SUPPLEMENTAL DATA

β_2-adrenergic agonists augment the air pollution-induced IL-6 release and thrombosis

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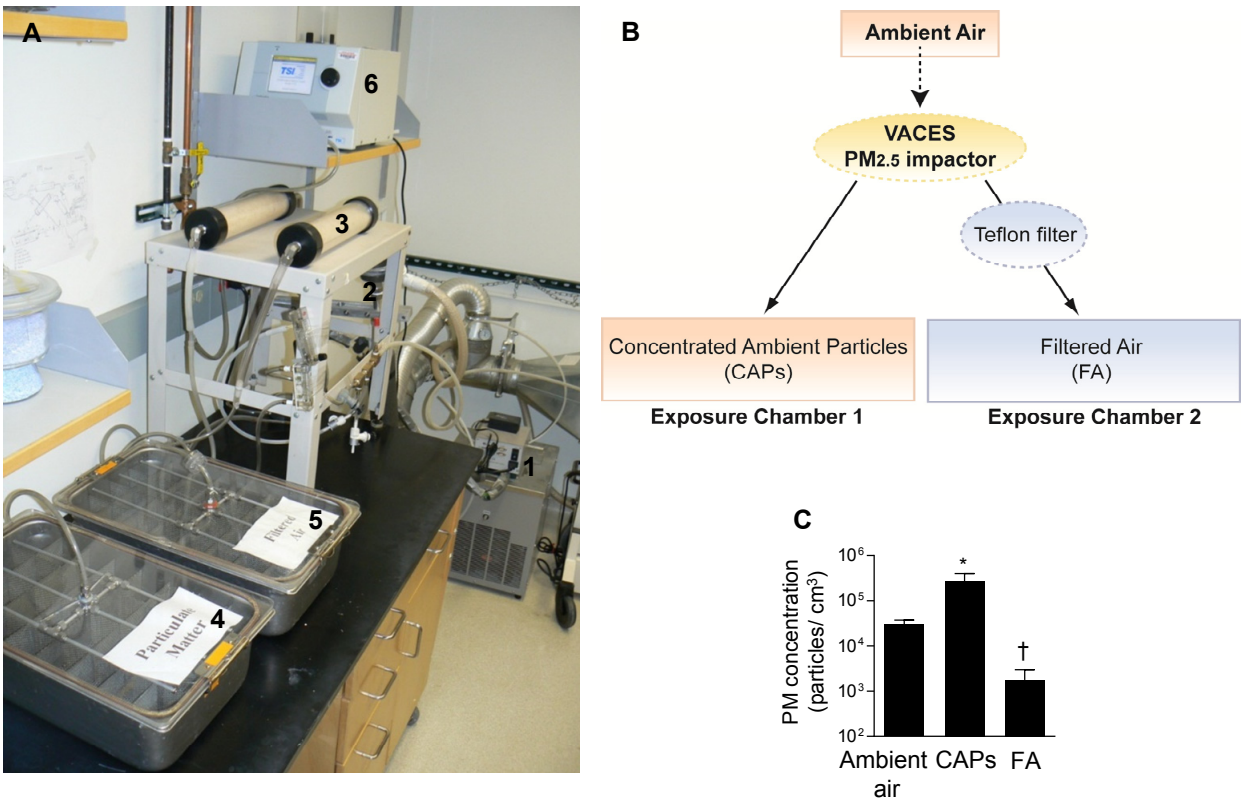
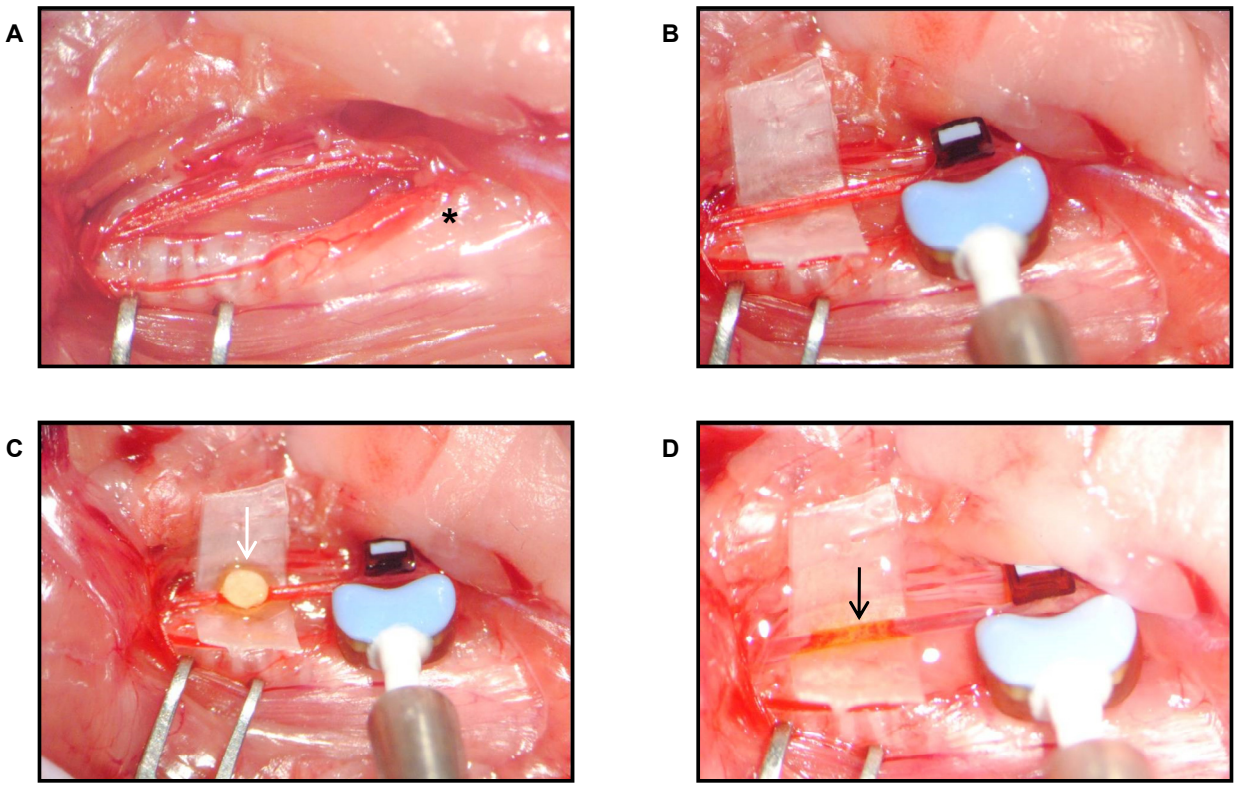
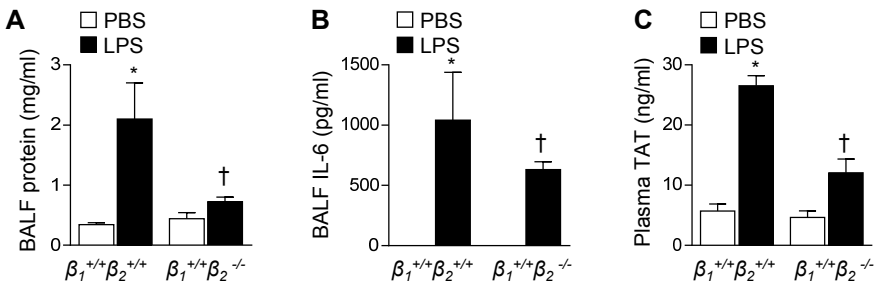


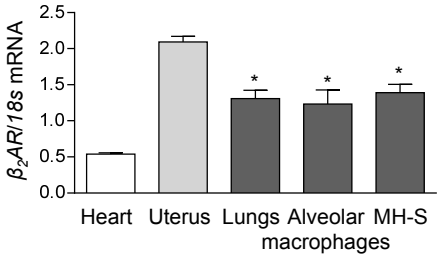
Supplemental Figure 1. Exposure to concentrated ambient particulate matter air pollution. (*10, 11, 34-36*) **(A)** Mice were exposed to concentrated ambient particles ($PM_{2.5}$) in diameter using a Versatile Aerosol Concentration Enrichment (VACES) system. (**1**) Filtered ambient air (110 L/min) was passed over a pool of warm water and the vapor saturated particles are grown to supermicron size by condensation in a cooled condenser (**2**) before being concentrated using a virtual impactor. (**3**) The concentrated particles were passed through a diffusion dryer to remove water, restoring them to their original size. (**4**) The particles were then delivered to specially designed murine chambers housing up to 32 mice each (with food and water) (**5**) Control mice were housed in an identical chamber, connected to the VACES with a teflon filter placed in the chamber inlet. (**6**) Ambient and delivered particle concentrations were measured using a TSI 3775 particle counter (Shoreview, MN).**(B)** Schematic of the VACES and chambers. **(C)** Particle concentrations are about 10-fold higher or lower than ambient levels in the CAPs and FA chambers, respectively. $p<0.05$, *CAPs and †FA vs. ambient air.



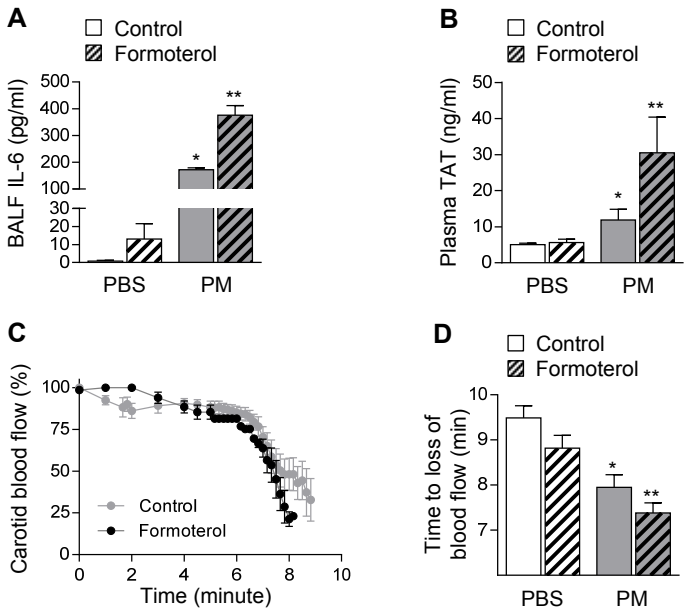
Supplemental Figure 2. Measurement of carotid thrombosis after $FeCl_3$-induced injury. (A) The neck was dissected to expose the left carotid artery (* indicates trachea, head is to the left). **(B)** Paraffin was placed behind the carotid artery to avoid injury to the surrounding tissues. **(C)** Filter paper, precisely cut to 1 mm diameter using a murine ear punch device was placed on the carotid artery and an ultrasonic flow probe (Transonic) was placed proximal to the injury. **(D)** Flow was recorded continuously as thrombus (arrow) formed in the artery.



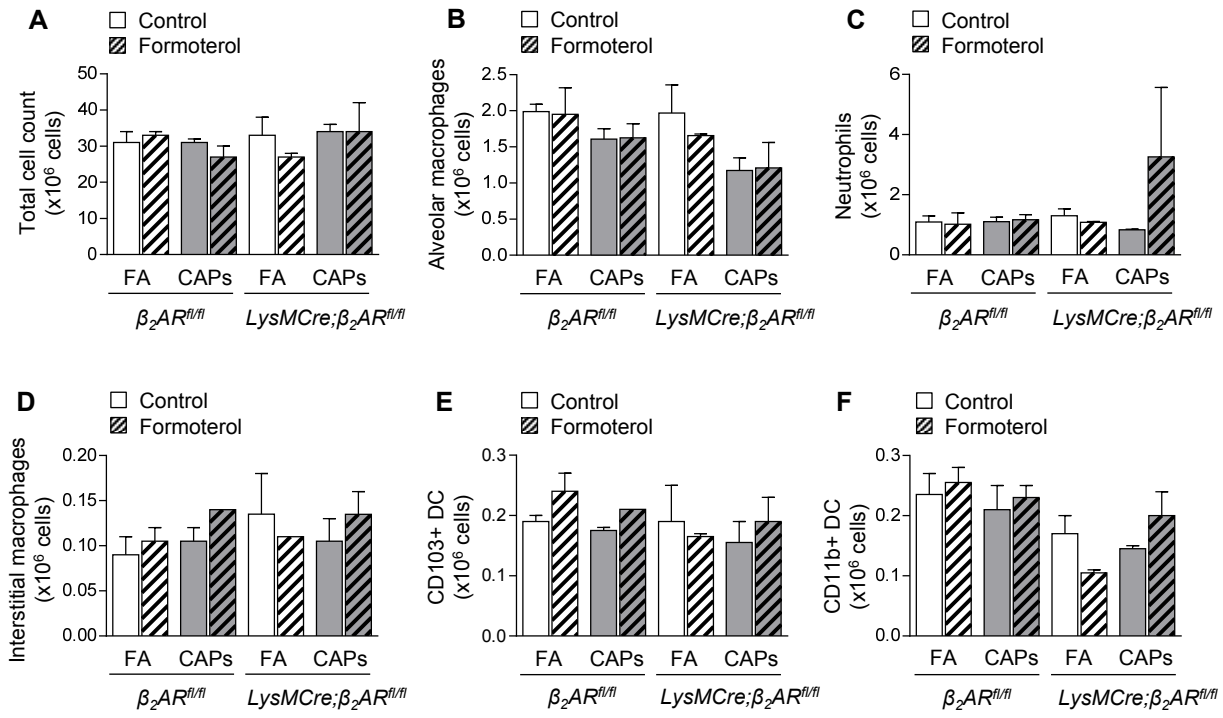
Supplemental Figure 3. Loss of β2-adrenergic receptors improves LPS-induced IL-6 release and thrombin generation. We treated mice intratracheally with either PBS or LPS (4 mg/kg) and 24 hours later measured **(A)** bronchoalveolar lavage fluid (BALF) protein concentration, **(B)** BALF levels of IL-6 levels and **(C)** plasma levels of thrombin-antithrombin complexes. * indicates P <0.05 for comparison between LPS and PBS and † indicates P <0.05 for comparison between $\beta_1^{+/+}\beta_2^{+/+}$ and $\beta_1^{+/+}\beta_2^{-/-}$.



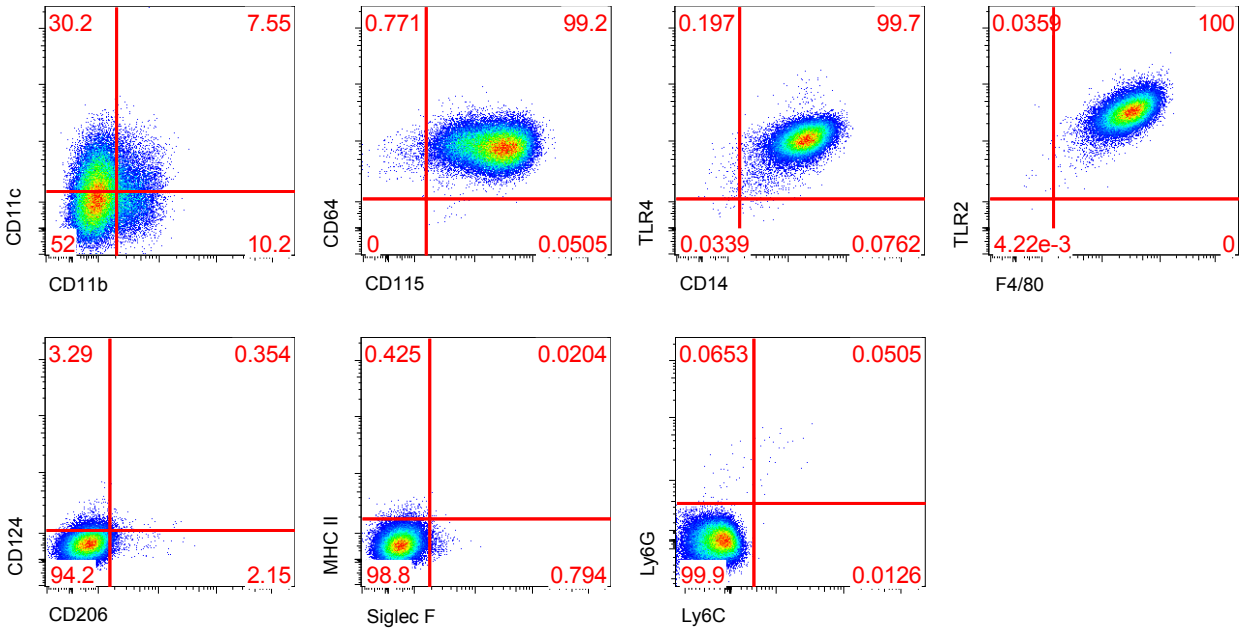
Supplemental Figure 4. Relative expression of β$_2$-adrenergic receptors in murine tissues and alveolar macrophages. Wild-type C57BL/6 mice were euthanized and total RNA was isolated from tissue homogenates of the heart, uterus and lungs. Total RNA was also isolated from lysates of freshly isolated primary alveolar macrophages and cultured MH-S cells. The levels of mRNA encoding the *β$_2$-adrenergic receptor* (*β$_2$AR)* gene were measured using qRT-PCR and normalized to mRNA encoding the18S ribosomal subunit (*18S*). * indicates P <0.05 for comparison with heart or uterus. N =4 for all measures.



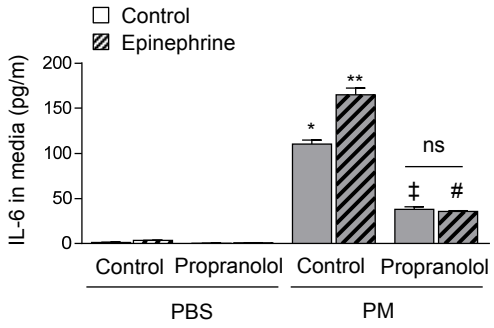
Supplemental Figure 5. Long-acting β_2-agonist therapy causes a further increase in PM-induced IL-6 release, thrombin generation and acceleration of arterial thrombosis. Wild-type C57BL/6 mice were treated with either PM or PBS intratracheally followed by either formoterol ($1x10^{-5}$ M) or vehicle (2% ethanol, control) via inhalation, every 12 hours starting 1 hour after the intratracheal instillation of PM or PBS. Twenty-four hours later, we measured levels of **(A)** IL-6 in bronchoalveolar lavage fluid (BALF), **(B)** thrombin antithrombin (TAT) complexes in plasma and **(C)** time to loss of blood flow after $FeCl_3$-induced carotid injury. **(D)** Time to complete loss of blood flow after PM or PBS exposure is shown. * indicates $P < 0.05$ for comparison between PBS and PM, ** indicates $P < 0.05$ for comparison between PM and PM + formoterol.



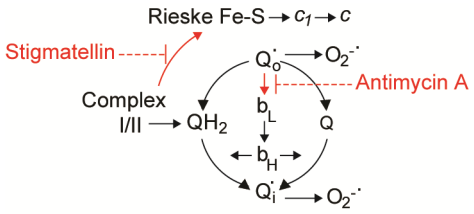
Supplemental Figure 6. Long-acting β$_2$-agonist therapy does not alter PM-induced changes in immune cells in the lung. We exposed *β2AR$^{fl/fl}$* and *LysM-Cre/β2AR$^{fl/fl}$* mice contemporaneously to CAPs ($PM_{2.5}$) or filtered air (FA) for 8 hours daily on 3 consecutive days and treated them with formoterol ($1x10^{-5}$ M) or control (2% ethanol) via inhalation, beginning 1 hour before the initial CAPs exposure and continuing every 12 hours until the end of the experiment and measured the number of **(A)** immune cells, **(B)** alveolar macrophages, **(C)** neutrophils, **(D)** interstitial macrophages, **(E)** CD103+ dendritic cells (DCs) and **(F)** CD11b+ DCs in whole lung homogenates using flow cytometry (75).



Supplemental Figure 7. Phenotype of MH-S cell line determined using flow cytometry. All MH-S cells expressed canonical macrophage markers (CD64, F4/80, CD115, CD14) as well as Toll-like receptors (TLR) 2 and 4. Similar to mouse alveolar macrophages, most MH-S cells did not express CD11b and MHC II, but expressed CD11c. MH-S cells were negative for Ly6G (marker of neutrophils) and Ly6C (marker of classical bone marrow-derived monocytes). In contrast to mouse alveolar macrophages MH-S cells did not express Siglec F or CD206.



Supplemental Figure 8. Epinephrine augments the PM-induced IL-6 release from alveolar macrophages. MH-S cells were treated with vehicle or PM and 1 hour later treated with epinephrine (10^{-10}M), or control vehicle. Media levels of IL-6 were measured 24 hours later. * indicates $P < 0.05$ for comparison between PBS and PM, ** indicates $P < 0.05$ for comparison between PM and PM + epinephrine. ‡ indicates $P < 0.05$ for comparison between PM vs. PM + propranolol, # indicates $P < 0.05$ for comparison between PM + epinephrine and PM + epinephrine + propranolol. $N \geq 4$ for all measures.



Supplemental Figure 9. The effects of stigmatellin and antimycin A on electron transport in the mitochondria.