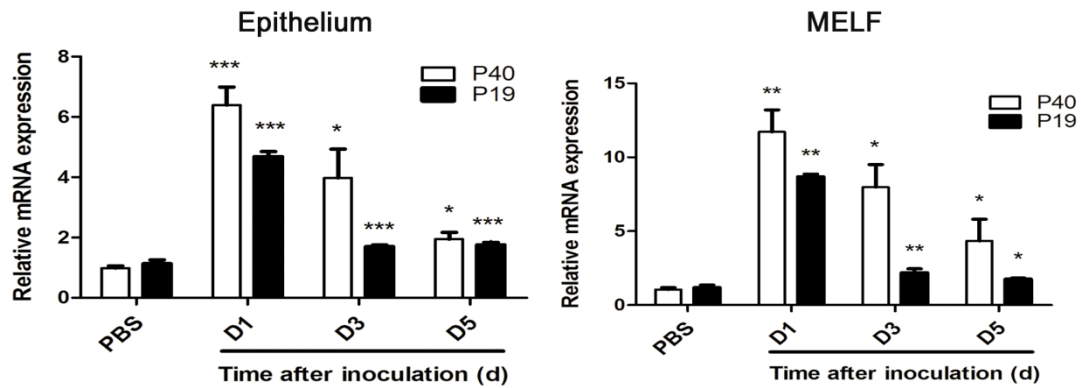
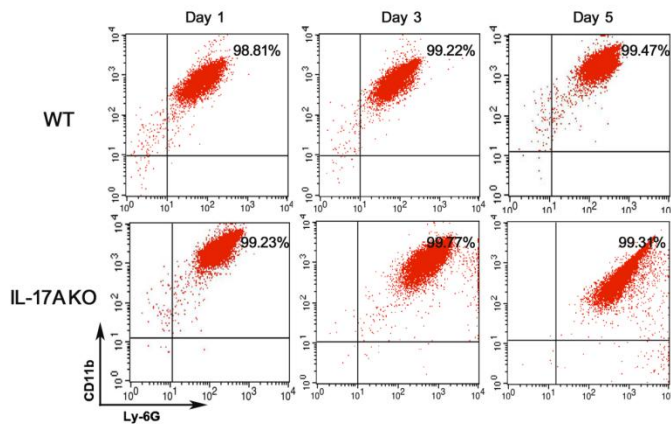


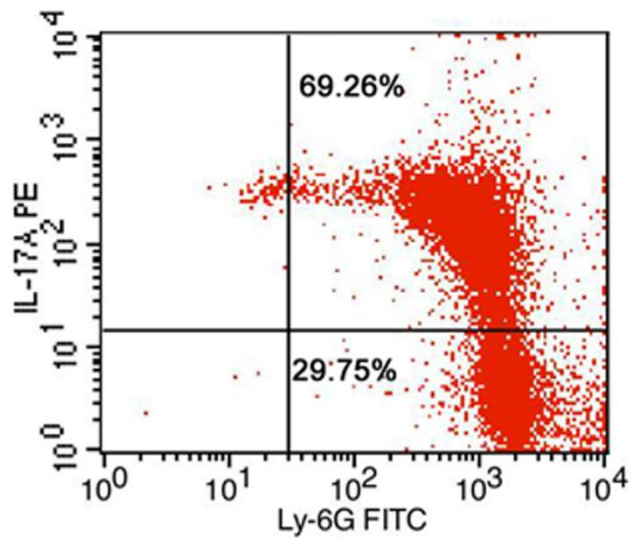
Supplement



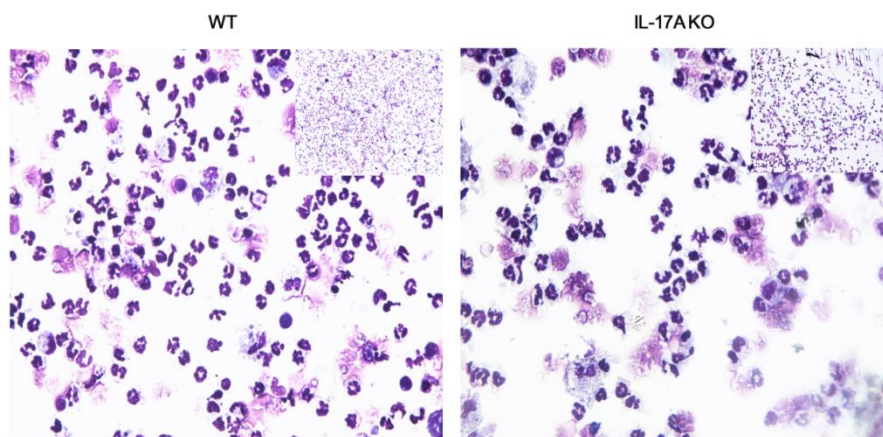
FigureS1. IL-23 is expressed in response to Spn during AOM. IL-23p40 and p19 expression of epithelium (A) and MELF(B) in the middle ear were analyzed by real time PCR method at designated time points postinfection. Values are expressed as relative gene expression (compared with GAPDH). Values represent means \pm SD. n=6; * P < 0.05; ** P < 0.01; *** P < 0.001, between two groups.



FigureS2. Recruitment of neutrophils into the middle ear at designated time points postinfection. The proportion of Ly6G⁺CD11b⁺ granulocytes in the MELF samples was determined by flow cytometry analysis of inflammatory cells from wild-type and IL-17A KO mice. n= 4-5. Data from a single set of experiments are shown but are representative of at least three such experiments.



FigureS3. Neutrophils are a source of IL-17A during AOM. Cells in MELF were Fc blocked, stained with a live/dead staining kit, and thereafter stained for Ly-6G, followed by fixation/permeabilization, and stained for intracellular IL-17A. Representative flow cytometric plots after gating on live cells followed by gating on Ly-6G⁺IL-17A⁺ cells are shown. Double-positive populations (Ly-6G⁺IL-17A⁺; square gates) were based on unstained controls. n= 4-5. Data from a single set of experiments are shown but are representative of at least three such experiments.



FigureS4. Recruitment of neutrophils into the middle ear during AOM. Cytospin preparations of MELF in WT mice and IL-17A KO mice were analyzed by Giemsa staining at day 1 postinfection. Original magnification, $\times 400$. n= 4-5. Data from a single set of experiments are shown but are representative of at least three such experiments.

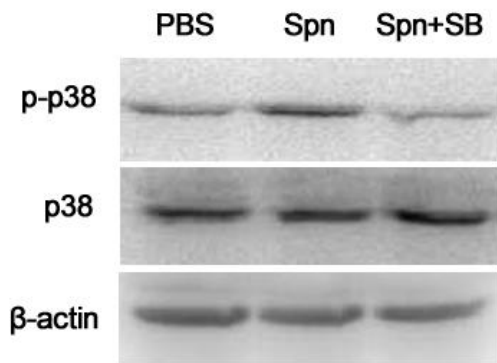


Figure S5. Inhibition of p38MAPK Signaling Pathway by SB203580 in vivo. The p-p38 of WT mice were analyzed by Western blotting following direct bilateral transtympanic inoculation of Spn or Spn plus SB203580 at day 3 postinfection. n= 4-5. Data from a single set of experiments are shown but are representative of at least three such experiments.

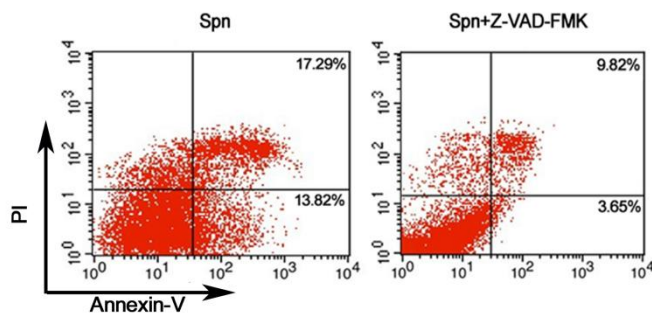


Figure S6. Inhibition of caspase by Z-VAD-FMK in vivo. The percentage of neutrophils apoptosis in MELF of WT mice were analyzed by flow cytometry following direct bilateral transtympanic inoculation of Spn or Spn plus Z-VAD-FMK(a general caspase inhibitor) at day 3 postinfection. n= 4-5. Data from a single set of experiments are shown but are representative of at least three such experiments.