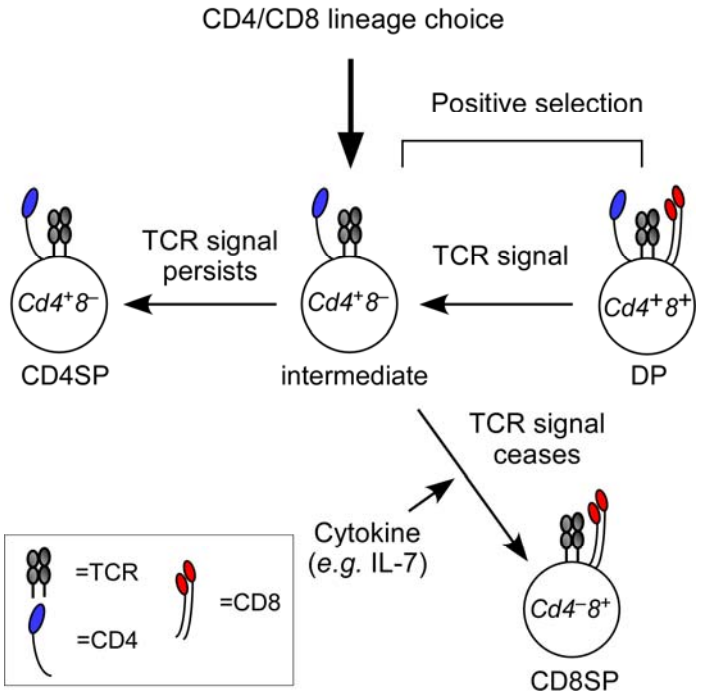
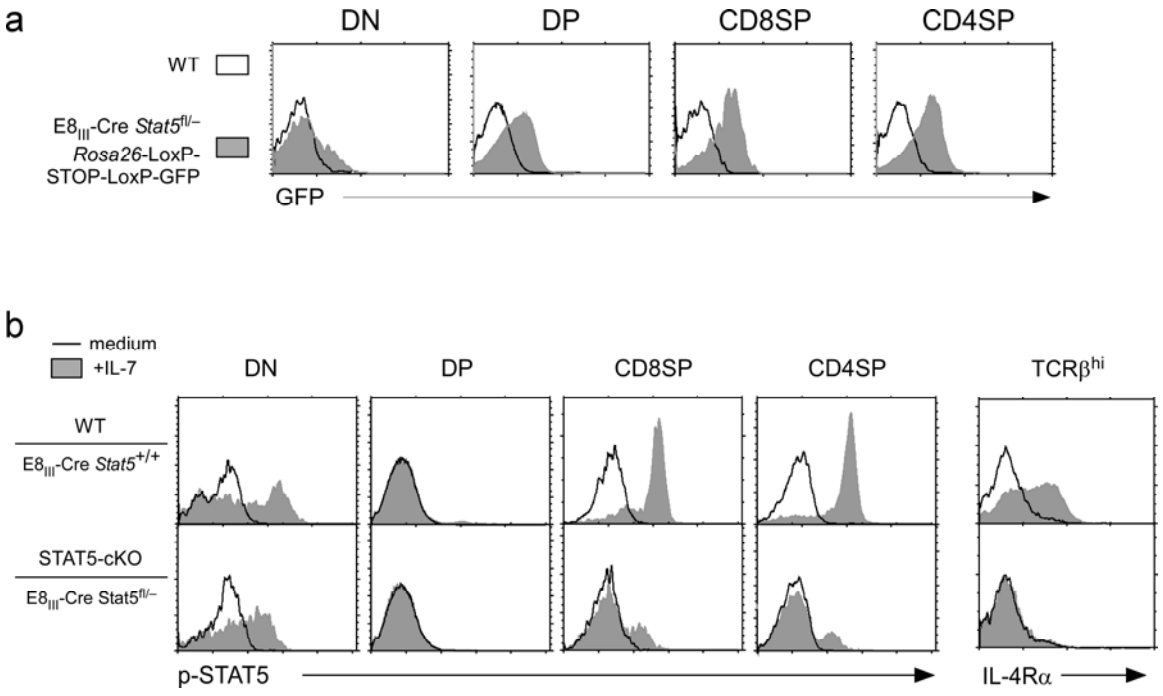
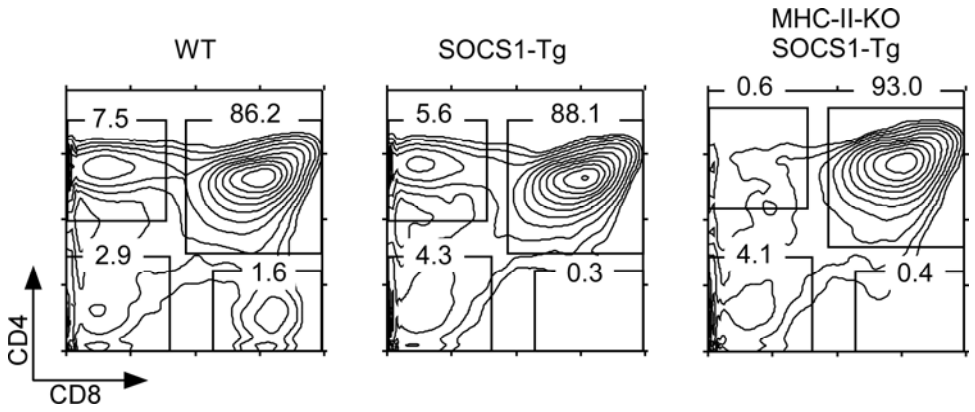


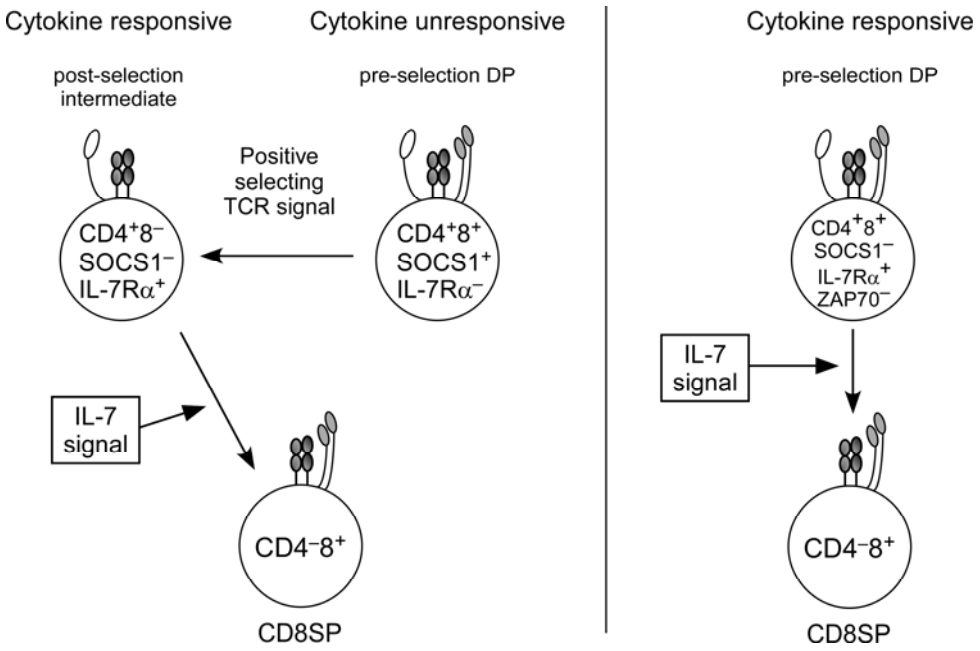
Supplementary Figure 1. Schematic presentation of the kinetic signaling model of CD4–CD8 lineage choice.



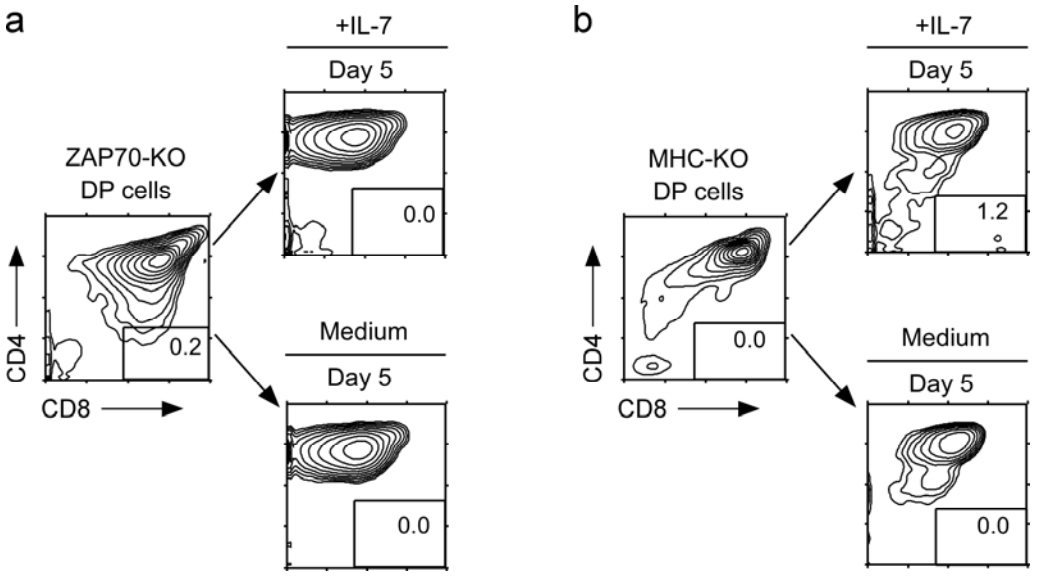
Supplementary Figure 2. $E8_{III}$-Cre transgene is expressed in DP thymocytes and terminates STAT5 activity in CD8SP and CD4SP thymocytes from *Stat5*$^{fl/-}$ mice. (a) Introduction of the $E8_{III}$-Cre transgene into Rosa26-loxP-STOP-loxP-GFP reporter mice reveals that the transgene induces Cre expression in DP thymocytes, as DP thymocytes and their developmental progeny (CD8SP and CD4SP thymocytes) are GFP$^+$. (b) IL-7 fails to induce p-STAT5 in mature thymocytes from STAT5-cKO mice. Freshly isolated thymocytes from WT ($E8_{III}$-Cre *Stat5*$^{+/+}$) and STAT5-cKO ($E8_{III}$-Cre *Stat5*$^{fl/-}$) mice were stimulated with IL-7 (shaded curve) or medium (open curve) for 30 min and assayed for intracellular p-STAT5 content (left). Surface IL-4Rα expression upon overnight IL-7 stimulation was determined on TCRβ^{hi}-gated thymocytes (right).



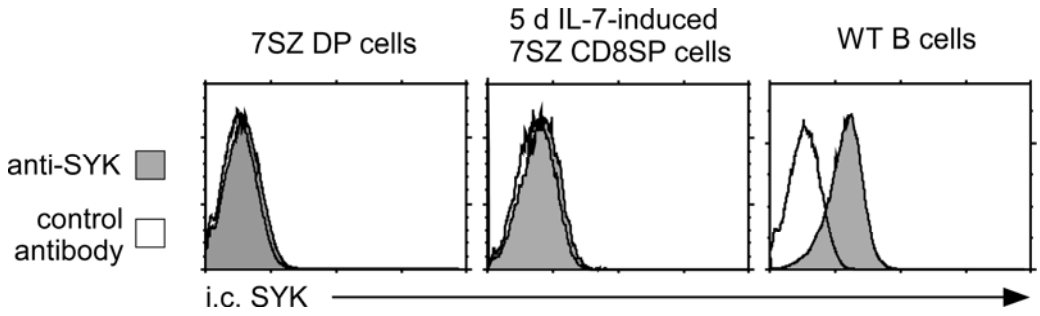
Supplementary Figure 3. SOCS1-Tg blocks generation of $CD8^+$ and MHC-I selected thymocytes. Thymocytes from WT, SOCS1-Tg, and MHC-II-KO·SOCS1-Tg mice were assessed for CD4 versus CD8 expression. Data are representative of 3 independent experiments.



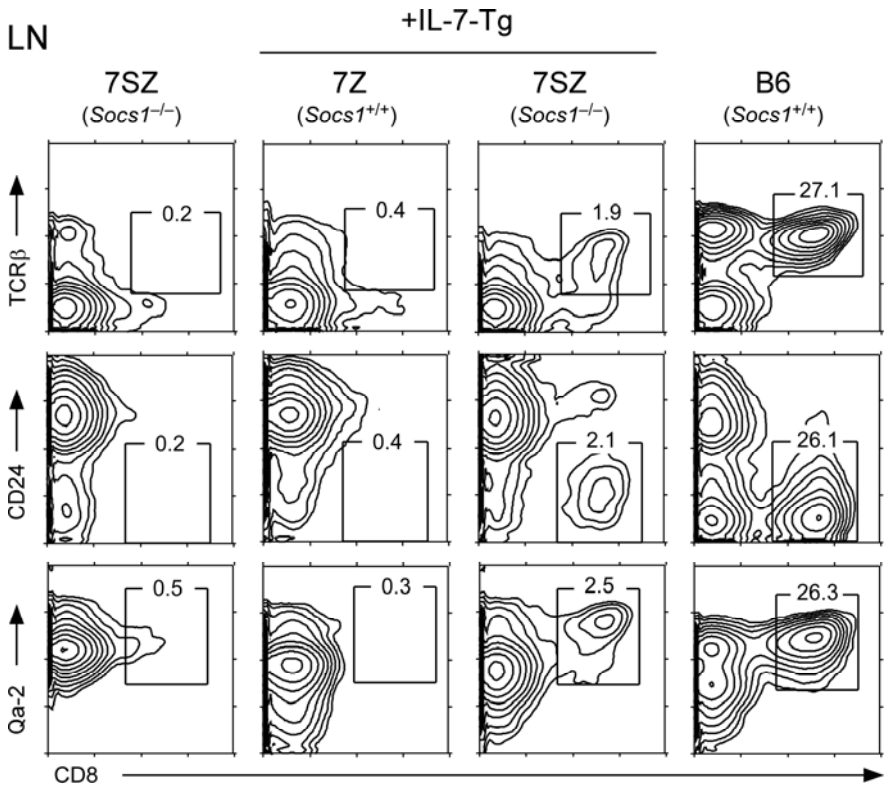
Supplemental Figure 4. Developmental implications of cytokine-unresponsive versus cytokine-responsive DP thymocytes. Pre-selection DP thymocytes are normally cytokine-unresponsive because they express high levels of SOCS1 and express little or no IL-7Rα (left panel). Consequently, TCR-mediated positive selection signaling is required to convert DP thymocytes into IL-7-responsive *Cd4*$^{+}$*Cd8*$^{-}$ intermediate thymocytes by terminating SOCS1 and inducing IL-7Rα expression, so that the intermediate cells can then be signaled by IL-7 to differentiate into mature CD8^{+} T cells (left). However, pre-selection DP thymocytes from IL-7Rα-Tg·SOCS1-KO mice are IL-7-responsive even in the absence of TCR signaling (right panel). To ensure that pre-selection DP thymocytes from IL-7Rα-Tg·SOCS1-KO mice had not been TCR-signaled, we additionally made them ZAP70-deficient, so that mice were IL-7Rα-Tg·SOCS1-KO·ZAP70-KO (simply referred to as 7SZ mice). IL-7 signaling of cytokine-responsive pre-selection 7SZ DP thymocytes would be predicted to induce their differentiation into mature CD8^{+} T cells, completely circumventing TCR-mediated positive selection (right panel).



Supplementary Figure 5. *In vitro* IL-7 has no effect on DP thymocytes from ZAP70-KO or MHC-KO mice. Purified DP thymocytes from ZAP70-KO (a) or MHC-KO mice (b) were cultured with either IL-7 or medium for 5 days and then assessed for surface CD4 and CD8 expression.



Supplementary Figure 6. SYK kinase is not expressed in IL-7 responsive DP or IL-7-induced CD8SP thymocytes from 7SZ mice. Intracellular (i.c.) SYK kinase expression was determined in freshly isolated 7SZ DP cells and in IL-7-induced 7SZ $CD8^+$ SP cells using anti-SYK monoclonal antibodies. LN B cells from B6 mice were used as positive control for SYK staining (right).



Supplementary Figure 7. $CD8^+$ T cells in LN of IL-7 transgenic 7SZ mice. Mature $CD8^+$ T cells were identified in lymph nodes of IL-7-Tg·7SZ mice. Mature CD8 T cells were identified as $TCR\beta^{hi}CD24^{-}Qa\text{-}2^+$ cells. Boxes identify mature $CD8^+$ T cells in each plot, and the frequency of cells in each box is shown.

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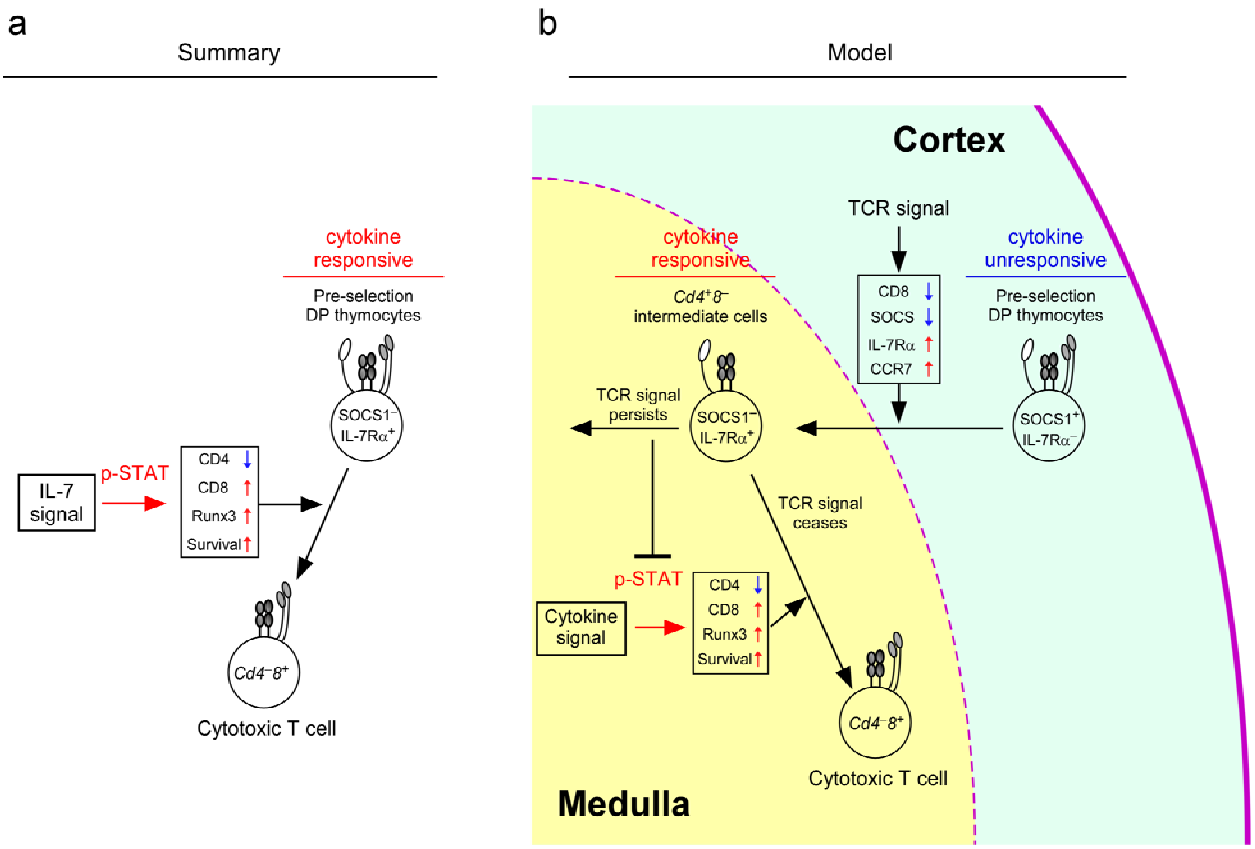

Supplementary Figure 8. CD8$^+$ T cell generation in the thymus requires sequential signaling by TCR and γc-cytokines. (a) A graphic summary of our results with cytokine-responsive DP thymocytes from 7SZ mice, revealing that IL-7 signaling is needed to induce CD8 lineage-specification and differentiation of TCR-unsignaled pre-selection DP thymocytes into mature CD8$^+$ T cells. (b) Synthesizing previous studies[13,34] with our current results reveals a new understanding of CD8$^+$ T cell differentiation and indicates that sequential signaling by TCR and IL-7 is required for DP thymocytes to differentiate into mature CD8$^+$ T cells. TCR signaling converts cytokine-unresponsive DP thymocytes into IL-7-responsive intermediate thymocytes that are transcriptionally *Cd4*$^+$*Cd8*$^-$ and *Ccr7*$^+$. Because they are transcriptionally *Cd4*$^+$*Cd8*$^-$, intermediate thymocytes specifically lose surface CD8 coreceptor expression, causing MHC-I-specific TCR signaling to cease and the cells to regain their ability to transduce cytokine signals. Cytokine-responsive intermediate thymocytes then encounter IL-7 by migrating to IL-7 richer areas of the thymus (cortico-medullary junction and medulla), which is facilitated by *e.g.* CCR7 expression. After encountering IL-7, STAT-mediated IL-7 signaling induces Runx3 expression which specifies CD8-lineage choice by mediating coreceptor reversal, *i.e.* the transcriptional conversion of *Cd4*$^+$*8*$^-$ intermediate thymocytes into *Cd4*$^-$*8*$^+$ cells, and by providing the survival signals required for differentiation of CD8-committed thymocytes into mature CD8$^+$ cytotoxic-lineage T cells.