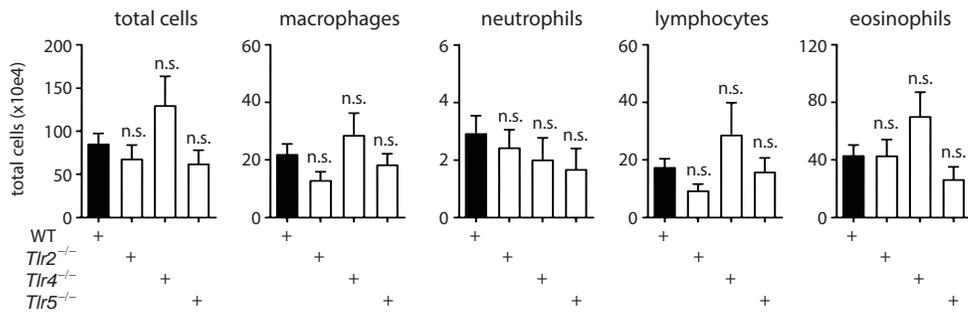
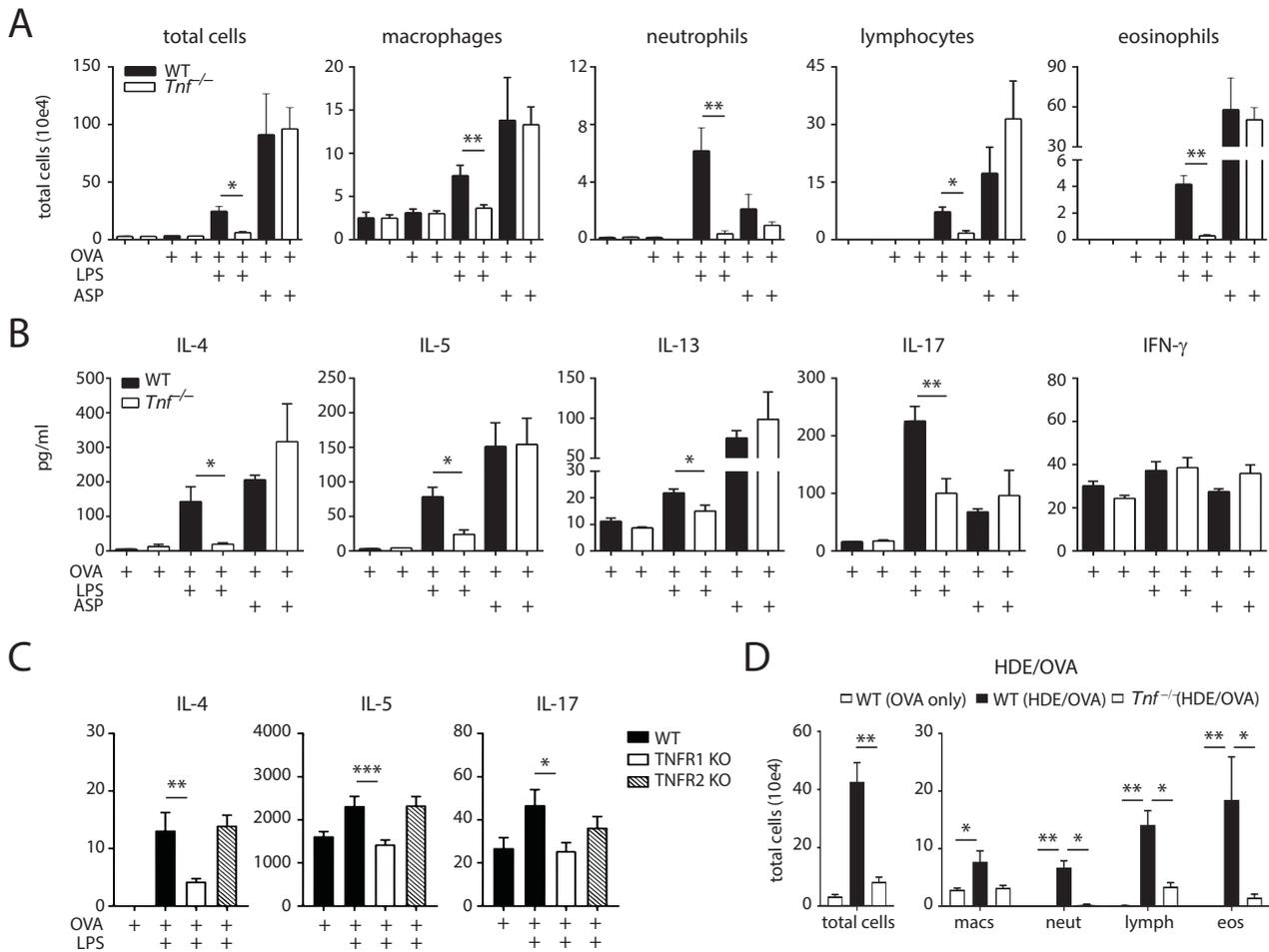


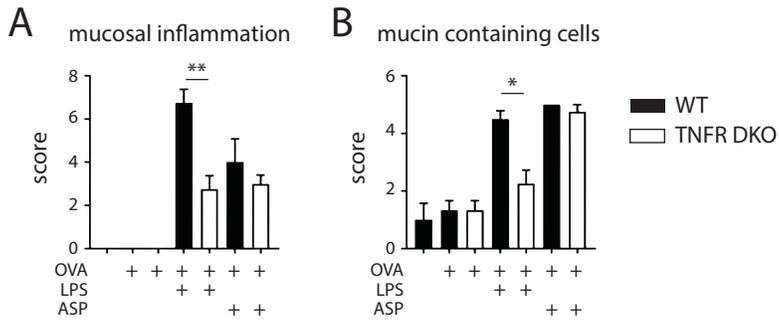
Supplemental Figure 1. Line histograms showing fluorescence in cells from mice treated with AF647-labeled OVA (solid lines) or unlabeled OVA (dashed lines). Numbers above bars denote percentages of cells staining for AF647-OVA in each sample.



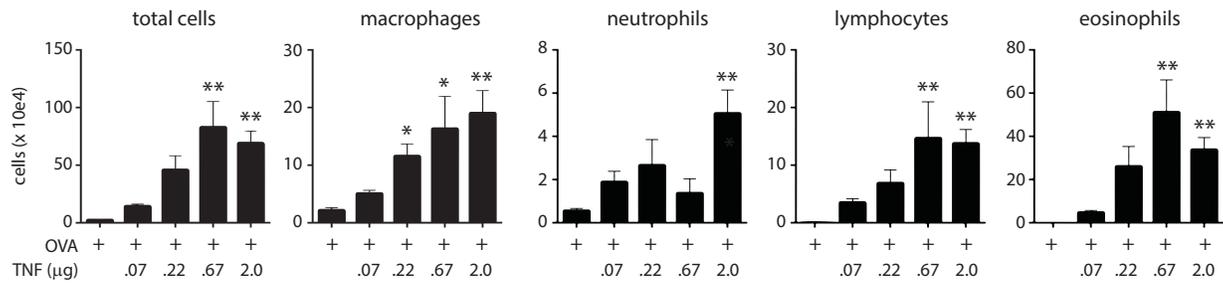
Supplemental Figure 2. TLR ligands do not contribute to protease-mediated allergic airway disease. Mean cell numbers \pm SEM in the airways of WT mice and the indicated strains of *Tlr* mutant mice that were sensitized with ASP/OVA, and subsequently challenged with aerosolized OVA ($n = 5 - 8$ mice/group). Data shown are from one of two experiments yielding similar results. n.s., not significant ($P > 0.05$) between WT and mutant mice; Kruskal-Wallis one-way ANOVA with Dunn's multiple comparison test.



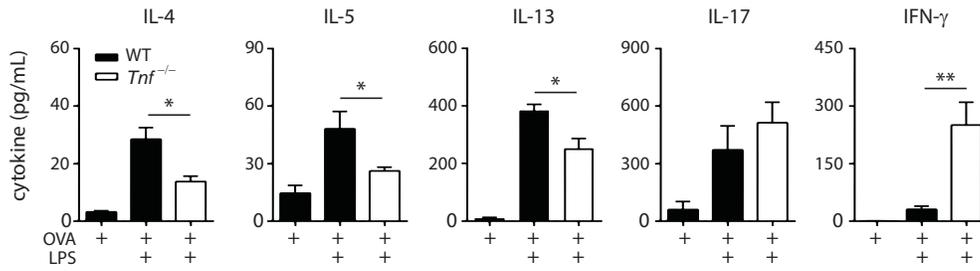
Supplemental Figure 3. TNF is required for LPS-mediated, but not a protease-mediated model of asthma. (A and B) WT and *Tnf*^{-/-} mice were sensitized to LPS/OVA or ASP/OVA, and subsequently challenged with aerosolized OVA. **(A)** Total cell numbers and **(B)** cytokine levels in BALF post-challenge ($n = 6$ mice per negative control groups; $n = 10 - 12$ mice per LPS/OVA and ASP/OVA treated groups). **(C)** WT, TNFR1 and TNFR2 single KO mice were sensitized to LPS/OVA and then challenged with aerosolized OVA. Shown are cytokine concentrations in supernatants of lung explants restimulated with OVA for 24 hrs ($n = 12$ mice/group). **(D)** Cell numbers for the indicated leukocyte types in BALF of WT and *Tnf*^{-/-} mice sensitized to OVA using HDE as an adjuvant, and subsequently challenged with aerosolized OVA ($n = 6$ mice/group). Values shown represent mean \pm SEM from two pooled experiments yielding similar results **(A, B, and C)** or from a single experiment **(D)**. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ for WT vs *Tnf*^{-/-} mice that were similarly treated **(A, B, and D)** or for WT vs TNFR1 or TNFR2 single KO mice that were sensitized with LPS/OVA; Kruskal-Wallis one-way ANOVA with Dunn's multiple comparison test.



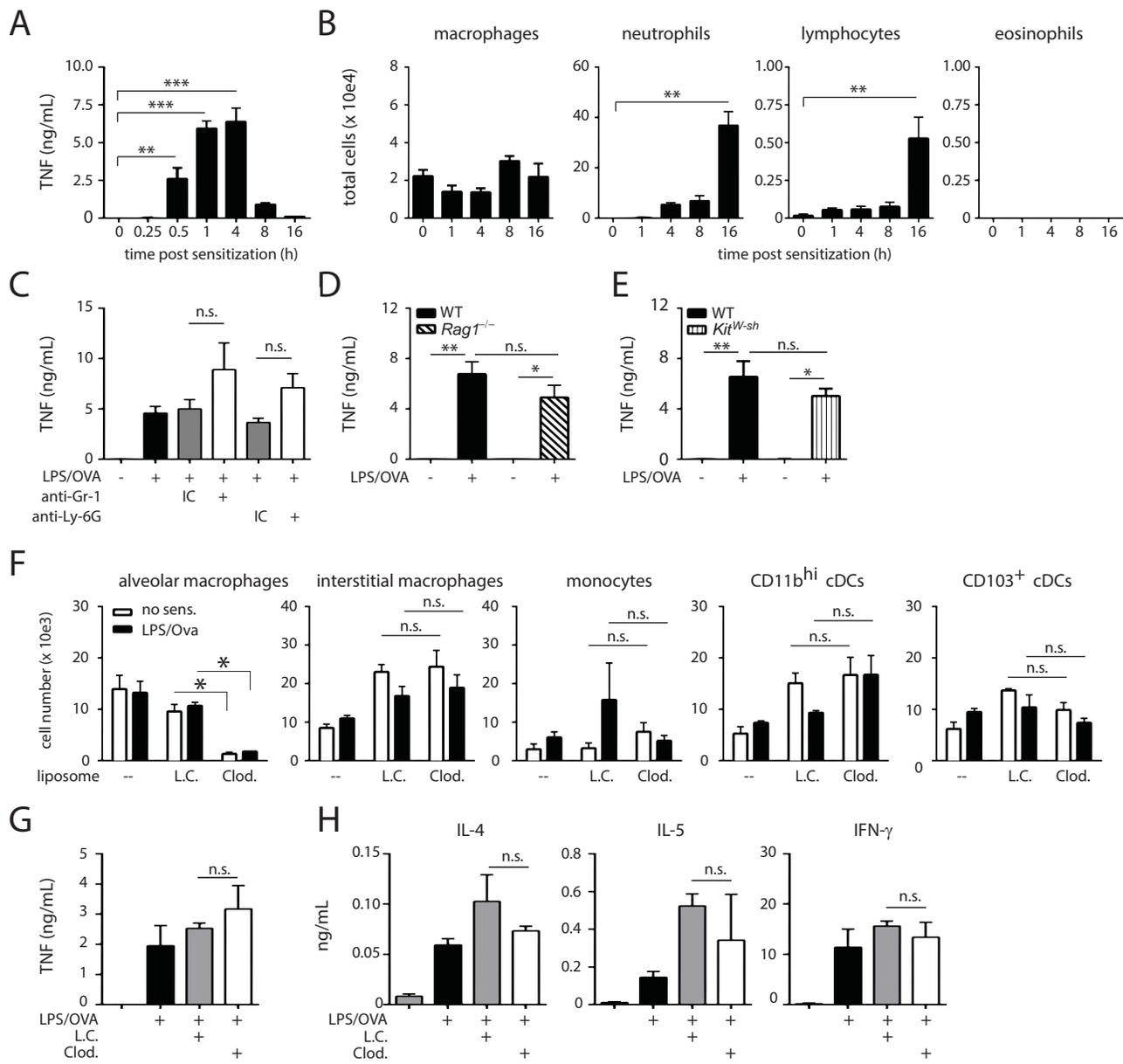
Supplemental Figure 4. TNF is required for LPS-mediated, but not protease-mediated, mucosal inflammation and mucus production. (A and B) WT and TNFR DKO mice were sensitized to OVA using the indicated adjuvants, and then challenged with aerosolized OVA. **A)** Mean inflammation score \pm SEM for H&E stained lung sections (ranked from 0 (no inflammation) to highest (8)). **B)** Mean score \pm SEM for mucin containing cells in AB/PAS stained sections (ranked from lowest (0) to highest (5)). Scores were based on the number and location of positively stained cells in the main bronchus and proximal branch, preterminal bronchioles and terminal bronchioles. Data shown are from one experiment ($n = 4$ mice/group). * $P < 0.05$, ** $P < 0.01$; WT vs TNFR DKO mice that were treated similarly; Kruskal-Wallis one-way ANOVA with Dunn's multiple comparison test.



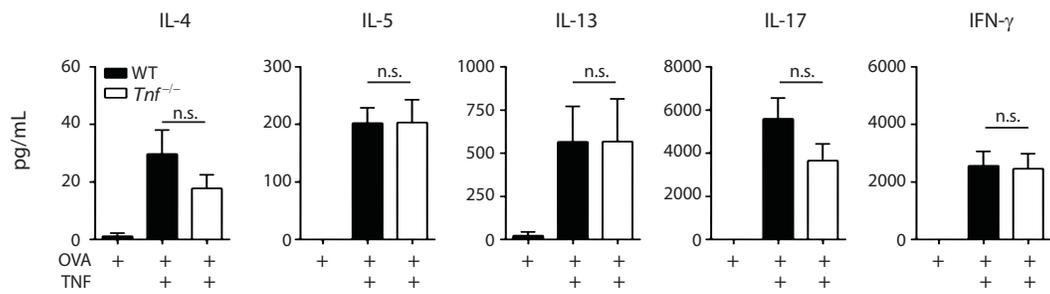
Supplemental Figure 5. Dose-response relationship between TNF and allergic airway inflammation. Mean number of leukocytes \pm SEM in the airways of mice ($n = 6$ mice/group) sensitized to OVA using the indicated amounts of rmTNF as an adjuvant, followed by challenge with aerosolized OVA. Data shown are from one of two experiments yielding similar results. * $P < 0.05$, ** $P < 0.01$ 'OVA only' vs TNF/OVA; Kruskal-Wallis one-way ANOVA with Dunn's multiple comparison test.



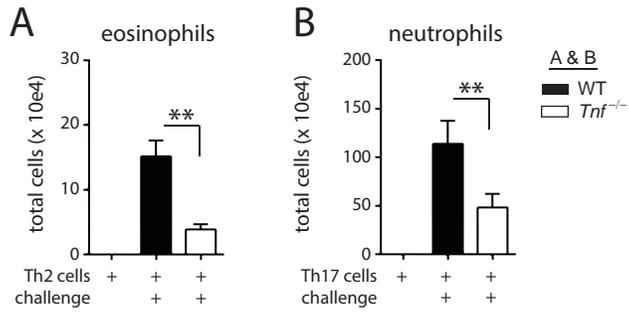
Supplemental Figure 6. TNF is required for production of type 2 cytokines, but not IL-17, in regional LNs after LPS-mediated allergic sensitization. Mean cytokine amounts \pm SEM in supernatants of OVA-stimulated mLN excised from WT and *Tnf*^{-/-} mice that received adoptive transfer of OT-II CD4⁺ T cells prior to sensitization with LPS/OVA ($n = 6$ mice/group). Data shown are from a single experiment. * $P < 0.05$, ** $P < 0.01$; WT vs *Tnf*^{-/-} mice that were sensitized to LPS/OVA; Kruskal-Wallis one-way ANOVA with Dunn's multiple comparison test.



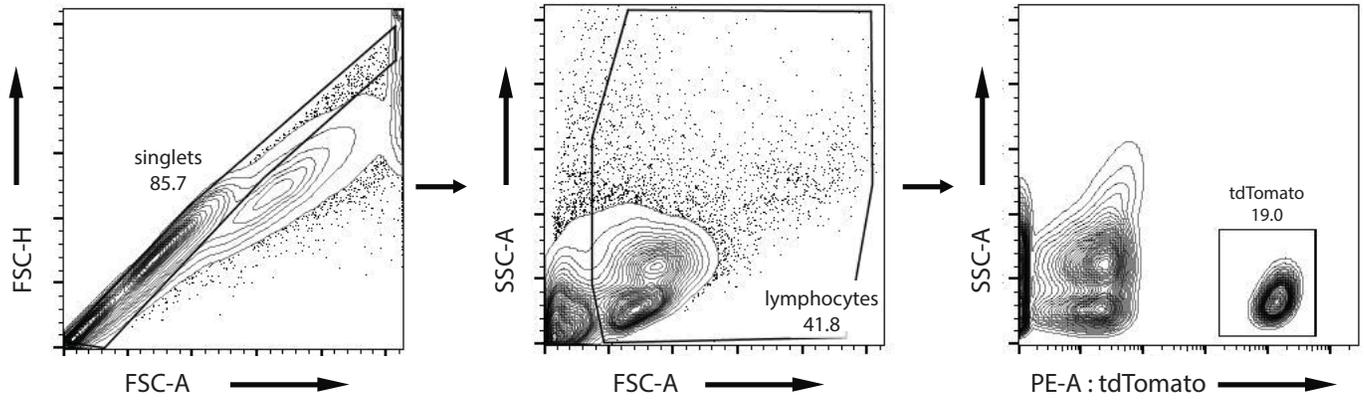
Supplemental Figure 7. Cellular sources of TNF. Time course for (A) TNF and (B) leukocyte accumulation in the airway following LPS/OVA inhalation ($n = 5$ mice/group). (C) TNF in the airway following antibody-mediated neutrophil depletion and LPS/OVA instillation ($n = 6$ mice/anti-GR-1 or -Ly-6G group, and $n = 4$ mice/isotype control (IC) groups). (D and E) TNF in the airways of (D) *Rag1*^{-/-} mice and (E) mast cell-deficient (*Kit*^{W-sh}) mice following LPS/OVA instillation ($n = 6$ mice/group). (F-H) Mice were treated with liposomes containing clodronate (Clod), or empty liposomes as control (L.C.). (F) Cell numbers for the indicated leukocytes in lungs of WT mice following Clod or L.C. treatment ($n = 3$ mice/group). (G) TNF levels in the airways of mice treated with Clod or L.C., then with LPS/OVA ($n = 6$ mice/group). (H) Cytokine concentrations in supernatants of cultured mLNs excised from mice treated with Clod. or L.C., then sensitized using LPS/OVA ($n = 4$ mice/L.C. group or $n = 6$ mice/Clod group). Values shown represent means \pm SEM from single experiments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ for the indicated comparisons; Kruskal-Wallis one-way ANOVA with Dunn's multiple comparison test.



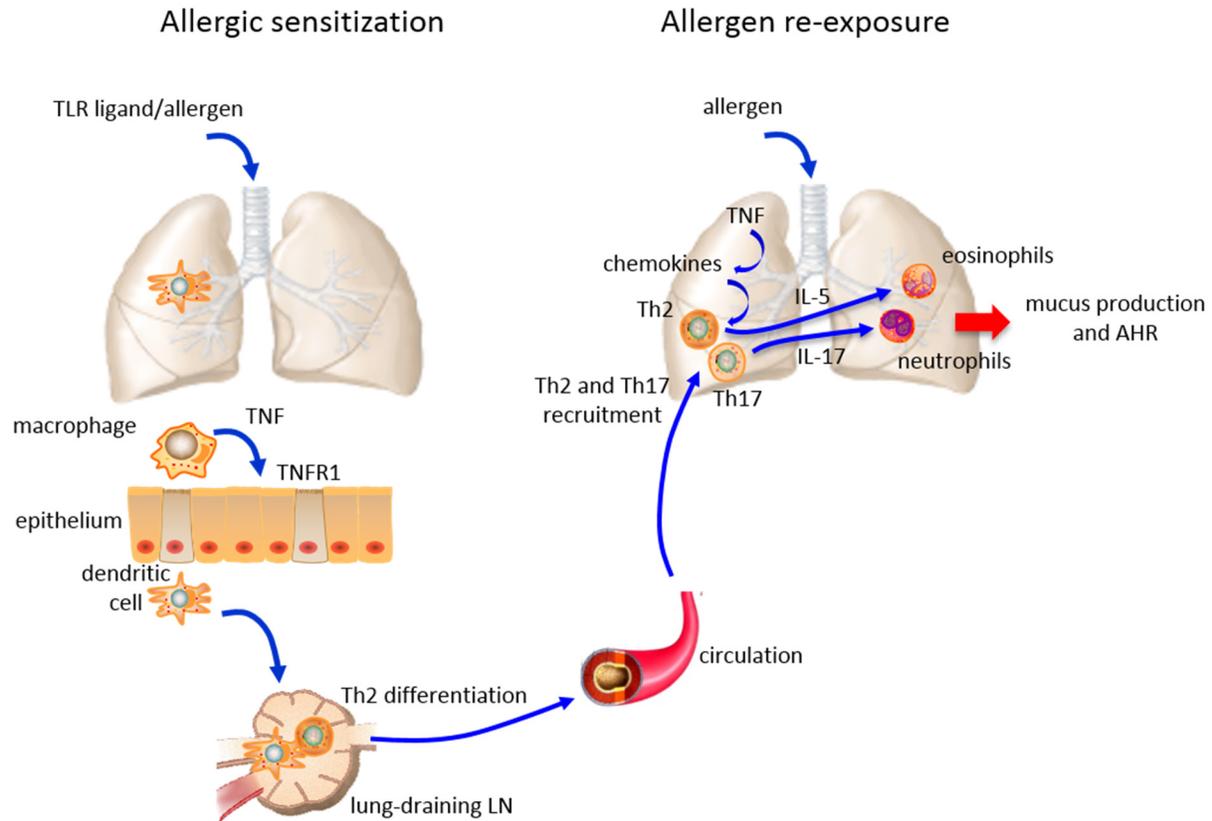
Supplemental Figure 8. TNF/OVA-induced cytokines in mLN of WT and *Tnf*^{-/-} mice. Mean concentrations of cytokines in cultures of OVA-stimulated mLN excised from WT and *Tnf*^{-/-} mice receiving OT-II CD4⁺ T cells prior to rmTNF/OVA inhalation ($n = 6$ mice/group). n.s. ($P > 0.05$) between WT and *Tnf*^{-/-} mice that were sensitized to rmTNF/OVA; Kruskal-Wallis one-way ANOVA with Dunn's multiple comparison test.



Supplemental Figure 9. TNF signaling is required for eosinophilic and neutrophilic inflammation following allergen challenge. Mean cell numbers \pm SEM in airways of WT and *Tnf*^{-/-} mice that received adoptive transfer of in vitro polarized, OVA-specific Th2 cells (A) or Th17 cells (B) prior to challenge with aerosolized OVA ($n = 4$, non-challenged controls; $n = 6$ mice, OVA-challenged mice). Data shown are from a single experiment. $**P < 0.01$; WT vs *Tnf*^{-/-} mice that received either Th2 or Th17 cells and then challenged; Mann-Whitney *U* test.



Supplemental Figure 10. Gating for tdTomato⁺ IL-17 fate mapping cells. Cytograms showing gating strategy for tdTomato⁺ Th17 cells. FSC, forward scatter; SSC, side scatter.



Supplemental Figure 11. Schematic representation of TNF function during TLR ligand mediated allergic inflammation. Inhaled TLR ligands induce TNF production by lung macrophages, which signal through TNFR1 on airway epithelial cells. This leads to dendritic cell-dependent induction of type 2 cytokine producing cells in the lung-draining LNs. Th17 development also occurs, but this is independent of TNF. Upon allergen re-exposure, TNF is again produced, leading to the production of chemokines, recruitment of Th2 and Th17 cells, and consequent eosinophilic and neutrophilic inflammation with accompanying mucus production and AHR.