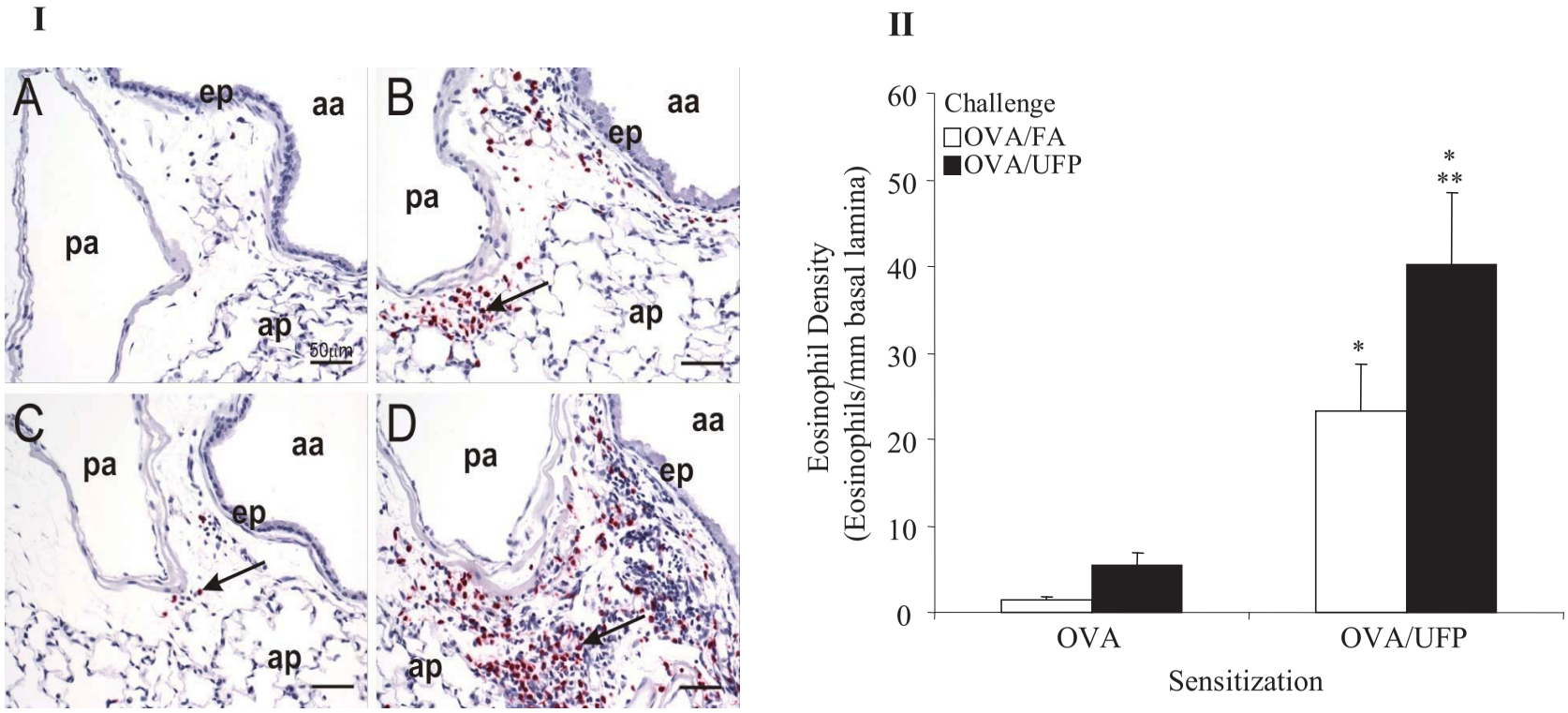
SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. UFP inhalation concurrent with OVA challenge increased eosinophil infiltration in the lung of OVA/UFP-sensitized mice compared to mice receiving FA at the same time of OVA challenge. **I**. Immunohistochemical staining of major basic protein (MBP) showing that OVA/UFP challenge induced a more prominent eosinophilic inflammation in the lung of OVA/UFP-sensitized animals. Arrows indicate MBP positive cells. **A and C**. Animals were sensitized by OVA alone and challenged with OVA/FA (A) and OVA/UFP (C) inhalation respectively. **B and D**. Animals were sensitized by OVA/UFP and challenged with OVA/FA (B) and OVA/UFP (D) inhalation respectively. **II.** Quantitative analysis of eosinophil infiltration in the lung by morphometry. *$p < 0.05$ compared to OVA-sensitized, **$p < 0.05$ compared to OVA/FA-challenged in the same sensitization group.

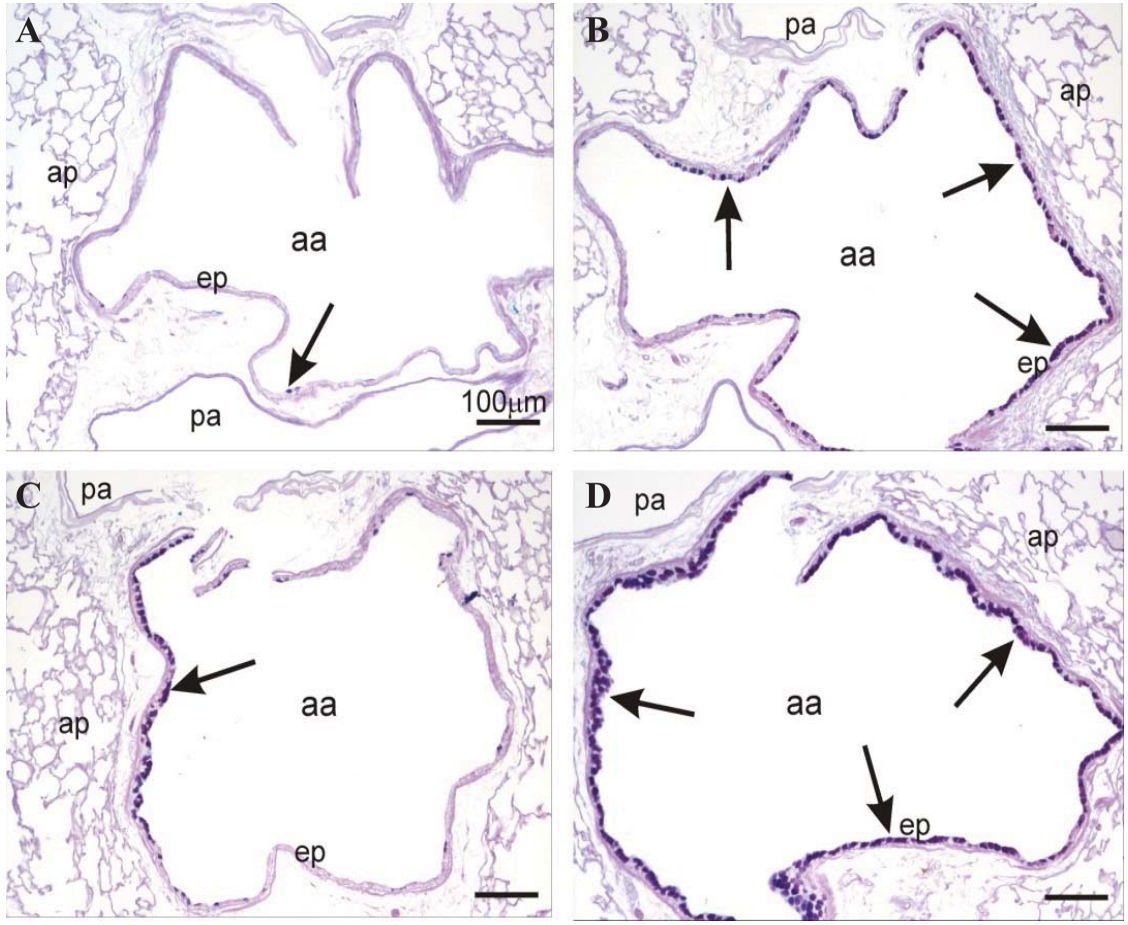
Supplemental Figure 2. Histochemical staining (Alcian Blue, pH 2.5/Periodic Acid Schiff sequence) for acidic and neutral mucosubstances (magenta stain; arrows) in mucus cells of the respiratory epithelium (ep) lining the axial airway (aa; generation 5) in the left lung lobe. Increased amounts of intraepithelial mucosubstances are present in the OVA/UFP-sensitized mouse after secondary challenge with OVA plus UFP. Arrows indicate mucus-secreting cells. **A and C**. Animals were sensitized by OVA alone and challenged with OVA/FA (A) and OVA/UFP (C) inhalation respectively. **B and D**. Animals were sensitized by OVA/UFP and challenged with OVA/FA (B) and OVA/UFP (D) inhalation respectively. Pulmonary artery, pa; alveolar parenchyma, ap.

Supplemental Figure 3. The high particulate OC content represented a typical traffic-related ambient UFP atmosphere in Los Angeles (see Materials and Methods for chemical analysis).

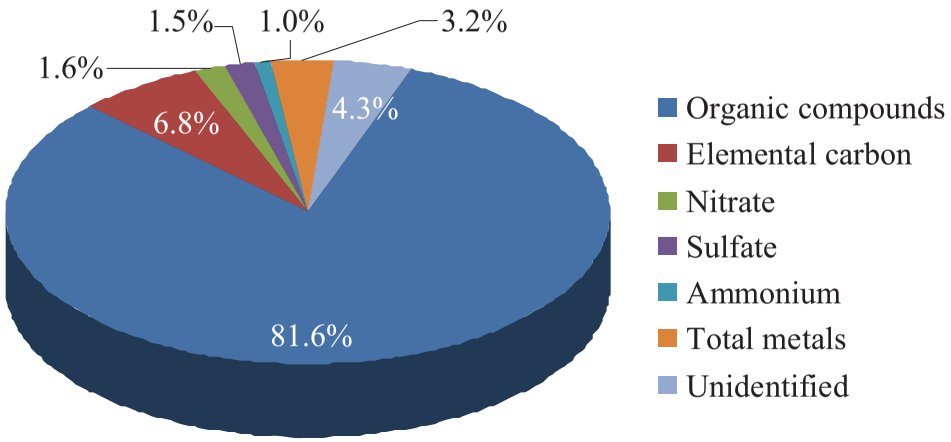
Supplemental Figure 4. Two UFP inhalation exposures during OVA challenge increased BAL eosinophil count and serum OVA-IgG1 level in animals sensitized by OVA/UFP. In a follow up experiment, we assessed whether a shorter inhalation exposure to ambient UFP could produce similar adjuvant effect on the secondary immune response in already sensitized animals. Two-inhalation exposures of OVA/FA or OVA/UFP were given to the animals sensitized by saline, OVA or OVA/UFP. Allergic sensitization, OVA challenge, inhalation exposure to FA or UFP and necropsy were conducted as described in the Materials and Methods. A. BAL eosinophil count. B. OVA-IgG1. $p < 0.05$ compared to respective saline-sensitization; **$p < 0.05$ compared to respective OVA-sensitization; $^{\#}p < 0.05$ compared to respective OVA/FA.



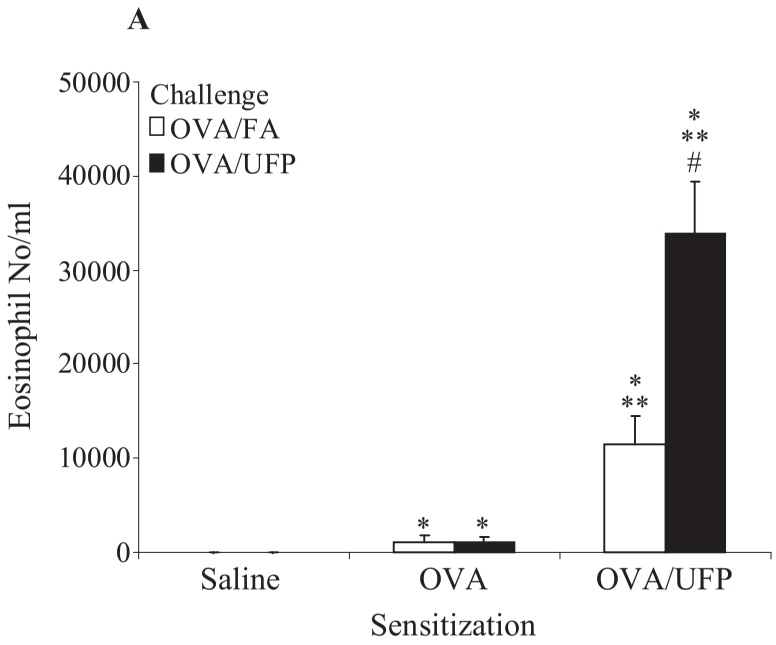
Supplemental Figure 1

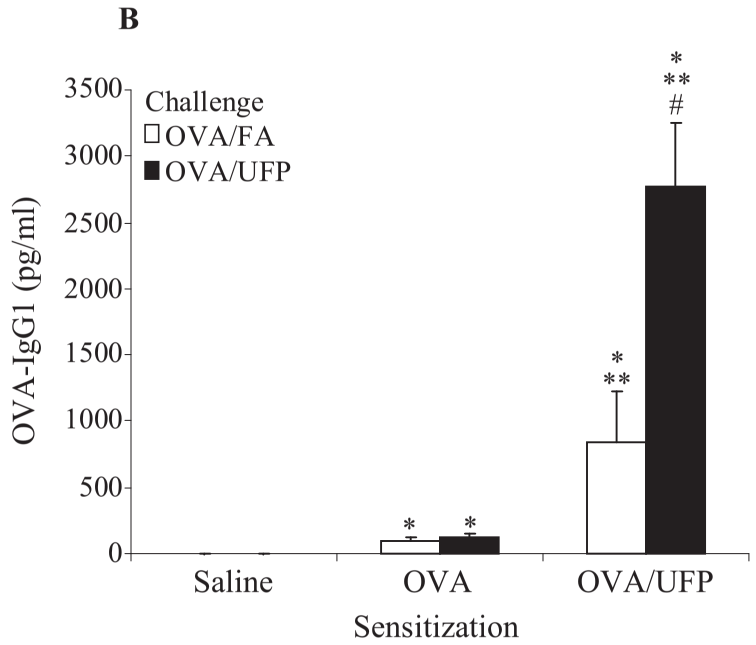


Supplemental Figure 2



Supplemental Figure 3





Supplemental Figure 4

Supplemental Table 1. Characteristics of Inhalation Exposure in the Mobile Laboratory

Experimental Parameter	
Exposure time (dates)	December 3-5, 8 and 9, 2008
Exposure time (hours)	20 hours
Total ambient particle no. (particles/cm^3)	$(1.15 \pm 0.18) \times 10^4$
No. concentration in FA chamber (particles/cm^3)	< 5000
No. concentration in UFP chamber (Particles/cm^3)	$(1.53 \pm 0.39) \times 10^5$
UFP chamber particle enrichment factor	13.3 ± 2.0
Mass in UFP exposure chamber ($\mu g/m^3$)	101.3 ± 5.1
UFP mass in ambient air ($\mu g/m^3$)	7.6 ± 0.4

Animals were exposed to FA or UFP in downtown Los Angeles as described in the Materials and Methods. Values are means ± SD.

Supplemental Table 2. Real-time PCR analysis of cytokine and chemokine gene expression in the lung

	Change in Gene Expression (Fold)*					
	Saline†		OVA2†		OVA/UFP2†	
Genes	**OVA/FA‡**	**OVA/UFP‡**	**OVA/FA‡**	**OVA/UFP‡**	**OVA/FA‡**	**OVA/UFP‡**
KC	1.0 ± 0.1	1.2 ± 0.2	1.1 ± 0.1	1.1 ± 0.1	2.3 ± 0.4[ab]	3.5 ± 0.7[abc]
TNFα	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	0.9 ± 0.0	1.3 ± 0.1[ab]	1.6 ± 0.1[ab]
IL-10	1.0 ± 0.2	0.8 ± 0.1	1.1 ± 0.1	1.0 ± 0.2	4.1 ± 0.6[ab]	6.3 ± 0.8[abc]
MCP1	1.0 ± 0.1	1.3 ± 0.1	1.0 ± 0.1	1.3 ± 0.1	3.2 ± 0.5[ab]	4.0 ± 0.5[ab]
MIP2	1.0 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	2.7 ± 0.4[ab]	3.6 ± 0.6[ab]
IL-6	1.0 ± 0.2	1.4 ± 0.2	1.0 ± 0.1	1.0 ± 0.1	2.1 ± 0.3[ab]	2.8 ± 0.4[ab]
IL-2	1.0 ± 0.1	1.0 ± 0.0	1.1 ± 0.1	1.0 ± 0.0	1.7 ± 0.1[ab]	1.9 ± 0.1[ab]
IL-4	1.0 ± 0.1	0.9 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	2.8 ± 0.3[ab]	3.7 ± 0.5[ab]
Ym2	1.0 ± 0.5	107 ± 105	47 ± 39	152 ± 136	2336 ± 962[ab]	4060 ± 826[ab]
Fizz 1	1.0 ± 0.3	1.0 ± 0.1	3.1 ± 1.0	4.7 ± 2.1	43 ± 8.5[ab]	73 ± 10.1[ab]
GOB5	1.0 ± 0.3	0.9 ± 0.3	55 ± 38	105 ± 78	696 ± 202[ab]	1518 ± 219[ab]
AMcase	1.0 ± 0.1	2.0 ± 0.4	1.2 ± 0.2	1.6 ± 0.5	13 ± 5.6[ab]	8.3 ± 2.5[ab]
β-actin	1.0 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	0.9 ± 0.0	1.0 ± 0.0	1.0 ± 0.0

Values are means ± SEM. *Relative to saline sensitization followed by OVA/FA challenge; †allergic sensitization; ‡challenge [a]Significantly different from respective saline sensitization group, ($p < 0.05$), [b]significantly different from respective OVA sensitization group, ($p < 0.05$), [c]significantly different from respective filtered air group, ($p < 0.05$).

Supplemental Table 3. Characterization of ambient UFP

Particle Properties	**Results**
Endotoxin (u/ml)	0.5
Oxidant Potential (DTT consumption, nmol/μg/min)	0.05

Endotoxin level was measured by LAL assay. DTT assay was conducted