Supplemental Figure 1. Gating strategy and flow cytometric analysis. (A) Lymphocytes were gated based upon expression of CD4 and CD25, to identify Foxp3 expression in the total CD4⁺, CD4⁺CD25⁺ and CD4⁺CD25⁻ fractions. (**B**) Flow cytometric analysis quantifying the percent increase in Foxp3 expression relative to the starting value in $CD4^{-}$ cells (n = 6 mice/group). (C) Suppression of CFSE-labeled T_{resp} cells in vitro either with or without transfection of modified Foxp3 mRNA, and percent increase in suppression following Foxp3 mRNA delivery. In vitro studies performed in triplicate and repeated in three independent experiments. (D) Percent increase in 3xFLAG-Foxp3 expression relative to starting value in various cell types by flow cytometry (n = 5 mice/group). (**B**, **D**) Values calculated as: ('% Foxp3 in a given cell type (x) for Foxp3 mRNA-injected mice' - '% Foxp3 in cell type x for PBS injected mice') / '% Foxp3 in cell type x for PBS injected mice' * 100. According to this calculation, PBS-injected mice show an increase of 0% for all cell types. * $P \leq 0.05$, ** $P \leq$ 0.01, *** $P \leq 0.001$ relative to PBS-injected mice or for the indicated comparisons between plotted groups. Data are represented as means ± SD.

Supplemental Figure 2. Injection schedule in OVA-induced model of allergic asthma. Timing of allergen sensitization and challenge, vector administration and endpoint assessment is depicted.

Supplemental Figure 3. Modified Foxp3 mRNA decreases IL-17A expression by CD4+ cells. BALB/c mice were administered either PBS or modified Foxp3 mRNA i.t. and monitored for the percent of CD4⁺IL-17A⁺ cells at 24 hours by intracellular cytokine staining (**A**). Representative dot plots for each group are shown (**B**). n = 5 mice / group.

Supplemental Figure 1



CD4+ CD4-CD25+ Alv. Μφ ATII PMN

Supplemental Figure 2



Supplemental Figure 3

