Expanded View Figures

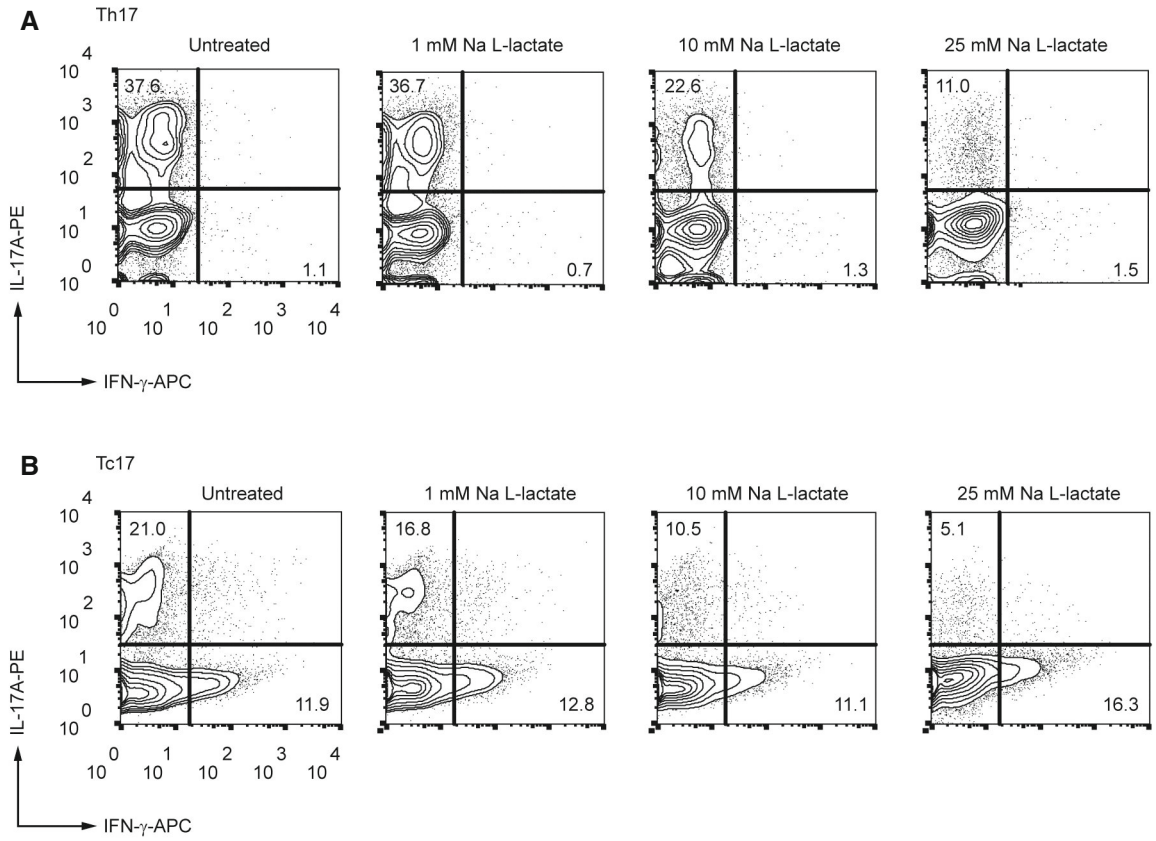

Figure EV1. Lactate suppresses production of IL-17A in T cells.

A, B $CD4^+$ (A) and $CD8^+$ (B) T lymphocytes were purified from spleens and LN of WT mice and differentiated under Th17-polarizing conditions for 3 days in the presence of increasing lactate concentrations, respectively. Representative contour plots indicate the percentage of $IL\text{-}17A^+$ and $IFN\text{-}\gamma^+$ cells, detected by flow cytometry (n = 3 biological replicates).

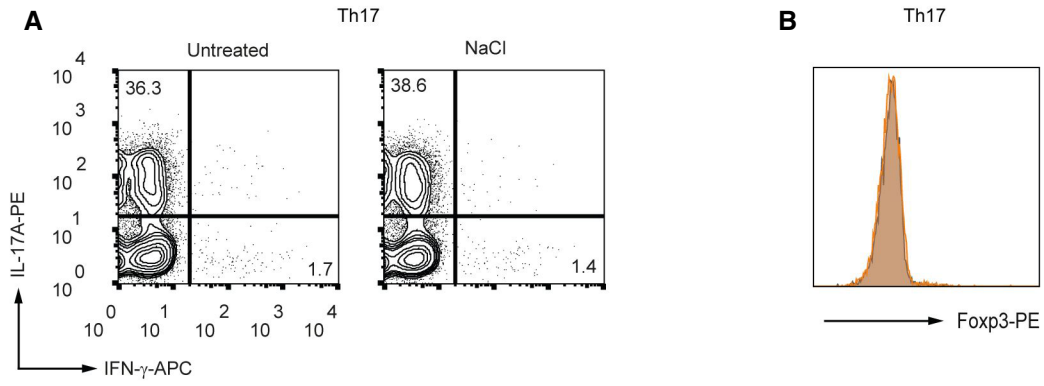

Figure EV2. Effect of NaCl on differentiation of Th17 cells.

A, B Murine $CD4^+$ T cells were cultured under Th17-inducing conditions in the presence or absence of NaCl (25 mM) for 3 days. The percentages of $IL\text{-}17A^+$ cells (A) and Foxp3 expression (B) were determined by flow cytometry (n = 3 biological replicates).

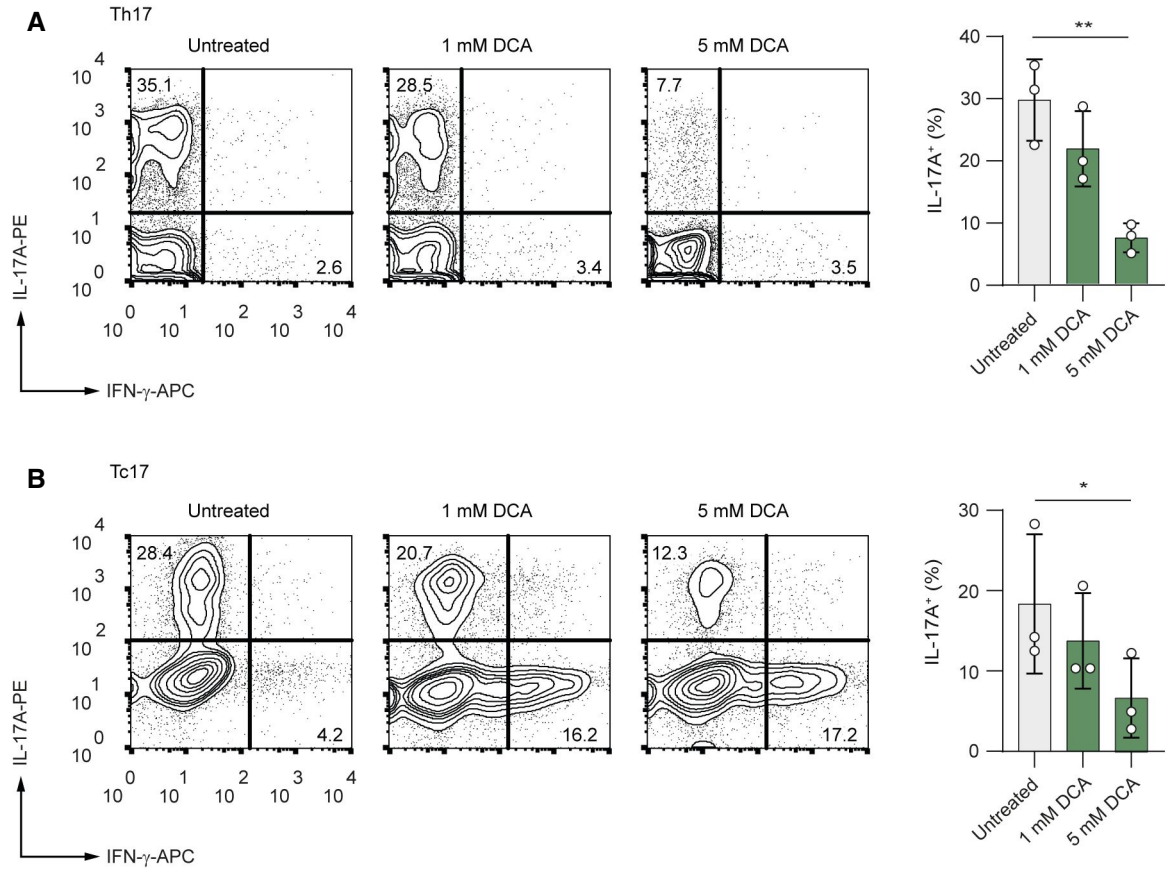

Figure EV3. DCA suppresses production of IL-17A in Th17 and Tc17 cells.

A, B $CD4^+$ (A) and $CD8^+$ (B) T cells were isolated from spleens and LNs of WT mice. Purified T cells were polarized under Th17-inducing conditions and treated with increasing concentrations of DCA for 3 days. Representative contour plots show the frequencies of $IL\text{-}17A^+$ and $IFN\text{-}\gamma^+$ cells analyzed by flow cytometry (n = 3 biological replicates; n.s., not significant; *P = 0.01–0.05; **P = 0.001–0.01; data are analyzed by the two-tailed unpaired Student's t-test).

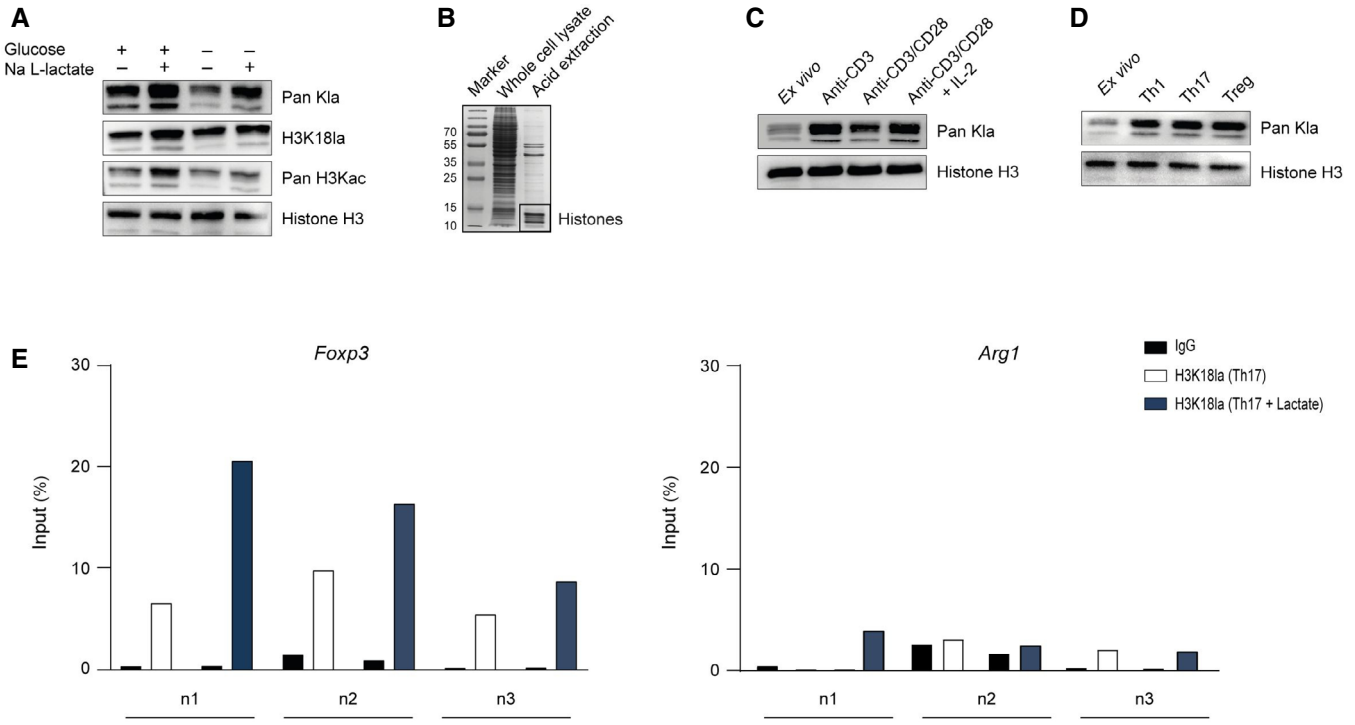

Figure EV4. Histone lactylation in CD4$^+$ T cells.

A Global histone (Kla) and specific H3K18 lactylation were analyzed by immunoblotting 24 h after stimulation of nonpolarized macrophages. Bone marrow-derived macrophages were cultured in the presence or absence of 25 mM glucose and 25 mM Na L-lactate. Immunoblotting of representative whole-cell extracts is shown (n = 3 biological replicates).

B Histone preparation by acid extraction from the whole-cell lysate of murine CD4$^+$ T cells, visualized by Coomassie blue staining.

C Western blots of acid-extracted histones from activated CD4$^+$ T cells showing global histone lactylation in the presence of glucose (25 mM) at 24 h after stimulation of cells. One of three similar experiments is shown.

D Immunoblotting of acid-extracted lactylated histones from differentiated Th1, Th17, and Treg cells on day 3 of differentiation. As control lymphocytes, *ex vivo* purified, nonactivated CD4$^+$ T cells were used.

E ChIP analysis of H3K18-lactylated histones at the *Arg1* and *Foxp3* promoter regions in the absence or presence of extracellular lactate (25 mM) was performed after 24 h of the cell culture for Th17 cells. Three independent experiment (n = 3 biological replicates) are shown (n1, n2, n3).

Source data are available online for this figure.

Figure EV5. Gating strategy for detection of Th17 cells.

Gating strategy used for flow cytometry analysis of Th17 cells. The purity of CD4$^+$ T cells, as well as IL-17A frequency and Foxp3 expression, is displayed.

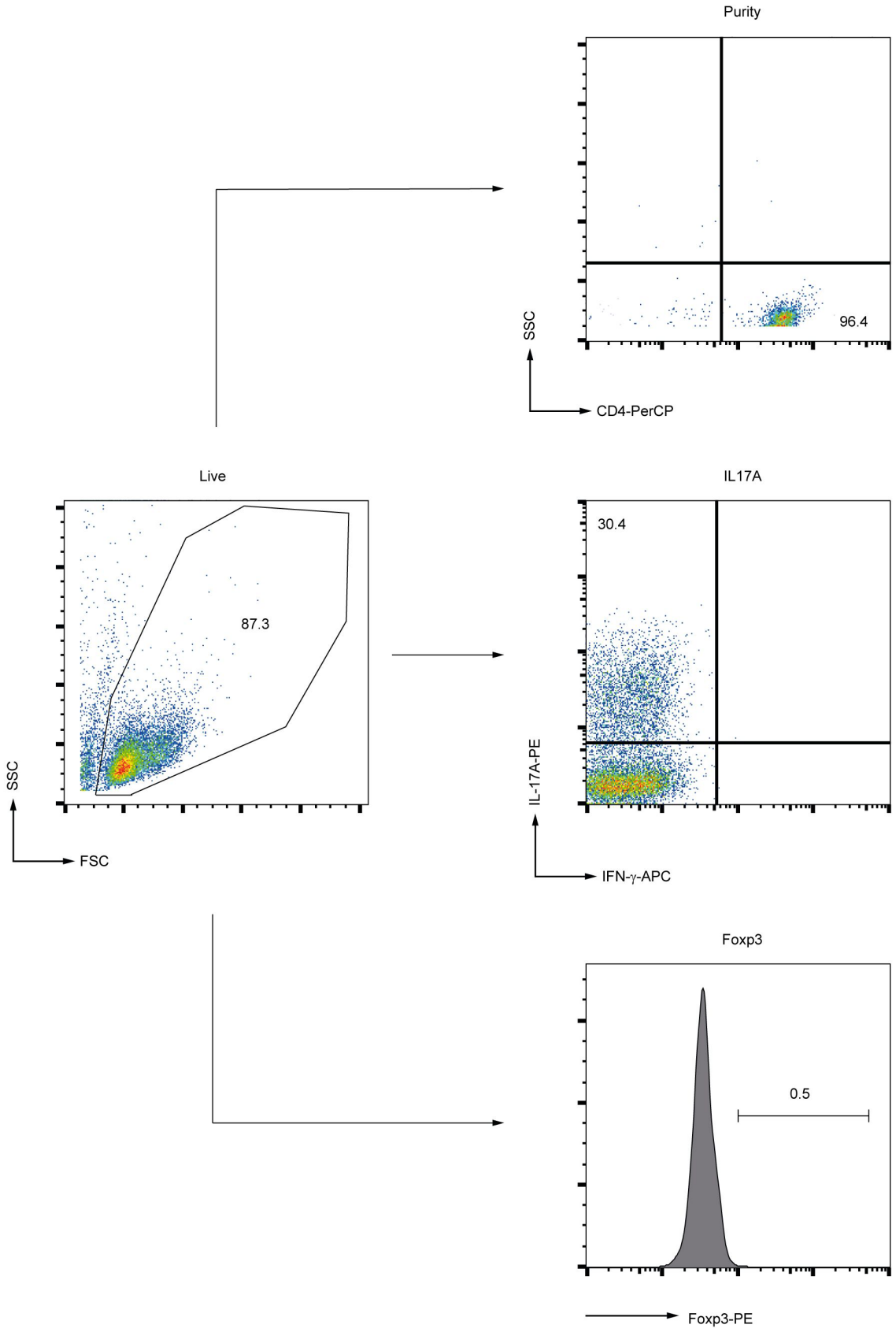
Purity
SSC
96.4
CD4-PerCP
Live
87.3
SSC
FSC
IL17A
30.4
IL-17A-PE
IFN-γ-APC
Foxp3
0.5
Foxp3-PE

Figure EV5.