Supporting Information

Di et al. 10.1073/pnas.0910133107

SI Materials and Methods

Histological Score of Colitis. Grade 0 indicates no changes; grade 1 indicates minimal scattered mucosal inflammatory cell infiltrates, with or without minimal epithelial hyperplasia; grade 2 indicates mild scattered to diffuse inflammatory cell infiltrates, sometimes extending into the submucosa and associated with erosions, with minimal to mild epithelial hyperplasia and minimal to mild mucin depletion from goblet cells; grade 3 indicates mild to moderate inflammatory cell infiltrates that were sometimes transmural, often associated with ulceration, with moderate epithelial hyperplasia and mucin depletion; grade 4 indicates marked inflammatory cell infiltrates that were often transmural and associated with ulceration, with marked epithelial hyperplasia and mucin depletion; and grade 5 indicates marked transmural inflammation with severe ulceration and loss of intestinal glands.

Colitis scores between samples were compared using unpaired one-tailed Student *t* tests and plotted using the *stripchart* function implemented in the R Statistical Package (http://www.r-project.org).

Intracellular Ca^{2+} Activity. Intracellular Ca^{2+} concentrations were determined as previously reported (1). Briefly, cells were loaded at 1×10^6 cells/mL with 5 μM Fura-2 AM ester (Molecular Probes) in RPMI medium for 30 min at room temperature, washed, and then resuspended in RPMI medium. Cells were attached to poly(L)lysine-coated coverslips for 20 min in an RC-20 bath flow chamber (Warner Instrument) and fura-2 fluorescence was recorded (Delta Ram; PTI) at excitation wavelengths of 340 and 380 nm. Cells were then perfused with the bath solution in the presence or absence of 2 mM extracellular Ca^{2+} and stimulated with 5 μg/mL of anti-CD3 antibody and then cross-linked with 5 μg/mL of rat antimouse IgG (BD Pharmingen). Background fluorescence was obtained by treating the cells with 100 mM $MnCl_2$ at the end of the experiment. Data are presented as the 340/380 ratio after background subtraction.

Purification of Th17 Cells. IL-23 receptor GFP reporter mice (kindly provided by V. K. Kuchroo and M. Oukka, Harvard Medical School) were immunized with 100 μg of MOG_{35-55} peptide immulsified in CFA. Day 8 after immunization, LN cells were isolated and cultured in vitro with 20 μg of MOG_{35-55} and 25 ng/ml rIL23. Seven days after in vitro stimulation, >90% of the GFP positive cells were Th17 cells (1).

1. Awasthi A, et al. (2009) Cutting edge: IL-23 receptor gfp reporter mice reveal distinct populations of IL-17-producing cells. *J Immunol* 182:5904–5908.
2. Srivastava S, et al. (2006) Histidine phosphorylation of the potassium channel KCa3.1 by nucleoside diphosphate kinase B is required for activation of KCa3.1 and CD4 T cells. *Mol Cell* 24:665–675.

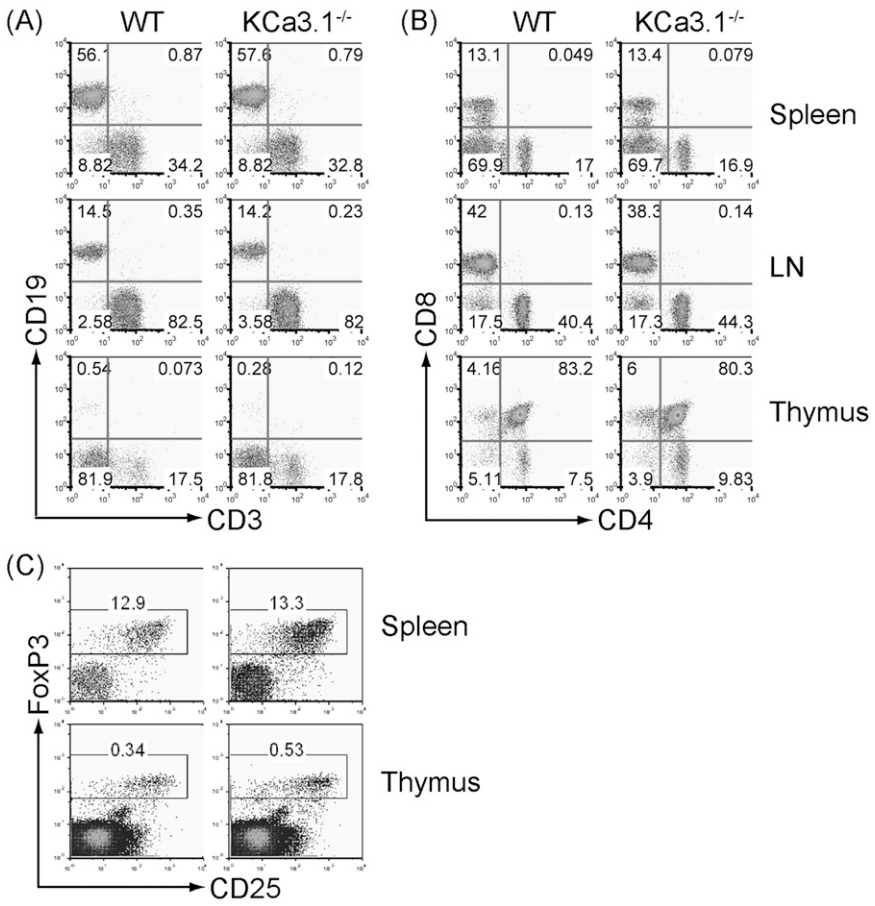

Fig. S1. T cell development is normal in *KCa3.1*$^{-/-}$ mice. Cells were isolated from spleen, thymus, and lymph nodes (*LN*) from *WT* and *KCa3.1*$^{-/-}$ mice, stained with antibodies to CD3 and CD19 (*A*), CD4 and CD8 (*B*), or CD4, CD25, and FoxP3 (*C*) followed by FACS analysis.

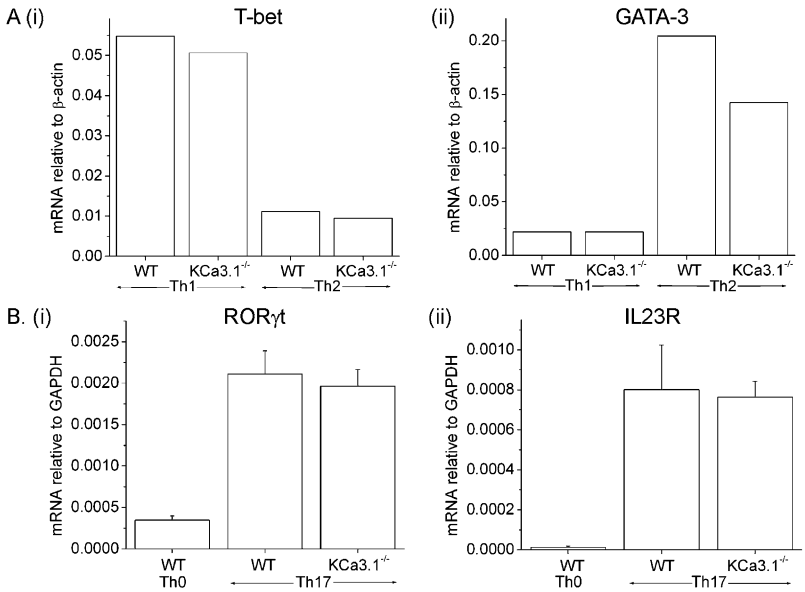

Fig. S2. Naïve CD4 T cells from *WT* and *KCa3.1*$^{-/-}$ mice were differentiated into Th1, Th2, and Th17 cells as described in *Materials and Methods*, and RT-PCR was performed. (*A*) Expression of T-bet (*i*) and GATA-3 (*ii*) mRNA expression was similar between WT and KCa3.1$^{-/-}$ cells, indicating KCa3.1 is not important for Th1 or Th2 differentiation. (*B*) In addition, RORγt (*i*) and IL23R (*ii*) was similar between WT and KCa3.1$^{-/-}$ Th17 differentiated cells, indicating that KCa3.1 is not required for Th17 cell differentiation.

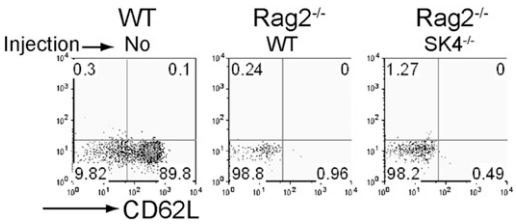

Fig. S3. Expression of CD62L was assessed by FACS analysis on WT CD4 spleen cells or cells isolated from spleen in Fig. 4*D(ii)* in the main text.