SUPPLEMENTARY DATA

Targeting Cellular Senescence as a Therapeutic Strategy to Attenuate Pulmonary Fibrosis Associated with Metabolic Syndrome

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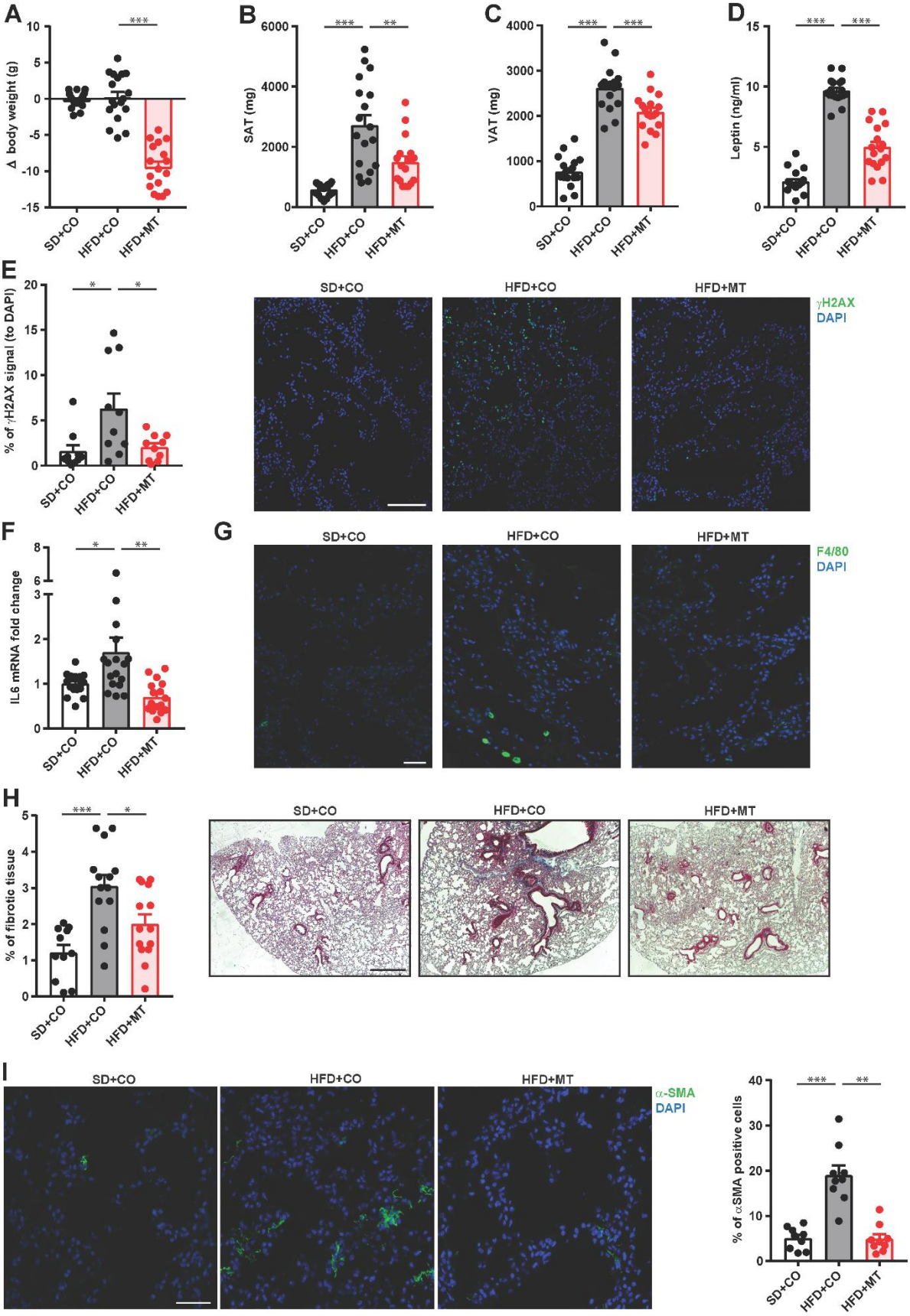
SUPPLEMENTARY DATA



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Supplementary Figure 1. C57BL/6 mice (8 months old) on standard (SD) or high fat diet (HFD) were divided into three groups, i.e. SD+CO: standard diet + corn oil; HFD+CO: high fat diet + corn oil; HFD+MT: high fat diet + MitoTam (n=17-18 per group). The mice were treated with MitoTam (2 mg/kg body weight) dissolved in 4 % ethanol in corn oil or the vehicle given i.p. twice per week for a period of 4 weeks. Body weight difference (Δ) was calculated as the difference between initial and final body weight **(A)** together with SAT **(B)** and VAT **(C)** weight at the end of the experiment. Postprandial serum leptin concentration was assessed by Luminex **(D)**. DNA damage in the lungs was assessed by immunohistochemistry using □H2AX staining. DAPI denoting cell nuclei. Representative pictures are shown. The bar indicates 100□ μm **(E)**. The level of *IL6* mRNA in lungs was assessed by qRT-PCR **(F).** Macrophage infiltration in the lungs was assessed by immunohistochemistry using F4/80 staining. DAPI denoting cell nuclei. Representative pictures are shown. The bar indicates 30 μm **(G)**. Histological samples of lungs were stained for fibrotic tissue using Masson's Trichrome staining. Representative pictures are shown. The bar indicates 100□M **(H)**. Fibrotic cells were documented by immunofluorescent staining using alpha-smooth muscle actin (□-SMA). DAPI denoting cell nuclei. Representative pictures are shown. The bar indicates 30 μm **(I)**. Data are presented as mean ± SEM. Statistical significance was assessed using one-way ANOVA followed by Tukey's multiple comparisons test. *$p < 0.05$; **$p < 0.005$; ***$p < 0.001$.

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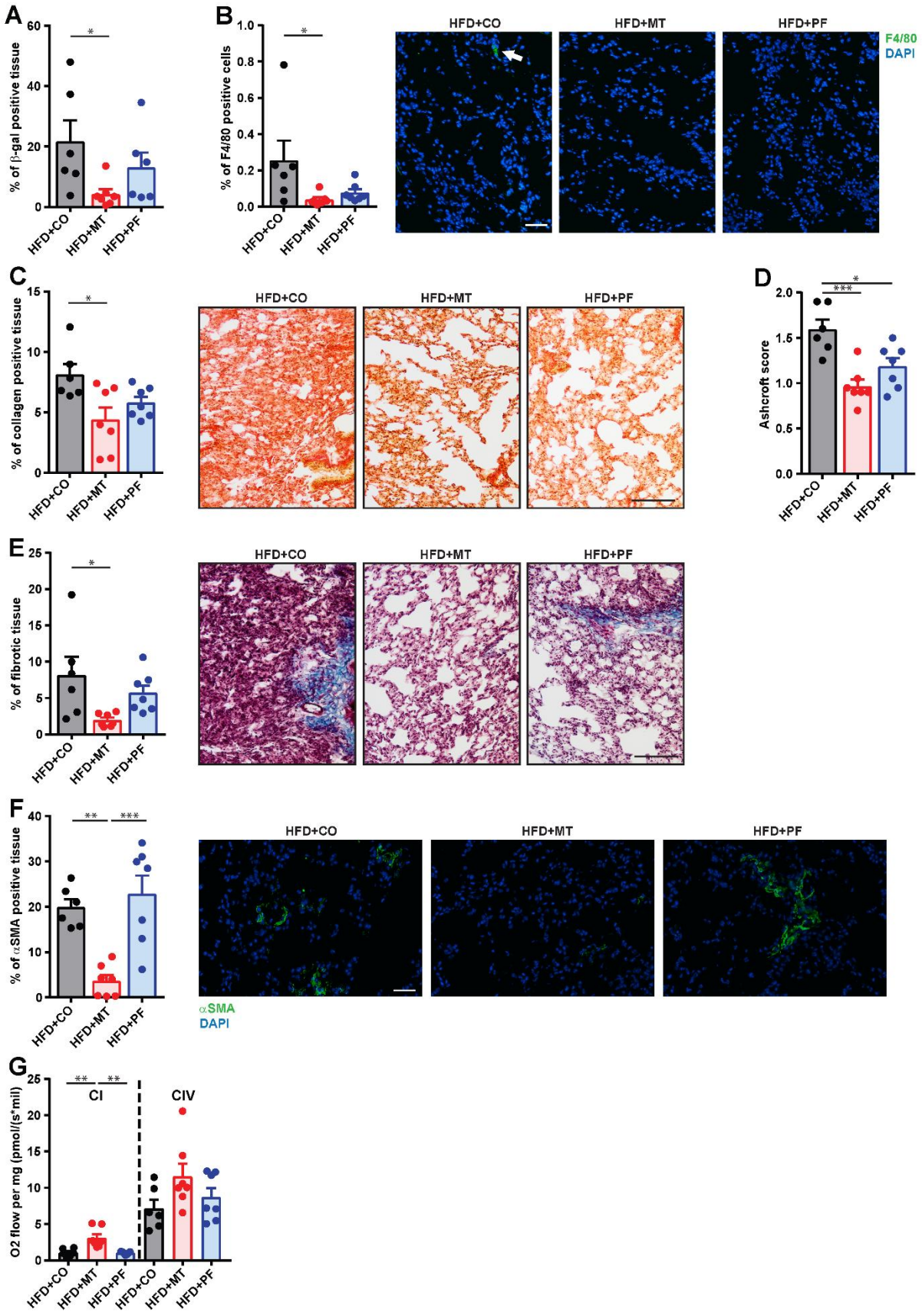

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Supplementary Figure 2. C57BL/6 male mice (10month old, n=6-7) fed with HFD (high fat diet) were treated with MitoTam (2 mg/kg body weight; MT) dissolved in 4 % ethanol in corn oil or the vehicle (CO) given i.p. twice per week for a period of 4 weeks. A separate group of HFD-fed mice was pair-fed (HFD+PF), receiving a reduced amount of food corresponding to the intake observed in HFD MT-treated mice. SA-β-gal positive tissue in lungs (%) is shown as bar graph **(A)**. Macrophage infiltration in the lungs was assessed by immunohistochemistry using F4/80 staining. DAPI denoting cell nuclei. Representative pictures are shown. The bar indicates 30 μm **(B)**. Histological samples of lungs were stained for collagen using PicroSirius Red staining **(C)**. Ashcroft score was assessed **(D)**. Histological samples of lungs were stained for fibrotic tissue using Masson's Trichrome staining. Representative pictures are shown. The bar indicates 100 μm **(E)**. Fibrotic cells were documented by immunofluorescent staining using alpha-smooth muscle actin (α-SMA). DAPI denoting cell nuclei. Representative pictures are shown. The bar indicates 30 μm **(F)**. Respiration via mitochondrial complex I (CI) and activity of mitochondrial complex IV (CIV) in lungs was measured by Oxygraph O2k **(G)**. Data are presented as mean ± SEM. Statistical significance was assessed using one-way ANOVA followed by Tukey's multiple comparisons test. *$p < 0.05$; **$p < 0.005$; ***$p < 0.001$.