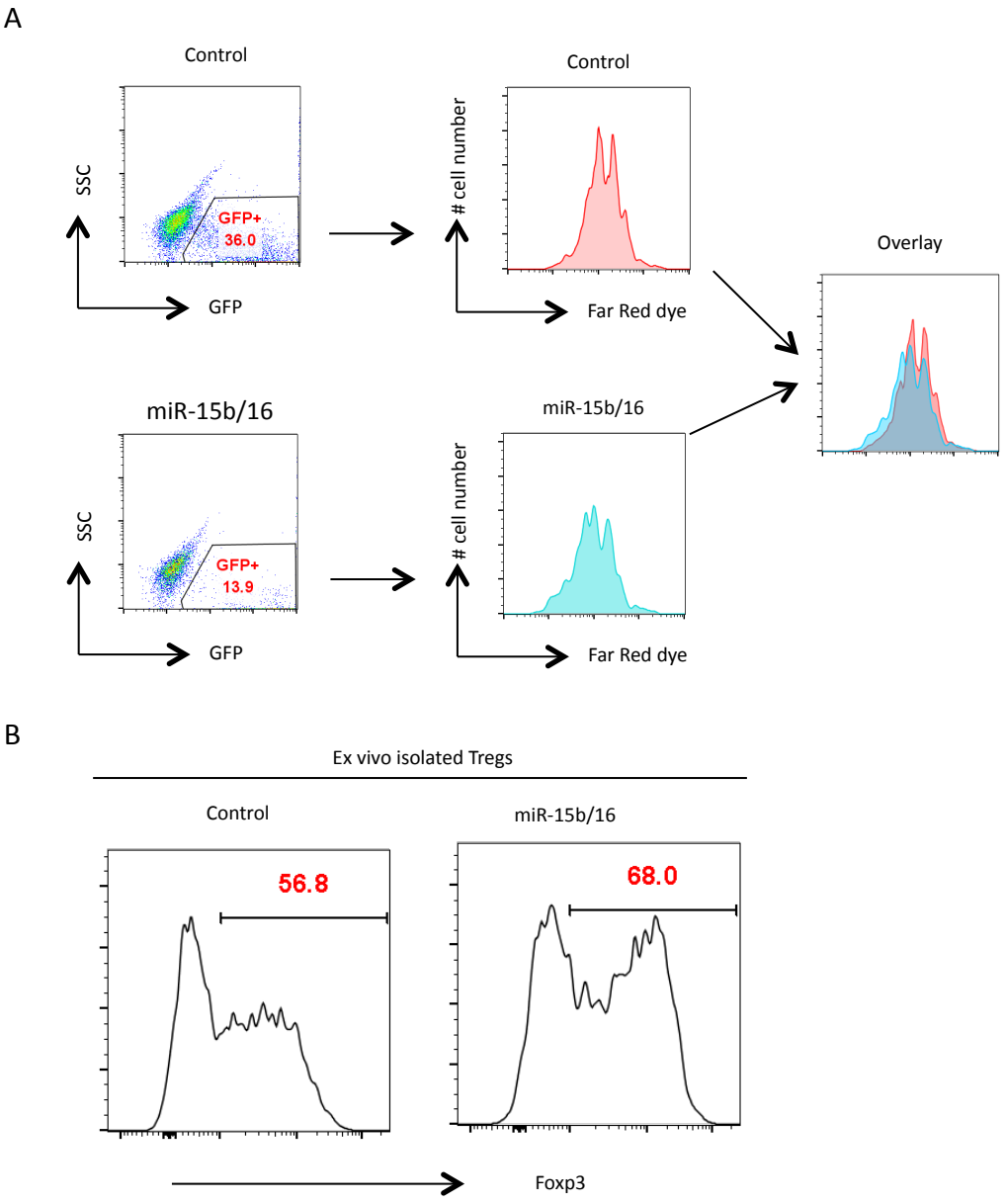
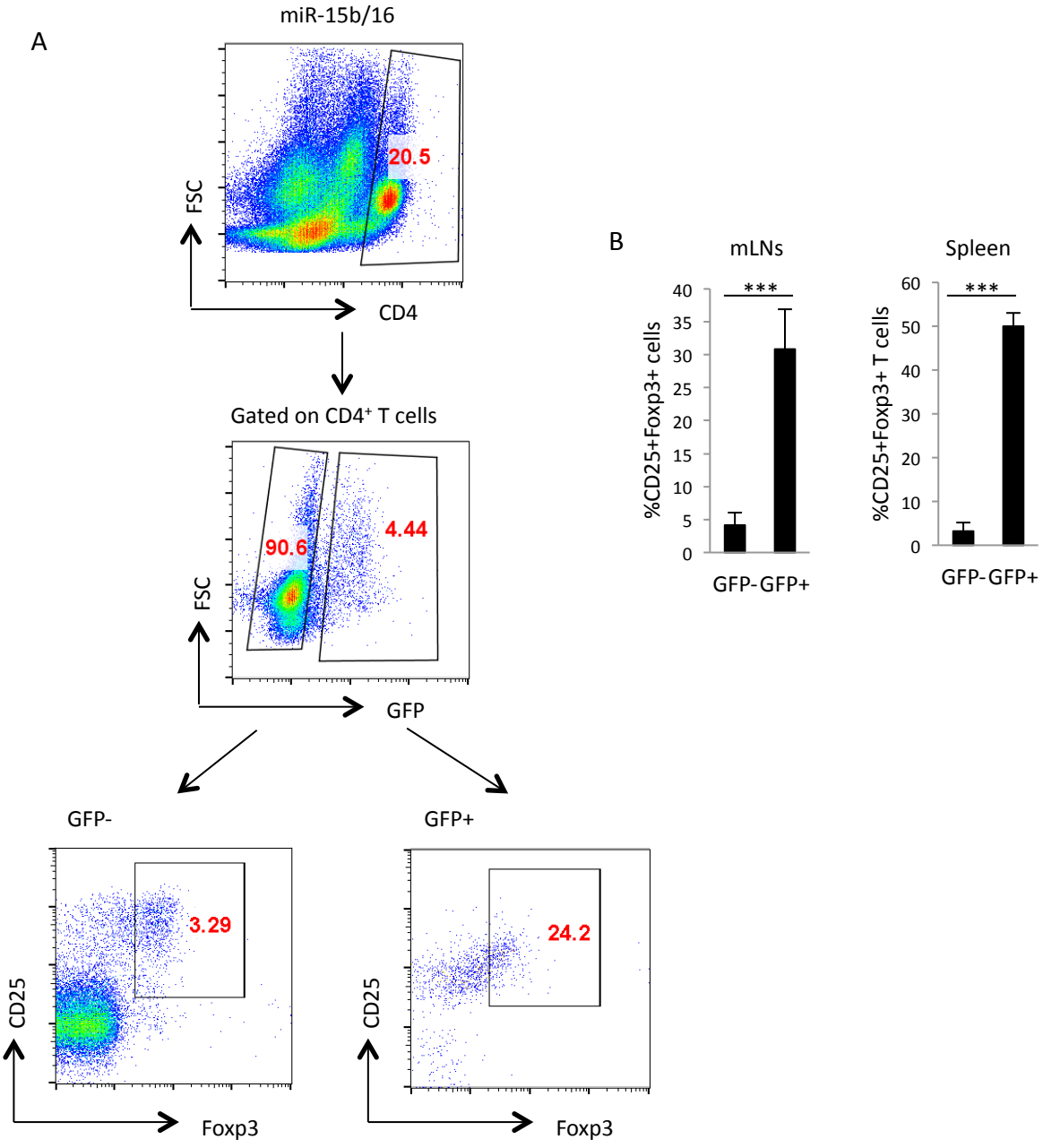
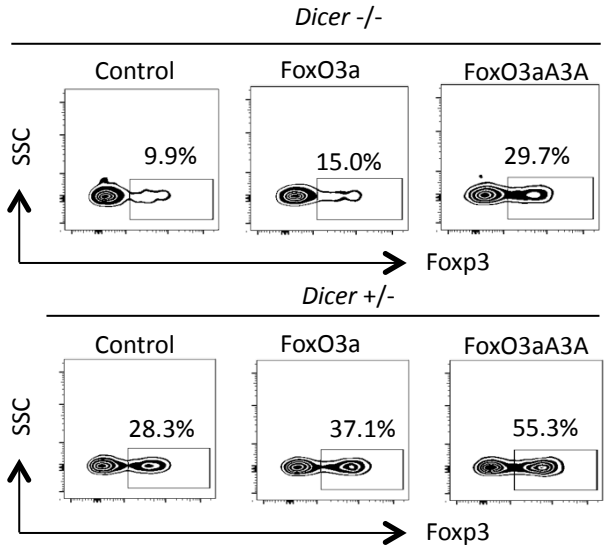


Supplemental Figure 1. Overexpression of miR15b/16 does not impact the growth of CD4⁺ T cells but affects stability of Foxp3 expression in cultured, *ex vivo*-derived Tregs. (A) Growth effect on CD4⁺ T cells. T cells were stained with 1mM Far-Red cell proliferation dye (Life Technologies) and cells were activated as described in Materials and Methods. After overnight activation, cells were transduced with control and miR-15b/16-expressing retroviruses, and three days later GFP⁺ cells were analyzed for proliferation by measuring dilution from cell division of the Far-Red signal in the APC (FL-4) channel. Overexpression of miR-15b/16 did not inhibit proliferation over this time course. (B) Effect on stability of Foxp3 expression. *Ex vivo* derived Tregs were transduced with control or miR-15b/16-expressing retroviruses and cultured in the presence of 40ng/ml IL-2, then analyzed for Foxp3 expression after three days.

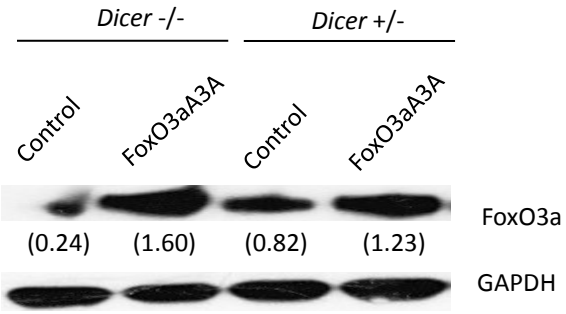


Supplemental Figure 2. Overexpression of miR-15b/16 has an intrinsic effect on the development of pTregs *in vivo*. CD4$^+$ T cells were transduced with a miR-15b/16 expressing retrovirus then unsorted cells (approximately 5% GFP$^+$) were adoptively transferred into *Rag2*$^{-/-}$ mice. After six weeks, mice were killed and cells were isolated from spleens and mesenteric lymph nodes and analyzed for Foxp3 expression in the CD4+ cells in both the GFP$^-$ and GFP$^+$ populations. (A) A representative experiment is displayed. (B) The mean and standard deviation values from four mice are displayed. Cells overexpressing miR15b/16 had significantly enhanced levels of pTregs in both mesenteric lymph nodes (*** p=0.004) and spleen (*** p=0.002), indicating that the overexpression of miR-15b/16 had an intrinsic effect on the development of pTregs.

A



B



Supplemental Figure 3. Overexpression of FoxO3a or a mutant protein containing Ala substitutions at all three AKT phosphorylation sites (A3A) enhanced the induction of iTregs in both *Dicer*$^{-/-}$ or *Dicer*$^{+/-}$ CD4^{+} T cells. (A) Shown is a representative of two independent experiments with similar results in each. (B) The expression levels of FoxO3a in one of the two overexpression experiments with the phosphorylation mutant (A3A). The relative level of FoxO3a compared to GAPDH is listed below each band. Similar results were obtained in each experiment, and they demonstrate that the relative level of FoxO3a does not determine the absolute level of iTreg induction so other signaling mechanisms must be affected in *Dicer*$^{-/-}$ CD4^{+} T cells.