INTERPLAY BETWEEN CIGARETTE SMOKING AND PULMONARY REVERSE LIPID TRANSPORT

SUPPLEMENTARY METHODS

Measurement of antibodies against MDCO-216

Wells of a 96-well plate were coated overnight with 1 μg/100 μl of MDCO-216 in PBS. Wells were blocked with 200 μl of PBS-0.05% Tween + 1% BSA (Sigma-Aldrich, Oakville, ON, Canada) for 1 hour. Wells were incubated at room temperature with diluted serum (1:2000 in PBS-0.05% Tween + 1% BSA) for 2 hours. Wells were then washed 5 times with PBS-0.05% Tween. For detection, wells were incubated with the goat anti-mouse IgG+IgM+IgA H&L coupled to biotin (0.25 μg/ml in PBS-0.05% Tween + 1% BSA; AbCam, Toronto, ON, Canada) for 1 hour, washed, incubated with Streptavidin-HRP (1:40; R&D systems, Minneapolis, MN, USA) for 30 minutes, washed and incubated with TMB substrate reagent BD OptEIA™ (BD Biosciences, San Jose, CA, USA) for 20 minutes. The reaction was stopped after 30 minutes with 2N H_2SO_4 and the absorbance at 450 nm was read using Synergy H1 Hybrid Reader (BioTek, Winooski, VT, USA).

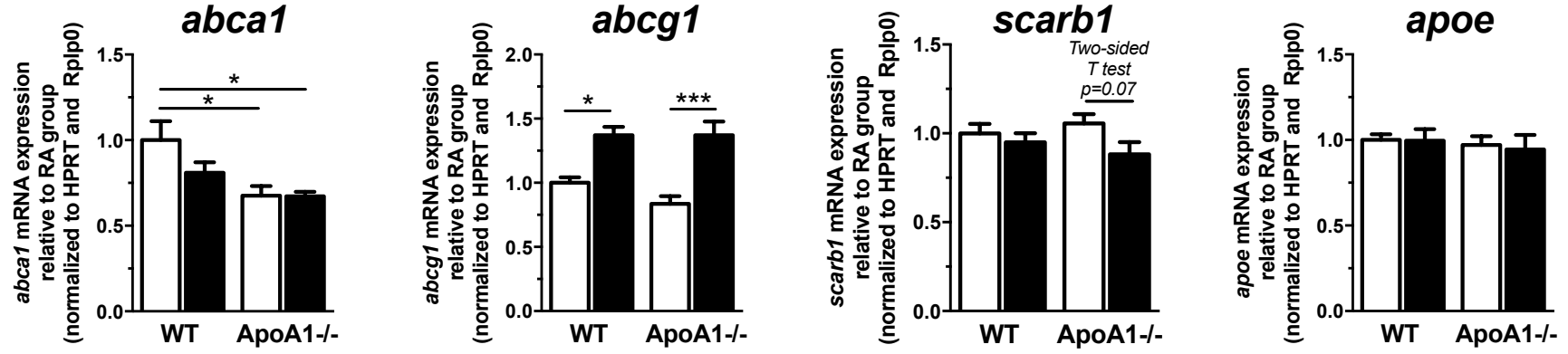
SUPPLEMENTARY FIGURES

FIGURE S1. Impact of ApoA-1 deficiency and MDCO-216 administration on the pulmonary expression levels of key genes involved in reverse lipid transport in response to cigarette smoke exposure. A) Pulmonary mRNA levels (qPCR) of *abcg1*, *abca1*, *scarb1* and *apoe* were assessed in C57BL/6J and ApoA1-deficient (C57BL/6J background) mice (n=4-5/group) that were exposed to room air or mainstream cigarette smoke for 8 weeks prior to sacrifice the day after the last exposure. **B**) Pulmonary mRNA levels (qPCR) of *abcg1*, *abca1*, *scarb1* and *apoe* were assessed in BALB/c mice (n=9-10/group) that were exposed to room air or mainstream cigarette smoke for 8 weeks,

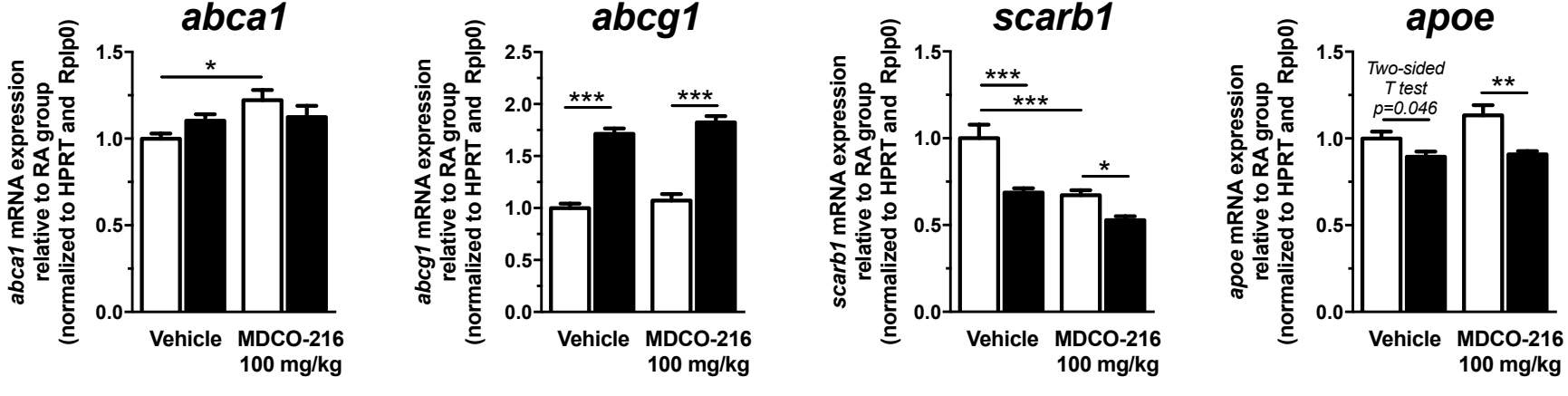
injected with vehicle or 100 mg/kg of MDCO-216 every second-day of weeks 5 to 8, and sacrificed 18h after the last exposure. *p<0.05; **0<0.01; ***p<0.001.

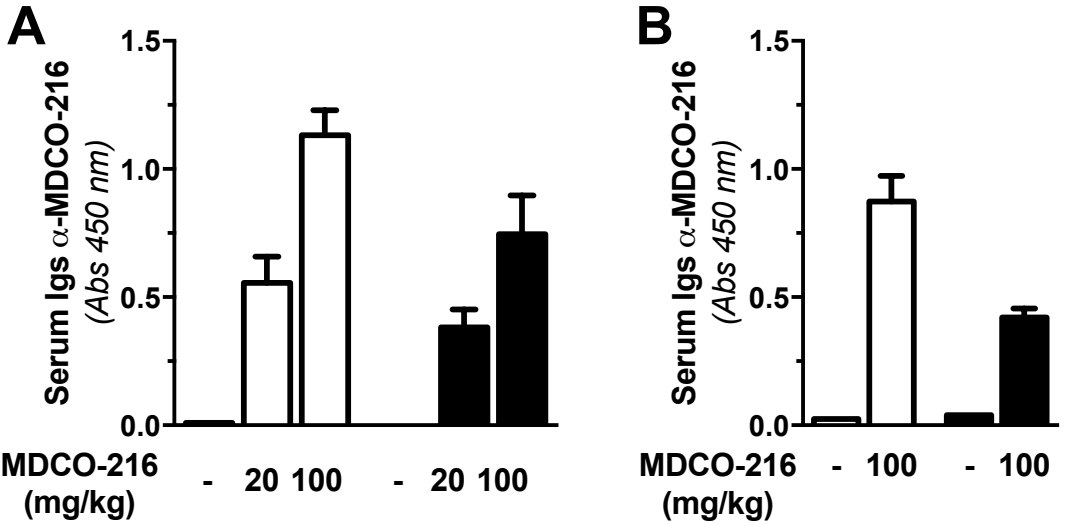
FIGURE S2. Presence of antibodies against MDCO-216 in mice chronically injected with the compound. Total immunoglobulins against MDCO-216 were measured in the serum (diluted 1:2000) of **A**) BALB/c mice (n=4-5/group) exposed to room air or mainstream cigarette smoke for 2 weeks, injected with vehicle, 20 or 100 mg/kg of MDCO-216 on weekdays and sacrificed 18h after the last exposure and **B**) BALB/c mice (n=9-10/group) exposed to room air or mainstream cigarette smoke for 8 weeks, injected with vehicle or 100 mg/kg of MDCO-216 every second-day of weeks 5 to 8, and sacrificed 18h after the last exposure.

ApoA-1 deficiency



MDCO-216 therapy




A
Serum Igs α-MDCO-216
(Abs 450 nm)
1.5
1.0
0.5
0.0
MDCO-216
(mg/kg)
-
20
100
-
20
100
B
Serum Igs α-MDCO-216
(Abs 450 nm)
1.5
1.0
0.5
0.0
MDCO-216
(mg/kg)
-
100
-
100