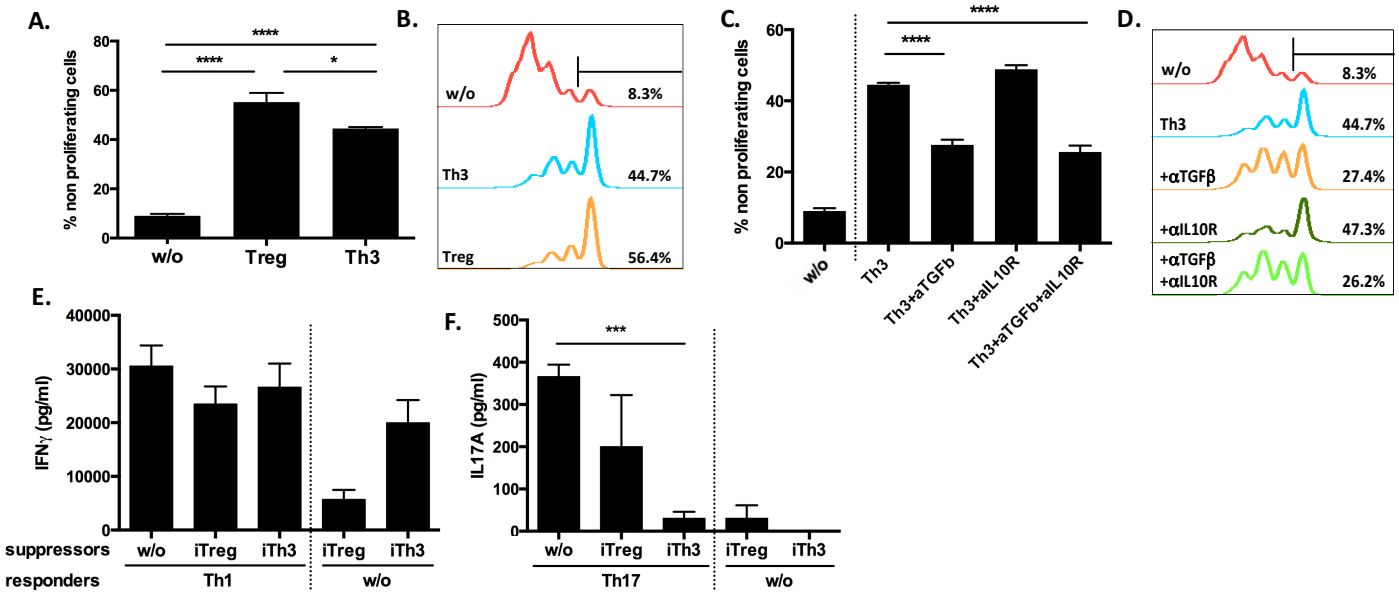
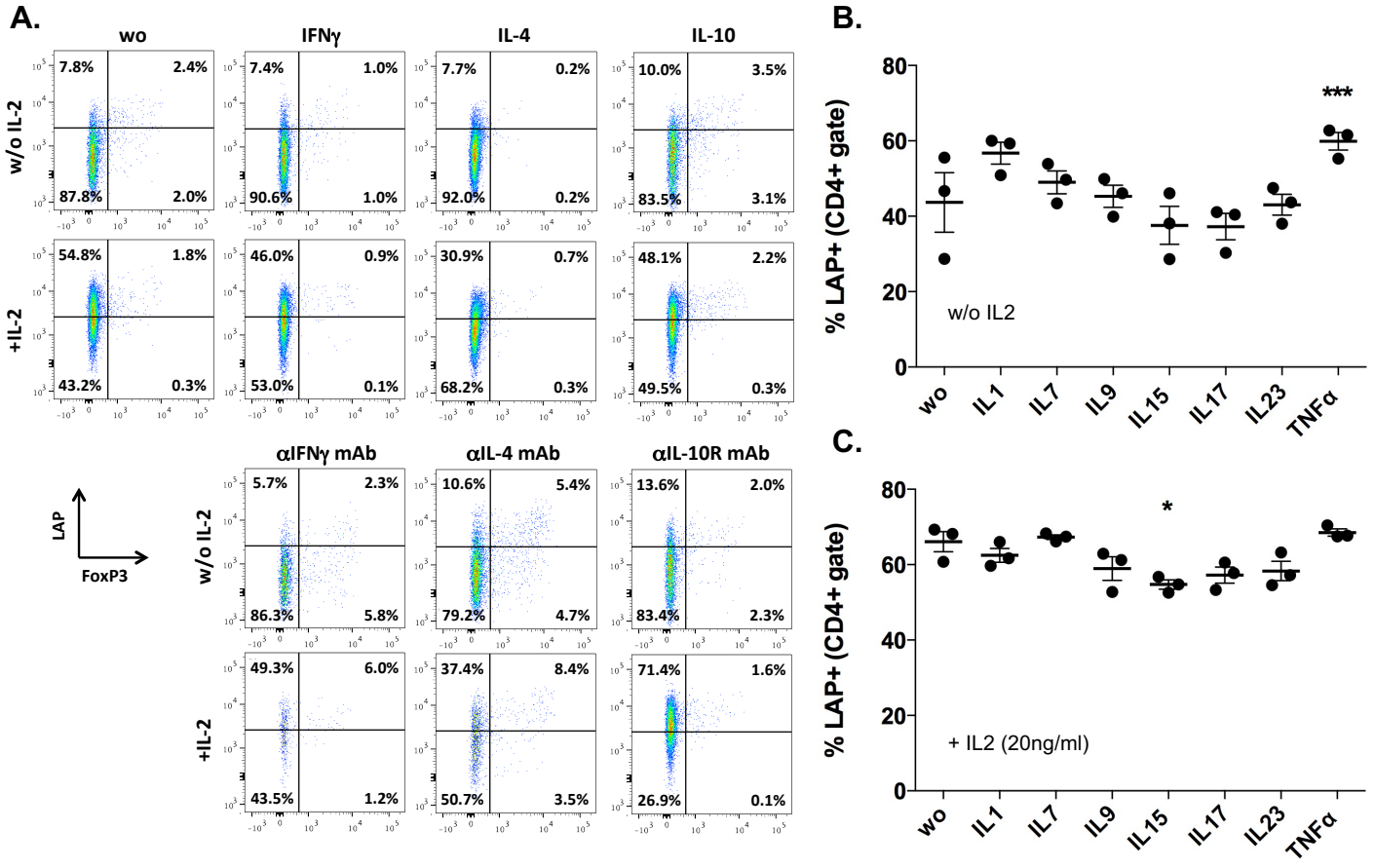


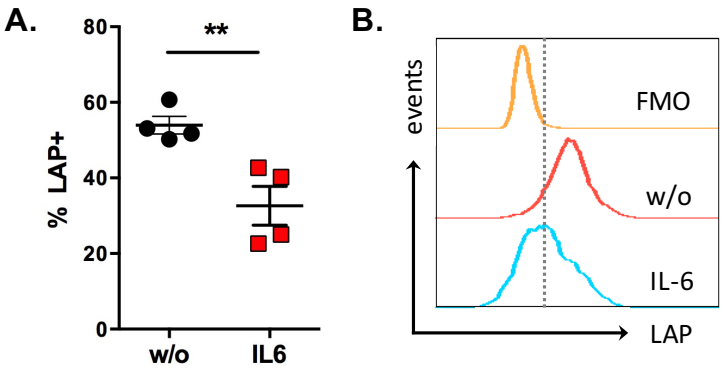
Supplementary figure 1. *In vitro* generated CD4$^+$LAP$^+$FoxP3$^-$ T cells inhibit proliferation of CD4$^+$ T cells and IL-17A secretion by Th17 cells. A-B. Representative experiment showing the percentage of non proliferating cells (left) or dilution of proliferation dye in responder cells (right) gated on life CD4$^+$CD45.1$^+$ cells after 3 days of culture in the absence (wo) or presence of *in vitro* generated CD4$^+$FoxP3$^+$ T cells (iTreg) or CD4$^+$LAP$^+$FoxP3$^-$ T cells (iTh3). **C-D.** Impact of blocking TGF-β, IL-10R or both on proliferation of responder cells using the culture system described in 3A. **E.** IFNγ production (pg/ml) after coculture of *in vitro* generated Th1 cells alone or together with iTreg or iTh3 cells (1:1 ratio) as compared to iTreg or iTh3 cells alone. **F.** IL-17 secretion (pg/ml) after coculture of *in vitro* generated Th17 cells alone or together with iTreg or iTh3 cells (1:1 ratio) as compared to iTreg or iTh3 cells alone. Bars in Figure A, C, E and F represent average values ± SEM (n=3). Histograms show representative data from one mouse. Statistical significant values are labeled: ***p<0.001, ****p<0.0001 (One-way ANOVA)

Supplementary figure 1.



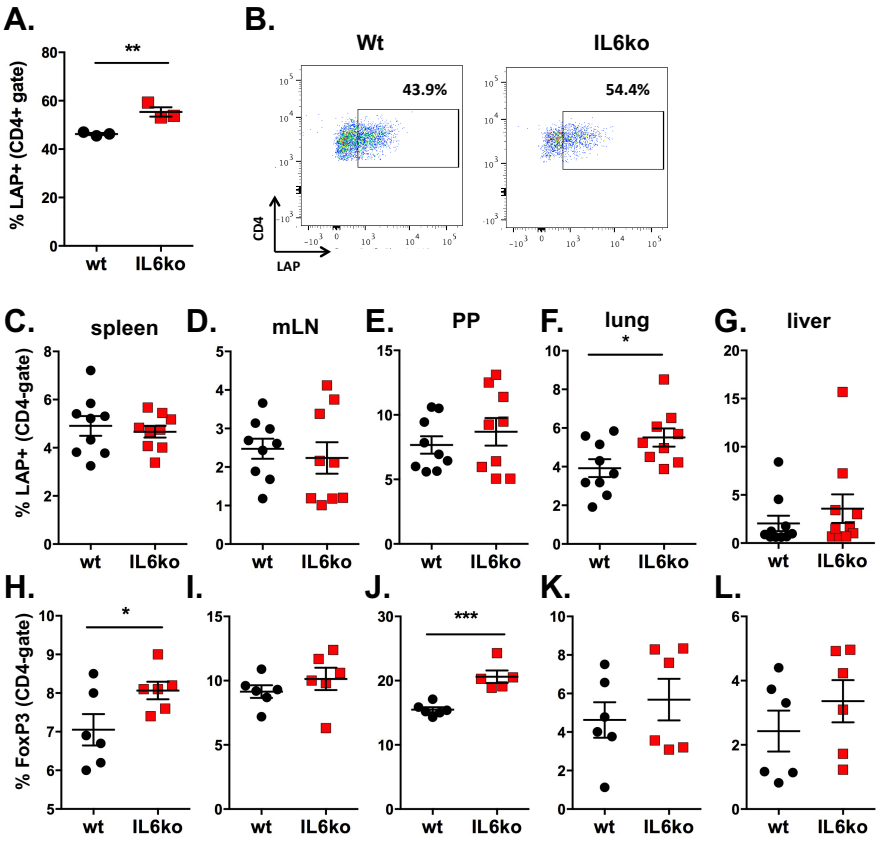
Supplementary figure 2. Influence of cytokines on *in vitro* induction of membrane bound TGF-b on naïve CD4+ T cells. A-C. Representative dot blots showing LAP and FoxP3 staining (A) and summary of representative experiments (B-C) showing the percentage of LAP expressing CD4+ T cells after stimulation of naïve CD4+ T cells with coated anti-CD3 mAb (1 mg/ml), anti-CD28 mAb (10 mg/ml) without (A, B) or with IL-2 (20 ng/m, A, CI) and indicated cytokines (20 ng/ml) or neutralizing antibodies (20ug/ml). Graphs in B and C show average values ± SEM for one representative experiment with three samples. Statistically significant values are labeled; *p<0.05, ***p<0.001. (Ordinary one-way ANOVA with Dunnett's multiple comparisons test)

Supplementary figure 2



Supplementary figure 3. LAP expression in response to antigen specific stimulation. A-B. Percentage of LAP+ cells (CD4-gate) after coculture of naïve CD4+ T cells from 2D2 mice with MOG primed DCs with IL-2 (red line; 20ng/ml) alone or together with IL-6 (blue line; 25ng/ml) for three days. Graph A shows 4 samples from 2 independent experiments and graph B shows representative histograms from the same two independent experiments.

Supplementary figure 3



Supplementary figure 4. Analysis of IL-6$^{-/-}$ mice. A, B. LAP expression in CD4+ T cells from IL6-/- mice (IL6ko) as compared to wild-type (wt) mice after 72 hours stimulation with anti-CD3 mAb, anti-CD28 mAb and IL-2 *in vitro*. **C-L.** Percentage of LAP (C-G) or FoxP3 (H-L) expressing CD4+ T cells in spleens (C, H), mesenteric lymph nodes (mLN; D, I), Peyer's patches (PP; E, J), lung (F, K) and liver (G, L) of IL-6$^{-/-}$ mice as compared to wild-type mice. Panel A shows average values ± SEM for one representative experiment (n=3), panel B shows a representative dot blot. C-L show FACS staining for at least five individual mice. Statistically significant values are labeled; *p<0.05, **p<0.01, ***p<0.001 (unpaired t-test).

Supplementary figure 4