Supplemental Data

Developmental and Molecular Characterization

of Emerging β- and γδ-Selected Pre-T Cells

in the Adult Mouse Thymus

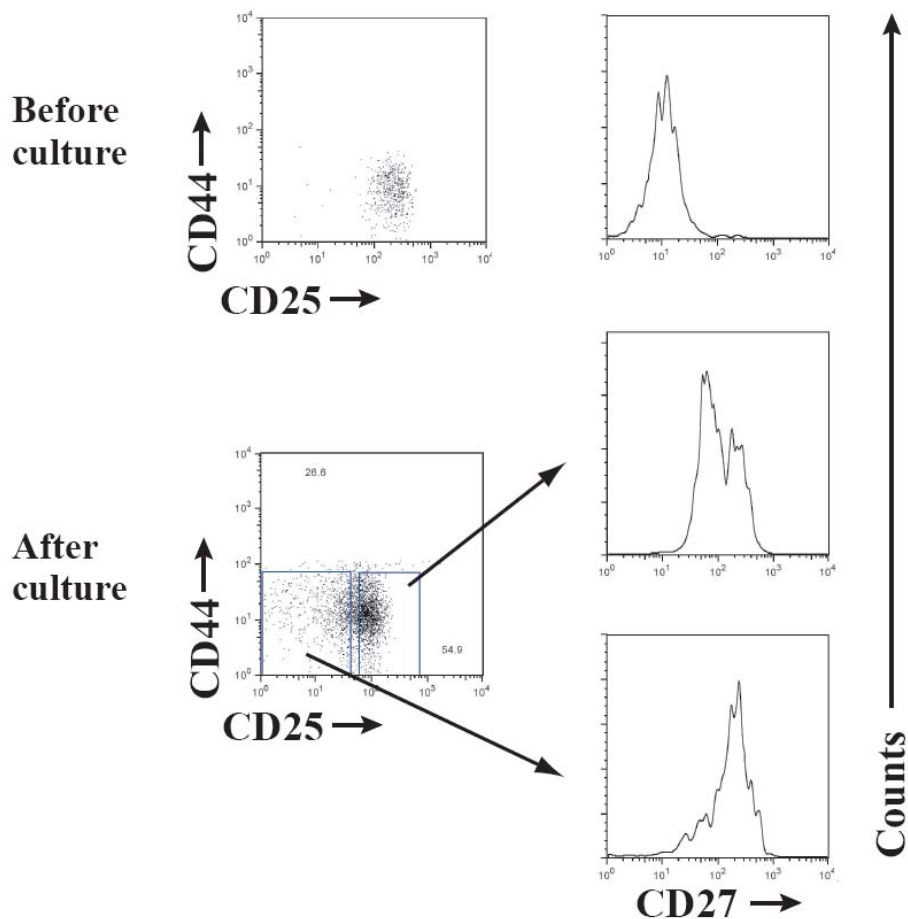Tom Taghon, Mary A. Yui, Rashmi Pant, Rochelle A. Diamond, and Ellen V. Rothenberg

Figure S1. DN3a Thymocytes Upregulate CD27 upon OP9-DL1 Culture
FACS analysis of DN3a thymocytes from C57BL/6 mice before and after 3 days of OP9 culture. Dot plots show CD44 and CD25 stainings. Histograms show intensities of CD27 expression in the corresponding populations. Data shown are representative of three independent experiments.
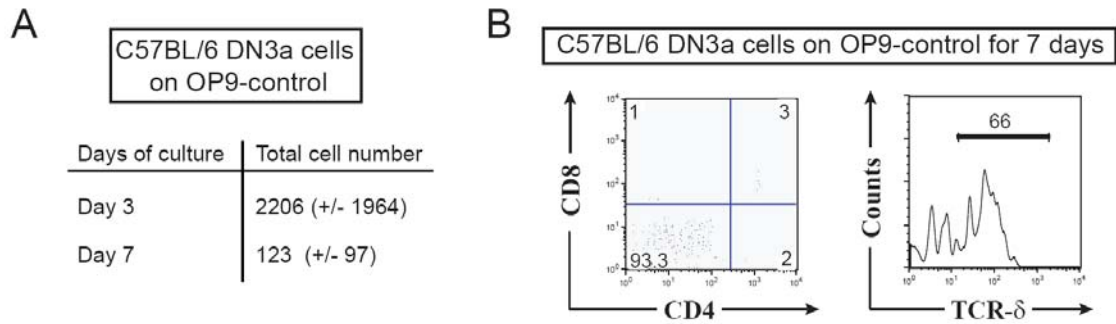
Figure S2. Only TCR-δ^+ Descendants of DN3a Cells Survive in the Absence of DL1
(A) Total number of cells in OP9-control cultures initiated with 5000 C57BL/6 DN3a thymocytes after 3 and 7 days of culture.
(B) FACS analysis of cells described in (A) after 7 days of OP9-control culture.
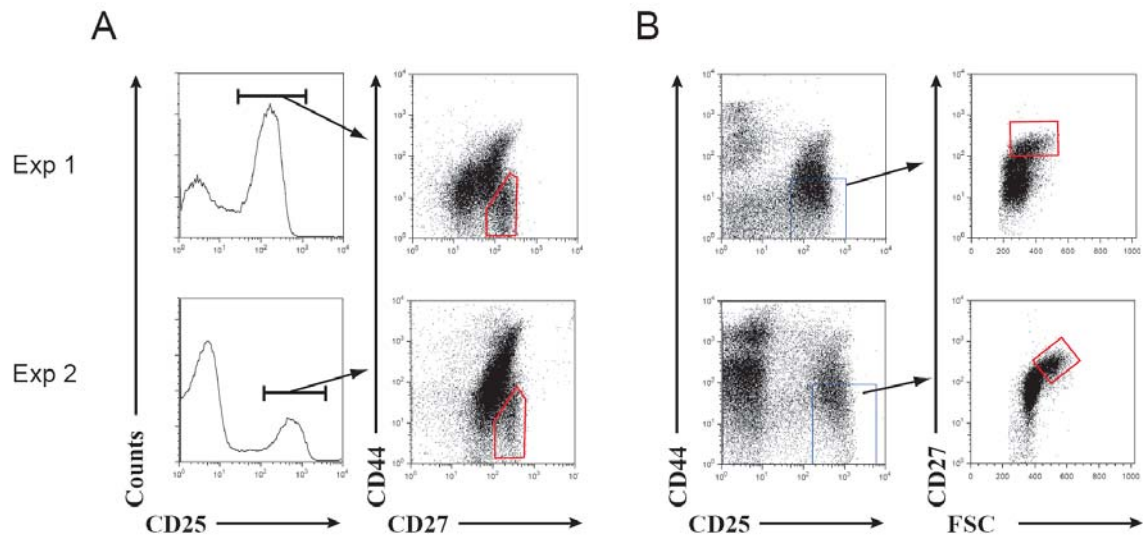

Figure S3. Alternative Methods to Identify DN3b Thymocytes
FACS analysis of CD3 and CD8 depleted C57BL/6 thymocytes. Dot plots show two independent experiments (Exp1, top row and Exp2, bottom row) with two different gating strategies (A and B) as compared to Figure 1 of the manuscript to identify DN3b thymocytes (red gates).
(A) Depleted thymocytes were initially gated on CD25$^+$ cells as shown in histograms and then analyzed for CD44 and CD27 expression.
(B) Depleted thymocytes were gated on DN3 thymocytes based on CD44 and CD25 expression and then further analyzed based on FSC and CD27 expression.
Additional hints:
The frequency of DN3b thymocytes seems to depend on the age of the animals and may decrease with aging. Also very important is the use of the CD27 antibody to get optimal separation of CD27^{low} and CD27^{high} DN3 thymocytes. We recommend using CD27-PE and search for optimal settings by changing the FL2 PMT to get the best resolution.

Table S1. Primer Sequences

Notch1 forward	CCACTGTGAACTGCCCTATGT
Notch1 reverse	TTGTTTCCTGGACAGTCATCC
Notch 3 forward	AGGGAGATGCAGATGCAGAC
Notch 3 reverse	GAAGGAGGCCAGCATAAGTG
Runx1 forward	CTCGGCAGAACTGAGAAATG
Runx1 reverse	GACGGTGATGGTCAGAGTGA
Runx3 forward	GGTTCAACGACCTTCGATTC
Runx3 reverse	GGTCCATCCACAGTGACCTT
Gfi1 forward	CTCATTCCTGGTCAAGAGCAA
Gfi1 reverse	GAGCCTCGGTAAGCTGAGAGT
Gfi1B forward	GAGATGTTGCTGAACCAGAGC
Gfi1B reverse	TGTAGGAGGAGGCCAAGGTAT
Herp1 forward	GAAGCGCCCTTGTGAGGAA
Herp1 reverse	TGTCGGTGAATTGGACCTCAT
Mybl2 forward	AAGGAGGTGCTCCGTTCTGA
Mybl2 reverse	CCAGAGACTTGCGGACCTTCT
Egr2 forward	CAGACTCAGCCTGAACTGGAC
Egr2 reverse	AGAATGCTGAAGGATCCTGGT
Egr3 forward	CTCGGTAGCCCATTACAATCA
Egr3 reverse	GCGAACTTTCCCAAGTAGGTC
Id3 forward	AGAGGAGCTTTTGCCACTGA
Id3 reverse	TGGAGAGAGGGTCCCAGAGT

Table S2. Ratios of TCR-δ+ T Cell Generation on OP9-DL1/OP9-Control Stromal Cells

DN3 Subset	TCR-δ$^+$ Ratio on OP9-DL1/OP9-Control Stromal Cells
C57BL/6 DN3a	2.46 (± 1.97)
TCR-β$^{-/-}$ DN3a	2.19 (± 0.24)
C57BL/6 DN3b	1.40 (± 1.03)
TCR-β$^{-/-}$ DN3b	1.12 (± 1.14)