Supporting Information

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SI Text

Mice. Wild-type C57BL/6 and congenic CD45.1, Thy1.1, GFP, and IL-7R$\alpha^{-/-}$ mice were purchased from Jackson Laboratories and Charles River. Mice were housed in specific pathogen-free conditions. GFP$^{+/+}$ mice were crossed with ST8Sia IV$^{-/-}$ mice, and F1 progeny were bred and selected for ST8Sia IV$^{-/-}$; GFP^{+} offspring. All experiments were approved by UC Berkeley's Animal Care and Use Committee. The generation of ST8Sia IV$^{-/-}$ mice has been described (Eckhardt et al., 2000). After initial production, the ST8Sia IV$^{-/-}$ mice were backcrossed to C57BL/6 for at least 6 generations before use in these studies.

Antibodies. Fluorescein-conjugated anti-CD3ε (145–2C11), anti-CD4 (GK1.5), anti-CD11b (M1/70), anti-CD25 (PC61.5), anti-TCRβ (H57–597), anti-Gr-1 (RB6–8C5), anti-TER119; phycoerythrin-Cy5-conjugated Sca-1 (D7), isotype control rat IgG2a; and allophycocyanin-conjugated secondary anti-mouse IgG were purchased from eBioscience.

Fluorescein-conjugated anti-CD8a (53–6.7), anti-TCR$\gamma\delta$ (GL3), anti-NK1.1 (PK136), anti-B220 (RA3–6B2), and isotype controls mouse IgG2a (G155–178), rat IgG1 (R3–34), rat IgG2a (R35–95), rat IgG2b (A95–1); phycoerythrin-conjugated anti-CD8a (53–6.7), anti-CD25 (PC61), anti-CD90.2/Thy1.2 (53–2.1), anti-CD117 (2B8), and isotype controls rat IgG1 (R3–34), rat IgG2a (R35–95); phycoerythrin-Cy5-conjugated anti-CD3 (17A2), anti-CD44 (IM7); and peridinin chlorophyll-a protein (PerCP)-conjugated anti-CD90.1/Thy1.1 (OX-7) were purchased from BD Biosciences.

Flow Cytometry/Thymocyte Characterization. Cells were isolated, counted by hemocytometer, and immediately incubated for 10 min with Mouse BD Fc Block (anti-FcγIII/II R; BD Biosciences), followed by the addition of antibodies for staining. After 20 min, cells were washed twice in PBS (Invitrogen) and analyzed on a FACSCalibur (BD Biosciences) instrument with CellQuest (BD Biosciences) software. Thymocyte phenotyping experiments were repeated 9 times (n = 16 and 18 for ST8Sia IV$^{-/-}$ and wild type, respectively).

Detection of Early T-lineage Progenitors. Leukocytes were isolated from bone marrow, blood, and thymus, counted by hemocytometer, and monitored by flow cytometry to detect lineage markers (including TER119, CD3, CD4, CD8, B220, NK1.1, Gr-1, TCRβ, TCR$\gamma\delta$, and CD11b). All lineage-positive cells were excluded from further analysis, and the remaining lineage-negative (Lin^{-}) population was assessed for expression of cKit and Sca-1. LSKs were defined as Lin^{-}, cKit^{+}, Sca-1^{+} that fell within the lymphocyte region of a forward- and side-scatter plot. LSKs (100–800 cells) were counted/bone marrow sample (experiment repeated 6 times, n = 12, ST8Sia IV$^{-/-}$; n = 15, wild type); 3–100 LSKs were counted/thymocyte sample (experiment repeated 5 times, n = 7, ST8Sia IV$^{-/-}$; n = 8, wild type); and 15–40 LSKs were counted/blood sample (experiment repeated twice, n = 3, ST8Sia IV$^{-/-}$; n = 4, wild type).

Table S1. Noncompetitive reconstitution of irradiated mice by wild-type and ST8Sia IV$^{-/-}$ bone marrow

Donor	Recipient	Total # Thymocytes (millions)	% Donor-Derived Thymocytes	# Donor-Derived Thymocytes (millions)
Wild type GFP^{+}	Wild type	136 ± 36	91 ± 2	123 ± 31
Wild type GFP^{+}	ST8Sia IV$^{-/-}$	139 ± 31	93 ± 1	130 ± 30
ST8Sia IV$^{-/-}$ GFP^{+}	Wild type	138 ± 27	91 ± 0.6	127 ± 24
ST8Sia IV$^{-/-}$ GFP^{+}	ST8Sia IV$^{-/-}$	144 ± 27	89 ± 1	129 ± 24

Recipient mice were injected intravenously with 1.2×10^6 donor bone marrow cells. After 4 weeks, thymocytes were analyzed for donor origin (identified by GFP expression). $n = 4$ recipient mice/group.