Online Supplemental Material for

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Supplemental Material and Methods

Mice. The CD2EP-*Pax5* transgenic construct was generated by inserting a full-length *Pax5* cDNA into EcoRI and SalI sites of the vector p29Δ2(Sal$^-$), which contains ~5 kb of the human CD2 promoter sequence, ~5.5 kb of 3′ human CD2 flanking sequences, the 3′ untranslated region and a polyadenylation signal of the hCD2 gene (Greaves, D.R., F.D. Wilson, G. Lang, and D. Kioussis. 1989. *Cell.* 56:979–986.). The genotype of the transgenic mice was determined by PCR analysis with the following primers: CD2 promoter 5′ (TGTAAAAGATGTAAAGAGAGGC) and Pax5 3′ (GATCCTGTTGATGGAGCTGACG). Igβ$^{-/-}$ mice were a gift from Dr. M. Nussenzweig (The Rockefeller University, New York, NY). All mice were bred and maintained under specific pathogen-free conditions.

Western Blot Analysis. Whole cell extracts were prepared from unfractionated bone marrow cells, splenocytes, and thymoctyes by boiling in 2× SDS gel-loading buffer (100 mM Tris-Cl, pH 6.8, 200 mM DTT, 4% SDS, 20% glycerol, and 0.2% bromophenol blue), separated by electrophoresis in 10% polyacrylamide–SDS gels, and transferred to polyvinylidene disulfluoride membrane (Millipore Corporation). Antibody against Pax5 (N-19; Santa Cruz Biotechnology, Inc.) was used in the immunoblotting. Horseradish peroxidase–anti–rabbit Ig (Amersham Biosciences) was used as secondary antibody.

RT-PCR. Total RNA was isolated from sorted DN thymoctyes using TriZol reagent (Invitrogen). Random hexamer-primed cDNA synthesis was performed with Omniscript reverse transcriptase (QIAGEN) according to manufacturer's specification. Quantitative real-time RT-PCR was done with an ABI Prism 5700 thermal cycler (PerkinElmer) according to the manufacturer's instructions. For real-time quantification of transcripts, each target was amplified in triplicate on the same plate with the reference, HPRT. Data are represented as transcript abundance relative to HPRT. PCR conditions were described previously (Hsu, L.Y., J. Lauring, H.E. Liang, S. Greenbaum, D. Cado, Y. Zhuang, and M.S. Schlissel. 2003. *Immunity.* 19:105–117.). All primers used in RT-PCR analysis are available upon request.

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