SUPPLEMENTAL MATERIAL

Scanlon et al., http://www.jem.org/cgi/content/full/jem.20110522/DC1

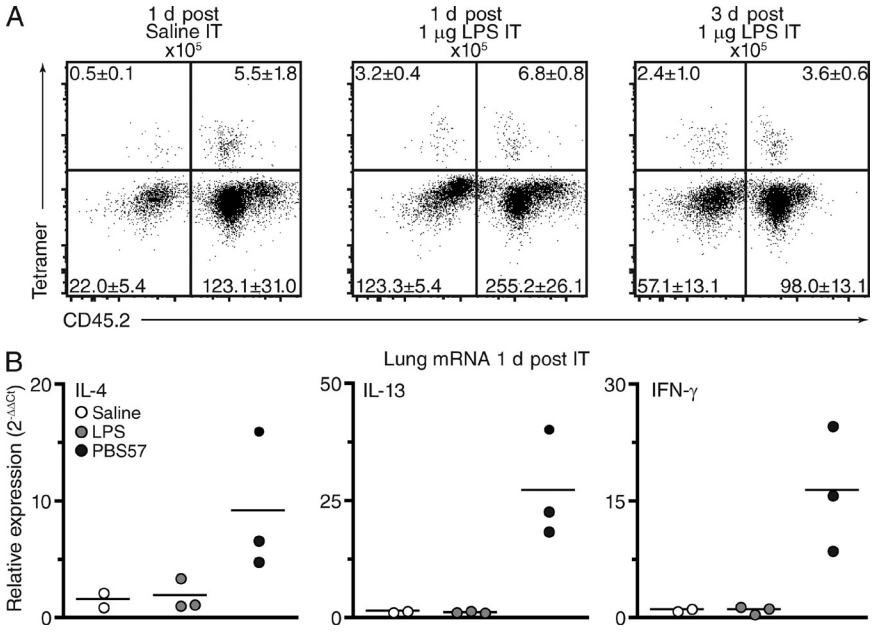

Figure S1. Comparison of 1 µg LPS and 100 ng PBS57 administered intratracheally. (A) High doses of LPS induce NKT cell extravasation measured by the intravascular anti–CD45-PE labeling assay. Numbers indicate mean percentage and SEM of three mice per group. (B) IL-4, IL-13, and IFN-γ messenger RNA in lung tissue 24 h after intratracheal (IT) saline, LPS, or PBS57 as indicated. Horizontal bars indicate the mean.

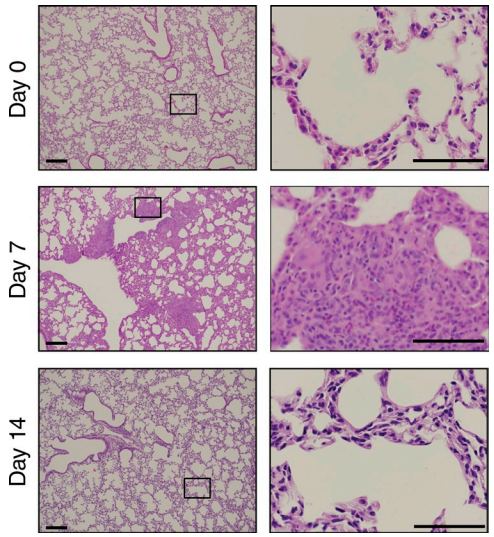

Figure S2. Extrinsic allergic alveolitis induced by PBS57. H&E staining of lung sections at days 0, 7, and 14 after intratracheal administration of 100 ng PBS57. Right column pictures are higher magnification images of the areas boxed in the left column. Data are representative of three lungs/group. Bars, 200 µm.

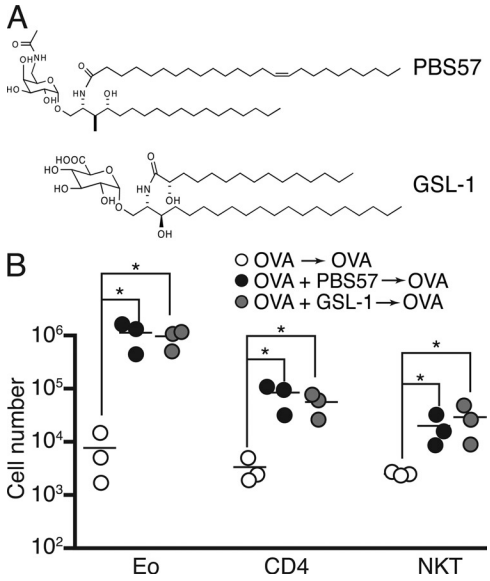

Figure S3. Microbial GSL-1 and the synthetic analogue PBS57 induce similar allergic airway inflammation. (A) Chemical structures of PBS57 and GSL-1. (B) Mice sensitized with OVA, OVA + 100 ng PBS57, or OVA + 100 ng GSL-1 as indicated were challenged with OVA alone. BAL cell counts of eosinophils (Eo), CD4 lymphocytes, and NKT cells at day 20 are shown for individual mice. Horizontal bars indicate the mean. *, $P < 0.05$.

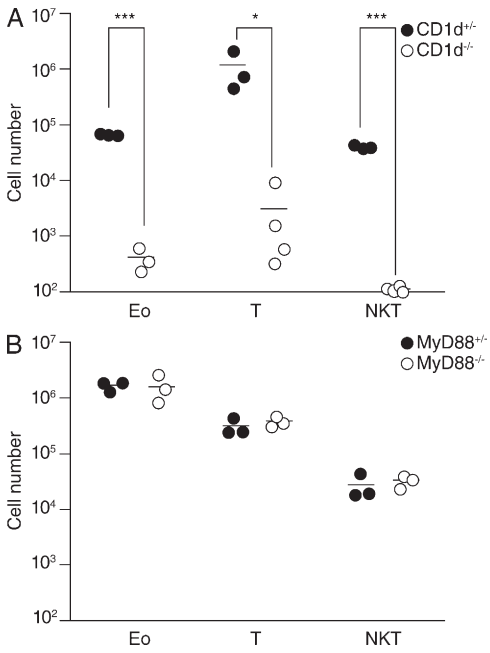

Figure S4. Allergic airway inflammation is CD1d dependent and MyD88 independent. (A) $CD1d^{+/-}$ and $CD1d^{-/-}$ littermates were sensitized with OVA + PBS57 at day 0 and challenged with OVA as described in Allergic airway inflammation model. Shown are BAL eosinophils (Eo), T cells, and NKT cells at day 20. (B) Similar experiment comparing $MyD88^{+/-}$ and $MyD88^{-/-}$ littermates. (A and B) Horizontal bars indicate the mean. *, $P < 0.05$; ***, $P < 0.001$.

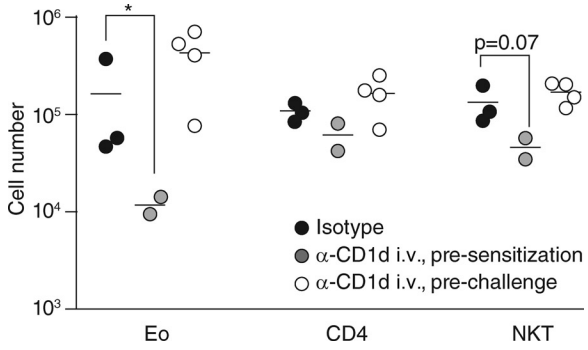

Figure S5. Effect of anti–CD1d blocking on allergic airway inflammation. Mice i.v. injected with 100 µg anti-CD1d antibody 20H2 either presensitization or prechallenge or with isotype control, as indicated, were assessed for allergic airway inflammation by counting eosinophils (Eo), CD4 T cells, and NKT cells in BAL. Horizontal bars indicate the mean. *, $P < 0.05$.

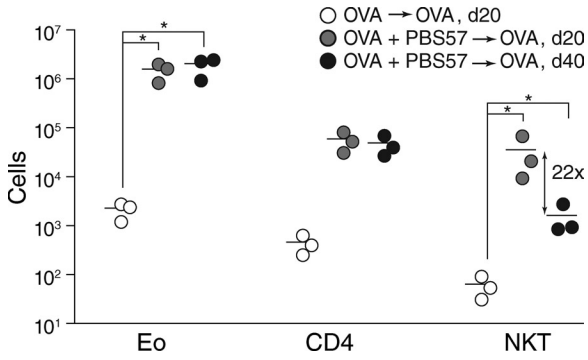

Figure S6. Decreased NKT cell expansion upon delayed OVA challenge. Mice received the indicated intratracheal sensitization and challenge before counting BAL eosinophils (Eo) and CD4 and NKT cells. Horizontal bars indicate the mean. *, $P < 0.05$.

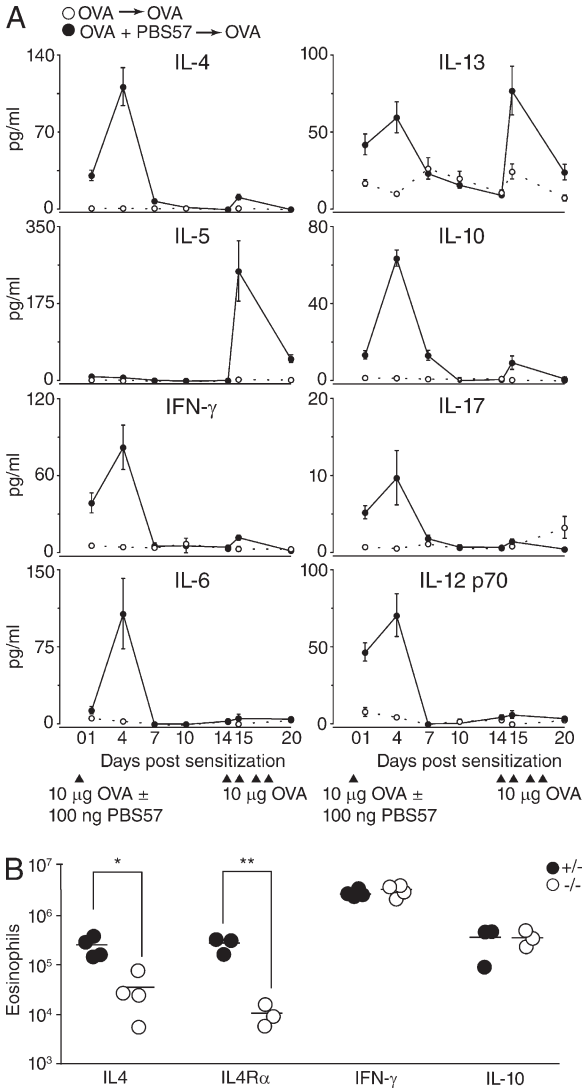

Figure S7. Mixed cytokines burst after sensitization resolves into a polarized Th2 response after challenge. (A) Quantification (mean ± SEM of three mice/group) of cytokines in BAL obtained at the indicated days. (B) Mice lacking IL-4 or IL-4Rα but not IFN-γ or IL-10 have impaired allergic airway inflammation assessed by BAL eosinophil counts at day 20. Horizontal bars indicate the mean. *, $P < 0.05$; **, $P < 0.01$.

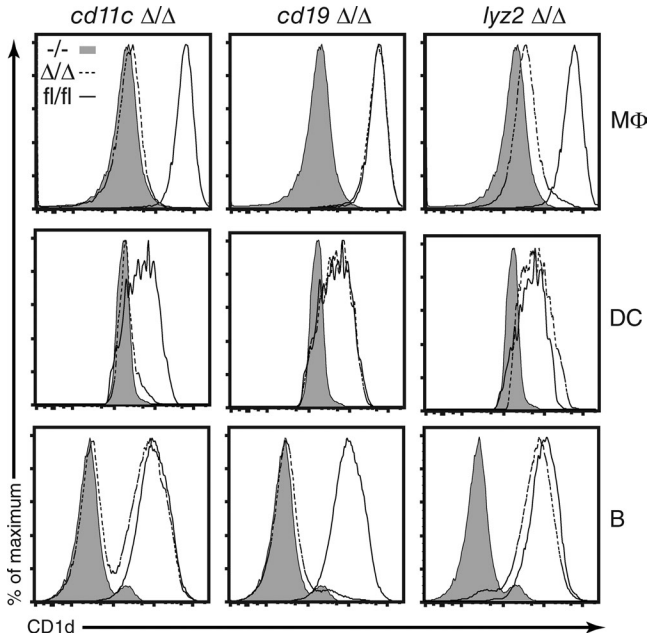

Figure S8. Cell type–specific CD1d depletion in CD1d1$^{fl/fl}$ mice. Representative histograms of CD1d expression in lung macrophages (MΦ), DCs, and B cells of CD1d1$^{fl/fl}$ mice (fl/fl), CD1d1$^{fl/fl}$ mice crossed to indicated Cre deleter strains (Δ/Δ), and CD1d$^{-/-}$ mice (−/−). Note that both macrophages and DCs lost CD1d expression in cd11c Δ/Δ mice and that a fraction of B cells lost CD1d expression in cd11c Δ/Δ mice. Data are representative of four mice/group.