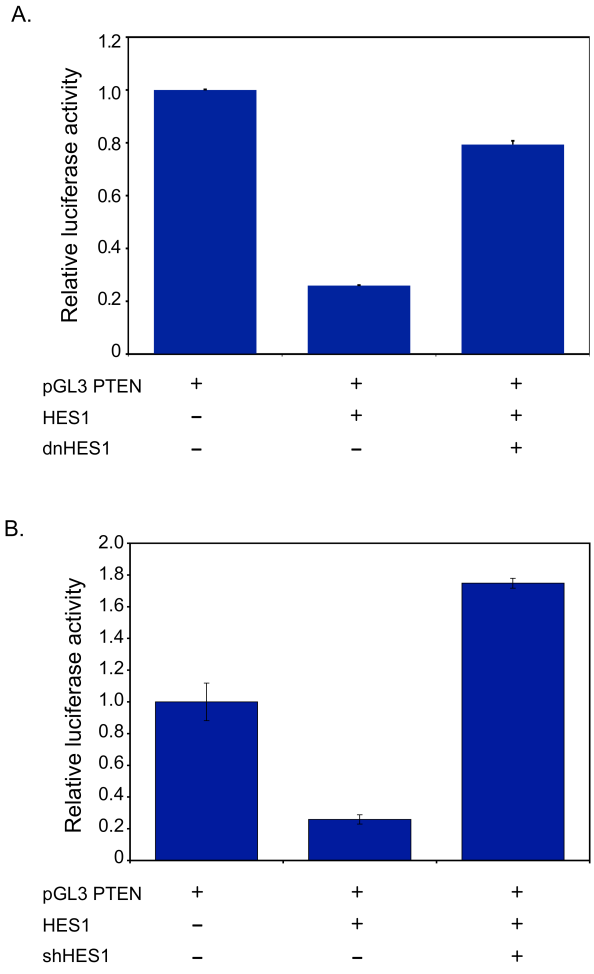
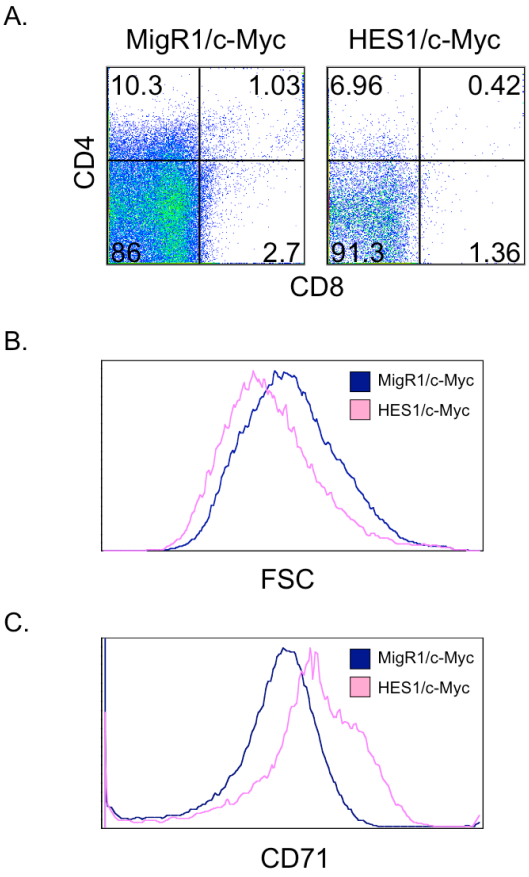
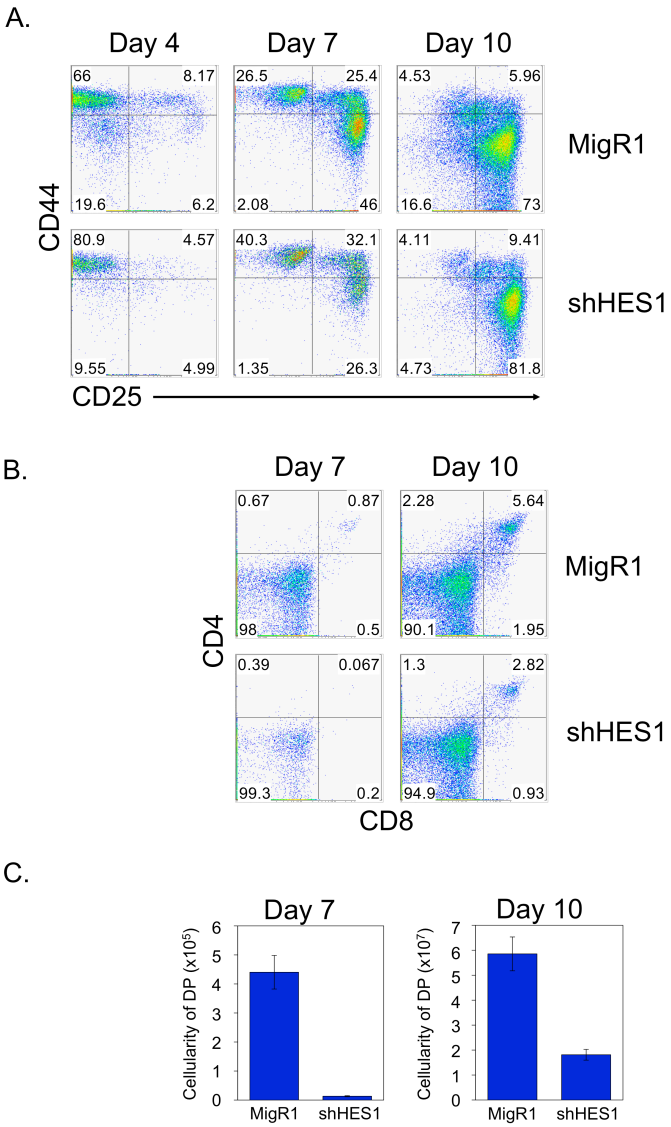
SUPPLEMENTAL FIGURES



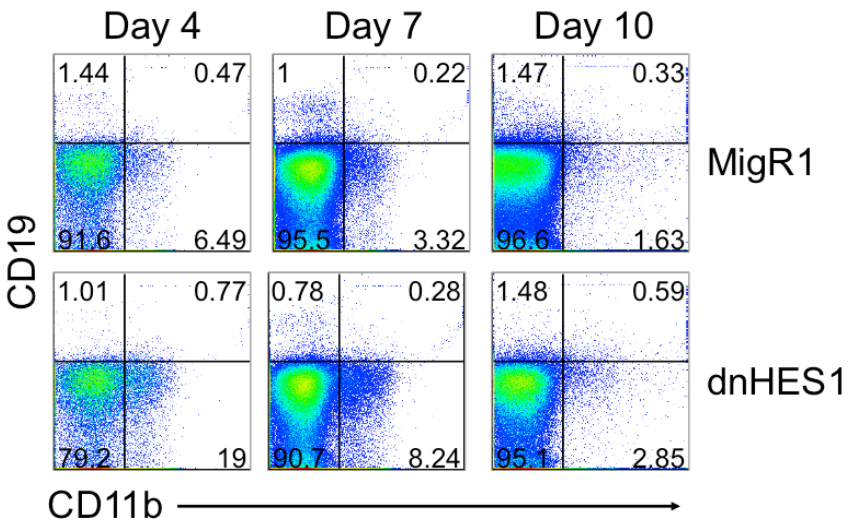
Supplemental Figure 1. Ectopic expression of HES1 inhibits *Pten* promoter activity. Luciferase reporter construct (pGL3) containing a ~2.7 Kb 5' upstream region of the *Pten* promoter (pGL3-PTEN) was used to measure the effects of HES1 and/or dnHES1, and/or shHES1 encoding constructs on *Pten* promoter activity. A) 293T cells were transfected with pGL3-PTEN, HES1, dnHES1, and pSV40-Renilla-luciferase, as indicated, and subsequently lysed and assessed for luciferase activity 48 h later. B) 293T cells were transfected with pGL3-PTEN, HES1, shHES1, and pSV40-Renilla-luciferase, as indicated, and subsequently lysed and assessed for luciferase activity 48 h later. Data are representative of three independent experiments.



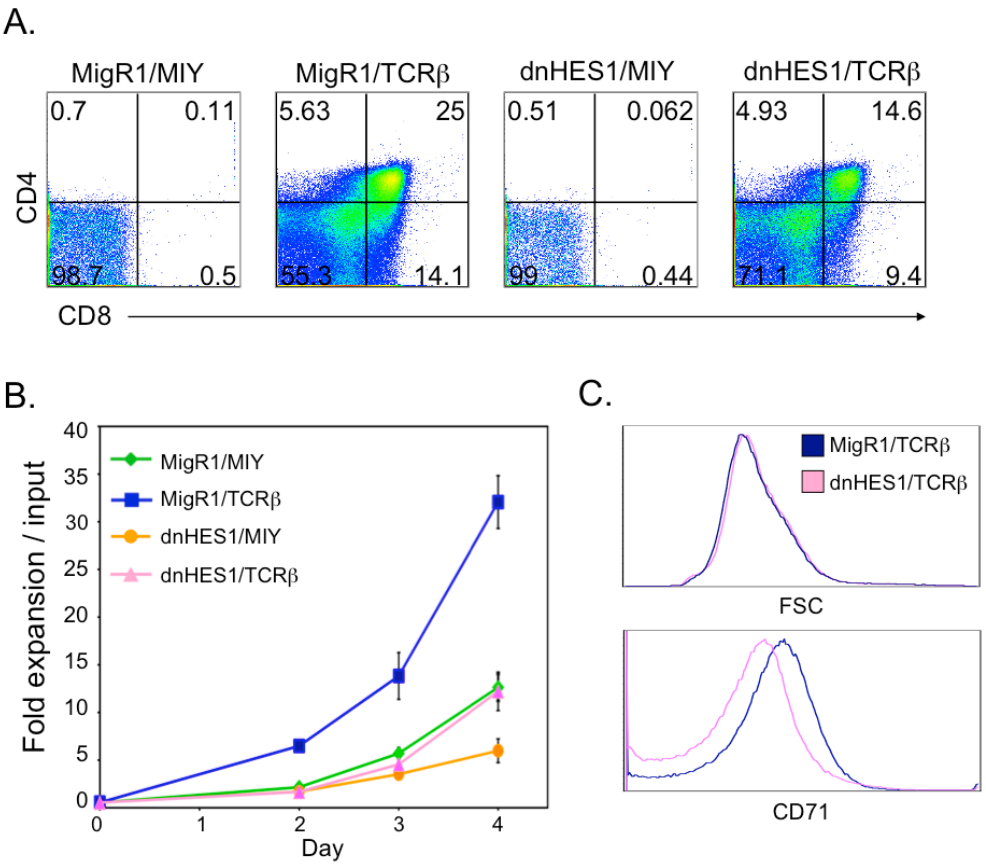
Supplemental Figure 2. Expression of HES1 and c-Myc are insufficient to promote differentiation of DN3 cells across the β-selection checkpoint in the absence of Notch signals. DN3a cells were retrovirally co-transduced to express HES1 and/or GFP (MigR1) and c-Myc (YFP^+) and cultured for 6 days on OP9-Ctrl cells. A) Flow cytometric analysis of CD4 and CD8 cell surface expression is shown for GFP^+, YFP^+, $CD45^+$ gated cells; while B-C) shows the corresponding cell size and CD71 expression, respectively, as indicated. Results are representative of three independent experiments.



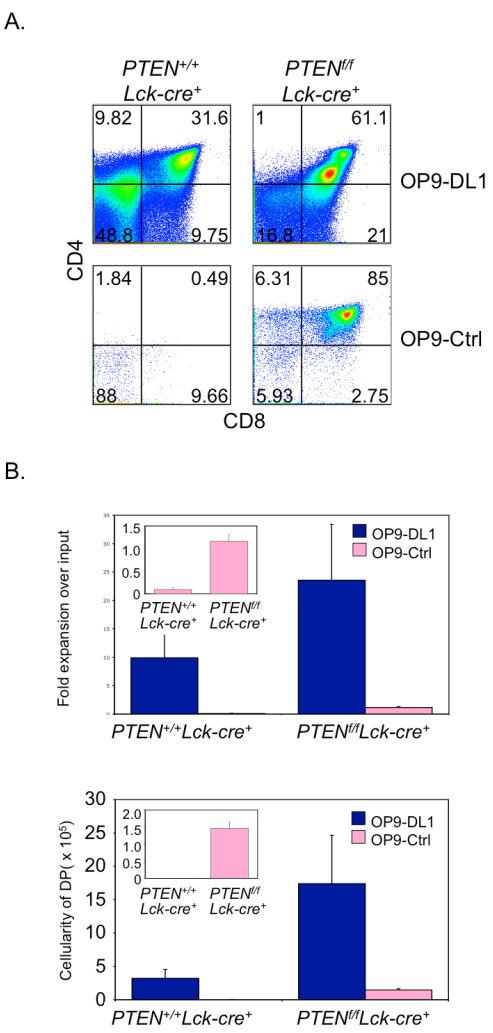
Supplemental Figure 3. Expression of an shRNA targeting *Hes1* (shHES1) leads to impaired T cell development. A) Developmental progression of FL-derived HPCs transduced to express shHES1 and/or GFP (MigR1) and subsequently cultured for 10 d with OP9-DL4 cells. Flow cytometric analysis of A) CD44 and CD25; and, B) CD4 and CD8, surface expression is shown for GFP^+ gated cells on days 4, 7, and 10 of co-culture, as indicated; while, C) shows the corresponding cellularity of DP cells present in the cultures, as indicated. DP cellularity was obtained by multiplying the total cellularity by the percentage of DP cells present in the cultures of each independent experiment. Data are representative of three independent experiments.



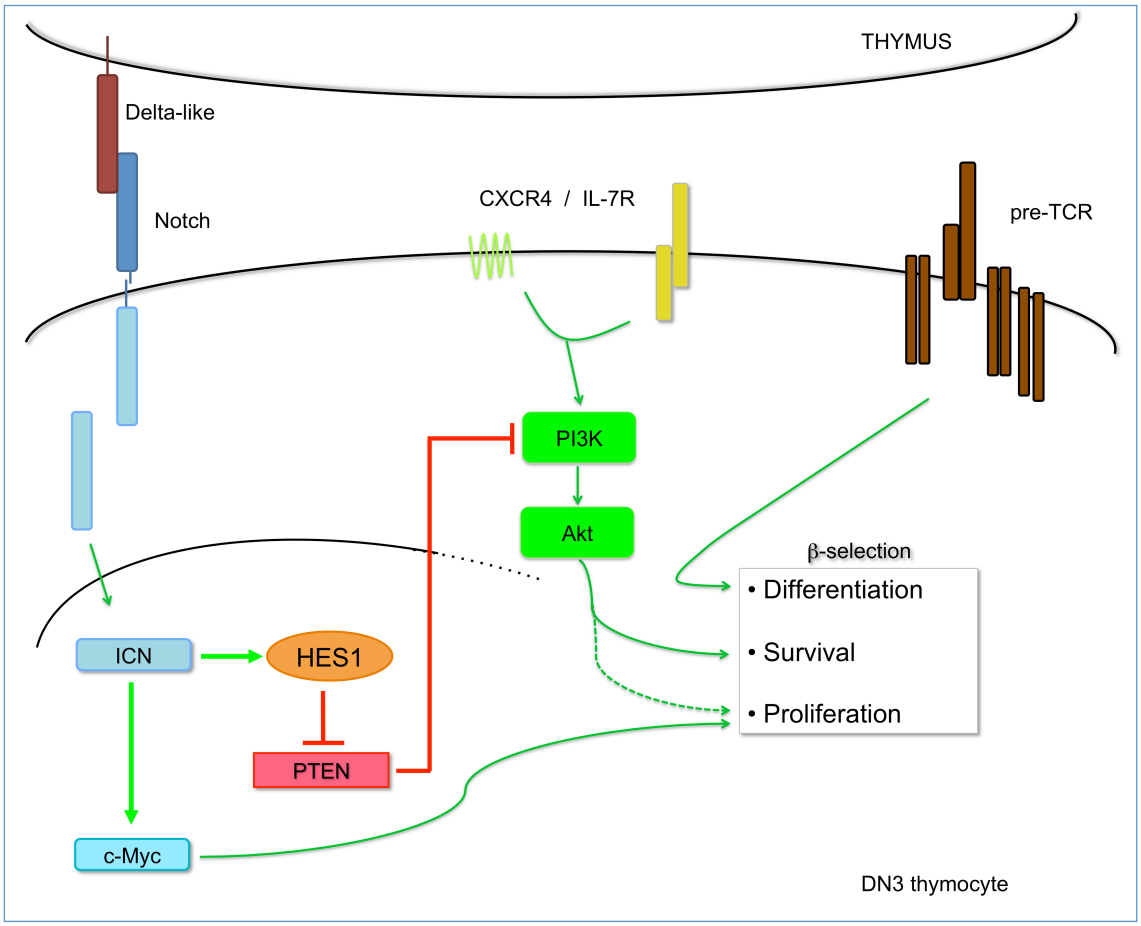
Supplemental Figure 4. Expression of a dominant negative form of HES1 (dnHES1) leads to CD11b^{+} non-T-lineage cell differentiation even in the presence of Notch signals. Developmental progression of FL-derived HPCs transduced to express dnHES1 and/or GFP (MigR1) and subsequently cultured for 10 d with OP9-DL4 cells. Flow cytometric analysis of CD19 and CD11b surface expression is shown for GFP^{+} gated cells on days 4, 7, and 10 of co-culture, as indicated. Data are representative of three independent experiments.



Supplemental Figure 5. Expression of a dominant negative form of HES1 (dnHES1) impairs T cell differentiation across the β-selection checkpoint. Developmental progression of in vitro-derived *Rag2*$^{-/-}$ DN3 cells co-transduced to express a TCRβ (TCRβ) and/or YFP (MIY) and dnHES1 and/or GFP (MigR1), and cultured with OP9-DL4 cells for 4 d. A) Flow cytometric analysis of CD4 and CD8 surface expression is shown for GFP^{+} and YFP^{+} gated cells after 4 d of co-culture, as indicated. B) The cellular fold expansion (total cellularity at each time point divided by the number of cells used at the start of the culture, input) observed in the cultures is shown for the indicated times and conditions. Data are representative of three independent experiments, with standard deviation of the mean shown as error bars. C) Corresponding histograms of cell size and CD71 expression for GFP^{+} YFP^{+} gated cells are shown, as indicated. Results are representative of at least three independent experiments.



Supplemental Figure 6. Conditional *Pten* deletion in DN3 cells allows for T cell differentiation across the β-selection checkpoint in the absence of Notch signals. Developmental progression of in vitro-derived DN3a cells cultured for 6 d with OP9-DL1 or OP9-Ctrl cells. Lin^- c-Kit^+ $Sca\text{-}1^+$ cells sorted from BM of $PTEN^{f/f;Lck\text{-}cre+}$ or $PTEN^{+/+;Lck\text{-}cre+}$ mice were cultured with OP9-DL1 cells for 14 d, sorted for DN3a cells, and returned to OP9-DL1 or OP9-Ctrl cells for 6 d. A) Flow cytometric analysis of CD4 and CD8 cell surface expression is shown for $CD45^+$ gated cells; while B) shows the corresponding fold expansion and DP cellularity as indicated. Fold expansion was obtained from the total cellularity divided by the number of cells used at the start of the culture (input), and DP cellularity by multiplication of the total cellularity by the percentage of DP cells present in the cultures. Inset graph (part of Figure 4) shows fold expansion and DP cellularity of $PTEN^{fl/fl}Lck\text{-}Cre^+$ or $PTEN^{+/+}Lck\text{-}Cre^+$ DN3a cells cultured with OP9-Ctrl cells. Results are representative of three independent experiments.



Supplemental Figure 7. A model of the effects Notch signaling via HES1 on PI3K/Akt pathway activity at the β-selection checkpoint. The figure depicts a thymus epithelial cell expressing Delta-like engaging and activating the Notch receptor (via the generation of intracellular Notch, ICN) on a DN3 thymocyte leading to the induction of HES1 and c-Myc expression. HES1, a transcriptional repressor, down regulates PTEN expression, thus decreasing PTEN's inhibition on PI3K/Akt pathway activity, occurring downstream of CXCR4 and/or IL-7R signaling. Although repression of PTEN by Notch, via HES1, is sufficient to induce survival and differentiation in pre-TCR^{+} DN3 thymocytes, c-Myc is additionally responsible for the proliferative effects downstream of Notch during β-selection.