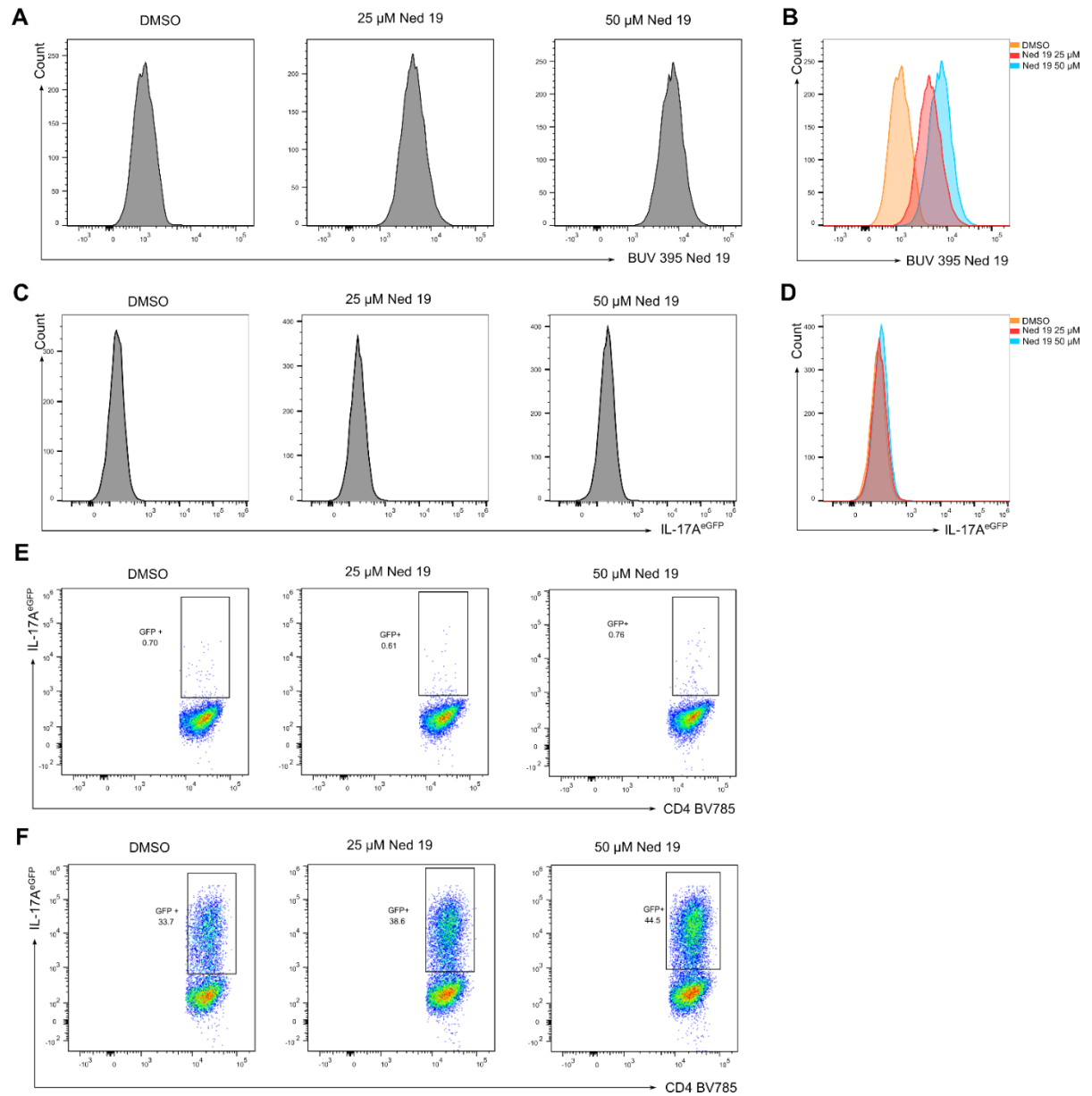
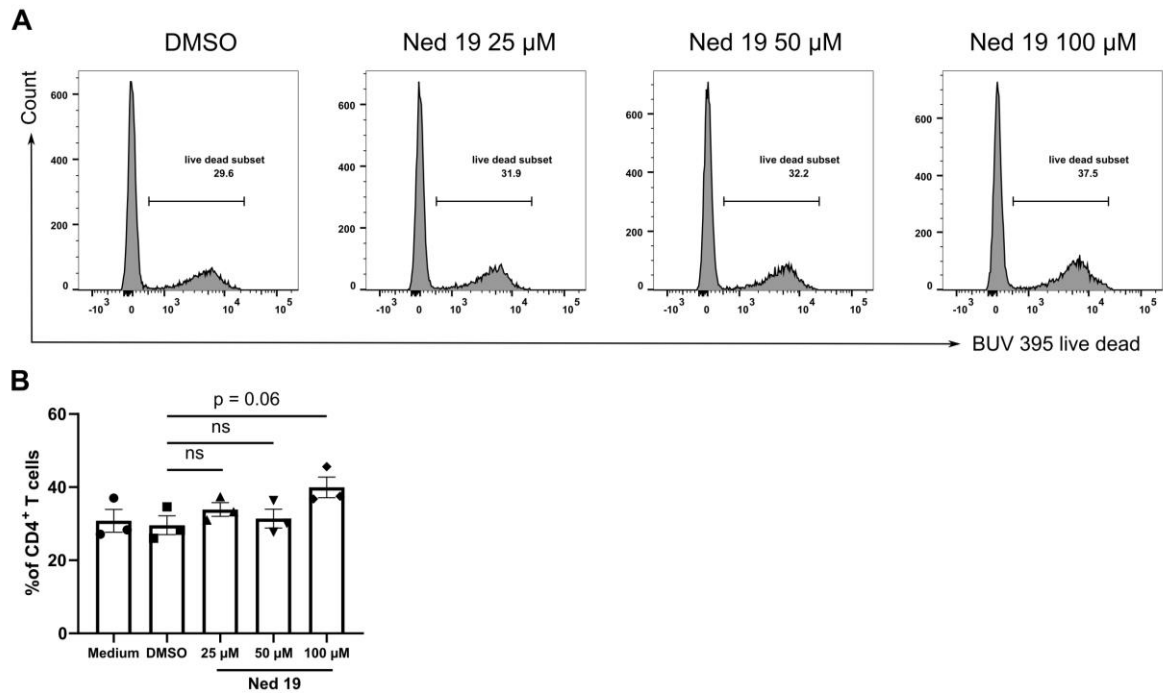
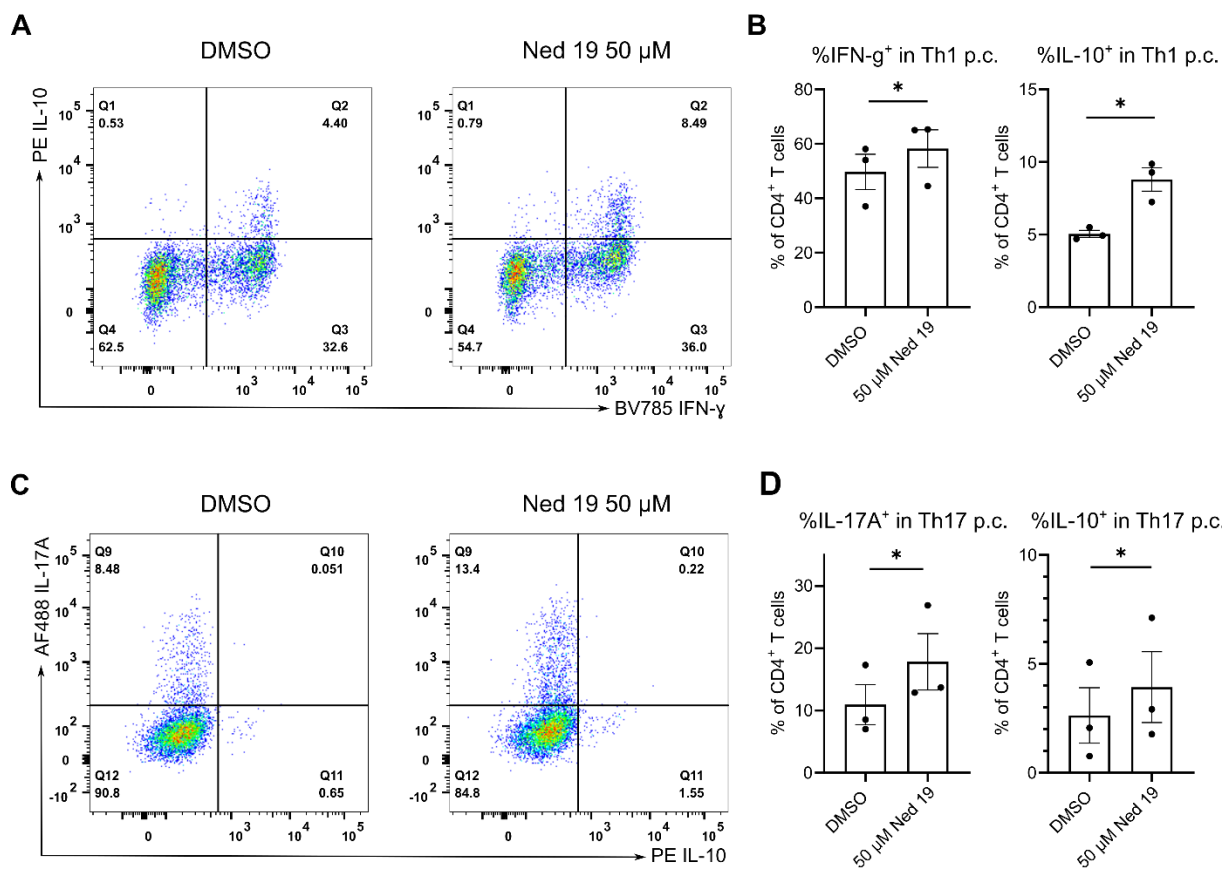




Supplementary Figure S1. Gating strategy used in flow cytometry experiment with trans-Ned 19. Naïve CD4⁺ T cells were isolated from and IL-17A^{eGFP} $\times$ IFN- γ ^{Katushka} $\times$ Foxp3^{RFP} reporter mice. Cells were incubated with DMSO or trans-Ned 19 and differentiated in vitro. **(A)** Mean fluorescence intensity in BUUV 395 channel. **(B)** Overlay of the MFI histograms in BUUV395 channels. **(C)** Mean fluorescence intensity in GFP channel. **(D)** overlay of the MFI histograms in GFP channels. **(E)** Gating of GFP⁺ events using GFP-negative condition. **(F)** Gating of GFP⁺ events in Th17 condition.

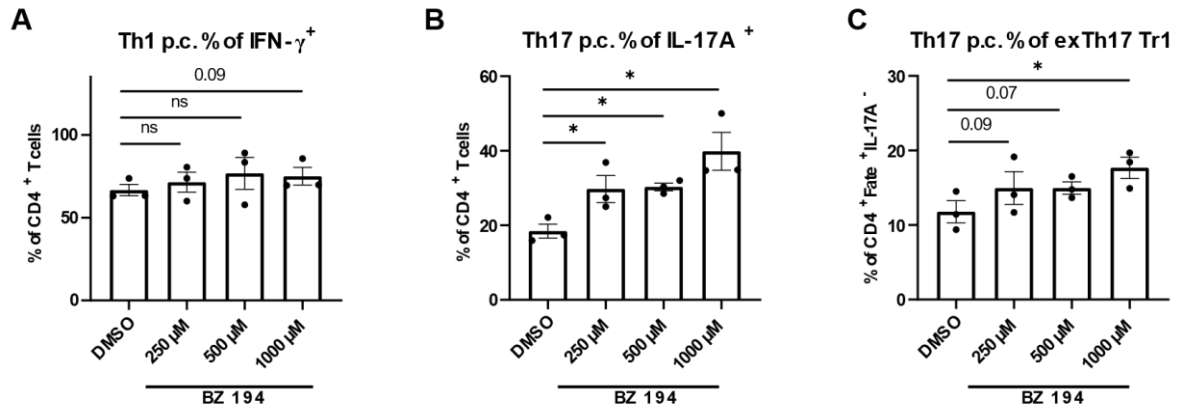


Supplementary Figure S2. NAADP inhibition by trans-Ned 19 does not decrease the viability of resting CD4⁺ T cells *in vitro*. The CD4⁺ T cells were freshly isolated from WT C57BL/6N mice, incubated with increasing concentrations of trans-Ned 19 for 1 h and cultured for 24 h in presence of IL-7 (10 ng/mL). **(A)** Representative histograms of live dead staining. **(B)** Summary statistics of viable cells. Presented data in B are mean $\pm$ SEM. The p-values were calculated with repeated measures ANOVA with Dunnett's multiple comparison test.



Supplementary Figure S3. NAADP inhibition by trans-Ned 19 promotes the differentiation of Th1 and Th17 cells upon stimulation with antigen. The naïve CD4⁺ T cells were isolated from OT-II mice and incubated with increasing concentrations of trans-Ned 19 for 1 h. Cells were stimulated with ovalbumin peptide and CD3-

depleted splenocytes under Th1 and Th17 polarizing conditions in presence of trans-Ned 19. (A) Representative staining for IFN- γ and IL-10 after differentiation under Th1 polarizing conditions. (B) Summary statistics of IFN- γ and IL-10 expression under Th1 polarizing conditions (p.c.). (C) Representative staining for IL-17A and IL-10 after differentiation under Th17 polarizing conditions. (D) Summary statistics of IL-17A and IL-10 expression under Th17 polarizing conditions. The data points in (B) and (D) are total percentages of IFN- γ^+ , IL-17A $^+$ and IL-10 $^+$ of CD4 $^+$ T cells. The p-values were calculated with paired Wilcoxon test. * $p < 0.05$.



Supplementary Figure S4. CD4 $^+$ T cell *in vitro* differentiation in the presence of NAADP antagonist BZ194. Naïve CD4 $^+$ T cells were freshly isolated from IL-17A eGFP $\times$ IFN- $\gamma^{Katushka}$ $\times$ Foxp3 RFP mice. Cells were incubated with increasing concentrations of BZ194 for 5 h and stimulated with plate bound anti-CD3 mAb, soluble anti-CD28 mAb and polarizing cytokine cocktails. (A) Summary statistics of Th1-cell differentiation (p.c. polarizing conditions). (B) Summary statistics of Th17-cell differentiation (C) Summary statistics of Treg differentiation. (D) Naïve CD4 $^+$ T cells were freshly isolated from Fate reporter mice (IL-17A Cre $\times$ Rosa26 STOP $^{fl/fl}$ YFP $\times$ 17A Katushka $\times$ IL-10 eGFP $\times$ Foxp3 RFP mice). Cells were incubated with increasing concentrations of BZ194 for 5 h, stimulated with plate bound anti-CD3 mAb, soluble anti-CD28 mAb and cultured for 96 h in the presence of BZ194 under Th17 polarizing conditions. Mean $\pm$ SEM are depicted on the graphs. P-values were calculated with repeated measures ANOVA with Dunnet's multiple comparison test. * $p < 0.05$.