Supporting Information

Pomié et al. 10.1073/pnas.1107136108

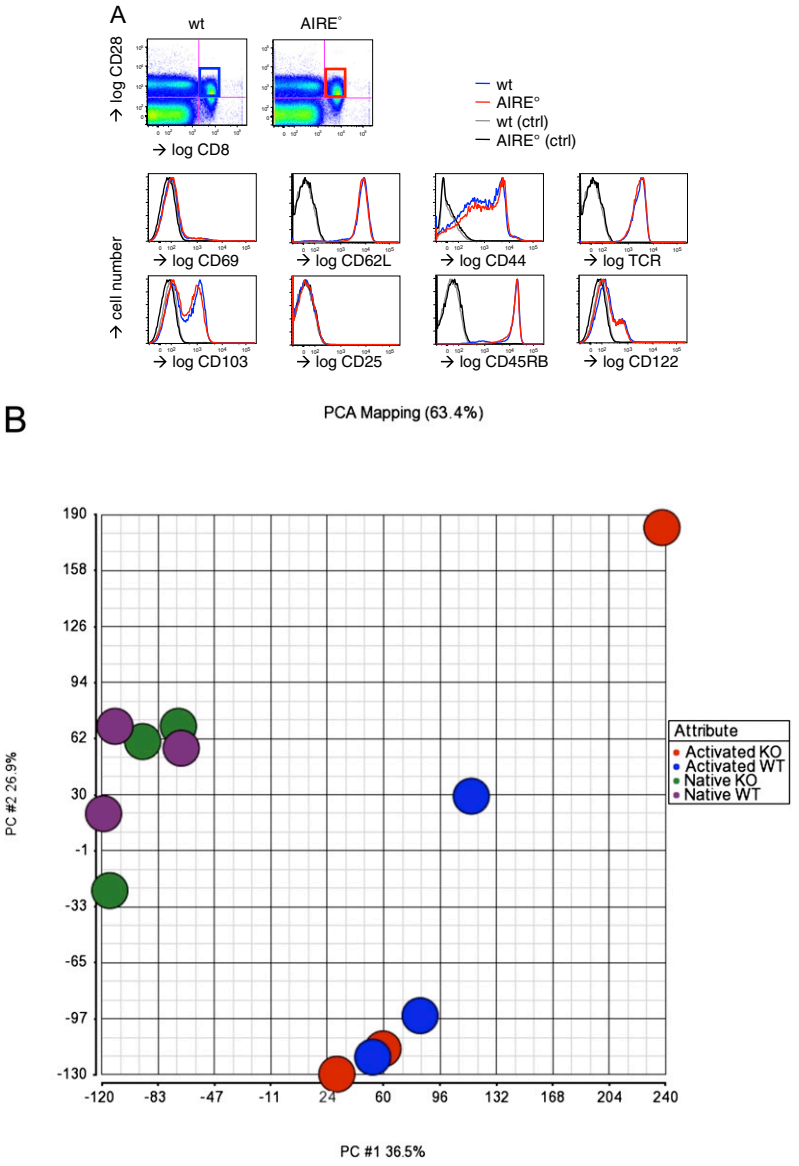

Fig. S1. (Continued)

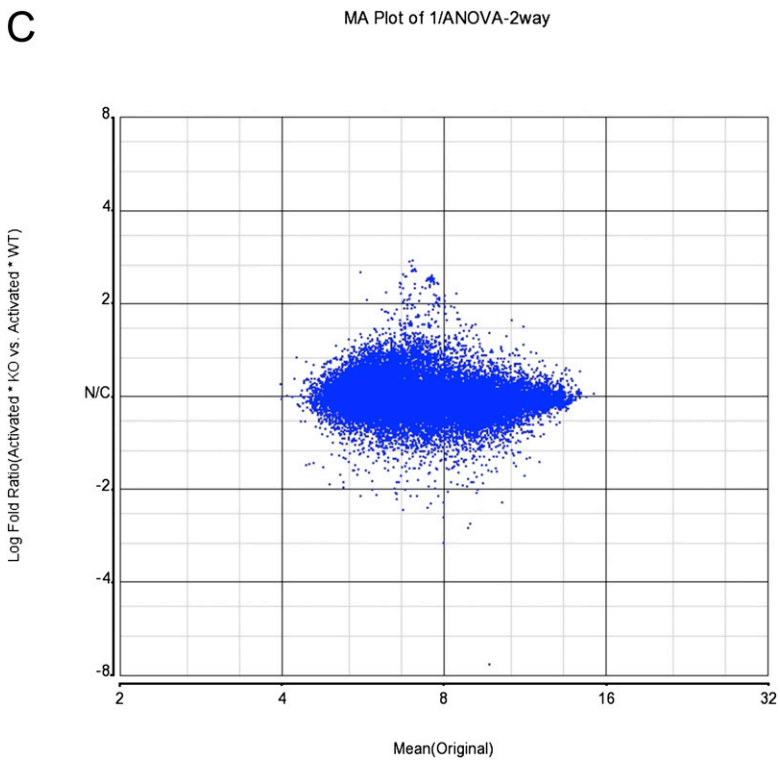

Fig. S1. (Continued)

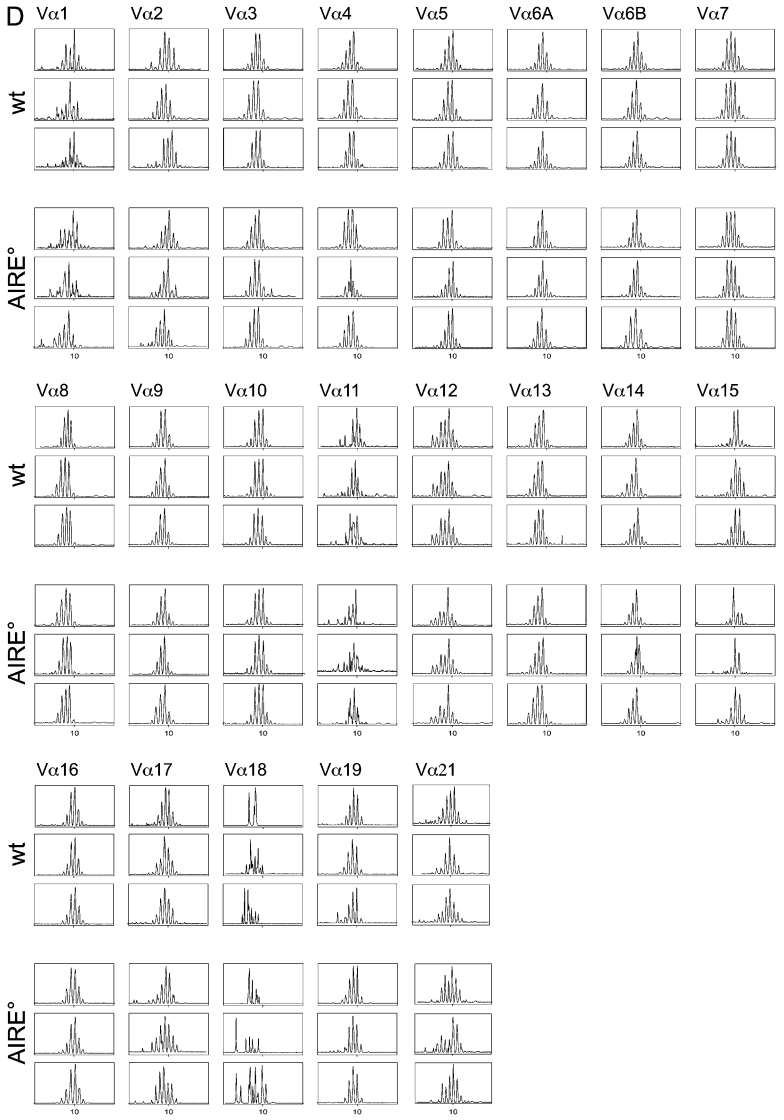

Fig. S1. (Continued)

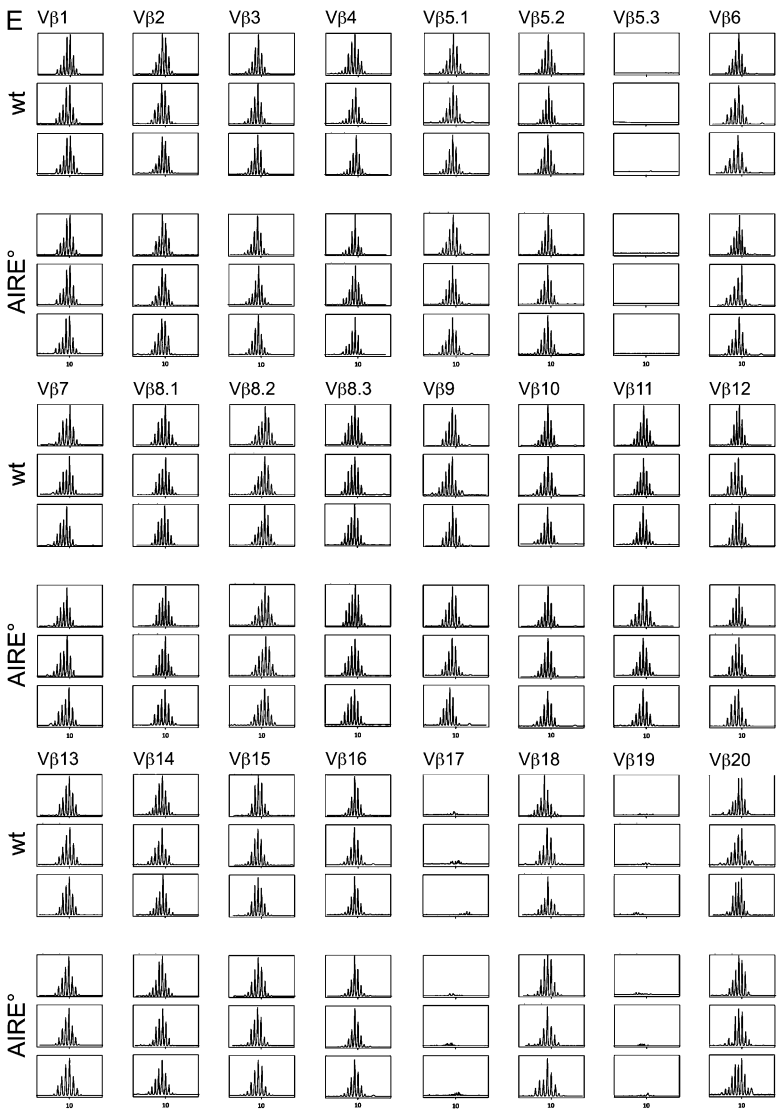

Fig. S1. $CD8^+CD28^+$ cells have identical phenotypes and $CD8^+CD28^{low}$ cells have indistinguishable transcriptional patterns in autoimmune regulator-deficient (AIRE°) versus WT mice. (*A*) AIRE° and WT $CD8^+CD28^+$ cells have identical phenotypes. Splenocytes from WT and AIRE° C57BL/6 mice were analyzed for expression of CD8 and CD28 by flow cytometry. $CD8^+CD28^+$ cells were electronically gated as shown (*Upper*) and expression profiles of indicated markers are depicted (*Lower*). Control (ctrl) stainings were performed using isotype-matched control antibodies. Similar results were obtained in three independent experiments. (*B*) AIRE° and WT $CD8^+CD28^{low}$ cells have indistinguishable transcriptional patterns. Principal components analysis plot of native and activated $CD8^+CD28^{low}$ regulatory T-lymphocyte populations (Treg) from WT and AIRE° mice. The first principal component (PC #1) is shown on the *x* axis and explains the maximum amount (36.5%) of variation in the gene expression data, in this case, activated versus freshly isolated cells. The second principal component (PC #2) is shown on the *y* axis and explains the second most amount of remaining variation in the gene expression data (26.9%), which is difficult to attribute to a known biological variable in this case, as there are no obvious groups of cells. (*C*) Multichip average plot for average gene expression in activated AIRE° versus activated WT $CD8^+CD28^{low}$ Treg. The log fold change for activated AIRE° versus activated WT cells is depicted on the *y* axis. These differences in average expression are not significant because adjusted *P* values for all values are approaching 1 (>0.99). (*D* and *E*) Immunoscope analysis of Vα (*D*) and Vβ (*E*) CDR3 reveals very similar patterns but suggests subtle differences between WT and AIRE° $CD8^+CD28^{low}$ Treg repertoires. CDR3 lengths of indicated Vα and Vβ segment containing T-cell receptors (TCRs) were analyzed as in Fig. 1*F*. The three superimposed plots represent three independently isolated Treg samples, each consisting of pooled cells from three mice. Note that the Vα12 patterns of WT versus AIRE° $CD8^+CD28^{low}$ Treg are reproducibly different.

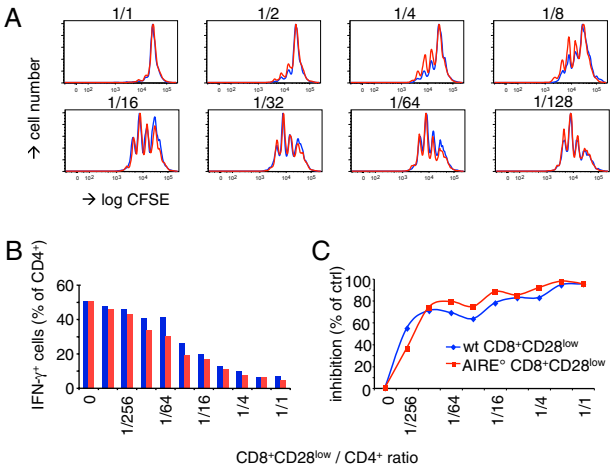

Fig. S2. In vitro prestimulated AIRE° and WT $CD8^+CD28^{low}$ cells have similarly increased in vitro regulatory capacity. (*A*) WT and AIRE° C57BL/6 $CD8^+CD28^{low}$ Treg were isolated as described in *Materials and Methods* and preactivated in vitro with DBA/2 APC. Isolated responder C57BL/6 $CD4^+$ T cells were 5(6)-carboxyfluorescein diacetate *N*-succinimidyl ester (CFSE)-labeled and cocultured with DBA/2 APC coated with an anti-CD3ε antibody in the presence of preactivated AIRE° (red lines) or WT (blue lines) $CD8^+CD28^{low}$ Treg at indicated regulatory:responder T-cell ratios. Proliferation of responder cells was assessed by FACS analysis of CFSE dilution on electronically gated $CD4^+$ cells. (*B*) As in *A*, but non-CFSE-labeled responder $CD4^+$ T cells were analyzed, by flow cytometry, for IFN-γ production. Shown results are representative of those obtained in three independent experiments. (*C*) Constant numbers of isolated C57BL/6 $CD4^+$ T cells were cultured with DBA/2 APC in the presence of titrated numbers of C57BL/6 AIRE° (red lines) or WT (blue lines) $CD8^+CD28^{low}$ Treg preactivated with DBA/2 APC. Proliferation in these mixed lymphocyte reactions was assessed by measuring incorporation of $[^3H]$thymidine. All results are representative of three independent experiments.

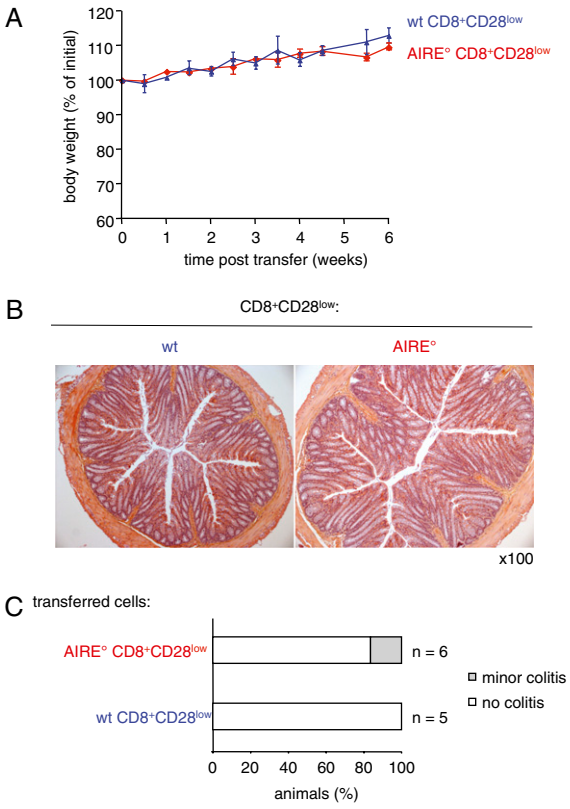

Fig. S3. AIRE° CD8$^+$CD28^{low} T cells are not colitogenic. RAG-2° hosts were i.v. injected with indicated syngeneic CD8$^+$CD28^{low} T cells. (*A*) Evolution of weight of animals. Shown is the mean weight of animals ± SD, depicted as percentage of weight at the start of the experiment (n = 5 for WT and n = 6 for AIRE° cells; two independent experiments). (*B*) Mice were euthanized 6 wk after injection of T cells. Microscopic sections of distal colon were stained with hematoxylin and eosin and examined for signs of colitis. Shown results are representative of those obtained in two independently performed experiments. (*C*) Colons of mice were examined as in *B* and clinical scores of colitis were attributed as described in *Materials and Methods* (n values as indicated, from two independent experiments).

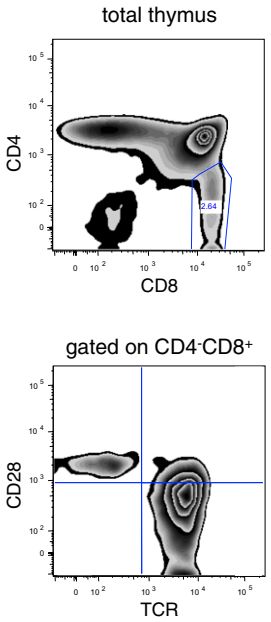

Fig. S4. Mature CD4$^-$CD8$^+$CD28^{low} cells are found in the thymus. WT C57BL/6 thymocytes were analyzed by flow cytometry for expression of CD4, CD8, TCRβ, and CD28. Mature CD4$^-$CD8$^+$ single positive cells were electronically gated using the indicated gate (*Upper*) and expression of TCR versus CD28 is depicted (*Lower*).