Supporting Information

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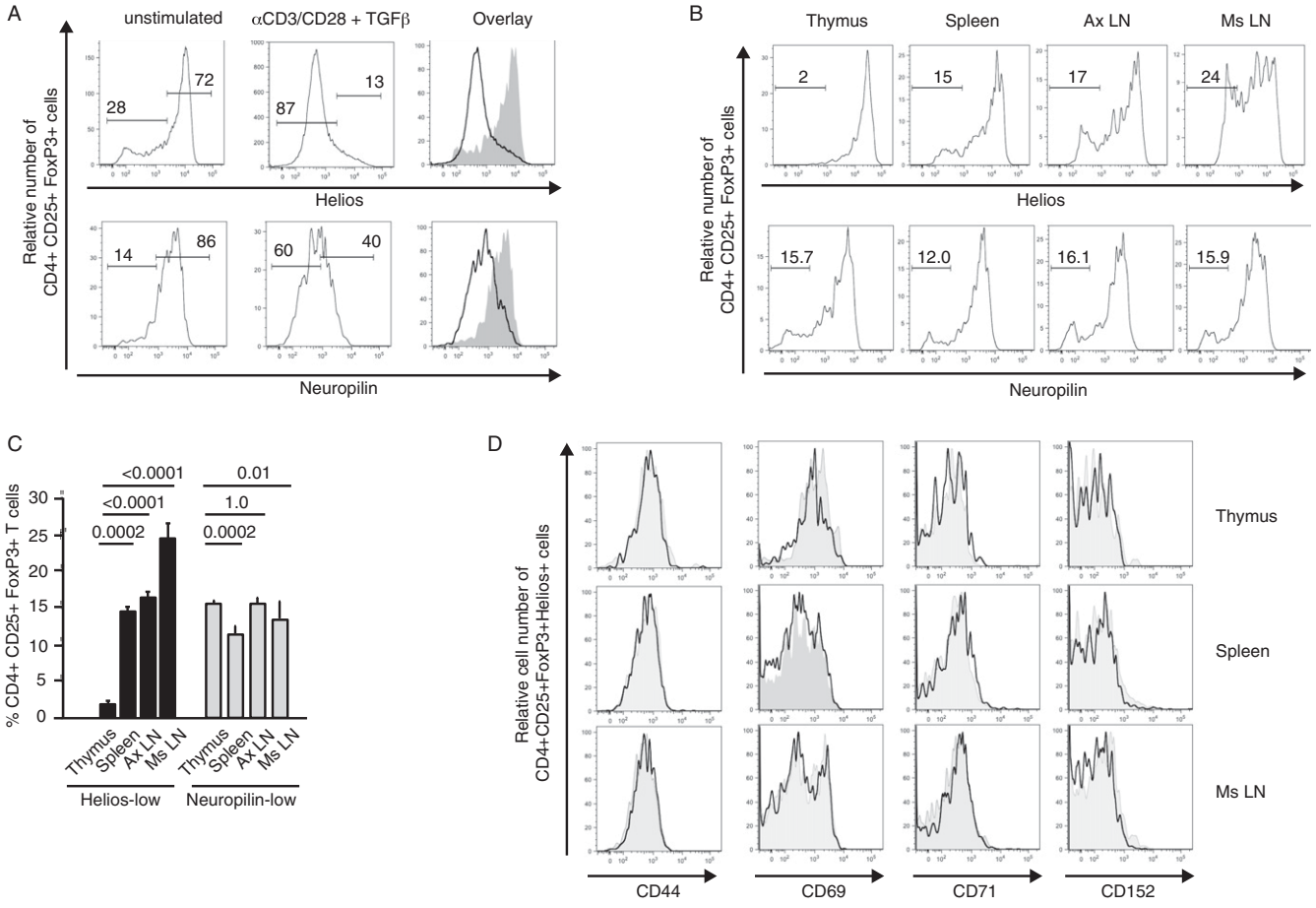

Fig. S1. Flow cytometric analysis of antibodies used to distinguish thymus-derived regulatory T cell (tTreg) vs. induced/peripheral Treg (i/pTreg) populations. (*A*) Intracellular expression of Helios (*Upper*) vs. surface expression of Neuropilin (*Lower*) after gating on $CD4^+CD25^+FoxP3^+$ splenic T cells. Representative overlay histograms are included (ex vivo generated iTregs = open histogram, splenic Tregs = gray histogram). All cells were derived from C57BL/6 mice. $n = 2$ experiments. (*B*) Intracellular staining for Helios expression (*Upper*) vs. surface expression of Neuropilin (*Lower*) on $CD4^+CD25^+FoxP3^+$ T cells under steady-state conditions. T cells were harvested from the thymus, spleen, mesenteric lymph nodes (Ms LN), and axillary lymph nodes (Ax LN) of C57BL/6 mice. The frequency of Tregs that expressed low levels of the respective markers is shown. $n = 4$ experiments. (*C*) Graph displaying the percentage of Tregs ($CD4^+CD25^+FoxP3^+$) that expressed low levels of Helios (black bars) vs. Neuropilin (gray bars). Data from four mice were averaged; error bars (SD) and *P* values comparing thymic and secondary lymphoid organs are included. $n = 4$ experiments. (*D*) Expression of activation markers on $Helios^{high}$ Tregs isolated from various tissues under homeostatic conditions. Select surface receptors that are typically up-regulated after T-cell activation were examined on $CD4^+CD25^+FoxP3^+Helios^{high}$ T cells freshly harvested from *Lck-cre; Klf2*$^{fl/fl}$ (black line) and littermate control (solid gray) mice and displayed as a histogram overlay. $n = 3$ mice per cohort.

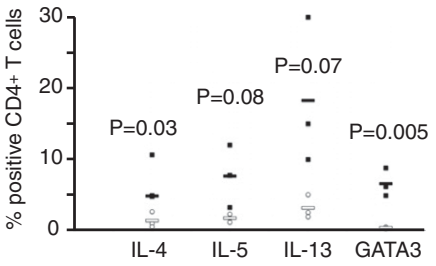

Fig. S2. Frequency of CD4$^+$ T cells expressing T_H2-associated cytokines and GATA3 transcription factor. Lymphocytes harvested from the mesenteric lymph nodes of wild-type (open circles) vs. *Lck-cre; Klf2$^{fl/fl}$* (black squares) mice were stimulated with phorbol 12-myristate 13-acetate (PMA) plus ionomycin for 6 h, then examined for intracellular expression of outlined factors. Averaged values (dashes) and *P* values are displayed. $n = 3$ mice per cohort.

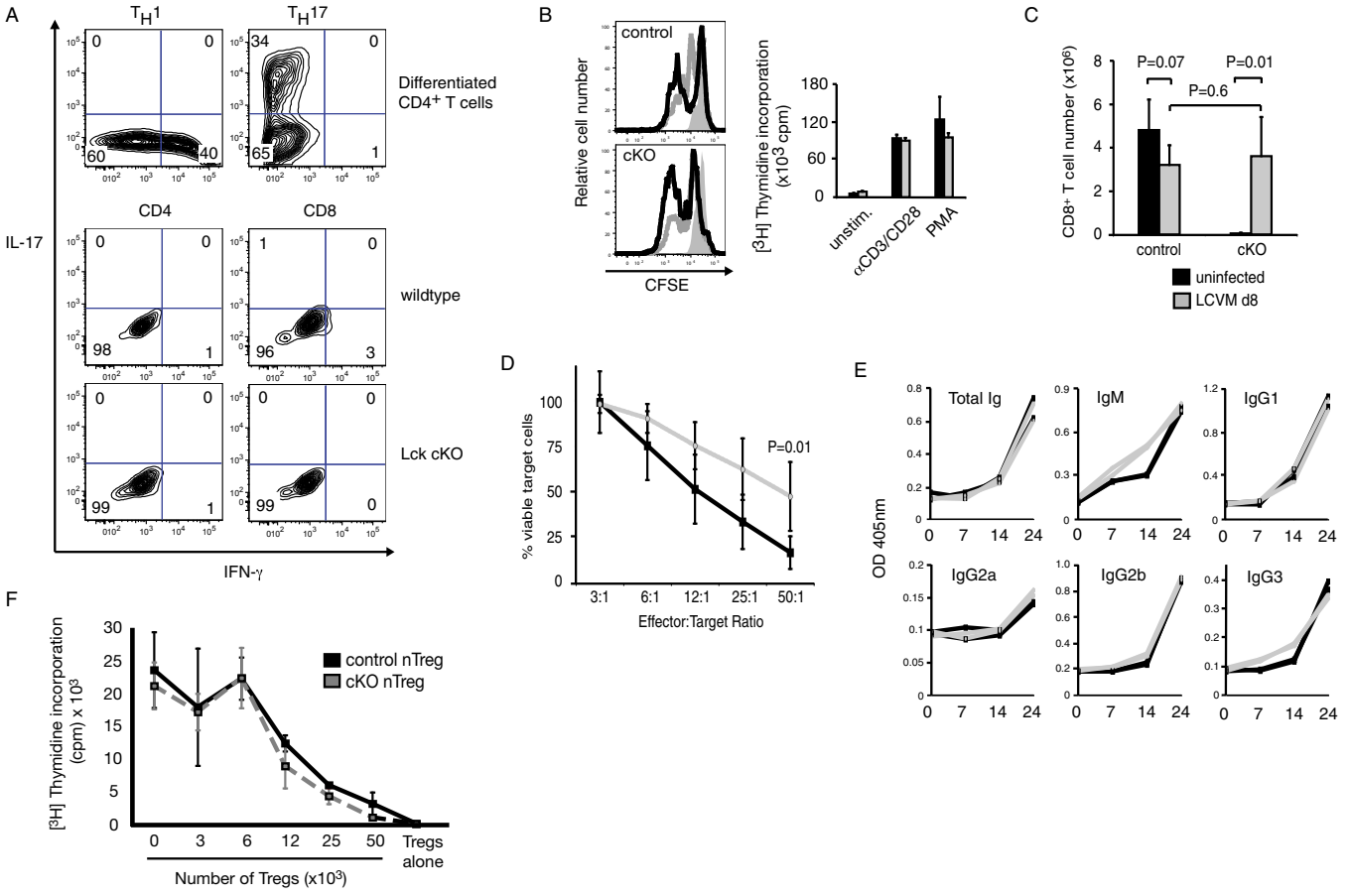

Fig. S3. *Lck-cre; Klf2$^{fl/fl}$* mice have a functional T-cell effector compartment. (*A*) T cells from wild-type vs. *Lck-cre; Klf2$^{fl/fl}$* (Lck cKO) mice were stimulated with PMA plus ionomycin to determine whether these cells had differentiated into pathogenic T_H1 (IFN-γ^+) or T_H17 (IL-17$^+$) effector cells in vivo. Ex vivo differentiated CD4$^+$ T cells (*Upper*) were included for staining purposes. $n = 3$ experiments. (*B*) Klf2-deficient T-cell proliferation as a function of carboxyfluorescein succinimidyl ester (CFSE) dilution (*Left*) and thymidine incorporation (*Right*). Splenic T cells from *Klf2$^{fl/fl}$* (control) and *Lck-cre; Klf2$^{fl/fl}$* (cKO) littermates were stimulated, and proliferation was analyzed by flow cytometry. Histogram overlay: solid gray, untreated; gray line, anti-CD3ε (0.5 μg/mL); black line, anti-CD3ε (5 μg/mL). $n = 3$ experiments. Alternatively, CD4$^+$ T cells from *Klf2$^{fl/fl}$* (black bars) and *Lck-cre; Klf2$^{fl/fl}$* (gray bars) littermates were stimulated as indicated, and [^{3}H]-thymidine incorporation was used to measure proliferation. Error bars indicate SD. $P > 0.1$ for all events. $n = 2$ experiments. (*C*) Quantification of splenic CD8$^+$ T cells in *Klf2$^{fl/fl}$* (control) vs. *Lck-cre; Klf2$^{fl/fl}$* (cKO) mice. T-cell numbers were averaged from cohorts of uninfected mice (black bars) or day-8 lymphocytic choriomeningitis virus (LCMV)-infected mice (gray bars). $n = 7$ mice per group, error bars are SD. (*D*) Cytolytic efficiency of CD8$^+$ T cells harvested from *Klf2$^{fl/fl}$* (solid black line) vs. *Lck-cre; Klf2$^{fl/fl}$* (dashed gray line) littermates. CD8$^+$ T cells from LCMV-primed mice were cocultured with RMA-S target cells that were precoated with an LCMV agonist peptide. Target cell lysis (release of intracellular and membrane-associated dyes) was measured by flow cytometry. Percent viable target cells = (% experimental CFSE$^+$PHK26$^+$ RMA-S cells/% CFSE$^+$PHK26$^+$ RMA-S cells cocultured with naïve CD8$^+$ T cells) × 100. Error bars show SD; *P* values >0.05 except as indicated. $n = 7$ mice per infected group. This experiment was performed once. (*E*) T-dependent Ig class-switching. Trinitrophenyl (TNP)-specific Ig concentrations in the sera of a *Klf2$^{fl/fl}$* mouse (black lines) vs. an *Lck-cre; Klf2$^{fl/fl}$* littermate (gray lines) after challenge with TNP-OVA. Mice were rechallenged with TNP-OVA at day 14. $n = 2$ experiments. (*F*) Suppression of Klf2$^+$ T-cell proliferation using CD4$^+$CD25$^+$ Tregs from *Klf2$^{fl/fl}$* (solid black line) vs. *Lck-cre; Klf2$^{fl/fl}$* (dashed gray line) mice. Averaged [^{3}H]-thymidine incorporation ± SD is shown. No significant variation ($P > 0.05$) between groups was detected. $n = 2$ experiments.

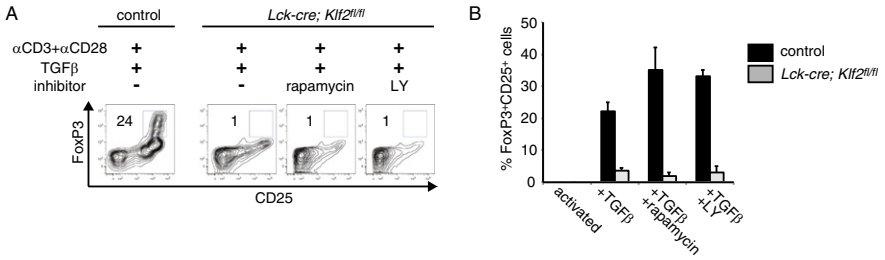

Fig. S4. Ex vivo generation of iTregs using $CD4^+CD25^-$ T cells harvested from KLF2-sufficient (control) or KLF2-deficient (*Lck-cre; Klf2*$^{fl/fl}$) mice. (*A*) Representative contour plots of live-gated, $CD4^+CD25^-$ T cells cultured under the specified conditions for 72 h. Frequency of $FoxP3^+CD25^+$ T cells is indicated. LY, LY294002. (*B*) Percent iTregs generated after 72 h. culture with activating media (αCD3+αCD28) alone or with additional reagents. These results are averaged from two independent experiments ($n = 1$ per condition); variance is indicated.

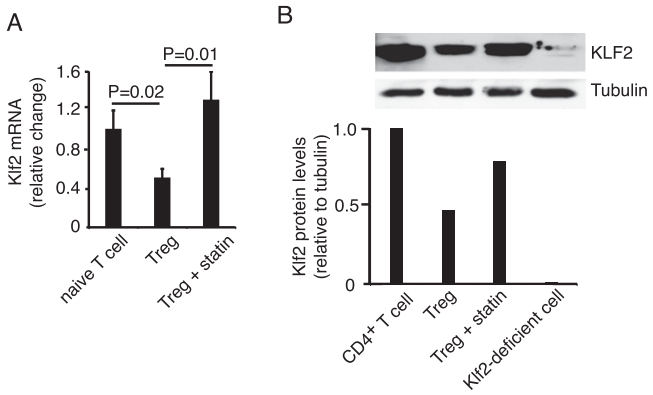

Fig. S5. Simvastatin elevates transcriptional and posttranslational levels of KLF2. (*A*) Relative *Klf2* mRNA expression in naive $CD4^+CD25^-$ T cells, $CD4^+CD25^+$ $FoxP3^+$ Tregs, or $CD4^+CD25^+FoxP3^+$ Tregs cultured with simvastatin for 24 h. Error bars (SD) and *P* values are shown. $n = 2$ experiments. (*B*) Immunoblot of KLF2/tubulin and corresponding densitometry plot using total cell lysate from $CD4^+CD25^-$ T cells, $CD4^+CD25^+FoxP3^+$ Tregs with or without simvastatin (24 h) or B cells from a *CD19cre; Klf2*$^{fl/fl}$ mouse (negative control for KLF2). $n = 3$ experiments.

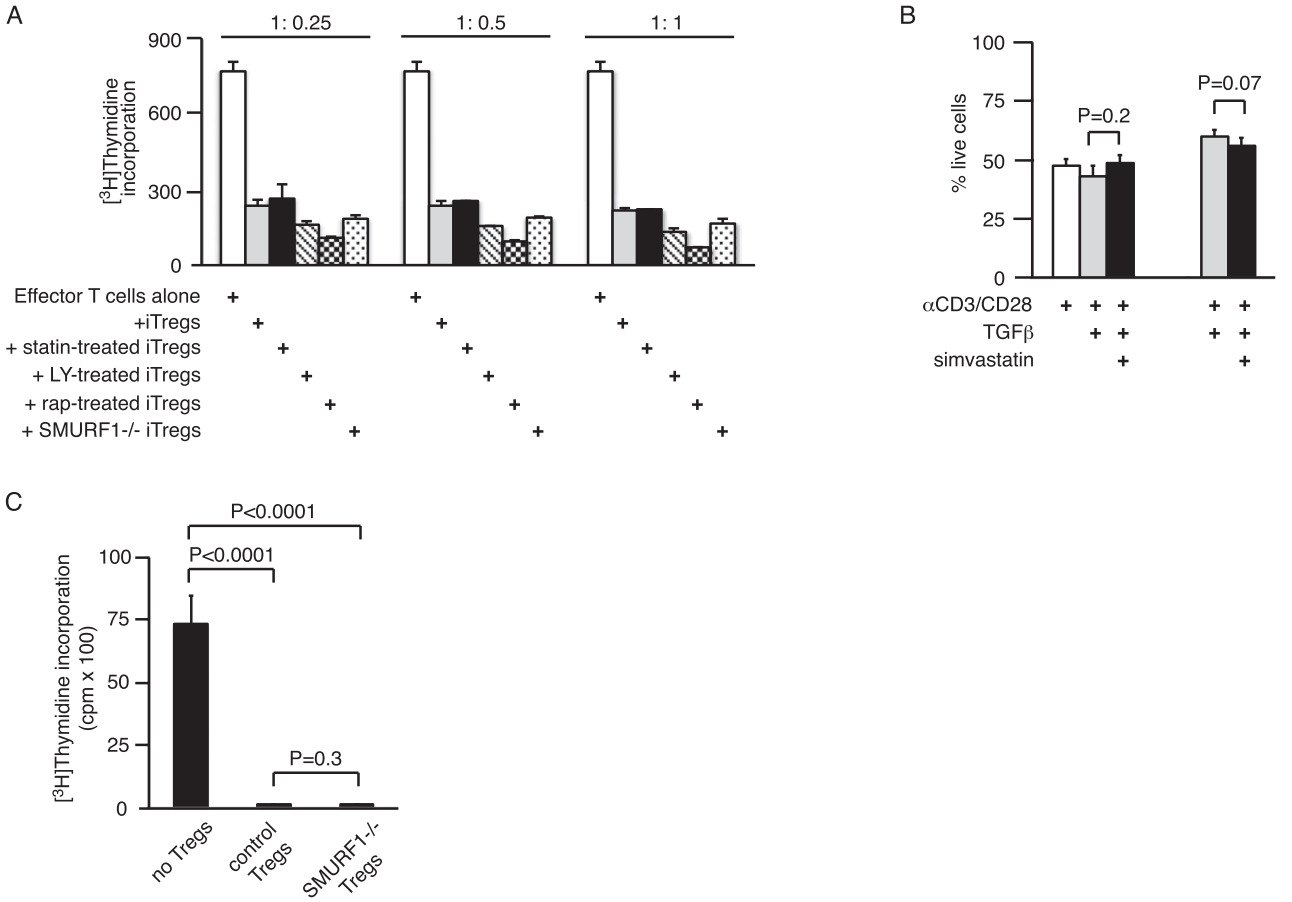

Fig. S6. Ectopic expression of KLF2 does not impair Treg function or survival. (*A*) Enhanced expression of KLF2 during the inductive stage of iTreg production does not negatively affect suppressor functions. Ex vivo suppressor assays were conducted using iTregs generated in the presence of αCD3+αCD28+TGFβ with or without simvastatin, rapamycin, or LY294002. Each condition was done in duplicate (mean and variance are shown) using three different ratios of effector T cells: iTregs. This experiment was performed twice. (*B*) Elevated KLF2 expression during iTreg production does not impact ex vivo cell survival. iTregs generated with or without simvastatin were cultured in standard media containing IL-2, and cell survival (cell membrane-intact, 7AAD-) was assessed 72 h later by flow cytometry. Initial input $CD4^+CD25^-$ T cells were harvested from WT (left columns) or $SMURF1^{-/-}$ (right columns) animals. This experiment was performed once in quadruplicate. Error bars (SD) and *P* values (Student *t* test) are shown. (*C*) Increased KLF2 expression does not impair SMURF1-deficient Treg suppressor activity. Ex vivo suppressor assays were conducted using splenic Tregs harvested from $SMURF1^+$ vs. $SMURF1^{-/-}$ animals. Error bars (SD) and *P* values (Student *t* test) are shown. $n = 2$ experiments performed in triplicate.