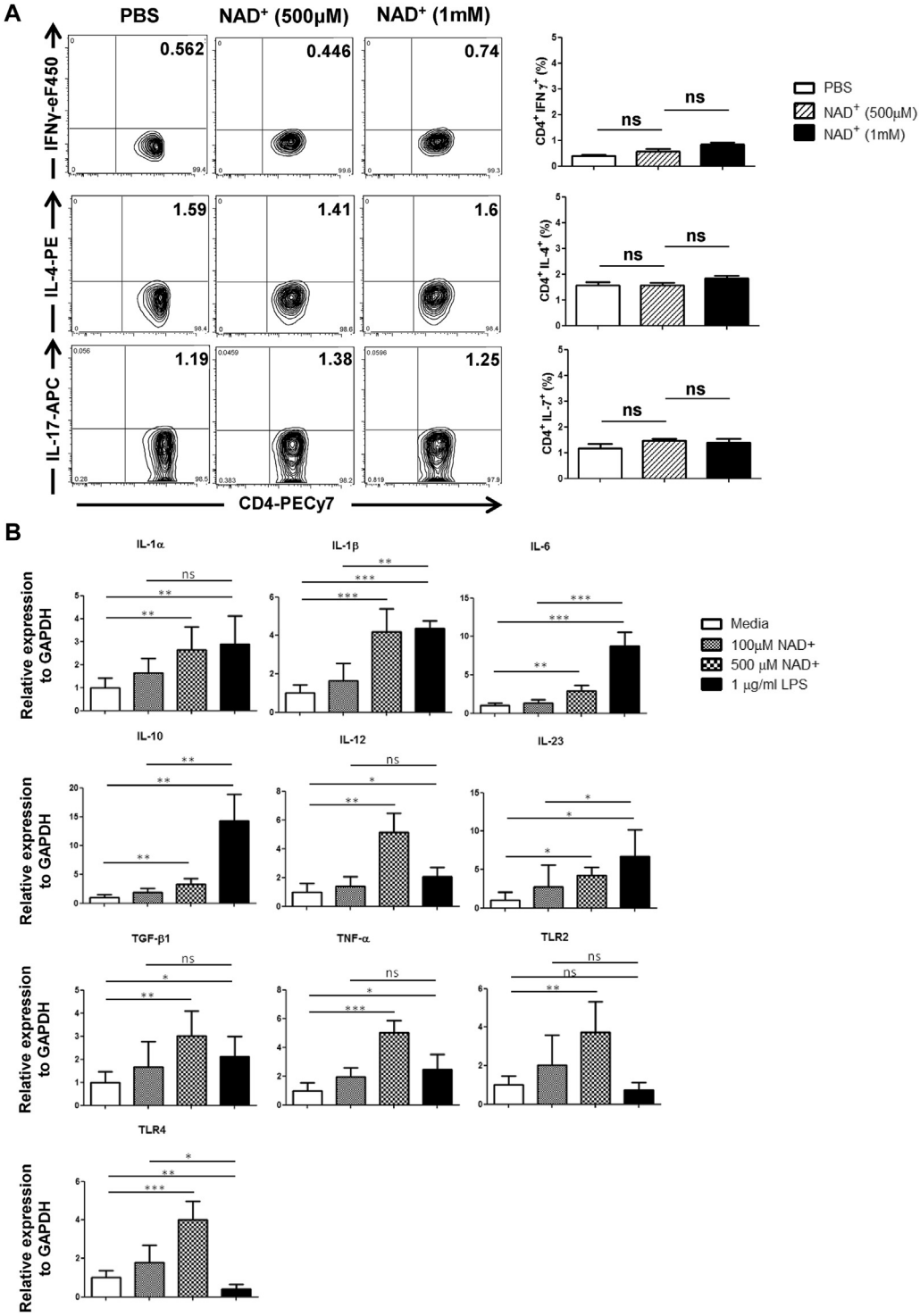

FIG E1. High doses of NAD^+ do not promote naive $CD4^+$ T-cell differentiation *in vitro*, and NAD^+ regulates $CD11b^+CD11c^+$ DC cytokine production *in vitro* in a dose-dependent manner. **A,** Sorted naive $CD4^+CD44^-CD62L^+$ T cells were isolated from spleens of C57BL/6 mice and cultured in complete media with increasing concentrations of NAD^+ (500 μmol/L and 1 mmol/L) or PBS. After 96 hours, frequencies of $CD4^+IFN\text{-}\gamma^+$, $CD4^+IL\text{-}4^+$, and $CD4^+IL\text{-}17A^+$ cells were assessed by using flow cytometry (n = 10). Data were derived from 3 independent experiments. **B,** Sorted $CD11b^+CD11c^+$ DCs were isolated from spleens of C57BL/6 mice and cultured (1×10^6 cells/well) in complete media and in the presence of increasing concentrations of NAD^+ (100 μmol/L and 500 μmol/L). As a positive control, $CD11b^+CD11c^+$ DCs were cultured in the presence of LPS (1 μg/mL). After 16 hours of culture, cells were collected, and mRNA expression levels of IL-1α, IL-1β, IL-6, IL-10, IL-12, IL-23, TGF-β, TNF-α, TLR2, and TLR4 were determined by using real-time PCR. Values are expressed as fold expression relative to the housekeeping gene glyceraldehye-3-phosphate dehydrogenase (*GAPDH*; n = 5). Data were derived from 2 different experiments. *ns*, Not significant. $^*P < .05$, $^{**}P < .01$, and $^{***}P < .001$, as determined by means of ANOVA, comparing the indicated groups. Data represent means ± SDs. *APC*, Allophycocyanin; *PE*, phycoerythrin.

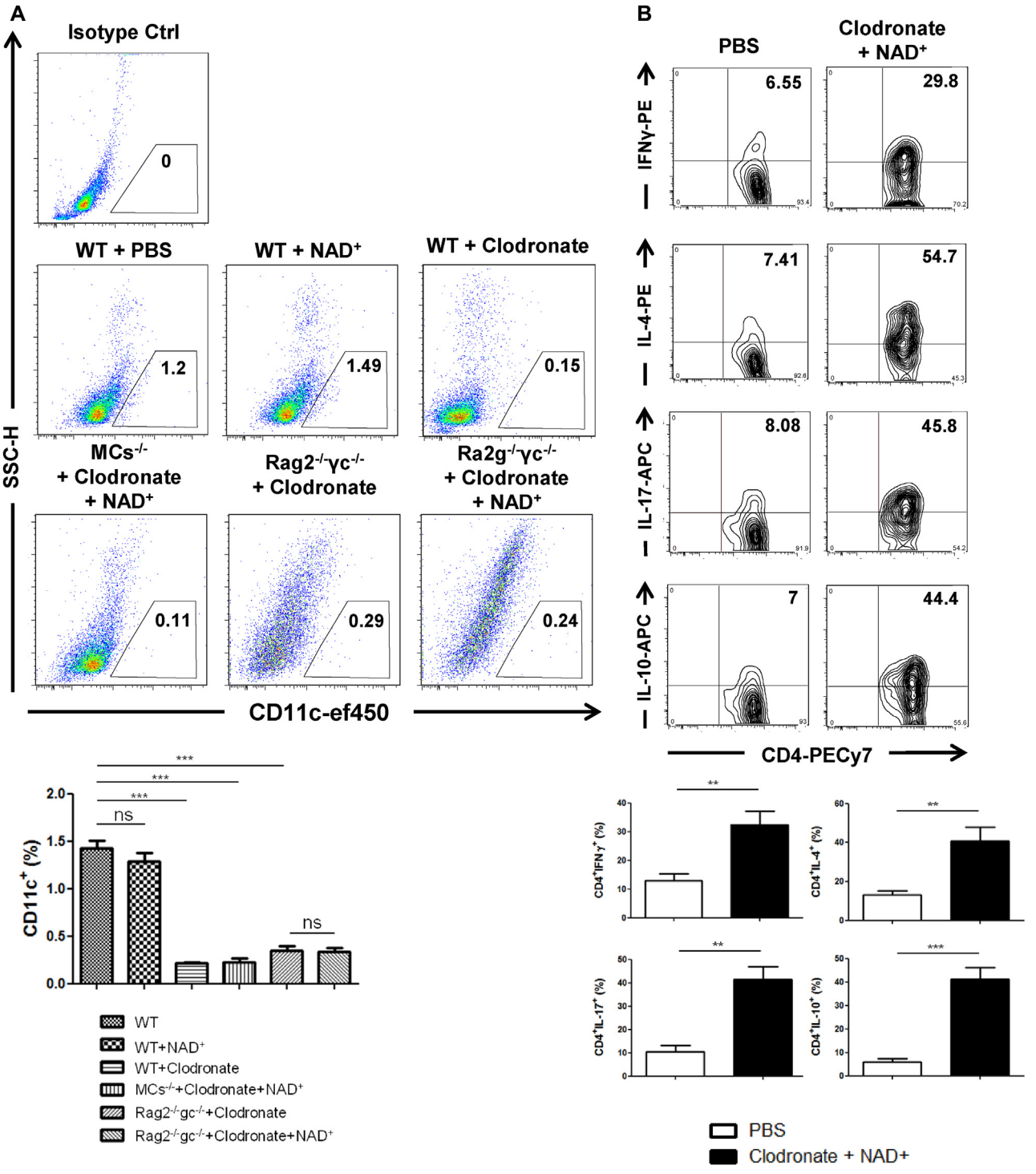

FIG E2. *In vivo* depletion of DCs by means of liposomal clodronate administration does not alter NAD^+-mediated $CD4^+$ T-cell differentiation. **A,** C57BL/6 WT, $MC^{-/-}$, and $Rag2^{-/-}\gamma c^{-/-}$ mice were treated intravenously with liposomal clodronate at −8 days, −5 days, and −1 day before NAD^+ treatment. Data derived from 2 independent experiments (n = 5 per group). **B,** C57BL/6 WT mice were treated intravenously with liposomal clodronate at −8 days, −5 days, and −1 day before NAD^+ treatment. Mice were then treated with daily intraperitoneal injections of 40 mg of NAD^+ or a placebo solution (PBS). After 7 days, mice were killed, and frequencies of $CD4^+$IFN-γ^+, $CD4^+$IL-4^+, and $CD4^+$IL-$17A^+$ cells were analyzed by using flow cytometry. Data were derived from 2 independent experiments (n = 10). Data represent means ± SDs. *ns*, Not significant. The Student *t* test and ANOVA were used to compare between groups: $^{**}P < .01$ and $^{***}P < .001$. *APC*, Allophycocyanin; *PE*, phycoerythrin; *SSC*, side scatter.

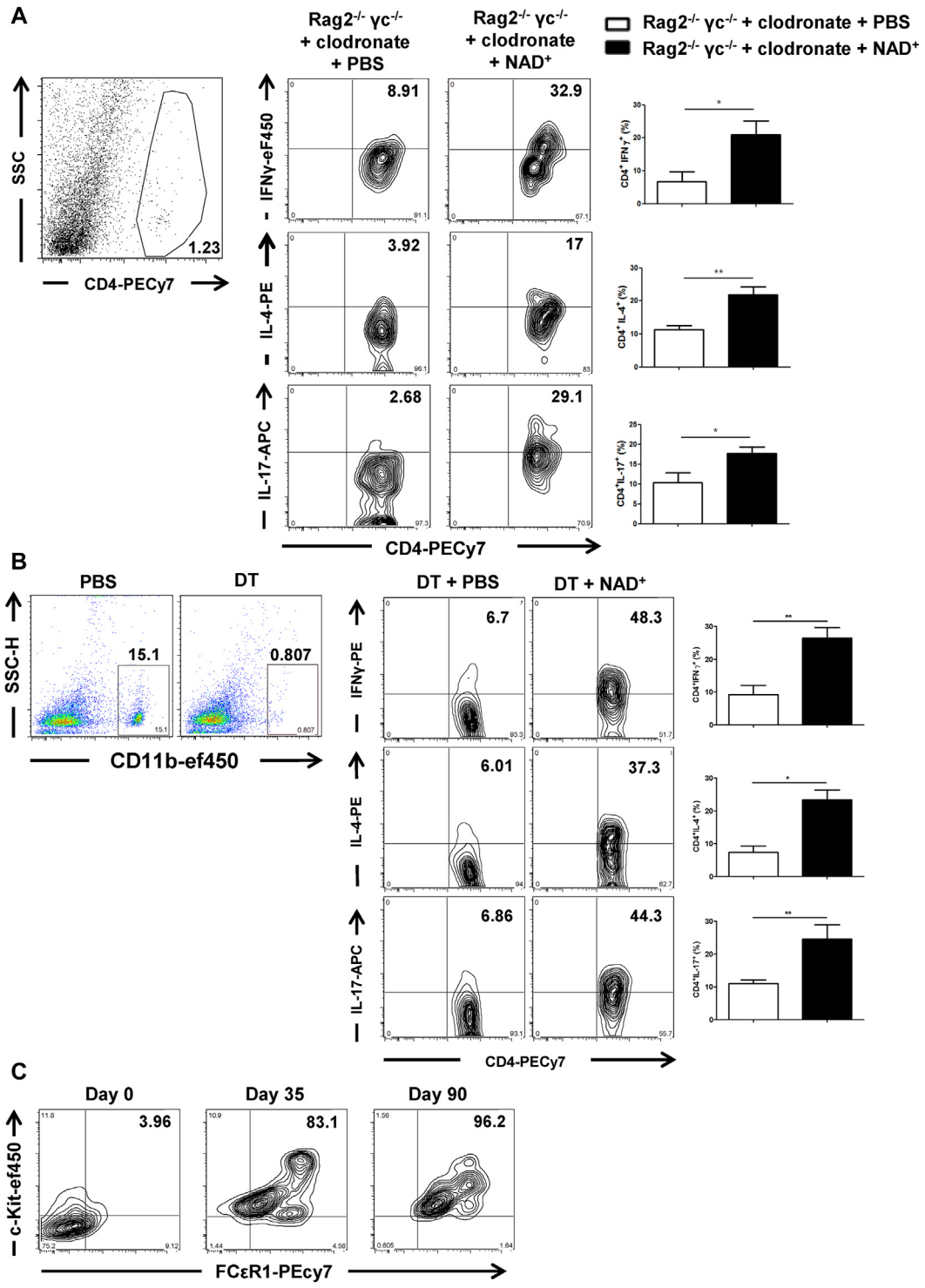

FIG E3. NAD^+ promotes T-cell differentiation in $Rag2^{-/-}\gamma c^{-/-}$ mice and flow cytometry of *in vitro* differentiation of BMMCs. **A,** $Rag2^{-/-}\gamma c^{-/-}$ mice were treated intravenously with liposomal clodronate at −8 days, −5 days, and −1 day before NAD+ treatment. After depletion, sorted naive $CD4^+CD44^-CD62L^+$ T cells from C57BL/6 WT mice sorted by means of fluorescence-activated cell sorting were adoptively transferred (3×10^6 cells per adoptive transfer). Animals were then treated with daily intraperitoneal injections of 40 mg of NAD^+ or a placebo solution (PBS). After 7 days, mice were killed, spleens were collected, and frequencies of $CD4^+IFN\text{-}\gamma^+$, $CD4^+IL\text{-}4^+$, and $CD4^+IL\text{-}17A^+$ cells were assessed by using flow cytometry. Data were derived from 2 independent experiments (n = 10). **B,** CD11b-DTR transgenic mice weighing 25 to 30 g were injected with diphtheria toxin (*DT*; 25 ng/g body weight) 24 hours before and 72 hours after beginning NAD^+ or PBS administration for depletion of $CD11b^+$ cells. After 7 days of treatment with PBS or NAD^+, frequencies of $CD4^+IFN\text{-}\gamma^+$, $CD4^+IL\text{-}4^+$, and $CD4^+IL\text{-}17A^+$ cells were assessed by using flow cytometry. Data were derived from 2 independent experiments (n = 10). **C,** BMMCs were obtained from femurs and tibias of 6- to 8-week-old C57BL/6 WT mice. BMMCs were cultured in WEHI-3–conditioned medium over 90 days. Purities of c-$Kit^+Fc\varepsilon RI^+$ MCs were then assessed by using flow cytometry. Data represent means ± SDs. The Student *t* test was used to compare between groups: $*P < .05$ and $**P < .01$. *APC*, Allophycocyanin; *PE*, phycoerythrin; *SSC*, side scatter.

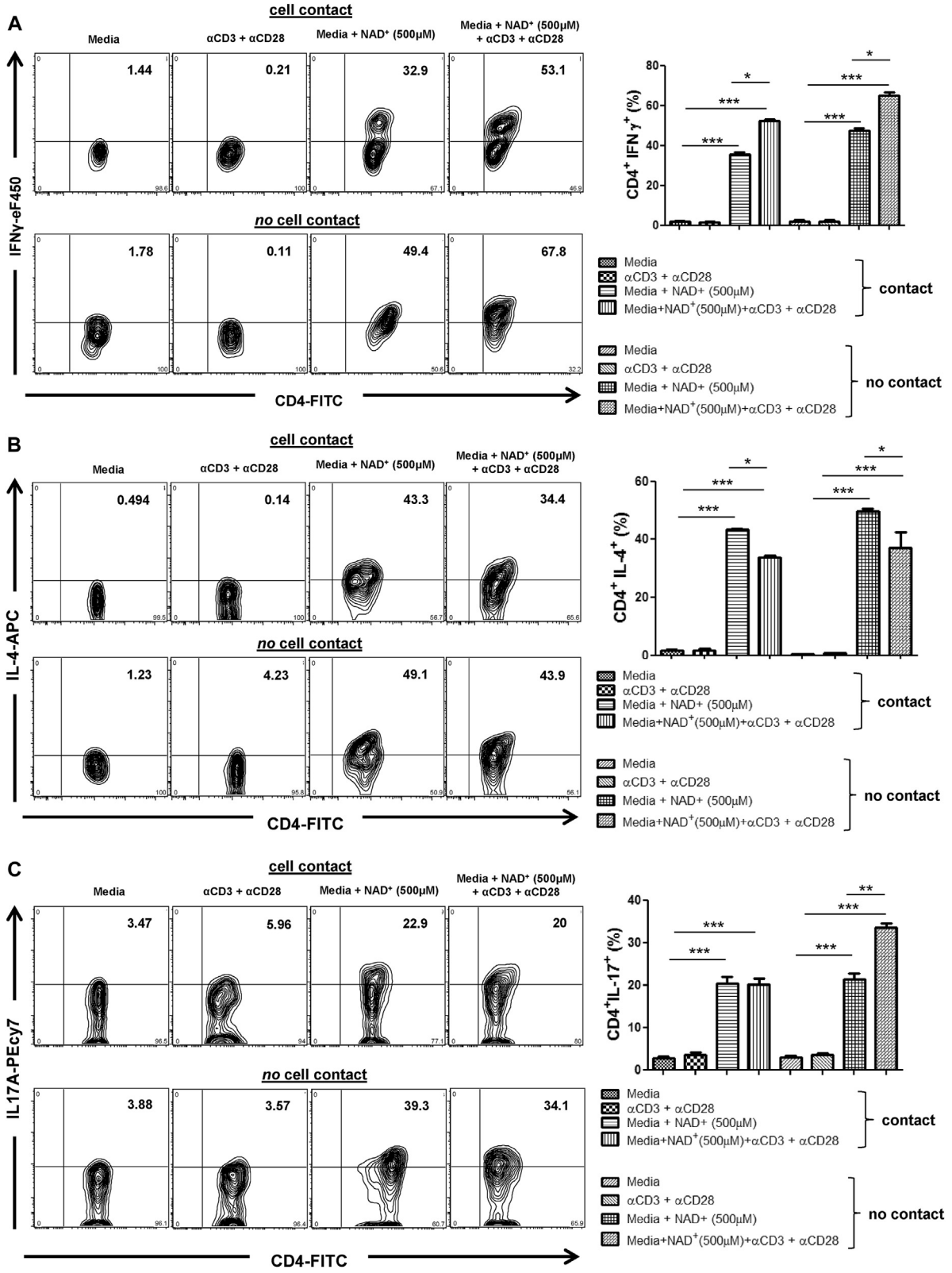

FIG E4. Murine MCs promote $CD4^+IFN\text{-}\gamma^+$, $CD4^+IL\text{-}4^+$, and $CD4^+IL\text{-}17A^+$ T-cell differentiation in the presence of NAD^+ both with and without cell-cell contact. BMMCs were cocultured with isolated naive $CD4^+CD44^-CD62L^+$ T cells from C57BL/6 mice (1:100 ratio) either in cell-cell contact or in separate compartments by using a transwell system. Cells were then treated with NAD^+ (500 μmol/L) or PBS. After 96 hours, frequencies of $CD4^+IFN\text{-}\gamma^+$ **(A)**, $CD4^+IL\text{-}4^+$ **(B)**, and $CD4^+IL\text{-}17A^+$ **(C)** cells were assessed by using flow cytometry (n = 6). Data were derived from 2 independent experiments. $^*P < .05$, $^{**}P < .01$, and $^{***}P < .001$, as determined by means of ANOVA, comparing the indicated groups. Data represent means ± SDs. *APC*, Allophycocyanin; *FITC*, fluorescein isothiocyanate; *PE*, phycoerythrin.

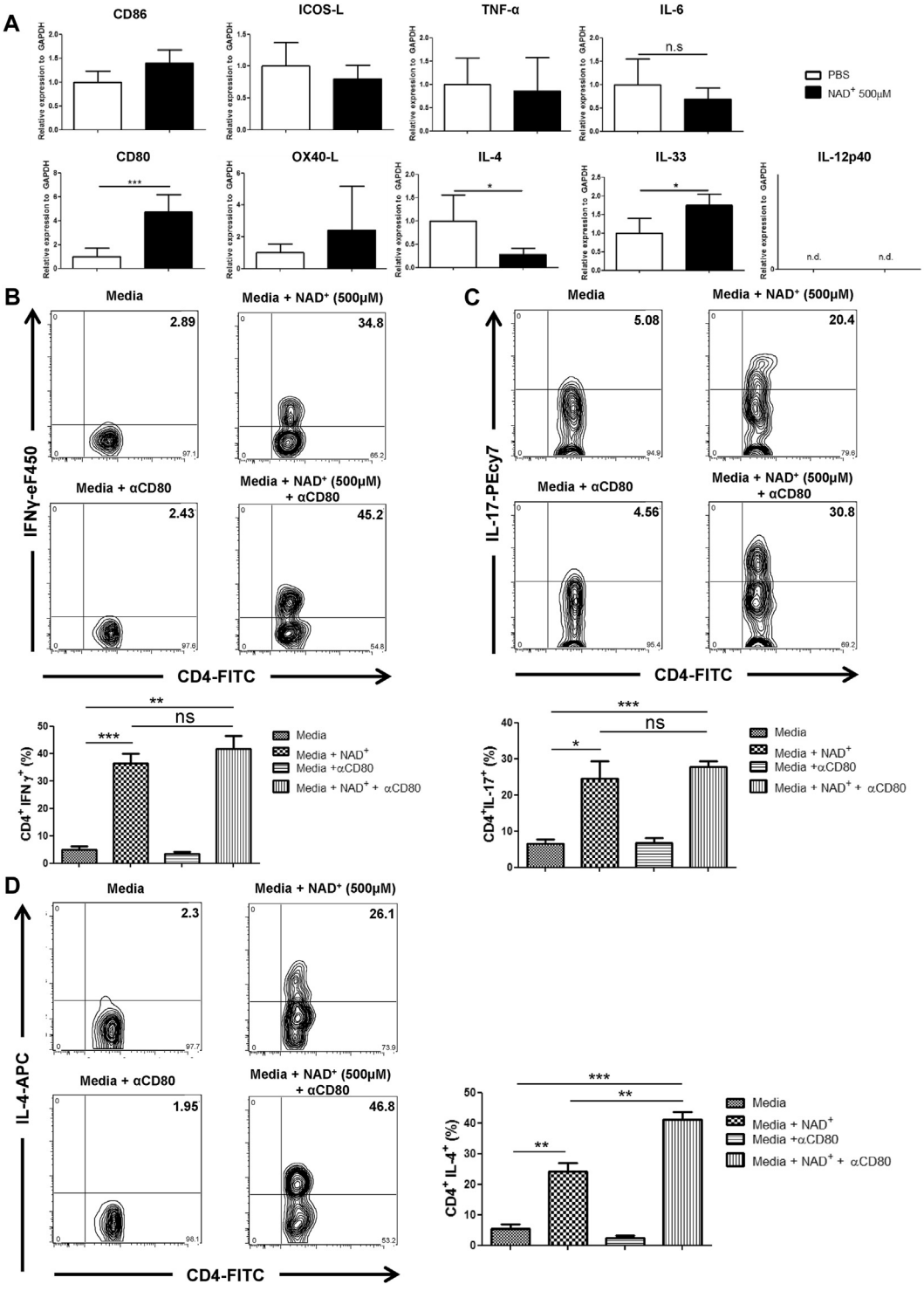

FIG E5. MCs do not regulate $CD4^+$ T-cell differentiation in the presence of NAD^+ through CD80. **A,** BMMCs from C57BL/6 mice were cultured in the presence of NAD^+ (500 μmol/L) or placebo (PBS). After 24 hours of culture, cells were collected, and mRNA was extracted. mRNA levels of CD86, CD80, TNF-α, IL-4, inducible costimulator ligand *(ICOS-L)*, OX40 ligand *(OX40-L)*, IL-6, and IL-33 were determined by using real-time PCR. Values are expressed as fold expression relative to the housekeeping gene glyceraldehye-3-phosphate dehydrogenase *(GAPDH)*. **B** and **C,** BMMCs were cocultured with isolated naive $CD4^+CD44^-CD62L^+$ T cells from C57BL/6 mice (1:100 ratio) in cell-cell contact conditions in the presence of α-CD80, NAD^+ (500 μmol/L), or placebo (PBS), as indicated. **B-D,** After 96 hours, frequencies of $CD4^+IFN\text{-}\gamma^+$ (Fig E5, *B*), $CD4^+IL\text{-}17A^+$ (Fig E5, *C*), and $CD4^+IL\text{-}4^+$ (Fig E5, *D*) cells were assessed by using flow cytometry (n = 6). Data were derived from 2 independent experiments. *ns*, Not significant. $^*P < .05$, $^{**}P < .01$, and $^{***}P < .001$, as determined by using the Student *t* test and ANOVA, comparing the indicated groups. Data represent means ± SDs. *APC*, Allophycocyanin; *FITC*, fluorescein isothiocyanate; *PE*, phycoerythrin.

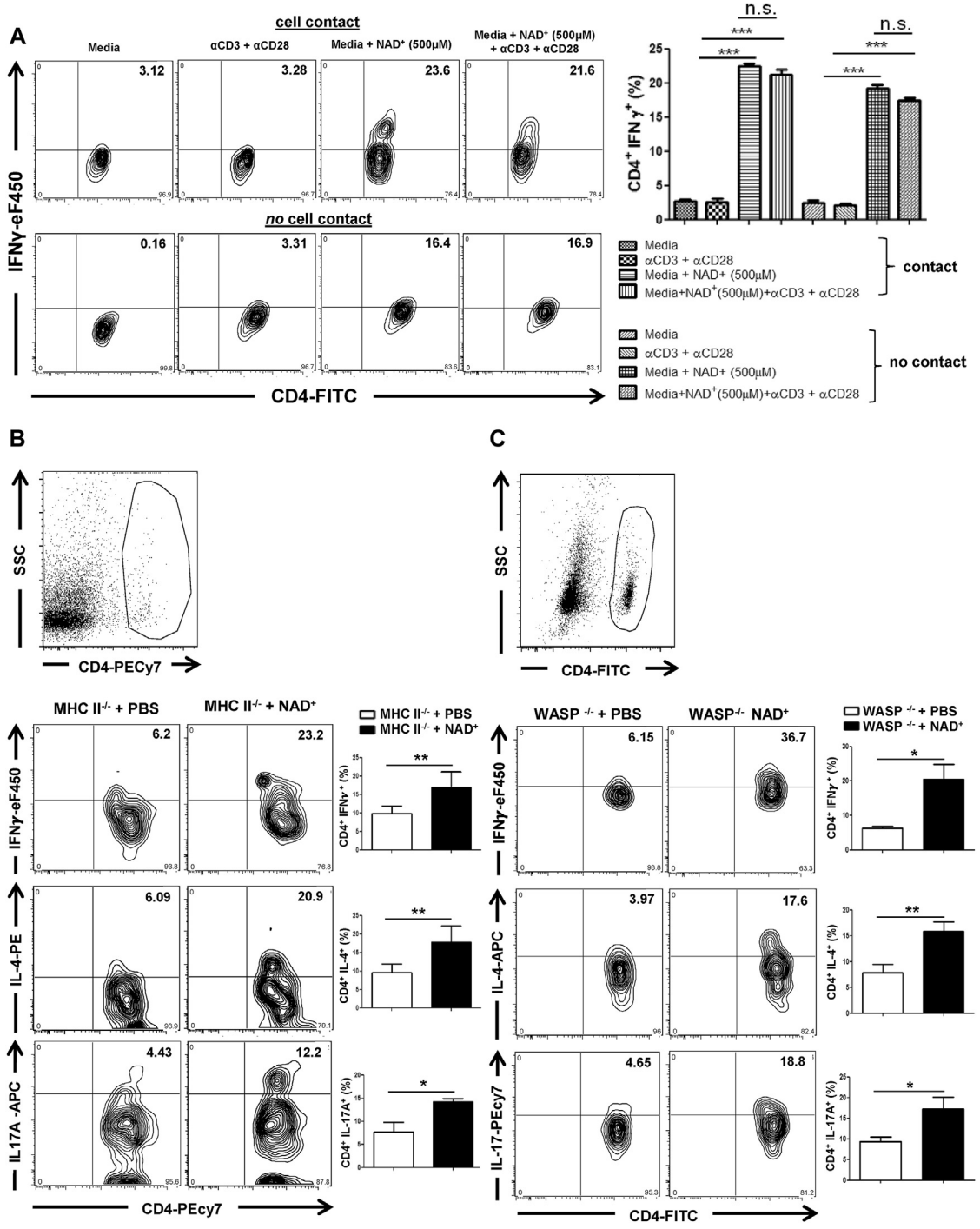

FIG E6. Conserved MC-mediated $CD4^+$ T-cell differentiation in the human MC line LAD-2 in the presence of NAD^+. NAD^+ induces T-cell differentiation in MHC class $II^{-/-}$ and $WASP^{-/-}$ mice. **A,** Human MC line LAD-2 cells were cocultured with isolated human naive $CD4^+$ T cells from healthy donors (1:100 ratio) either in cell-cell contact or in separate compartments by using a transwell system. Cells were then treated with NAD^+ (500 μmol/L) or PBS. After 96 hours, frequencies of $CD4^+IFN\text{-}\gamma^+$ cells were assessed by using flow cytometry (n = 6). Data were derived from 2 independent experiments. **B,** MHC class $II^{-/-}$ (B6.129S-$H2^{dlAb1\text{-}Ea}$) mice were treated daily with intraperitoneal injections of 40 mg of NAD^+ or a placebo solution (PBS). After 7 days, mice were killed, and splenocytes were harvested. Systemic frequencies of $CD4^+IFN\text{-}\gamma^+$, $CD4^+IL\text{-}4^+$, and $CD4^+IL\text{-}17A^+$ cells were analyzed by means of flow cytometry. Data were derived from 2 independent experiments (n = 5). **C,** $WASP^{-/-}$ (B6.129S6-Was^{tm1Sbs}/J) mice were treated daily with intraperitoneal injections of 40 mg of NAD^+ or a placebo solution (PBS). After 7 days, mice were killed, and frequencies of $CD4^+IFN\text{-}\gamma^+$, $CD4^+IL\text{-}4^+$, and $CD4^+IL\text{-}17A^+$ cells were assessed by means of flow cytometry. Data were derived from 2 independent experiments (n = 5). *ns*, Not significant. $^*P < .05$, $^{**}P < .01$, and $^{***}P < .001$, as determined by using the Student *t* test and ANOVA, comparing the indicated groups. Data represent means ± SDs. *APC*, Allophycocyanin; *FITC*, fluorescein isothiocyanate; *PE*, phycoerythrin; *SSC*, side scatter.

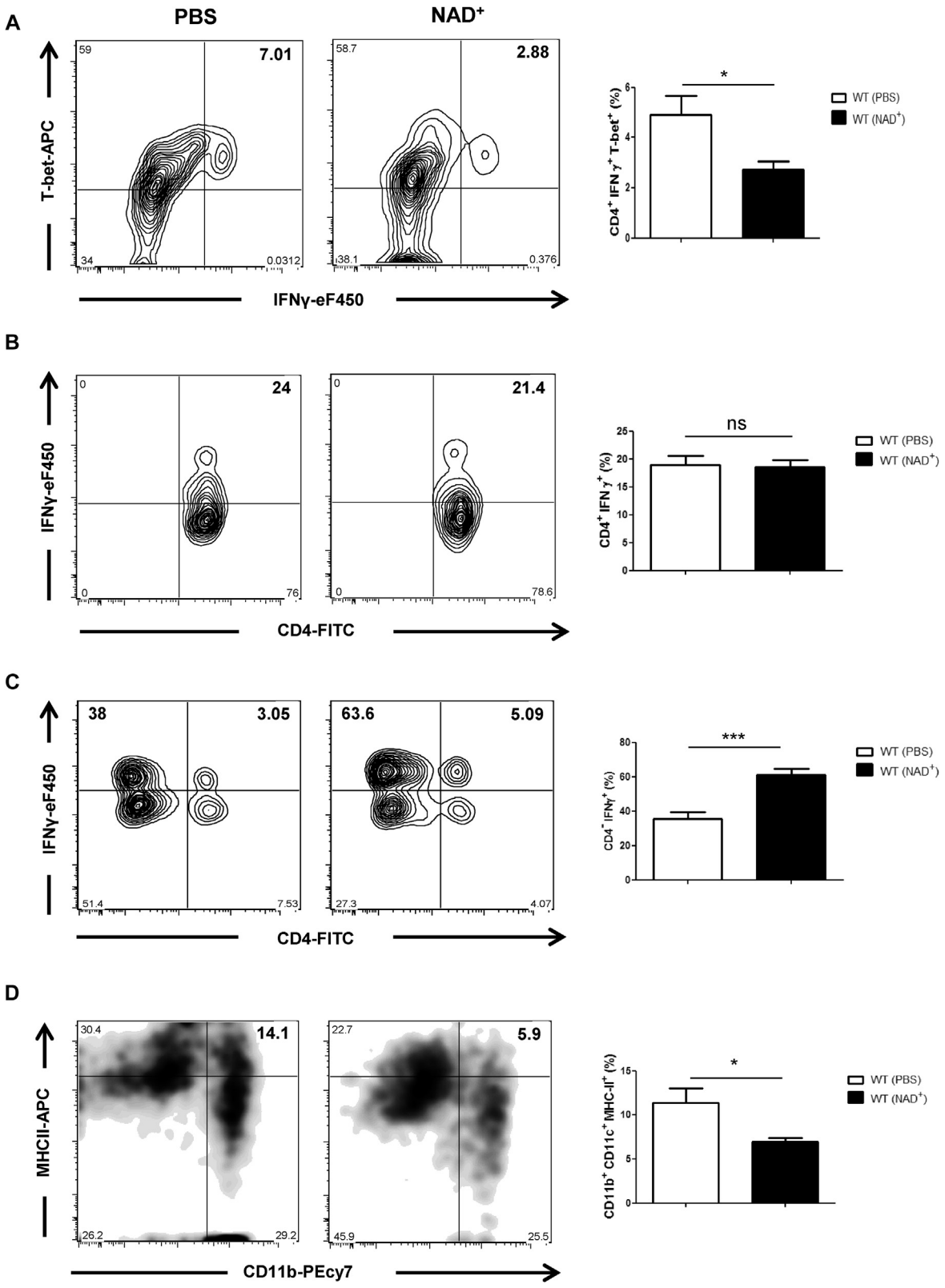

FIG E7. NAD^+ alters systemic frequencies of $CD4^+$T-bet^+IFN-γ^+ cells and $CD11c^+$MHC class II^+ DCs after *L monocytogenes* infection. C57BL/6 mice were treated for 5 days with daily intraperitoneal injections of NAD^+ (40 mg) or placebo solution (PBS). After 5 days, mice were infected with a nonlethal dose of *L monocytogenes* (1×10^7 colony-forming units) and killed 3 days later. Spleens were collected, and frequencies of $CD4^+$T-bet^+IFN-γ+ **(A)**, $CD4^+$IFN-γ^+ **(B)**, and $CD4^-$IFN-γ^+ **(C)** T cells and $CD11b^+CD11c^+$MHC class II^+ DCs **(D)** were assessed by using flow cytometry. Data were derived from 2 independent experiments (n = 5). Data represent means ± SDs. *ns*, Not significant. The Student *t* test was used to compare between groups: $*P < .05$ and $***P < .001$.