Figure S1: Ultrasound-guided injection of a mouse thymus.

Upper panel: Ultrasound image pre-injection with tip of needle in the right thymic lobe. Blue line outlines the thymus. Lower panel: Ultrasound image post-injection of right thymic lobe. White dots represent air bubbles.
Figure S2: T cell reconstitution from intrathymically injected syngeneic HSPCs is dose-dependent. Sublethally irradiated (625 cGy) C57BL/6 recipients received 0, 500, 1,000, or 2,000 syngeneic CD45.1+ LSK cells via intrathymic injection. Thymuses and spleens were harvested 2 months after injection and analyzed for thymocytes and CD3+ splenic T cells of host and donor origin. Mean and SEM are presented (n=4).
Figure S3: Intrathymically injected syngeneic LSK cells persist for at least 15 months after syngeneic BMT.

Lethally irradiated C57BL/6 recipients were transplanted with C57BL/6.CD45.1\(^+\) Lin\(^-\) BM cells and received 3,000 C57BL/6.Thy1.1\(^+\) luciferase-expressing LSK cells via ITI two hours after irradiation. The whole-body distribution of LSK-derived cells at the indicated time points after BMT was monitored using *in vivo* BLI. Pseudocolor images superimposed on conventional photographs are shown.
Figure S4: Intrathymically injected allogeneic LSK cells persist as committed hematopoietic progenitor cells after allogeneic BMT.

Lethally irradiated BALB/c recipients were transplanted with C57BL/6 TCD BM cells and received 5,000 C57BL/6.CD45.1<sup>+</sup> LSK cells via ITI two hours after irradiation. Thymuses and spleens were harvested two months after BMT and analyzed for multipotent hematopoietic stem and progenitor cells of BM donor and LSK donor origin. Mean and SEM are presented (n=4).
Figure S5: IL-7 dosage affects DN population of developing thymocytes.

BALB/c mice were intrathymically injected with either PBS or three different doses of IL-7 cytokine: 20 ng, 100 ng or 500 ng. Sublethal irradiation was performed on day 1 post-injection. Thymuses were harvested on day 28. Mean and SEM are presented (n=5).
Figure S6: Thymic conditioning pre-irradiation with KGF and IL-7 improves thymic recovery

Nine month-old BALB/c mice received a sublethal dose of TBI and a single intrathymic injection with either 2.0 µg of KGF on day –28 or 500 ng of IL-7 on day –1, or both. Age-matched control mice received no injection. Thymuses and spleens were harvested on day 30 after irradiation. Thymuses were analyzed for CD45−EpCAM+MHC class II+ thymic epithelial cells (A), and for single positive thymocyte subsets (B). Mean and SEM of one of two independent experiments are presented (n=4-6).