Supplementary Information

Methods

Toluidine blue staining of lung mast cells

5μM thick paraffin embedded lung tissues were cut and stained with toluidine blue. Toluidine blue positive mast cells in lungs were quantified according to area (mm^2). NIH Image J software was used to scale tissue area in the captured images. Mast cell number was expressed as the total number of mast cells counted per tissue area.

IVIS imaging necropsy

OVA/TNP was labeled with Qdot® 800 by Qdot® 800 Antibody Conjugation Kit (Invitrogen). Qdot® 800 labeled OVA/TNP was further conjugated to 1μm Polybead® carboxylate polystyrene microspheres as described above. To perform the airway challenge, mice were anesthetized and then intratracheally and intranasally instilled with 50μg and 10μg of Q dot® 800 labeled allergens, respectively. At 1 hour following the airway challenge, mice were sacrificed and lungs were taken out for necropsy. The lungs were viewed and photographed in the IVIS Xenogen imaging instrument (Caliper LifeSciences). To quantify fluorescence intensity in sub-anatomic regions of interest, the area was circled and the efficiency of the fluorescent intensity in the indicated areas was quantified by Xenogen Living Image 3.0 program.

Confocal microscopy to test pAg and MCs in lungs

Mice were actively sensitized as described. On day 36, mice were challenged with 200μg pAg, containing the indicated allergen coupled to 1μm Fluoresbrite™ carboxylate YG polystyrene microspheres (Polyscience). At 6 hours after the challenge, lungs were removed from sacrificed mice and frozen in OCT (Tissue-TEK). 20μM sections of each lung were prepared, fixed in acetone and blocked in 5%BSA in 1X PBS. Lung sections were then stained with TRITC-avidin (Sigma) for 30 minutes to detect MCs. After washing, slides were sealed with Pro-Long antifade reagent (Invitrogen). Confocal microscopy was then performed as described.

Passive immunization of mice

Mice were passively immunized with 10μg anti-TNP IgE or anti-TNP IgG1 (B.D.) in 100μl saline through retro-orbital injection. After 24 hours, the passively sensitized mice were challenged with saline, 200μg of sAg or pAg, or blank particles. At 48 hour after the challenge, mice were sacrificed and subjected to AHR and lung eosinophilia analysis.

ELISA to measure cytokines in BMMCs cultures

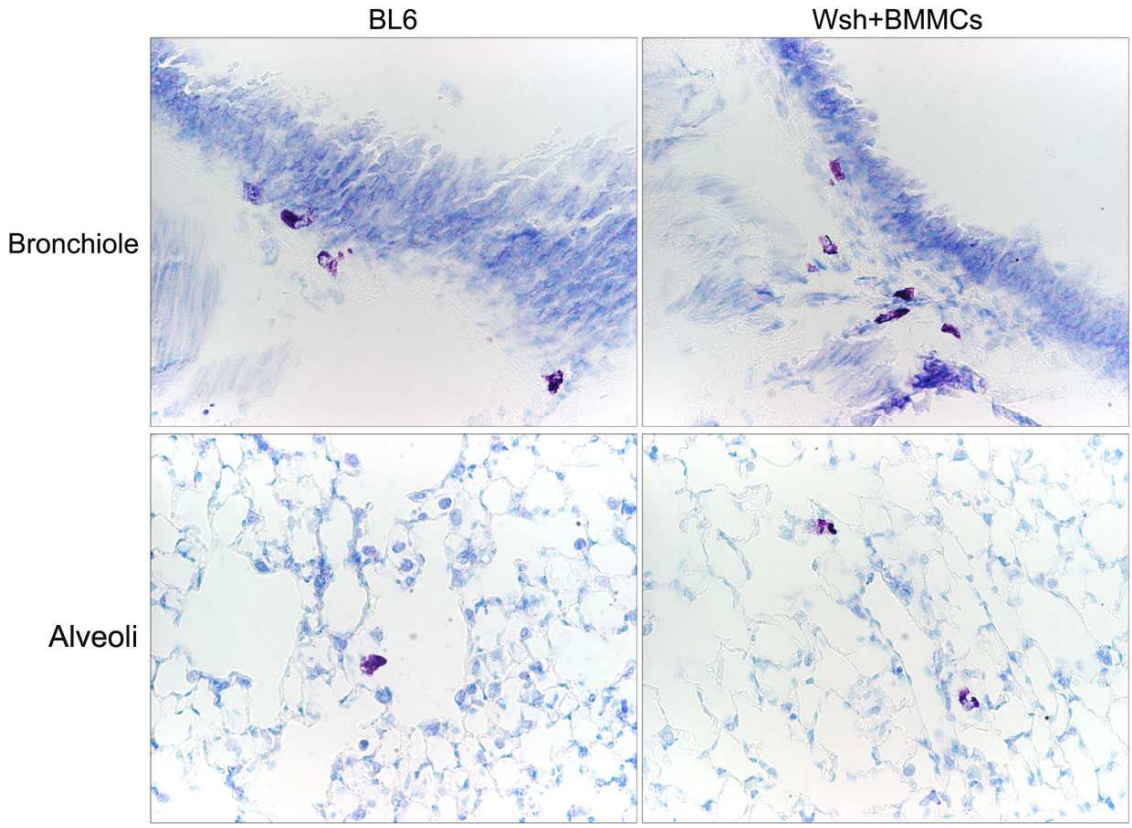
Cultures from $2x10^6$ BMMCs were harvested at 24 hours after the challenge of allergens, and IL4 level was detected using mouse IL4 ELISA set (B.D.).

Western blot analysis of tyrosine phosphoylated Syk

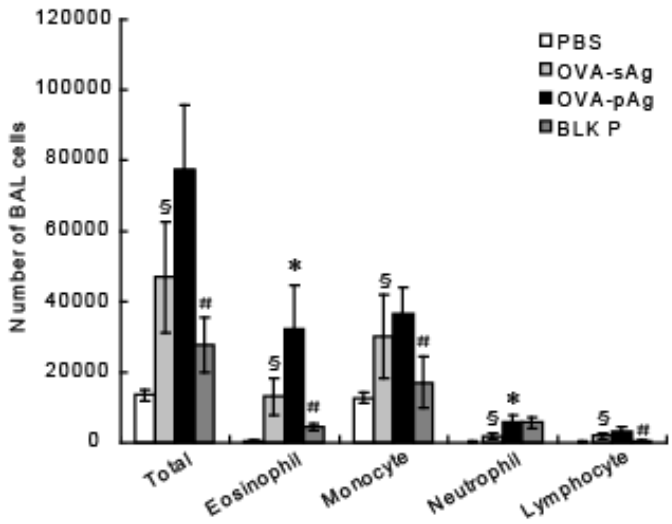
Protein was prepared from $1X10^6$ challenged BMMCs as described in manuscript. To

probe the phosphorylation status of individual tyrosine residues of the Syk protein, protein on blots were probed with primary antibodies to Syk, phospho-Syk (Tyr323), phospho-Syk (Tyr525/526), and phospho-Syk (Tyr 352)/Zap-70(Tyr 319) (Cell Signaling), followed by detection with a secondary HRP-linked antibody and Super Signal West Pico Chemiluminescent kit (Pierce).

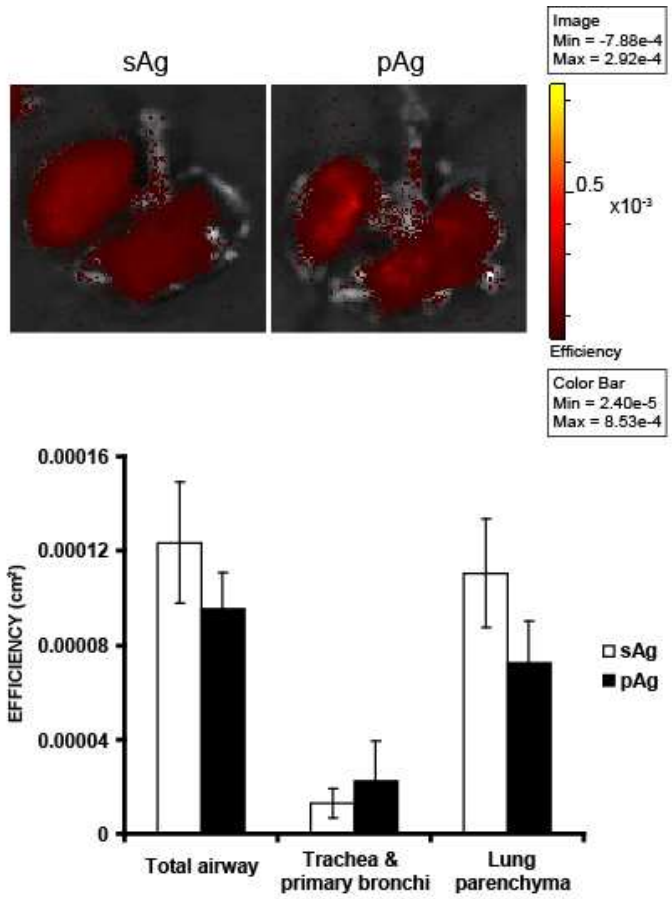
Figures and figure legends



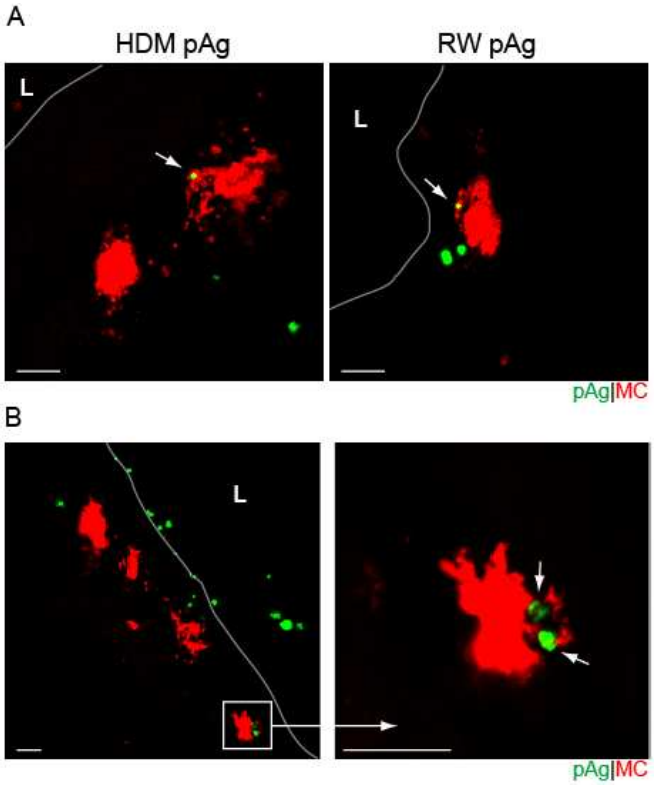
Supplementary Figure 1. Toluidine blue stained MCs in the lung. Paraffin fixed lung tissues from WT and Wsh mice repleted with BMMCs were sectioned and stained with toluidine blue. The top panels show the MCs around bronchioles. The bottom panels show MCs in the alveolar area.



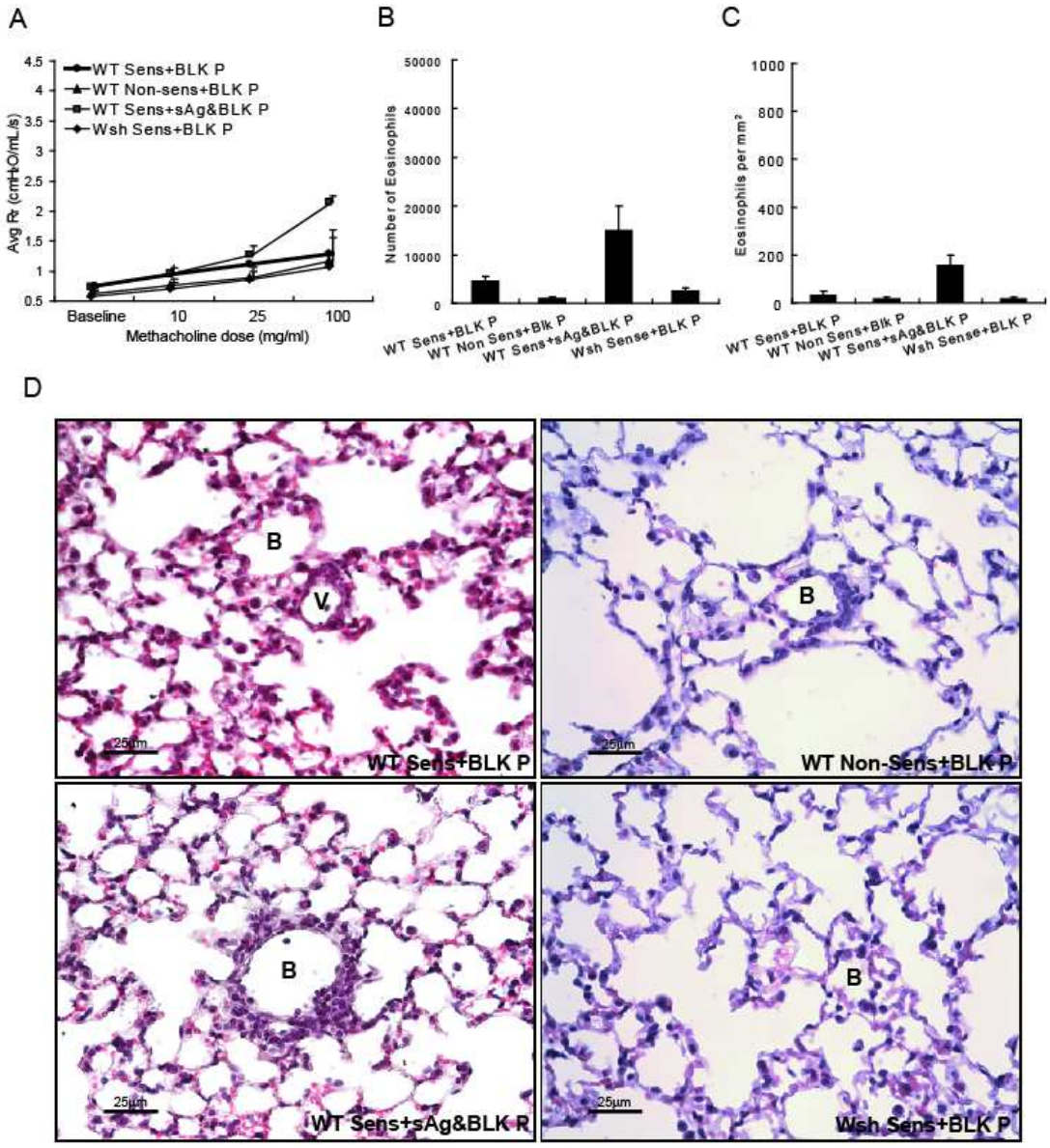
Supplementary Figure 2. Quantification of total cell and individual cell populations in BAL. H&E stained BAL fluid cells from WT mice were counted as total cells and differentiated cells. N=3-5, $^{\S}p<0.05$, sAg vs PBS; $^{*}p<0.05$, pAg vs sAg; $^{\#}p<0.05$, pAg vs BLK P. The data showed that, except for eosinophil counts, other cell types did not show specific elevation to pAg (the response to pAg is not significantly different from both sAg and BLK P). As for the number of total cells, although there is an elevation of total cells in response to pAg challenge when compared to sAg, however, the difference is not significant.



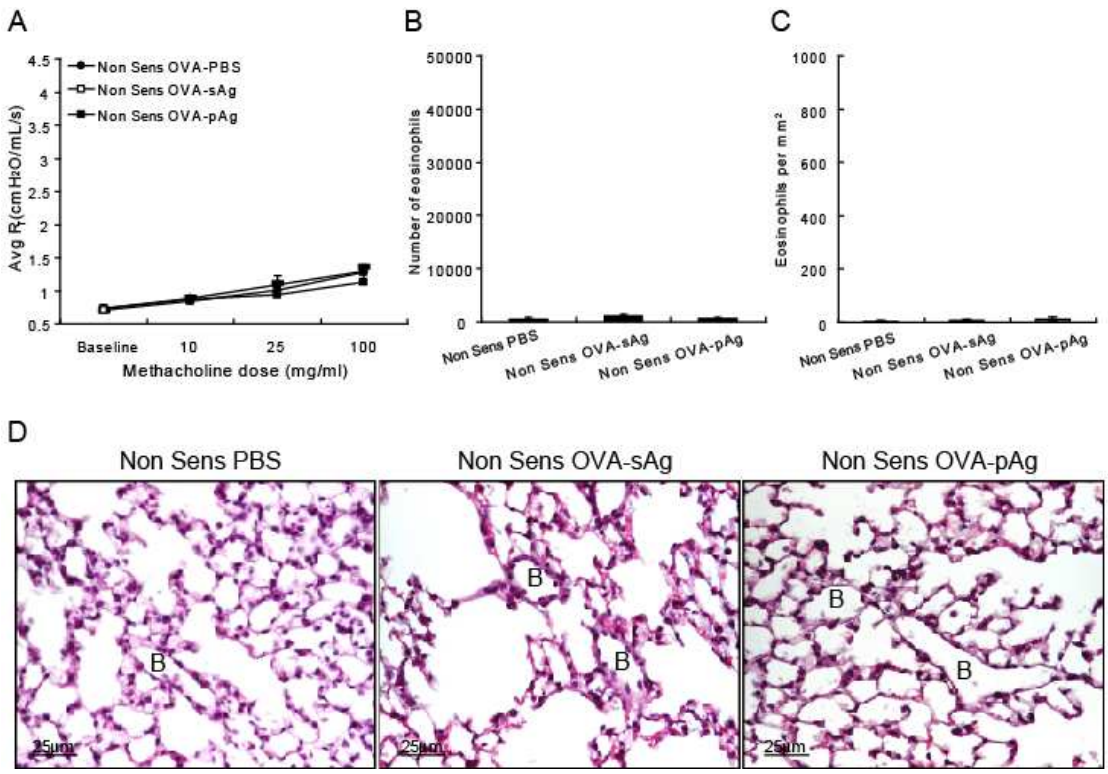
Supplementary Figure 3. Distribution of sAg and pAg in lungs. Equal amounts of Qdot® 800 (red) labeled sAg and pAg were instilled through the intranasal and intratracheal routes. After 1 hour, IVIS live imaging necropsy was performed on excised lungs. The image shows the representative photograph of lungs challenged by either sAg or pAg. The histogram shows the average efficiency of the fluorescent intensity in the whole airway, trachea and primary bronchi, and lung parenchyma from 4 mice in each group. Considering the intensity and distribution of fluorescence, there is no significant difference between the lungs challenged by sAg or pAg.



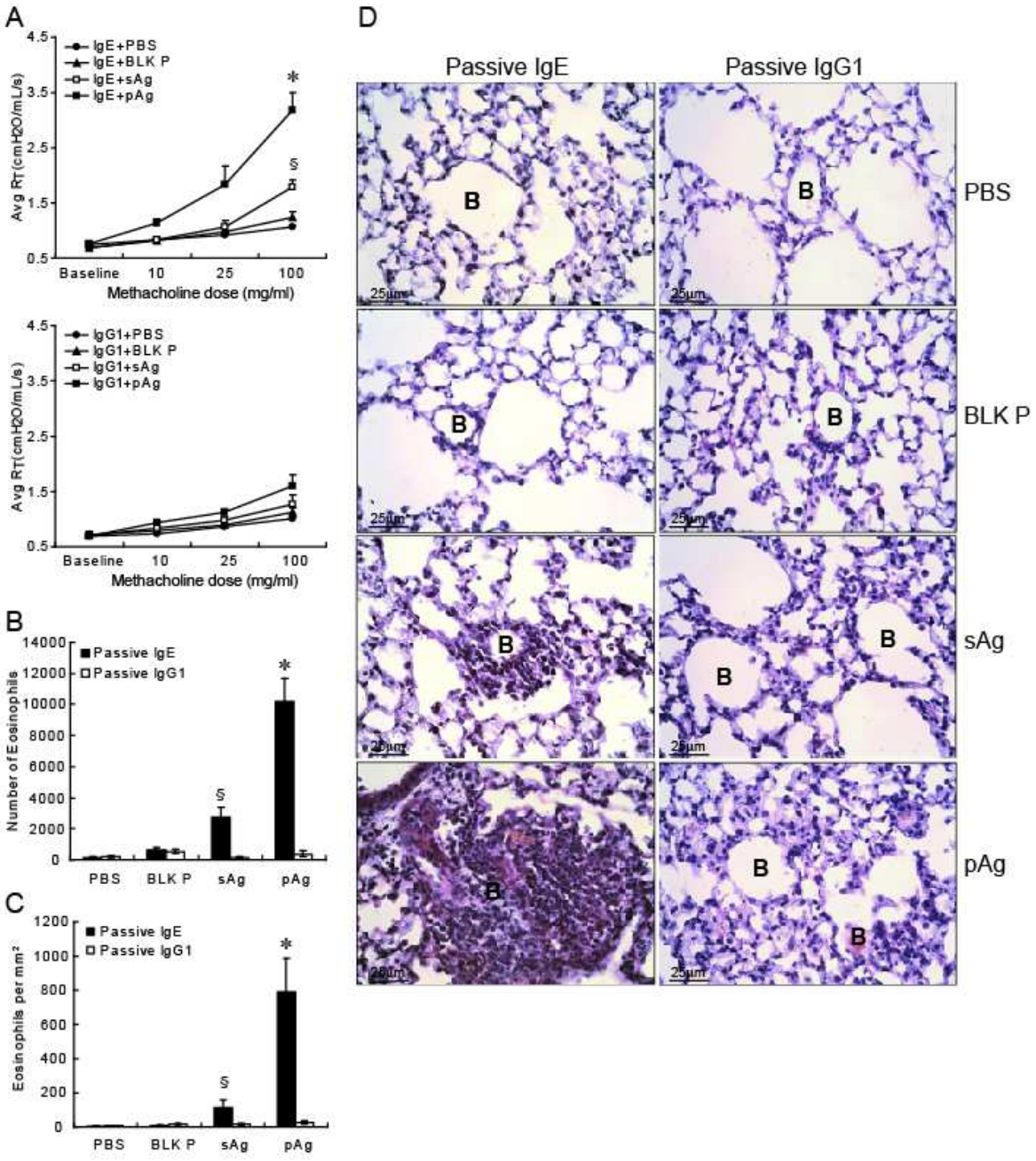
Supplementary Figure 4. Airway MCs interact with allergen conjugated particles in the lungs of sensitized mice. (**A**) Confocal images of lung tissues from mice sensitized with HDM or RW and challenged with fluorescent HDM-pAg or RW-pAg (shown in green). Tissues were stained for MCs (red). The confocal images show MCs can interact and retain natural allergen conjugated particles inside. **(B)** Sensitized mice were challenged with OVA/TNP-conjugated fluorescent particles (shown in green). Sections of lung were prepared and stained for MCs (red). Images were generated with confocal microscopy. The left confocal image shows the positioning of MCs underneath the airway epithelium, and that pAg can penetrate the epithelium to engage MCs. Scale bar = 20μm. The boxed region shows a MC interacting with pAg, which is displayed with higher magnification in the panel on the right. And arrows show the particles internalized in MCs. Scale bar = 20μm. White line depicts the position of airway epithelium, L denotes lumen. Scale bars = 20μm.



Supplementary Figure 5. Airway responses to blank particle formulations. Blank particles (BLK P) or sAg mixed with blank particles (sAg&BLK P) were used to challenge the lungs of OVA-TNP sensitized WT and Wsh mice. Naïve WT mice were used as controls. After 48 hours, mice were evaluated for AHR responses (**A**), BAL eosinophil influx (**B**) and tissue eosinophilia (**C**) as described. Representative H&E stained lung tissues (**D**) are from 4 mice in each group. Scale bars=25 μm. All these data demonstrated that the heightened airway responses are specific to pAg and not simply due to the deposit effect of particles.

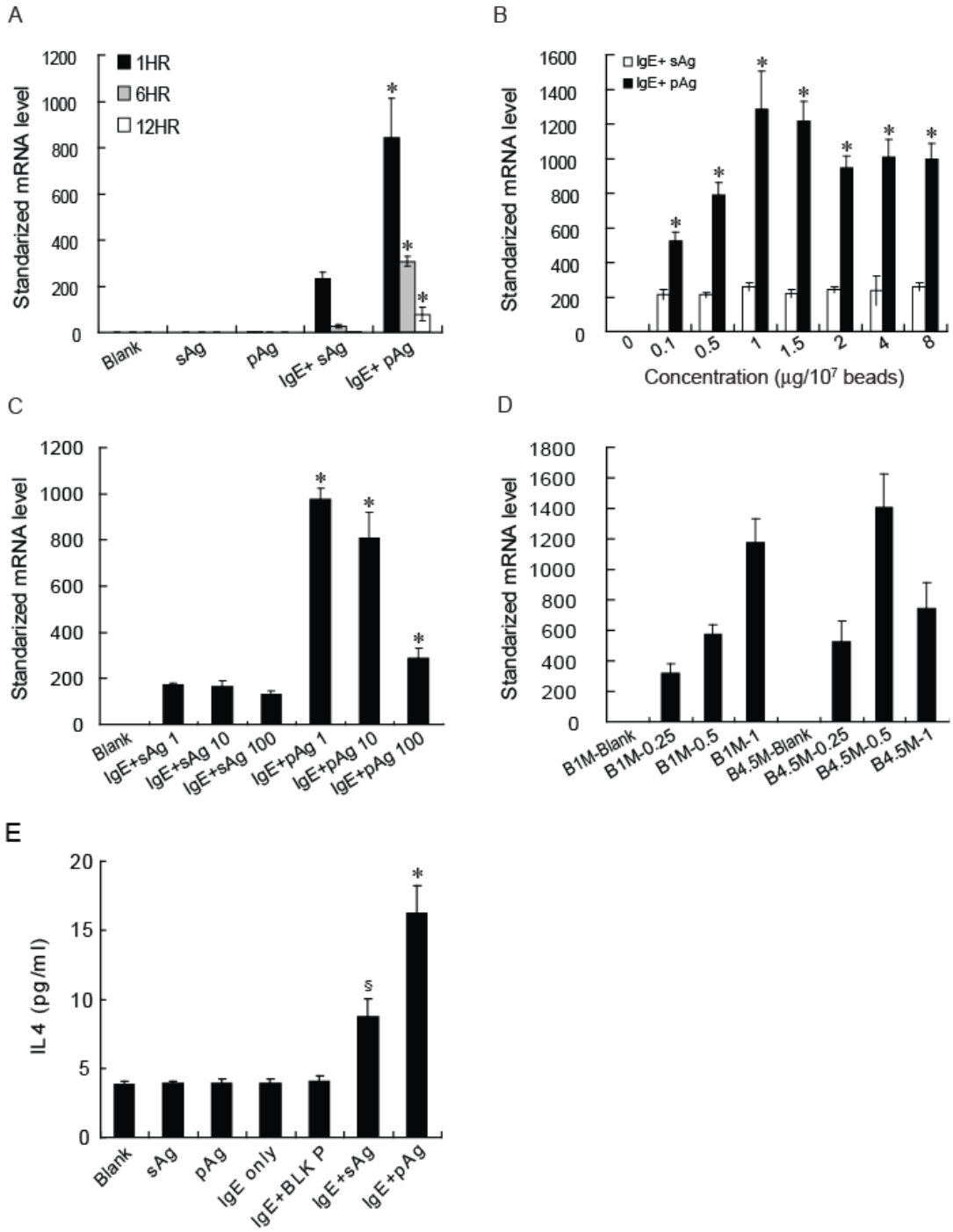


Supplementary Figure 6. Naïve mice do not respond to sAg and pAg. Naïve C57BL/6 mice without OVA/TNP sensitization had minimal AHR (**A**), BAL eosinophil (**B**), and lung eosinophil recruitment (**C**) in exposure to sAg or pAg. H&E stained lung tissues (**D**) accordingly showed no visible eosinophil accumulation around the small bronchioles (B). Scale bars = 25μm. All data are from 4 mice in each group.



Supplementary Figure 7. The differential pulmonary pathological responses to sAg and pAg are IgE specific. Mice were passively sensitized with 10μg TNP-specific IgE or TNP-specific IgG1. After 24 hours mice were challenged with saline, 60μg sAg or pAg, or blank particles (BLK P). At 48hour after the allergen challenge, AHR (**A**), BAL eosinophil influx (**B**), and lung eosinophil recruitment (**C**) were examined. In mice passively sensitized with IgE, sAg induced significantly

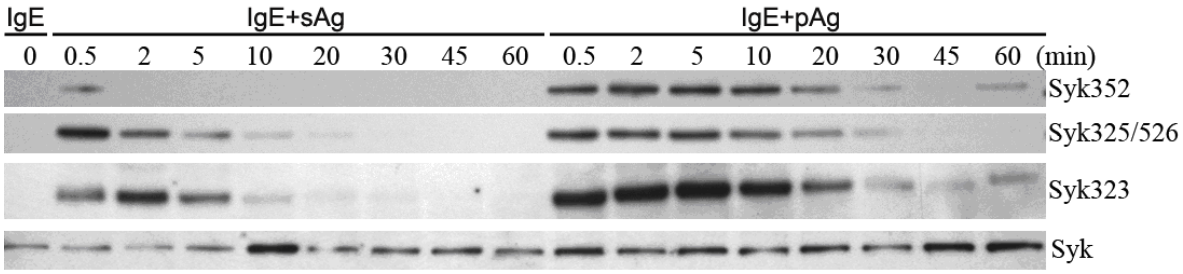
higher responses than saline and BLK P controls (n=4, $^{\S}p<0.01$, sAg vs PBS and BLK P), and pAg induced significantly higher airway responses than sAg (n=4, *p<0.01, pAg vs sAg). While in mice passively sensitized with IgG1, sAg and pAg induced minimal airway inflammatory responses. The representative H&E stained lung tissues (**D**) show that in IgE sensitized mice but not in IgG1 sensitized mice, both sAg and pAg induced airway eosinophil accumulation around bronchiole (B), and the pAg induced inflammation was heightened than sAg. Scale bars = 25μm.



Supplementary Figure 8. pAg induce heightened cytokine production compared to sAg under different experimental conditions. (**A)** mRNA of IL-4 in sensitized BMMCs at various time points following exposure to equal amounts (1μg) of sAg or pAg was detected by real time PCR (n=4, *p<0.01). (**B-D)** mRNA of IL-4 was

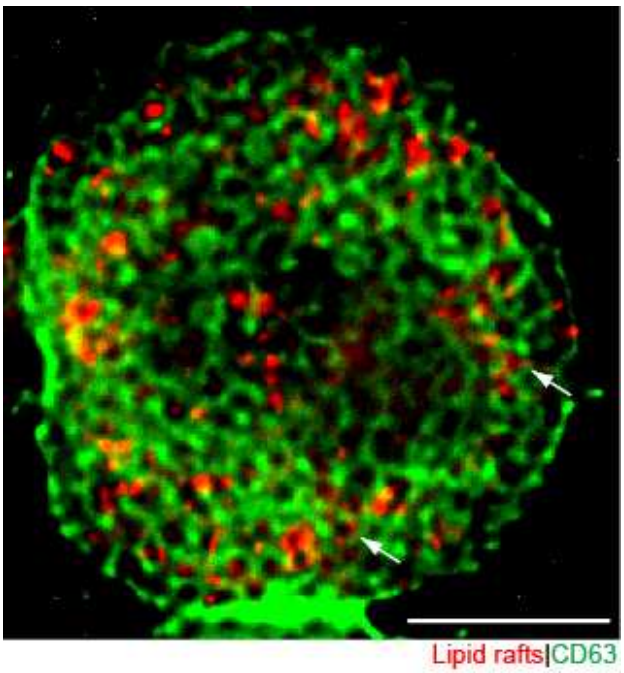
detected 1hr following allergen exposure to sensitized BMMCs by real time PCR. (**B)** Effect of increasing amount of sAg and pAg on IL-4 responses of BMMCs (n=4,*p<0.01). (**C)** Effect of particle/BMMC ratio on the IL-4 responses of BMMCs. pAg were prepared by conjugating 1μg of OVA/TNP on 10^7 particles. The ratios of particles to cells used were 1:1, 10:1 and 100:1. In a parallel experiment, comparable amounts of sAg were placed onto $1X10^6$ BMMCs. Altering the dose of sAg revealed little or no change in IL-4 production. However, increasing the ratio of pAg to BMMC from 10:1 to 100:1 markedly reduced the IL-4 response. Nevertheless, regardless of the amounts of allergen present, the IL-4 responses to pAg were consistently more significant than the responses elicited by comparable amounts of sAg. (**D)** Effect of particle size on the IL-4 responses of BMMCs to pAgs. Various amounts of OVA/TNP (0.25μg, 0.5μg, 1μg) were conjugated on $1X10^7$ polystyrene particles of 1μm size or 4.5μm size. A dose dependent increase in IL-4 production was observed with 1μm sized particles. However, when the larger 4.5μm sized particles were employed, a dose dependent effect was observed with the lower concentrations of allergen but not at the highest (1μg). Based on these data, it was determined that for all subsequent experiments involving pAg the optimal experimental conditions will be to expose $1X10^6$ BMMCs to $1X10^7$ 1μm sized particles conjugated with 1μg of OVA/TNP. (**E**) IL4 production in BMMC cultures. TNP-specific IgE sensitized BMMCs were challenged with 1μg sAg or pAg. 24hour after exposure to allergens, IL4 in BMMC culture supernatants was detected by standard ELISA. Controls included sensitized BMMCs challenged with vehicle or

blank particles (BLK P), unsensitized BMMCs challenged with vehicle, sAg or pAg. At the protein level, sAg triggered significantly enhanced IL4 production than controls (n=4, $^{\S}p<0.05$, sAg vs controls), and pAg triggered a higher IL4 production than sAg (n=4, *$p<0.05$, pAg vs sAg).



Supplementary Figure 9: pAg induces sustained tyrosine phosphorylation of Syk.

$1X10^6$ BMMCs were sensitized by TNP-specific IgE for 18 hours, followed by exposure to sAg (OVA/TNP) or pAg (OVA/TNP conjugated particles). The phosphorylation patterns on three different tyrosine residues of Syk were examined by western blot. The image is representative of three separate experiments. The data demonstrates that pAg induces significantly prolonged phosphorylation on all three tyrosine residues compared to the phosphorylation induced by sAg.



Supplementary Figure 10. Lipid raft enriched domains of BMMCs are encased in CD63 positive subcellular compartments. BMMCs were stably transfected with GFP-CD63 (green). These BMMCs were fixed and probed for lipid raft components employing Alexa-647 conjugated cholera toxin subunit B (red). The image reveals that lipid raft enriched areas are encased within CD63 positive compartments (arrows show the co-localization of lipid raft components within CD63 positive compartments.)

Tables

Supplementary Table 1: Number of mast cells per mm^2 in lung tissues

Mice	Mean	SD
WT	1.05	0.57
Wsh	0.21	0.20
Wsh+BL6 BMMCs	4.57	1.57*
Wsh+Cav BMMCs	3.54	0.65*
Wsh+WT BMMCs	3.41	0.41*

* $p<0.05$, n=3-5, BMMCs repleted Wsh mice vs WT mice.