

Figure S1: (A) $Arl4d^{+/+}$ or $Arl4d^{-/-}$ splenic $CD4^+$ T cells were differentiated into T_H1 , T_H2 , or T_H17 cells using a commercial kit. After differentiation, $CD4^+$ T cells were stimulated with PMA and ionomycin and stained intracellularly for IL-2. (B) Expression of various surface markers associated with T_{reg} suppressive activity in *in vitro*-induced $Foxp3^+$ $CD4^+$ T cells from $Arl4d^{+/+}$, $Arl4d^{-/-}$, $CD4-Cre^+xAr14d^{+/+}$, $CD4-Cre^+xAr14d^{\Delta/\Delta}$, $Foxp3-Cre^+xAr14d^{+/+}$, and $Foxp3-Cre^+xAr14d^{\Delta/\Delta}$ animals.

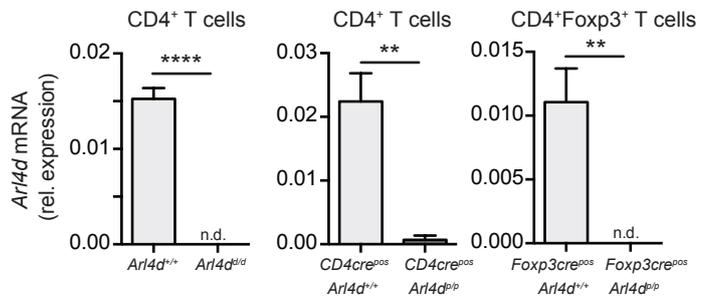


Figure S2: Successful knockdown of *Arl4d* mRNA in CD4-Cre x *Arl4d*^{fl/fl} and Foxp3-Cre x *Arl4d*^{fl/fl} mice. Splenic CD4⁺ T cells and Foxp3⁺ (YFP^{pos}) CD4⁺ T cells were isolated by magnetic beads and flow cytometric sorting, after which RNA was isolated for qPCR of *Arl4d* mRNA. As a control, splenic CD4⁺ T cells from global *Arl4d*-deficient mice were used. Statistical significance was determined by Student's *t* test, ** $p \leq 0.01$, **** $p \leq 0.0001$, n.d.= not detectable.