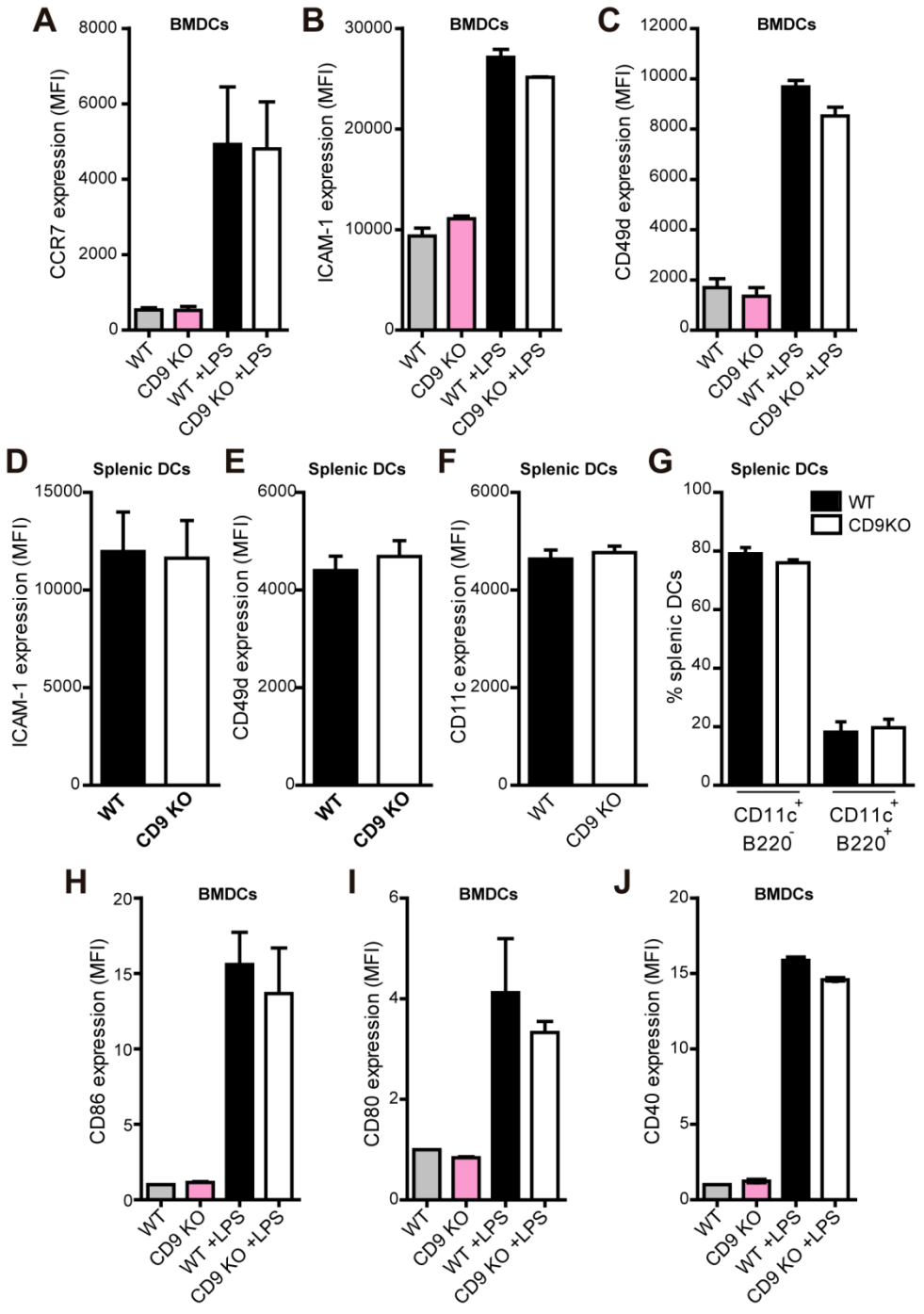
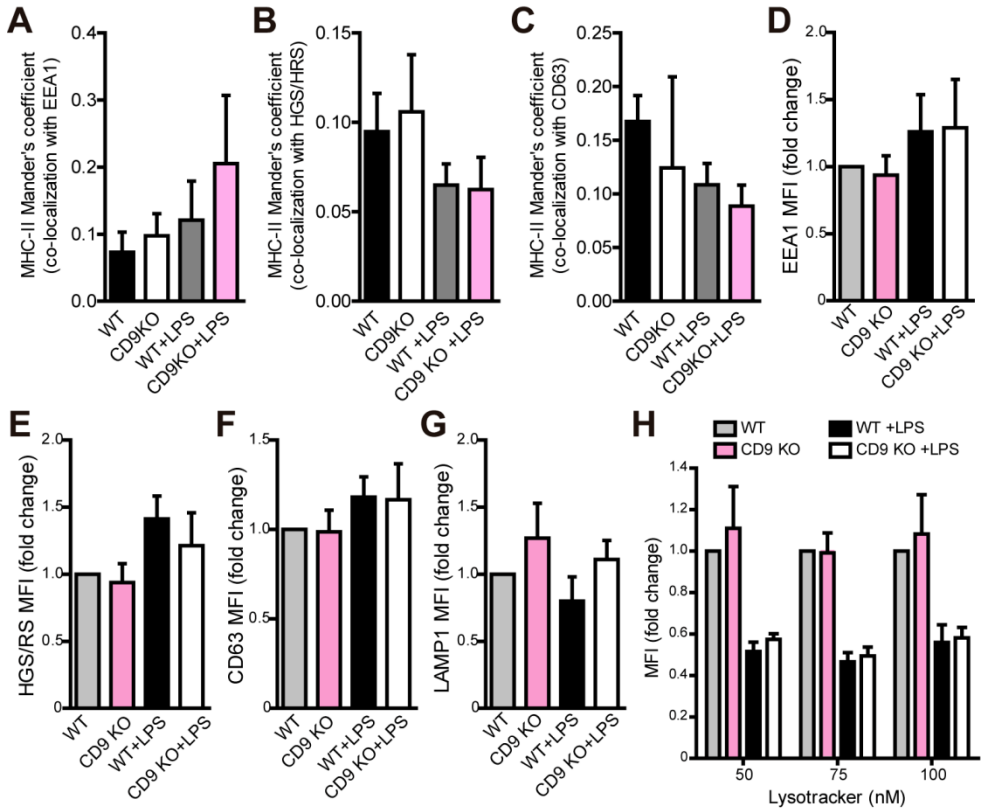
SUPPLEMENTAL FIGURES AND LEGENDS



Supplemental Figure 1

Supp. Fig. 1. CD9 deficiency does not affect surface expression of several surface markers. Immature and LPS-matured CD11c^{+} WT and CD9 KO GM-CSF-dependent BMDCs (thereafter called MoDCs) were stained for with different antibodies against **(A)** CCR7, **(B)** ICAM-1, **(C)** CD49d, **(H)**

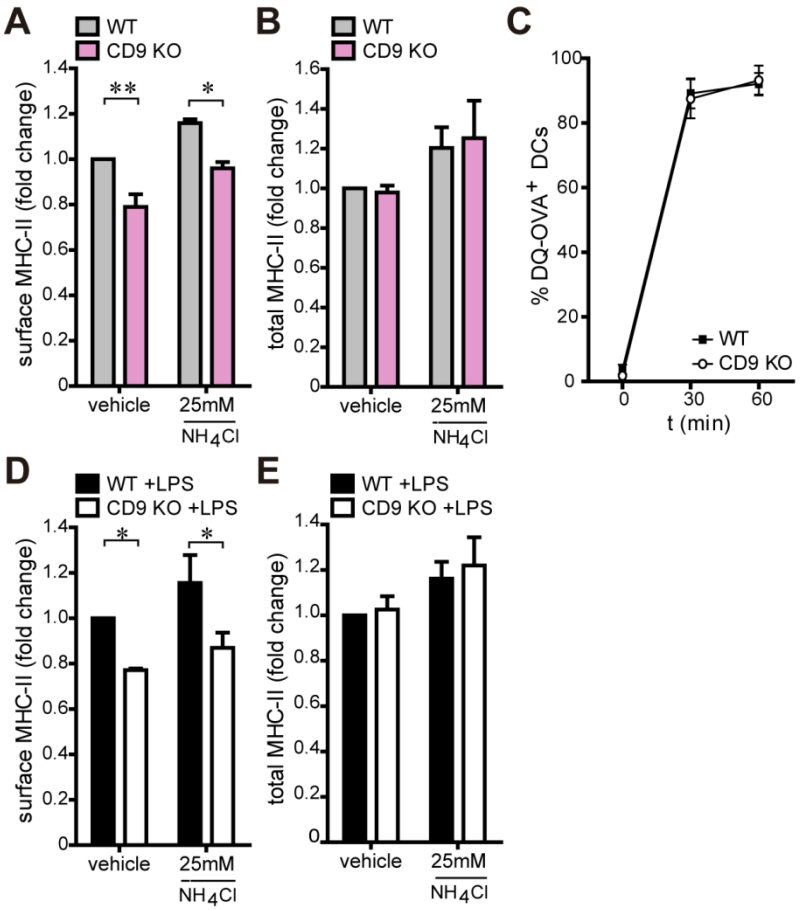
CD86, **(I)** CD80, and **(J)** CD40, and analyzed by flow cytometry. Splenic WT and CD9 KO DCs were stained for with different antibodies against **(D)** ICAM-1, **(E)** CD49d, **(F)** CD11b, and **(G)** CD11c and B220 to differentiate plasmacytoid and conventional DCs. (A-C; H-J) Data are mean-fold induction ± SEM from two independent experiments analyzed by one-way ANOVA with Tukey's post-hoc multiple comparison test, and (D-G) means ± SEM of two independent experiments analyzed by Student *t*-test.



Supplemental Figure 2

Supp. Fig. 2. CD9 deficiency does not modify the expression levels of different markers of intracellular vesicles. (A-C) Immature or LPS-matured WT and CD9 KO MoDCs were plated onto PLL-coated coverslips, fixed, permeabilized, stained for with antibodies against MHC-II and **(A)** EEA1, **(B)** HGS/HRS, or **(C)** CD63, and analyzed by confocal microscopy. Graphs show the quantification of (A) EEA1-MHC-II, (B) HGS/HRS-MHC-II, or (C) CD63-MHC-II co-localization performed in 3D stack confocal microscopy images, and quantified by Mander's coefficient. Data are means ± SEM of three independent experiments (A, at least n=100 immature and 40 mature cells; B, at least n=85 immature and mature cells; C, at least n=25 immature and 20 mature cells) analyzed by one-way ANOVA with Tukey's post-hoc multiple comparison test. **(D-G)** Immature and LPS-matured $CD11c^{+}MHC\text{-}II^{+}$ WT and CD9 KO

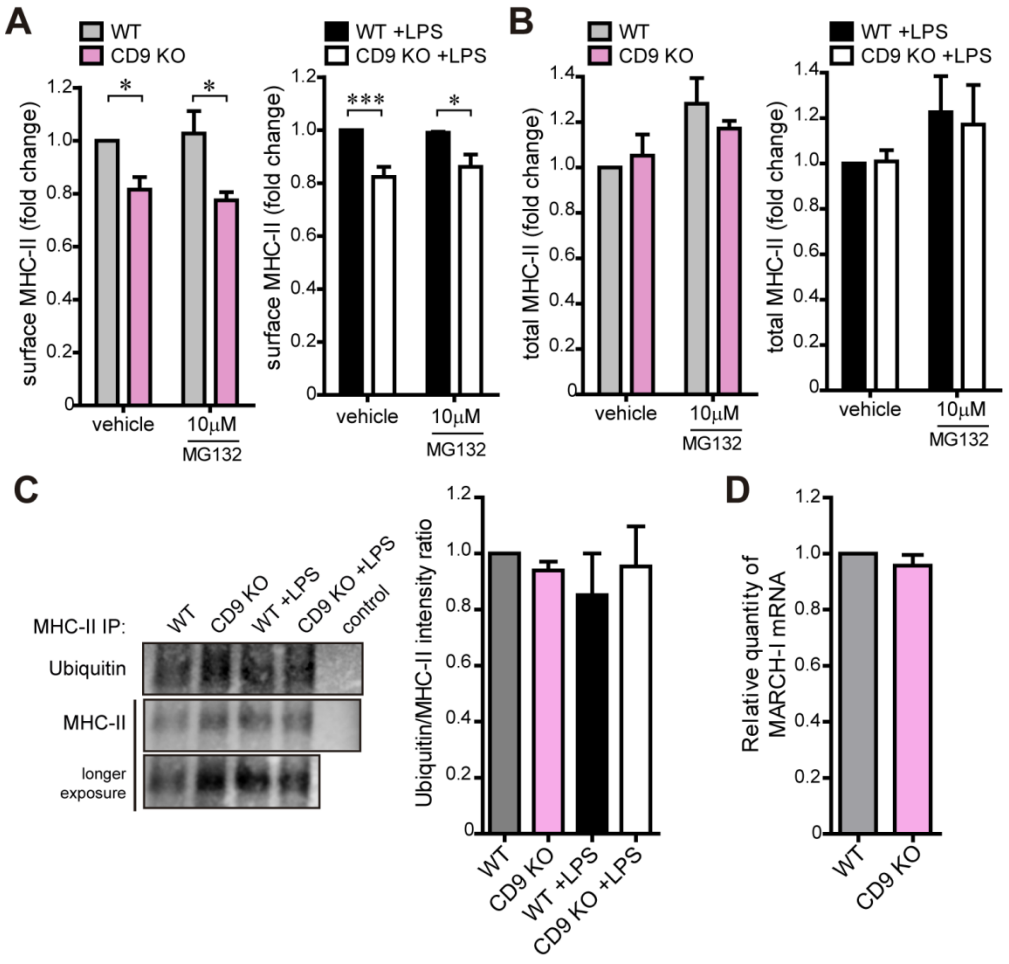
DCs were fixed, permeabilized, stained for with different antibodies against **(D)** EEA1, **(E)** HGS/HRS, **(F)** CD63, **(G)** LAMP1, and analyzed by flow cytometry. Graphs show the mean-fold induction ± SEM of three independent experiments analyzed by one-way ANOVA with Tukey's post-hoc multiple comparison test. **(H)** Immature and LPS-matured WT and CD9 KO DCs were loaded with different concentrations of Lysotracker Red for 30min at 37ºC, washed, stained for with antibodies against CD11c and MHC-II, and analyzed by flow cytometry. Data are mean-fold induction ± SEM from three independent experiments analyzed by one-way ANOVA with Tukey's post-hoc multiple comparison test.



Supplemental Figure 3

Supp. Fig. 3. Antigen proteolytic processing is not affected in CD9 KO MoDCs. (A-B) Immature WT and CD9 KO MoDCs were treated with 25mM of NH_4Cl or with the vehicle for 1h at 37ºC, and (A) surface and (B) total MHC-II expression in $CD11c^+$ cells was analyzed by flow cytometry. Data are mean-fold induction ± SEM from three independent experiments analyzed by one-way ANOVA with Tukey's post-hoc multiple comparison test. **(C)** Immature WT and CD9 KO DCs were incubated for

different times with DQ-OVA, washed and the percentage of DQ-OVA$^+$ cells was analyzed by flow cytometry. Data are means ± SEM from four independent experiments analyzed by two-way ANOVA with Bonferroni's post-hoc multiple comparison test. **(D-E)** LPS-matured WT and CD9 KO DCs were analyzed as in A-B. Data are mean-fold induction ± SEM from three independent experiments analyzed by one-way ANOVA with Tukey's post-hoc multiple comparison test.



Supplemental Figure 4

Supp. Fig. 4. CD9 deficiency does not affect MHC-II ubiquitination. (A-B) Immature and LPS-matured WT and CD9 KO MoDCs were treated with 10μM of MG132 or with the vehicle for 4h at 37ºC, and (A) surface and (B) total MHC-II expression in CD11c$^+$ cells were analyzed by flow cytometry. Data are mean-fold induction ± SEM from three independent experiments analyzed by one-way ANOVA with Tukey's post-hoc multiple comparison test. **(C)** Cell lysates from immature and LPS-matured WT and CD9 KO MoDCs were immunoprecipitated with an anti-MHC-II antibody (M5/114), and ubiquitinated proteins detected after membrane incubation with anti-ubiquitin antibodies. Membranes were re-probed

with MHC-II antibody for loading measurement. In the control lane the beads were incubated only with cell lysates from WT DCs. The blots shown are from a representative experiment out of three. Graph shows the mean-fold induction ± SEM from three independent experiments analyzed by one-way ANOVA with Tukey's post-hoc multiple comparison test. **(D)** Total mRNA was extracted from immature WT and CD9 KO DCs pellets. The amount of MARCH-I mRNA was quantified by RT-qPCR relative to β-actin and YHAWZ controls. Data are mean-fold induction ± SEM from two independent experiments analyzed by Student *t*-test.