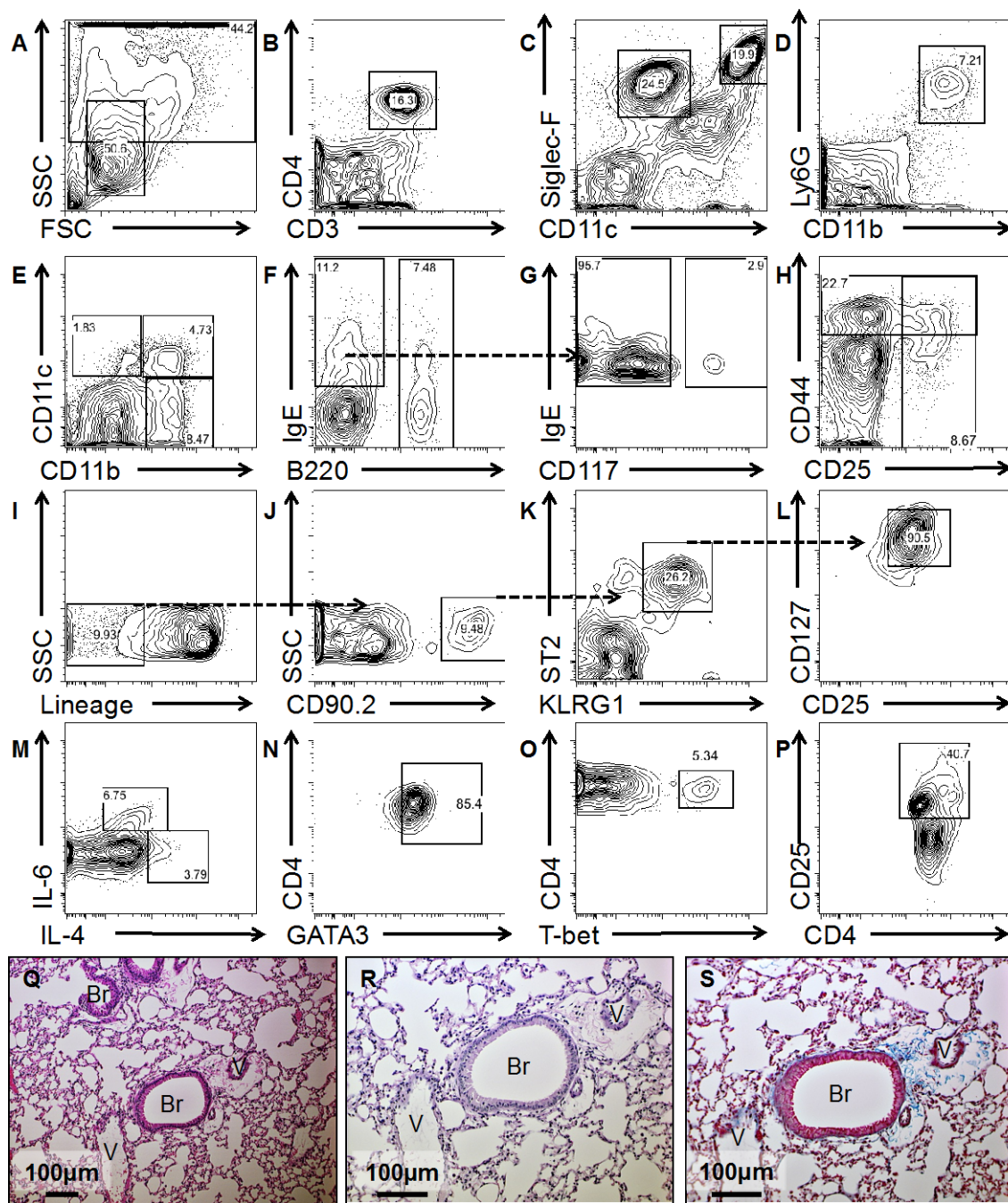


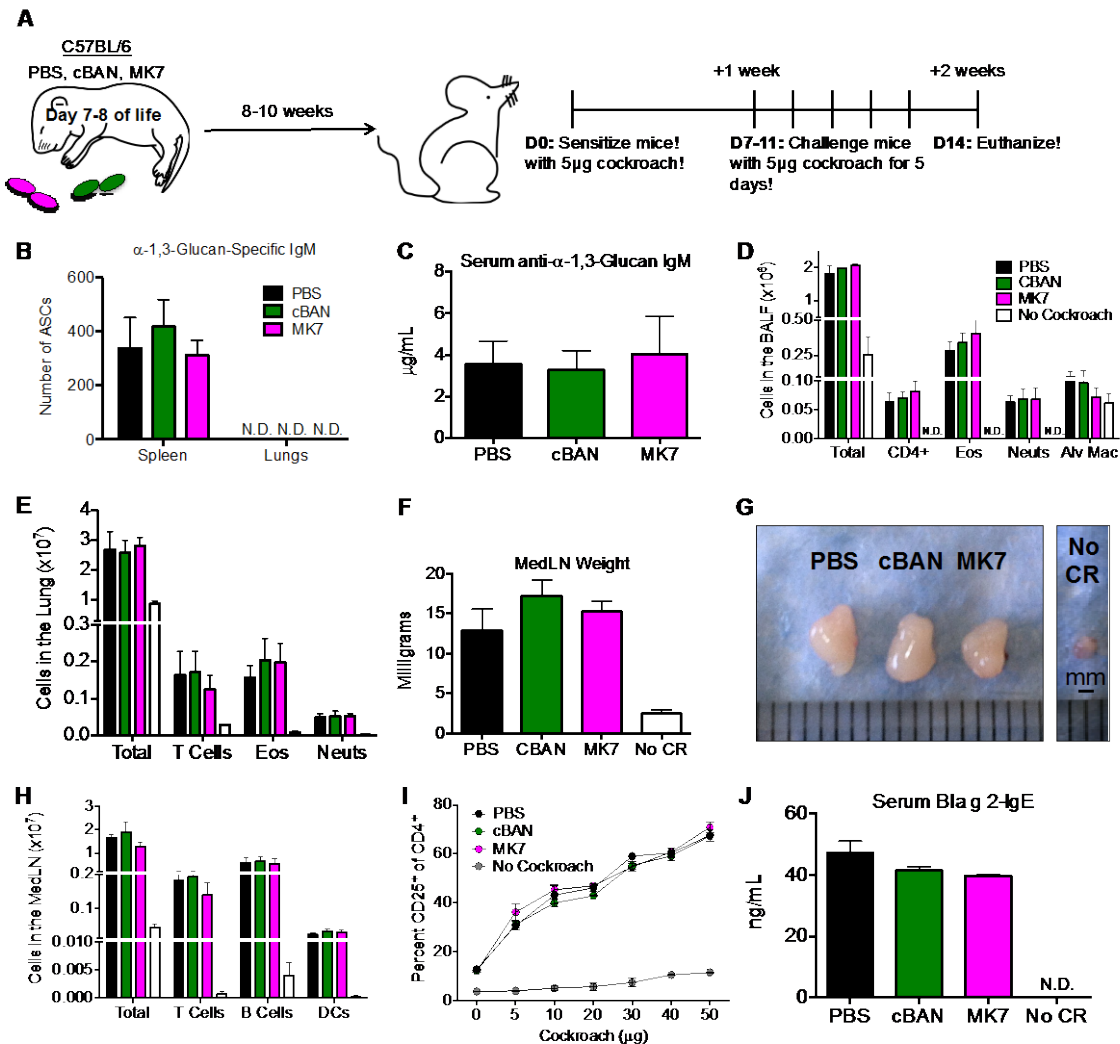
## Supplemental Figure 1



1  
 2 **Supplemental Figure 1: Representative gating scheme for cells detected by flow**  
 3 **cytometry and histological stains of paraffin-embedded lungs from mice that have**  
 4 **not received any allergens. Cells from suspensions of BALF, lungs, and MedLN were**

5 analyzed by flow cytometry to detect specific markers as described below: (A)  
6 representative forward scatter (FSC) and side scatter (SSC) for cells from the lungs (B) T  
7 cells ( $CD3^+CD4^+$ ), (C) alveolar macrophages ( $Siglec-F^+CD11c^+$ ) and eosinophils ( $Siglec-$   
8  $F^+CD11c^-$ ), (D) neutrophils ( $CD11b^+Ly6G^+$ ), (E) dendritic cells ( $CD11b^-CD11c^+$ ),  
9 immature dendritic cells ( $CD11b^+CD11c^+$ ), and macrophages ( $CD11b^+CD11c^-Ly6G^-$ ) (F)  
10 B cells ( $B220^+$ ) and IgE-bound non B cells ( $B220^-IgE^+$ ), (G) basophils ( $B220^-IgE^+Ckit^-$ ),  
11 and mast cells ( $B220^-IgE^+Ckit^+$ ). (H) T cells ( $CD3^+CD4^+$ ) were further phenotyped as  
12  $CD44^{high}$  or  $CD25^+$ . (I) ILC2s were identified by excluding lineage cells expressing CD3,  
13 CD4, CD8, CD19, B220, CD11b, CD11c, F4/80, GR1, TER119, and DX5. From the  
14 remaining cells, ILC2s were identified as those that expressed (J) CD90.2, (K) ST2 and  
15 KLRG1, (L) CD127 and CD25, and CD117 (not shown).  $CD4^+$  T cells were further  
16 characterized as (M)  $IL-4^+$  or  $IL-6^+$ , (N)  $GATA3^+$  (O)  $T-bet^+$ , or (P)  $CD25^+$ . Paraffin-  
17 embedded lungs from mice receiving no allergens were stained with (Q) Hematoxylin and  
18 Eosin, (R) Periodic Acid-Schiff, (S) or Masson's Trichrome stains.

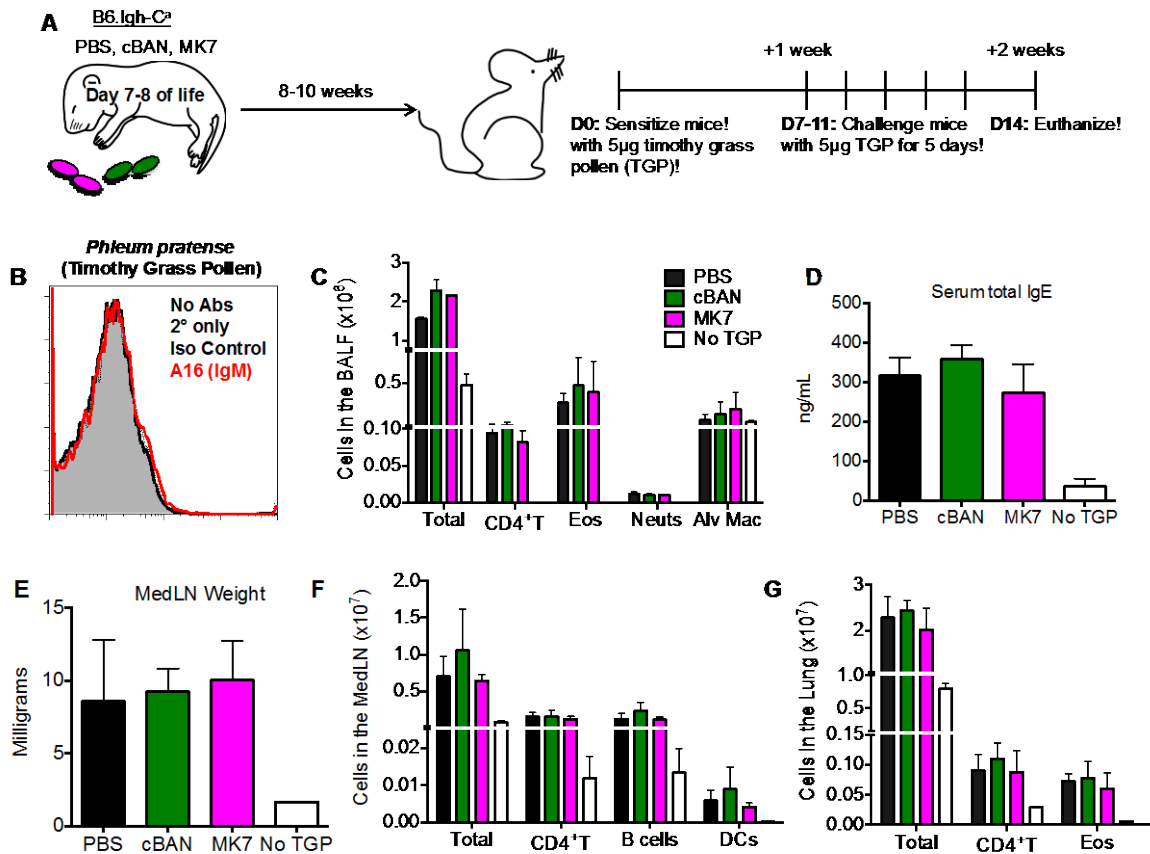
## Supplemental Figure 2



19

20 **Supplemental Figure 2: Immunization of neonatal C57BL/6 mice (Igh-C<sup>b</sup>) with MK7**  
 21 **does not result in suppressed development of cockroach allergy** (A) Neonatal C57BL/6  
 22 mice (B6.Igh-C<sup>b</sup>) were immunized i.p. with  $5 \times 10^7$  of cBAN or MK7, or treated with PBS.  
 23 At 8 to 10 weeks of age, these mice were sensitized and challenged i.t. with cockroach  
 24 allergen. (B) The number of  $\alpha$ -1,3-glucan-specific IgM- and IgA- secreting cells from the  
 25 lungs and spleen were enumerated and (C) anti- $\alpha$ -1,3-glucan-IgM from the serum was  
 26 quantified by ELISA. (D, E) Cells were identified from the BALF and lungs as  
 27 documented in Supplemental Figure 1 and enumerated. (F-H) MedLNs were  
 28 photographed, weighed, and the CD4<sup>+</sup> T cells, B cells, and DCs were quantified. (I)  
 29 300,000 MedLN cells were incubated with indicated amounts of cockroach allergen for  
 30 five days and the percent of CD4<sup>+</sup> T cells expressing CD25 was determined. (J) ELISA  
 31 was used to measure levels of serum anti-Bla g 2-IgE. Values represent the mean  $\pm$  SEM  
 32 from 3 independent experiments with 5-10 mice per group. Values that were not  
 33 detectable are noted as "N.D." Data were analyzed by ANOVA and statistically significant  
 34 differences are indicated as \* $p < 0.05$ , \*\* $p < 0.01$ , and \*\*\* $p < 0.001$

## Supplemental Figure 3



35

36 **Supplemental Figure 3: B6.Igh-C<sup>a</sup> mice immunized with MK7 as neonates are not**  
 37 **protected against the development of  $\alpha$ -1,3-glucan-free timothy grass pollen (TGP)**  
 38 **allergy.** (A) Neonatal B6.Igh-C<sup>a</sup> mice were immunized i.p. with 5x10<sup>7</sup> cBAN or MK7 or  
 39 treated with PBS. At 8 to 10 weeks of age, these mice were sensitized with TGP, rested  
 40 for seven days, challenged for 5 consecutive days, and euthanized two days later for  
 41 analysis. (B) TGP was stained with monoclonal anti- $\alpha$ -1,3-glucan IgM antibody, A16, or  
 42 isotype control antibody, MD4, and antibody binding was determined by flow cytometry.  
 43 (C) Cells were enumerated from the BALF and identified by flow cytometry as  
 44 documented in Supplemental Figure 1. (D) ELISA was used to determine levels of IgE  
 45 in the serum. (E) MedLNs were weighed and (F) cells were enumerated from the MedLN  
 46 and (G) pulmonary parenchyma. Values represent the mean  $\pm$  SEM from 2 independent  
 47 experiments with 5-10 mice per group. Data were analyzed by ANOVA and statistically  
 48 significant differences are indicated as \* $p$ <0.05, \*\* $p$ <0.01, and \*\*\* $p$ <0.001.