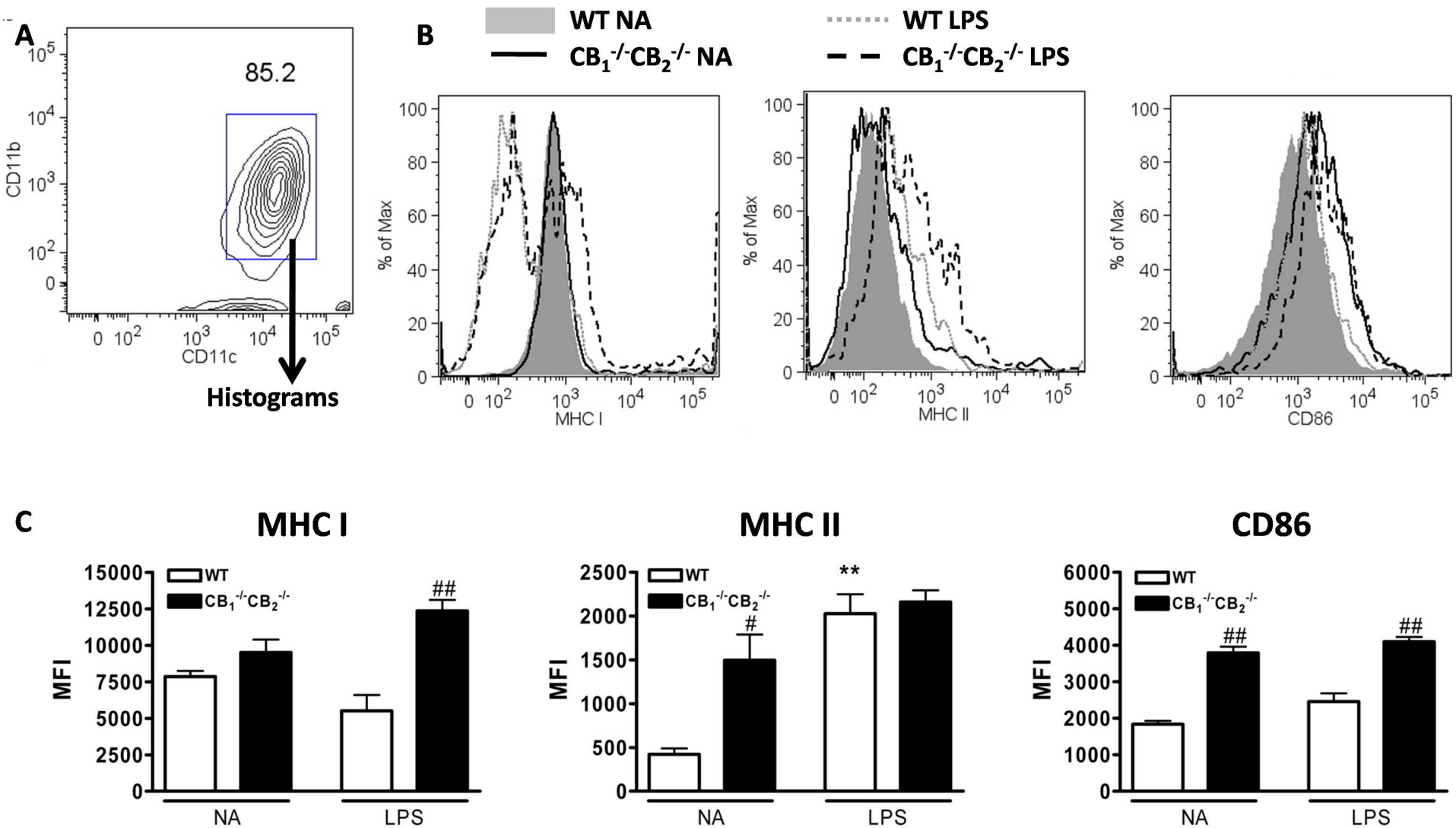


Supplemental Figure 1. Greater Neutrophils and Macrophage Recruitment after Influenza Infection in CB1^{-/-}CB2^{-/-} mice. Lungs of C57Bl/6 and CB1^{-/-}CB2^{-/-} mice (n=5) were lavaged with saline to collect bronchoalveolar lavage fluid (BALF). Total cell number was determined by counting of cells in BALF and DiffQuick staining was performed to distinguish between immune cell populations, which were enumerated by based on the percentage found on the slide using light microscopy (B, C). * (p = 0.05) ** (p = 0.01) marks a significant change induced by influenza within a timepoint, while # (p = 0.05) ## (p = 0.01) marks a significant difference between genotypes.



Supplemental Figure 2. Alveolar Macrophages isolated from BALF are mature in CB1^{-/-}CB2^{-/-} but not WT. Alveolar Macrophages were isolated by flushing the lungs with saline, washed and plated in the presence or absence of LPS (1 μ g/mL) (n=3). Maturation was assessed by staining for MHC I, MHC II, and CD86 on the surface of alveolar macrophages. A, alveolar macrophages were gated as CD11b⁺ CD11c^{hi} cells. B, histograms depicting changes in fluorescence intensity of MHC I, MHC II, and CD86 in alveolar macrophages obtained from lavage fluid of WT and CB1^{-/-}CB2^{-/-} mice. C, summary of MFI data, statistical significance is indicated as ** p = 0.01 WT-NA vs. WT-LPS, # p = 0.05 and ## p = 0.01 WT vs CB1^{-/-}CB2^{-/-} within groups. The results shown are representative from two identical experiments.

Supplemental Materials and Methods

BALF analysis and differential cell counts. Prior to mechanical disruption of lungs, saline (2 x 0.9 mL) was used to lavage lungs and collect the broncho-alveolar lavage fluid (BALF). Total cell counts were obtained by counting an aliquot of the total obtained BALF using a hemacytometer. Differential cell counts were prepared by centrifuging BALF onto glass slides using a Shandon Cytospin 3 centrifuge (Block Scientific, Nutley, NJ) at 600 rpm for 10 minutes. The slides were stained using the Diff-Quik kit (Dade Behring, Newark, DE) following manufacturer's instructions. The stained slides were counted for at least 200 discernable cell entities including lymphocytes, eosinophils, macrophages and neutrophils, and percentages were calculated. The total cell count was used to enumerate differential cell populations.

Isolation of alveolar macrophages from BALF. BALF was obtained as described above from naïve WT and CB1^{-/-}CB2^{-/-} mice. Cells were washed twice and counted with a Coulter Counter and incubated at 0.5×10^5 cells/mL in 2 mL in a 12 well dish. BALF cells were stimulated with and without (NA) LPS at 1 μ g/mL for 24 hours. To assess maturation, MHC I, MHC II, and CD86 staining was performed in addition to CD11b and CD11c to gate on macrophage populations. Analysis of fluorescence intensity of maturation markers was exclusively performed on the alveolar macrophage populations (CD11b^{mid}CD11c^{hi}), which constituted the majority of cells obtained from the BALF.