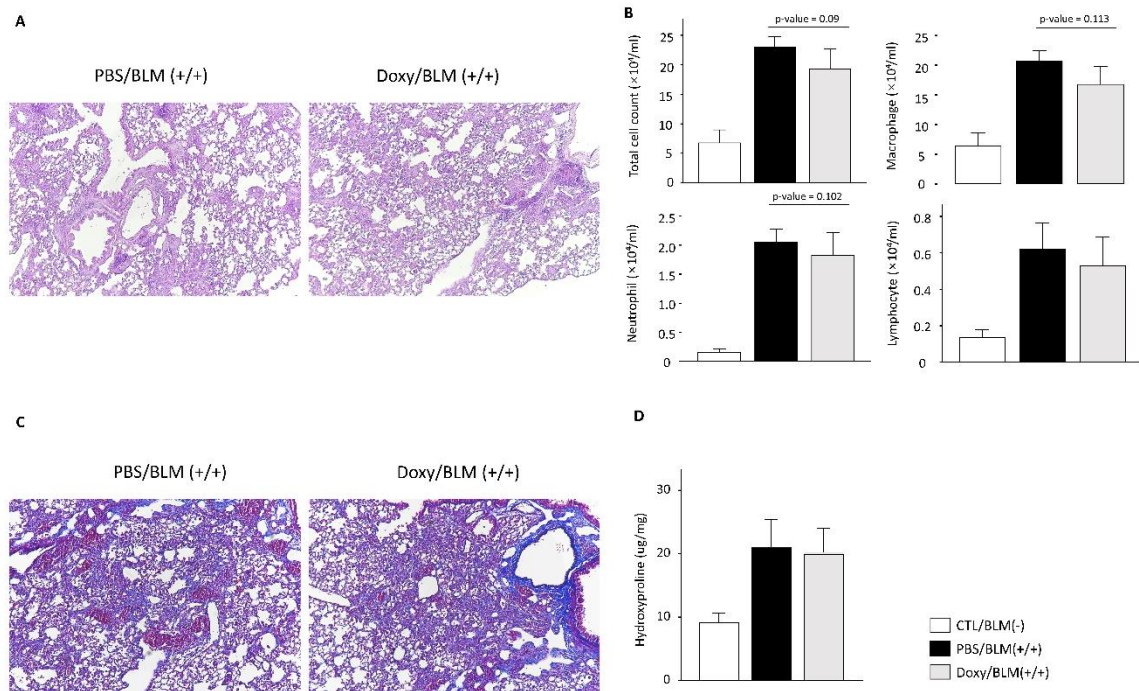
*<0.05 , BLM vs BLM + OA.

Supplementary Figure 3.



Treatment with oleic acid diminished BLM induced lung inflammation and fibrosis in mice. Oleic acid (3mg/ kg) was administered immediately to mice every 2 days after BLM treatment (3U/kg). (A) H&E staining of control, BLM, BLM +OA and OA -treated mouse lungs (n = 6 per group). Original magnification $\times 10$; ; original magnification $\times 100$ (B) Cell counts in BALF, collected on day 21. The total number of cells was counted using a hemocytometer. Differential cell counts in BALF were analyzed from 500 cells stained with Diff-Quick (n = 6 per group). (C) Masson's trichrome staining in the lungs of four groups of mice. Original magnification $\times 200$. (D) Collagen measurement by the hydroxyproline assay in the lungs of four groups of mice (n = 6 per group).

Supplementary Figure 4.



Doxycycline did not significantly diminish BLM-induced increased inflammation and fibrosis in the mouse lung. C57BL/6 mice (8 weeks, Male) were treated to BLM intratracheally with or without doxycycline-containing water for 3 weeks. (A) H&E staining of BLM-exposed mouse lungs with or without doxycycline treatment. Original magnification $\times 200$. (B) Numbers of inflammatory cells in BAL fluid between doxycycline-treated and non-treated mice treated to BLM ($n = 6$ per group). (C) Masson's trichrome staining in the lungs of the mice. Original magnification $\times 200$ (D) Collagen measurement by the hydroxyproline assay in the lungs of the mice ($n = 6$ per group).

Supplementary Figure 5. Uncropped blots from figure of the manuscript

Figure. 1B

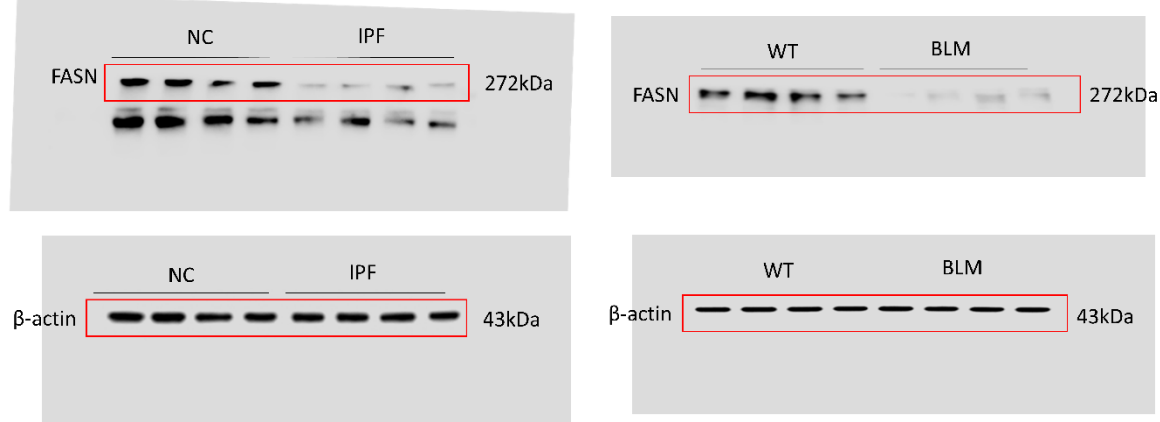


Figure. 2B

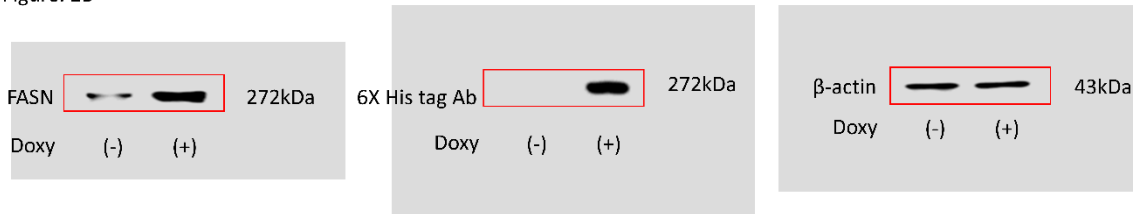


Figure. 3B

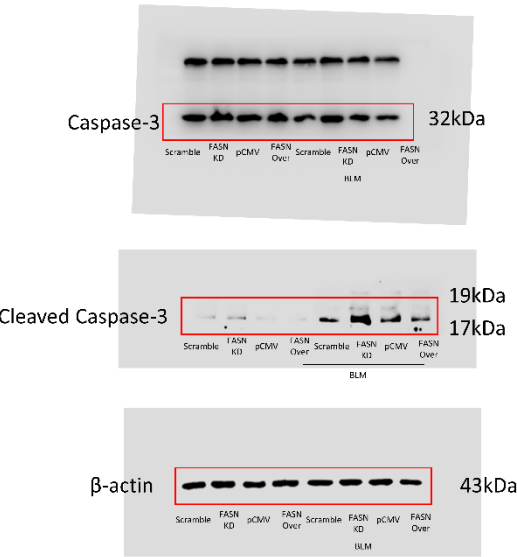


Figure. 4B

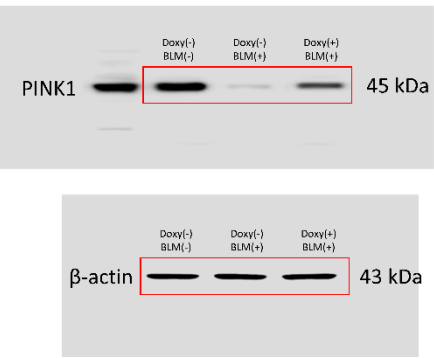
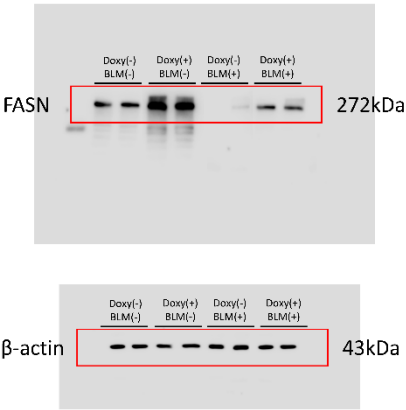
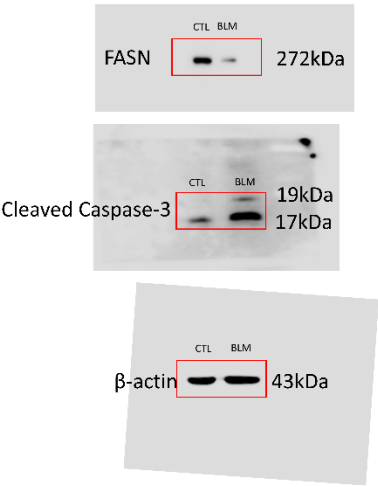


Figure. 7A



Supplementary Figure. 1A



Supplementary Figure. 1B

