Science Signaling
AAAS

www.sciencesignaling.org/cgi/content/full/6/303/ra101/DC1

Supplementary Materials for

Diacylglycerol Kinase ζ Limits the Generation of Natural Regulatory T Cells

Amanda M. Schmidt, Tao Zou, Rohan P. Joshi, Theresa M. Leichner, Matthew A. Pimentel, Connie L. Sommers, Taku Kambayashi*

*Corresponding author. E-mail: taku.kambayashi@uphs.upenn.edu

Published 26 November 2013, *Sci. Signal.* **6**, ra101 (2013)
DOI: 10.1126/scisignal.2004411

The PDF file includes:

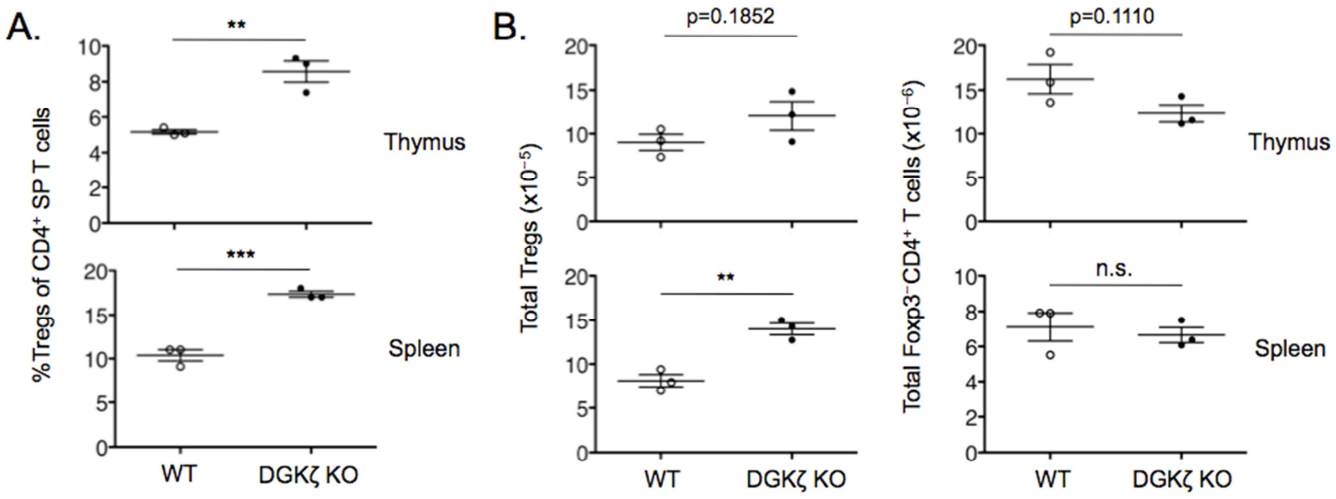

Fig. S1. DGKζ$^{-/-}$ mice exhibit increased percentages and numbers of thymic and peripheral T_{reg} cells at 3 weeks of age. (**A** and **B**) The thymi (top) and spleens (bottom) of 3-week old WT (open circles) and DGKζ$^{-/-}$ (closed circles) mice were analyzed by flow cytometry to detect Foxp3 and CD25. (A) Scatter plots show the means ± SEM of the percentages of T_{reg} cells within the population of live CD4 SP T cells. (B) Absolute numbers of T_{reg} cells (left) and Foxp3^{-}CD4^{+} T cells (right). Shown are data for all mice compiled from one experiment, with each circle representative of a single mouse. $^{**}P < 0.01$, $^{***}P < 0.001$, by unpaired two-tailed student's t-test. n.s., not significant.

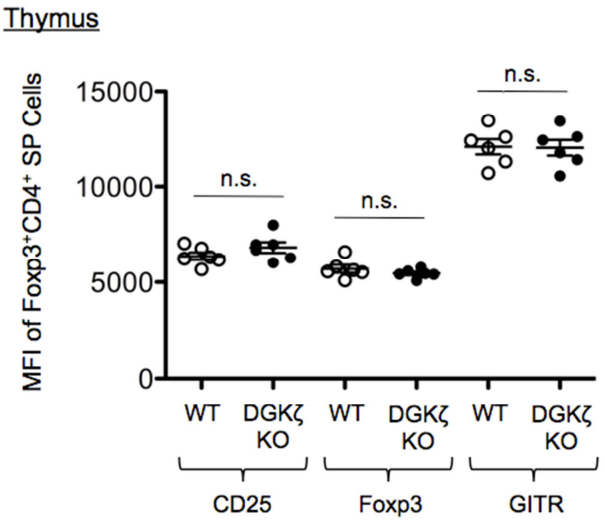

Fig. S2. DGKζ$^{-/-}$ and wild-type T$_{reg}$ cells are phenotypically similar. Thymic T$_{reg}$ cells from the indicated mice were assessed for the abundances of CD25, GITR, and Foxp3 protein by flow cytometry. A scatter plot shows the means ± SEM of the mean fluorescence intensity (MFI) of each protein in WT (open circles) and DGKζ$^{-/-}$ (closed circles) thymocytes, previously gated on live, Foxp3^{+} CD4 SP cells. Data are depicted for all mice compiled from two experiments, with each circle representative of a single mouse. n.s., not significant by unpaired two-tailed student's t-test.

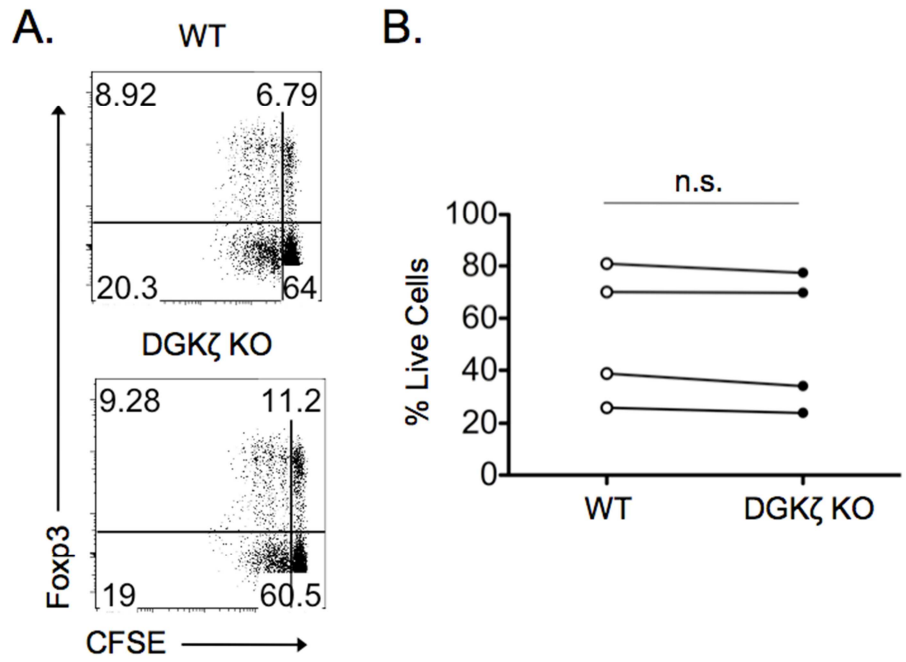

Fig. S3. Immature CD4 SP thymocytes from wild-type and DGKζ$^{-/-}$ mice exhibit similar cell division and death during 72 hours of culture. (**A** and **B**) Immature CD4 SP thymocytes (Foxp3^{-}CD25^{-}CD44^{lo}CD69^{hi}) sorted from the thymi of WT or DGKζ$^{-/-}$ Foxp3.GFP reporter mice were labeled with CFSE and cultured in the presence of IL-2 and anti-CD3 antibody. (A) Flow cytometric profiles of WT (top) and DGKζ$^{-/-}$ (bottom) thymocytes previously gated on CD4^{+} live singlets show dilution of CFSE and production of Foxp3 after 72 hours of culture. Data are from a single experiment and are representative of three independent experiments. (B) Scatter plots show the means ± SEM of the percentages of live cells observed by flow cytometry for cultured WT and DGKζ$^{-/-}$ thymocytes from four independent experiments. n.s., not significant by paired two-tailed student's t-test.

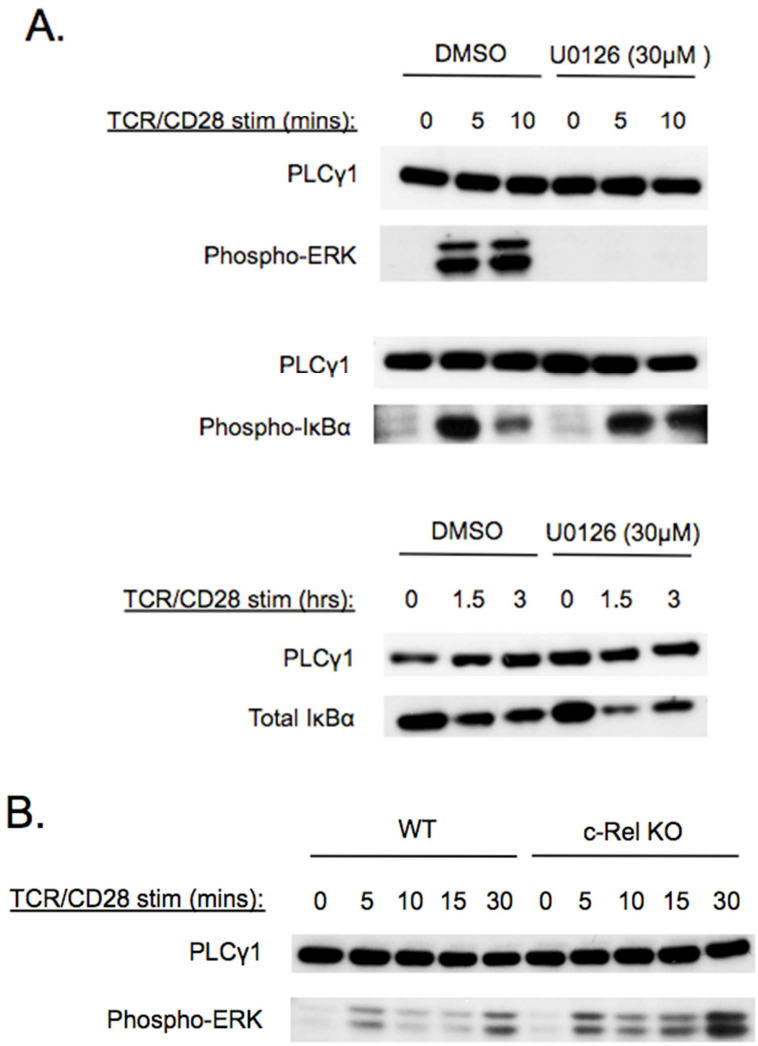

Fig. S4. ERK phosphorylation and NF-κB activation are not interdependent in DGKζ$^{-/-}$ mice. (**A**) FACS-sorted splenic Foxp3^{-}CD44^{lo}CD4^{+} DGKζ$^{-/-}$ T cells were pretreated with or without 30 μM U0126 for 1 hour and then were stimulated with through the TCR and CD28 for the indicated times. Cell lysates were analyzed by Western blotting to detect pERK (top) and pIκBα (bottom). Total PLC-γ1 protein served as a loading control. (**B**) MACS-sorted splenic CD4^{+} T cells from WT or c-Rel$^{-/-}$ mice were stimulated through the TCR and CD28 for the indicated times. Cell lysates were analyzed by Western blotting to detect pERK. Total PLC-γ1 served as a loading control. Western blots are representative of two independent experiments.