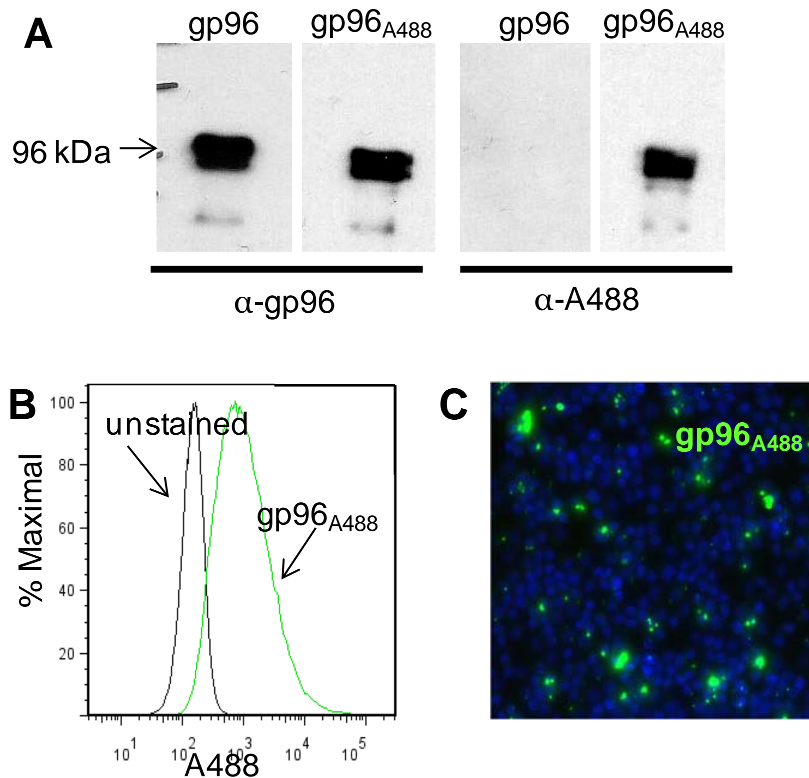
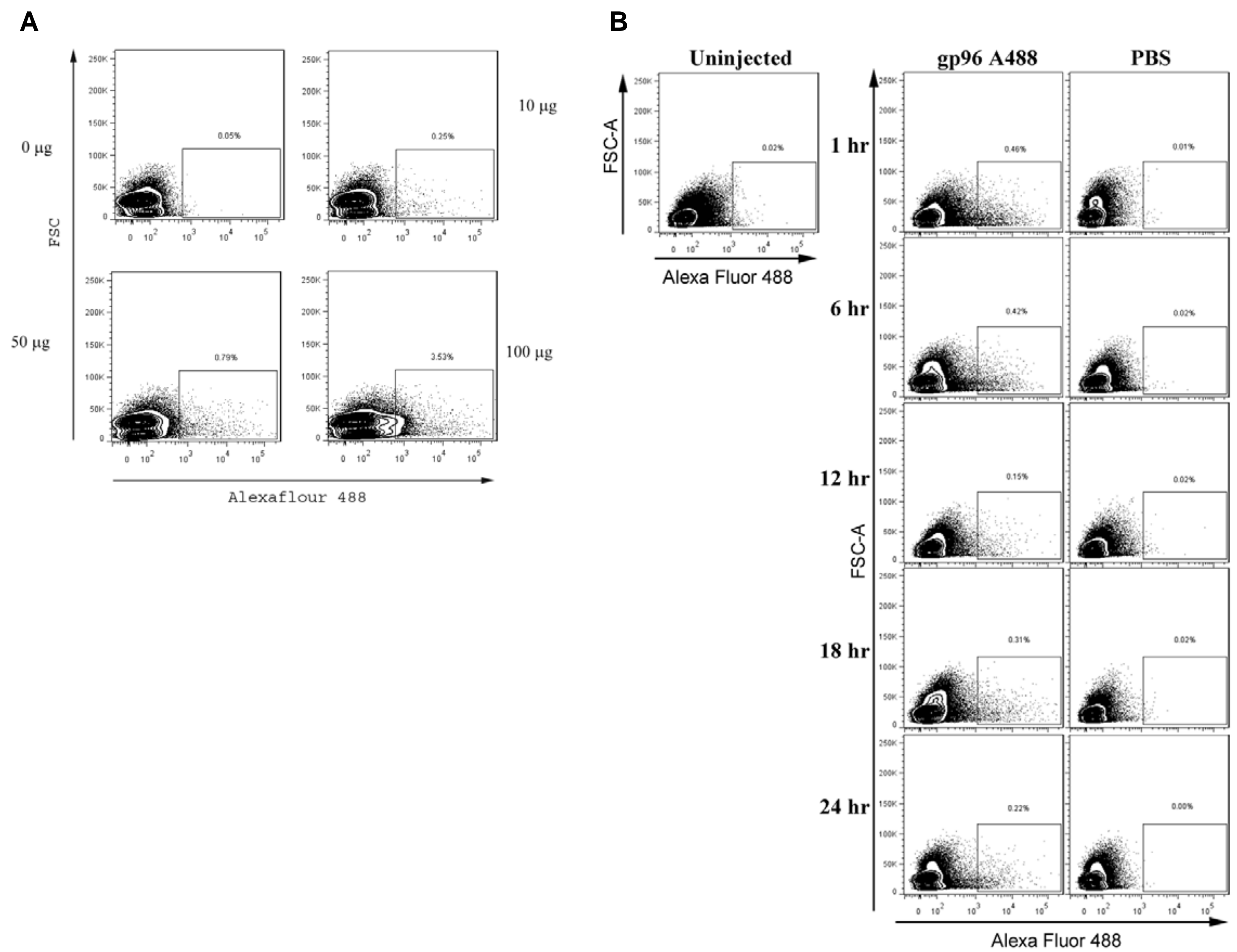
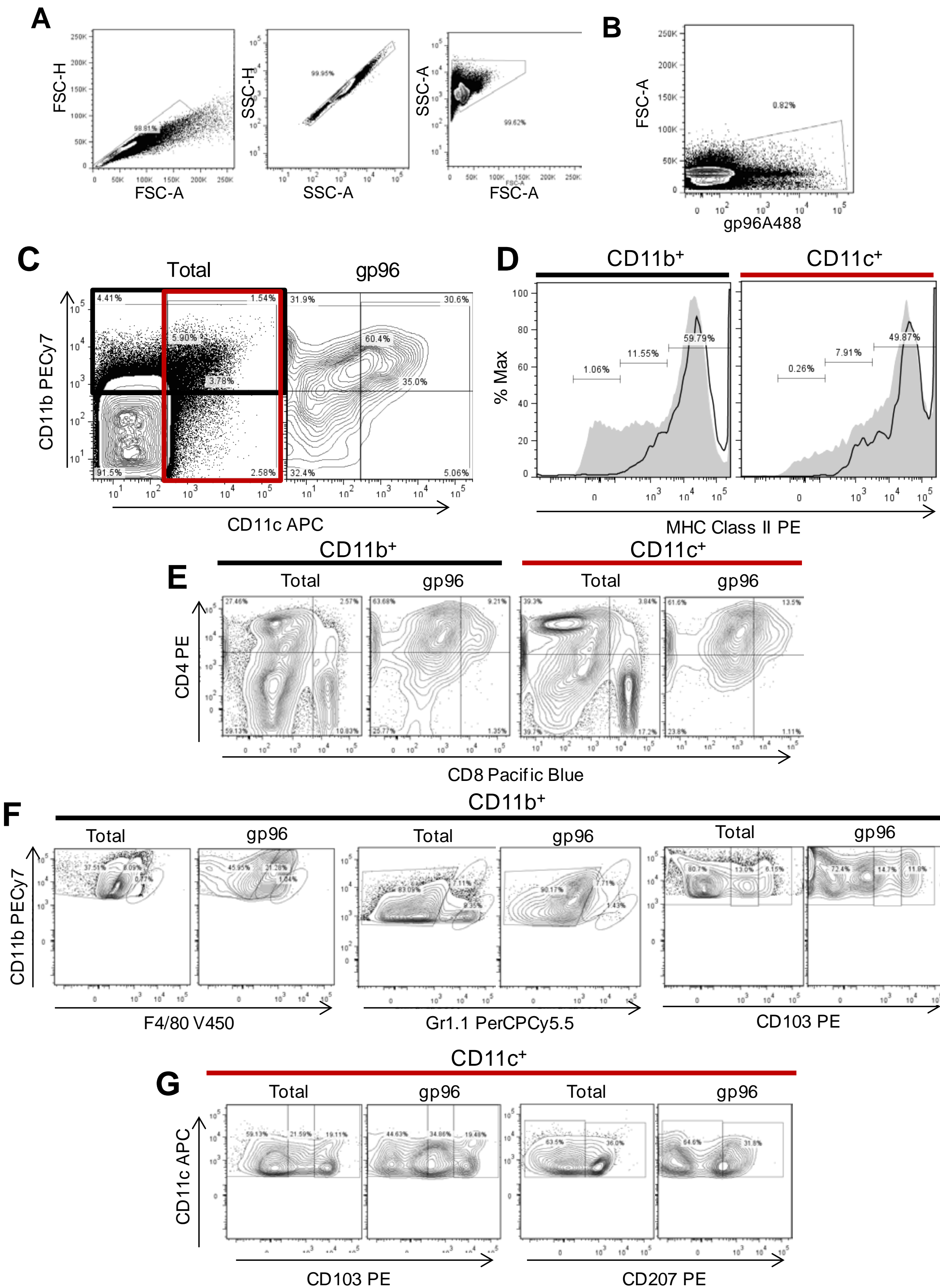


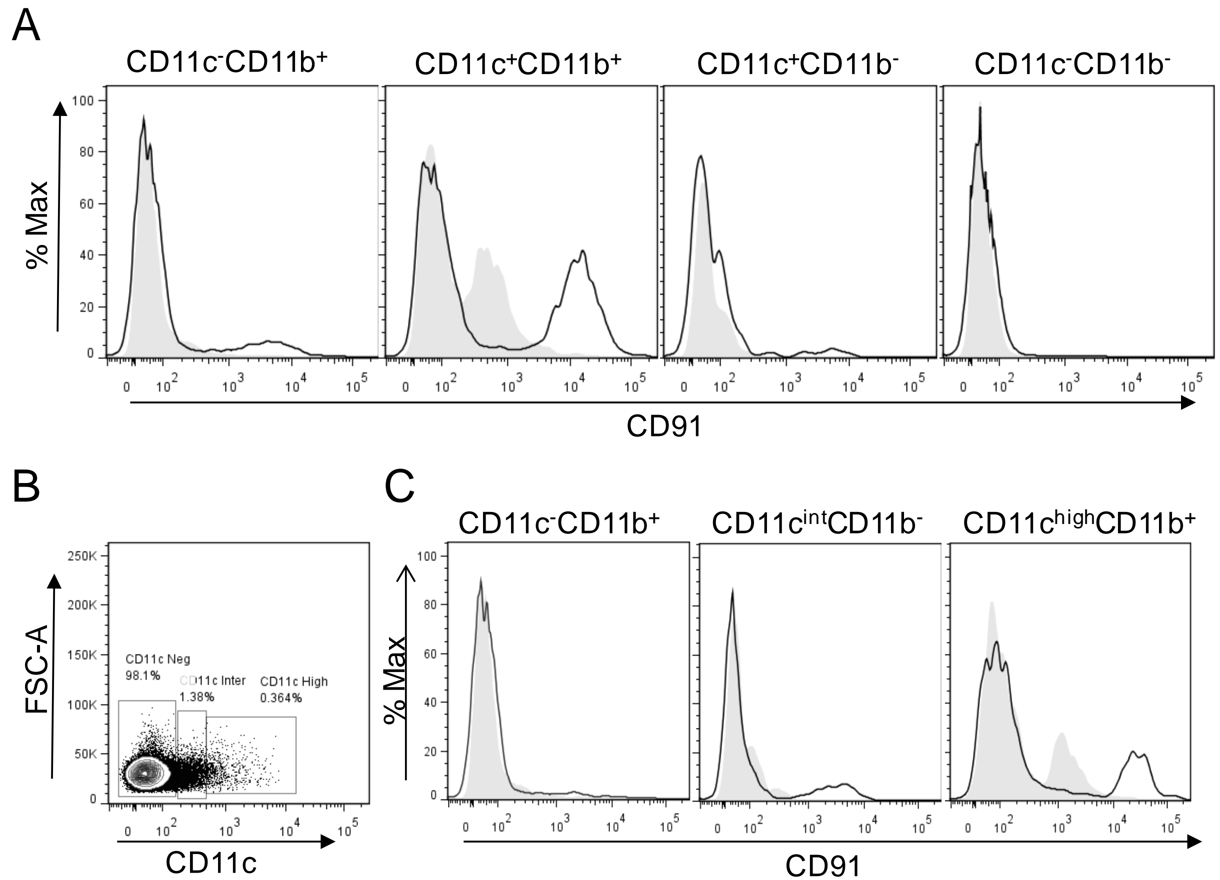
Supplemental Figure 1: Purification and labelling of gp96 with Alexafluor 488. (A) gp96 was purified to homogeneity, labeled with Alexafluor 488 and analyzed by SDS-PAGE and western blotting. (B,C) Biological integrity of $gp96_{A488}$ was verified by testing its endocytosis by $CD91^{+}$ RAW264.7 cells. RAW264.7 were incubated with $gp96_{A488}$ for 30 mins and analyzed by (B) flow cytometry and (C) microscopy.



Supplemental Figure 2: Flow dot plots show the change in number of A488^{+} cells over time and dose. (A) C57BL/6 mice were treated with a titrated dose of gp96$_{A488}$ doses delivered via intradermal injection. Axillary/brachial and inguinal lymph nodes (LN) were harvested 8 hours later and processed into single cell suspensions for flow cytometric analysis. (B) Mice were treated with 10 µg gp96$_{A488}$, and lymph nodes were harvested at the indicated times and processed into single cell suspensions for flow cytometry. Data are representative of three experiments, and were quantified in figure 1D. Data are representative of 3 independent experiments, and were quantified in Fig.1.



Supplemental Figure 3: Gating strategy for phenotypic analysis of LN cells. (A) Gating of lymph node cells based on forward scatter (FSC) and side scatter (SSC) properties to reduce doublets and cell debris, with the final FFC-A by SSC-A gate termed "Total". Total lymph node cells were then analyzed directly for surface marker expression or further gated on A488$^+$ cells (B). (C) Cells were gated on CD11b (solid line) or CD11c (red line). (D) CD11b$^+$ and CD11c$^+$ cells were analyzed for their MHC class II expression (filled, grey histogram is total Lymp node; open, black line histogram is gp96A488$^+$). (E) CD11c$^+$ or CD11b$^+$ cells were analyzed separately for expression of CD4 or CD8. (F) Additional markers analyzed on CD11b$^+$ cells were F4/80 and Gr1.1, with 3 gates (high, med, negative) for each as shown. (G) Additional markers analyzed on CD11c$^+$ cells were CD103 and CD207. Gates for all samples were based on single color controls as well as "fluorescence minus one." Shown are representative plots from a minimum of 3 mice for each test. Frequencies were quantified and averages are shown in Figure 2.



Supplemental Figure 4: Expression of CD91 on subsets of APCs in the lymph node. (A) Lymph node cells were analyzed by multi-color flow cytometry for CD11b and CD11c. (B) Alternative gating on CD11c high, intermediate, or negative cells for further analysis of CD91 expression. (C) Populations in (B) were further subtyped for CD11b. The indicated population was then stained with anti-CD91 antibody (solid line) or with an isotype control (filled histogram). The Average geometric mean was quantified for each population for Figure 5. (B) CD91 expression was analyzed on cells expressing CD11c (intermdiate or high) with or without CD11b.