

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⁺*Arl4d*^{+/+}, CD4-Cre⁺*Arl4d*^{Δ/Δ}, Foxp3-Cre⁺*Arl4d*^{+/+}, and Foxp3-Cre⁺*Arl4d*^{Δ/Δ} animals.

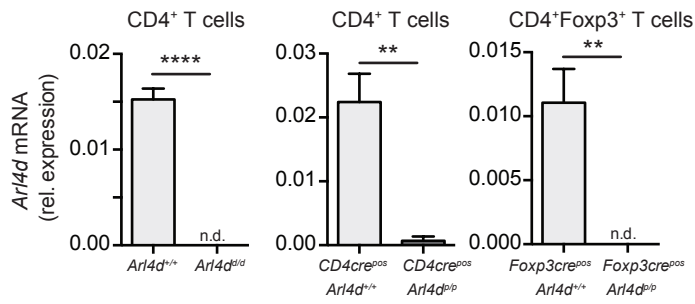


Figure S2: Successful knockdown of *Arl4d* mRNA in CD4-Cre x *Arl4d*^{fl/fl} and Foxp3-Cre x *Arl4d*^{fl/fl} mice. Splenic CD4⁺ T cells and Foxp3⁺ (YFP^{pos}) CD4⁺ T cells were isolated by magnetic beads and flow cytometric sorting, after which RNA was isolated for qPCR of *Arl4d* mRNA. As a control,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