Supplementary Information for

Cultured Thymus Transplantation Promotes Donor-specific Tolerance to Allogeneic Heart Transplants

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> Supplementary text
> Figs. S1 to S7
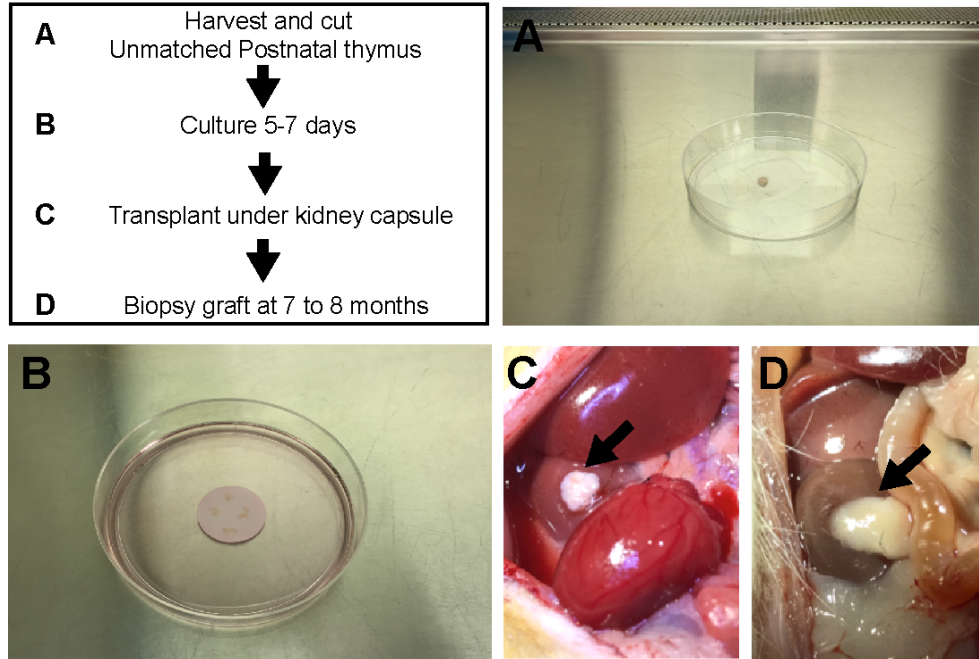> Captions for Figs. S1 to S7

Fig. S1. In vitro thymus culture and transplantation under the rat kidney capsule. A. Thymus was harvested from 3-day-old F1 (LWxDA) rat and cut into 4 pieces for in vitro culture. B. The thymus pieces were cultured on sterile nitrocellulose filters with thymus organ medium for 5-7 days in a 37ºC CO_2 incubator. C. Cultured thymus was transplanted under the kidney capsule of an LW rat. One stitch of 10-0 silk suture was applied to the incision site of the capsule to secure the graft. D. This thymus graft was harvested early at 6 months after transplantation. Arrows indicate the CTTT under the kidney capsule.
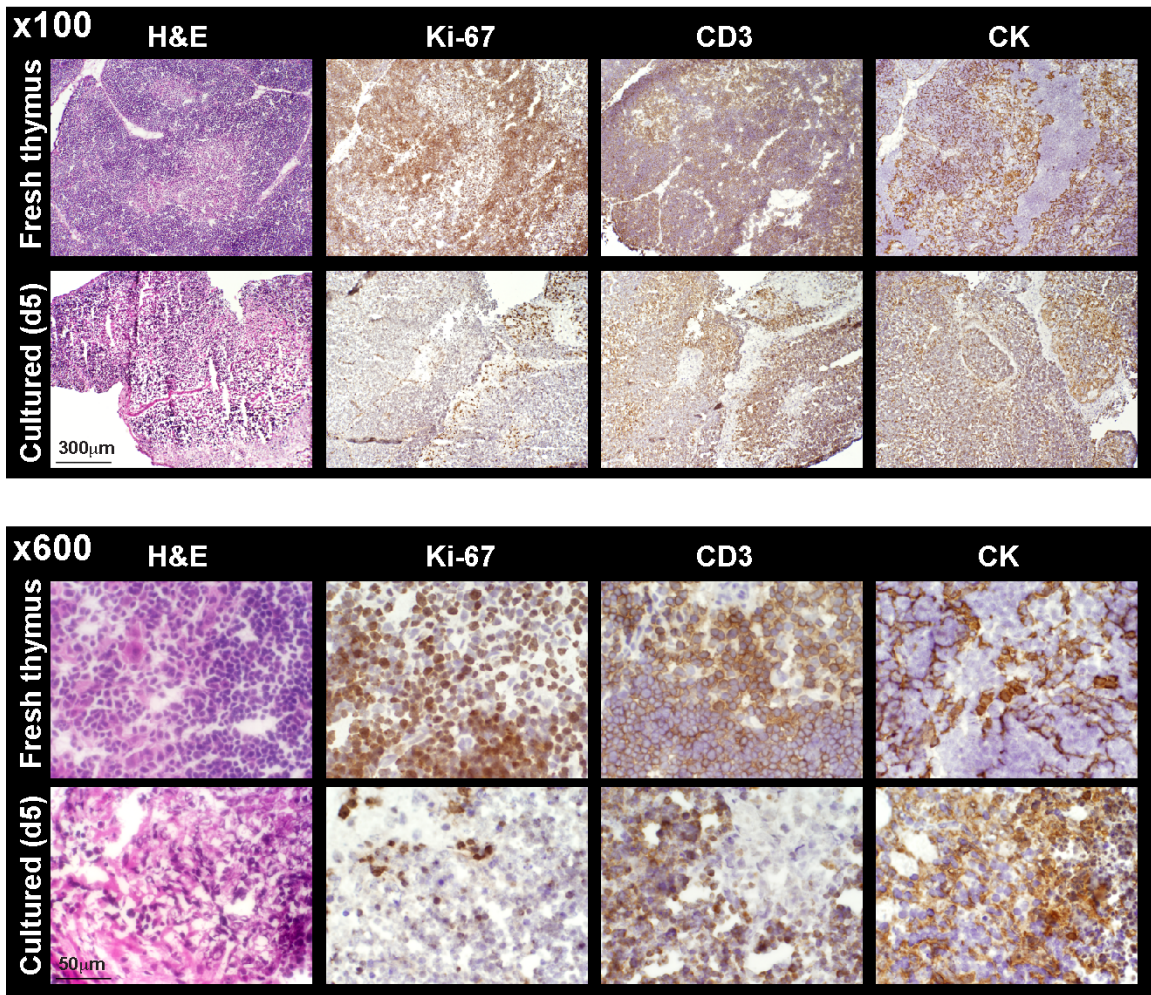
Fig. S2. Histologic appearance of fresh thymus and in vitro cultured thymus tissue. H&E staining shows medullary differentiation. Cytokeratin staining showed typical lacey pattern from both fresh and in vitro cultured thymus tissues. The Ki-67 staining is positive on cortical thymocytes. As expected, with culture, T cells die and/or are depleted from the thymus tissue with the result that there is very little Ki-67 staining.   The antigen stained by the CD3 antibody is very stable. The CD3 Ab, after 5 days of culture, likely represents some viable cells plus the detritus of dead T cells that have not washed out of the tissue. Representative images were shown as low (×100) and high magnification (×600).
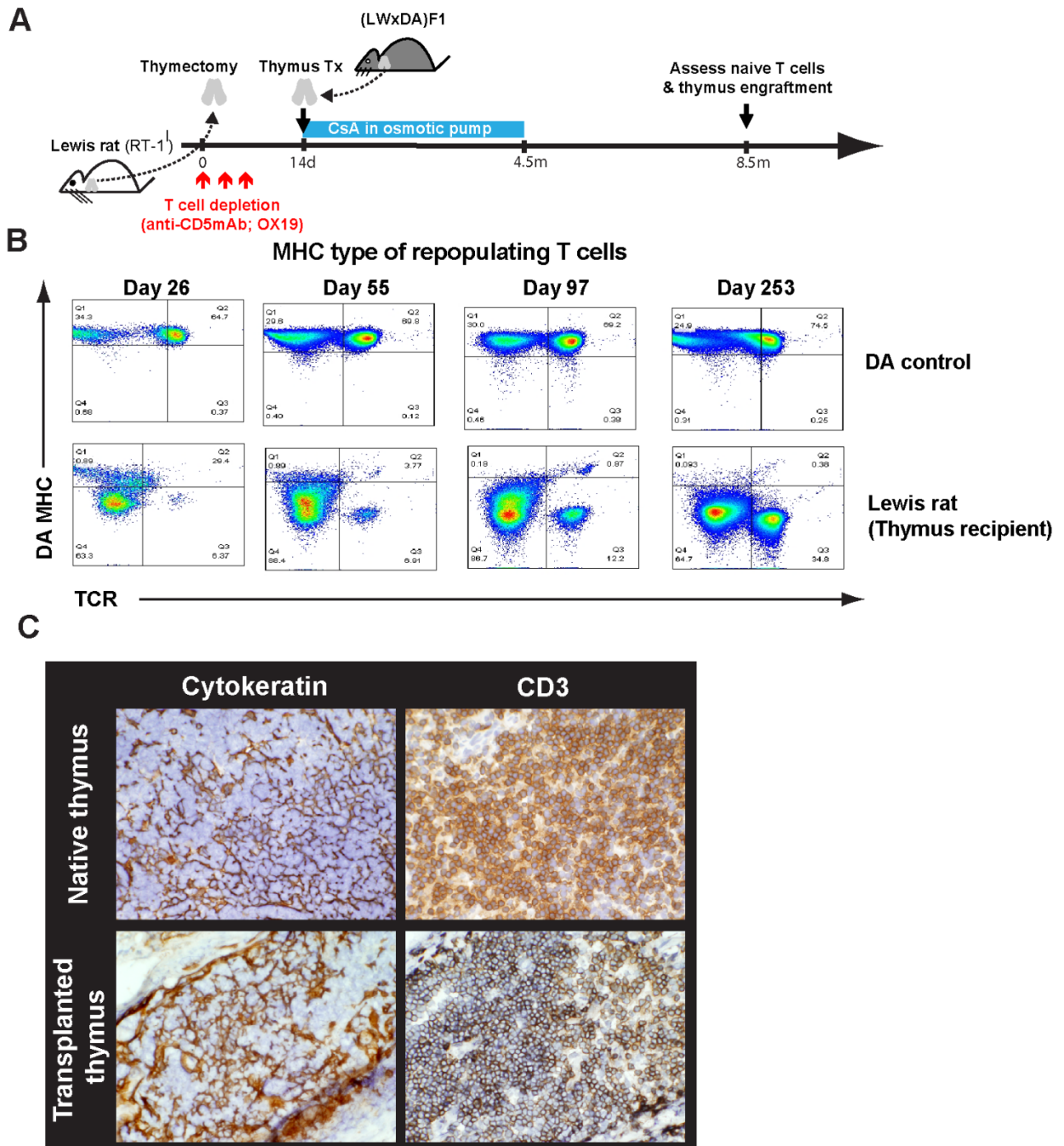
Fig. S3. CTTT supports thymopoiesis of recipient T cells in the thymectomized host. A Schematic representation of surgical procedures and treatment schedule. All recipients received CTTT after complete thymectomy followed by T cell depletion with anti-CD5mAb (OX19). B After CTTT, repopulating recipient-type T cells are seen in the lower right quadrant. C Immunohistochemical analysis of engrafted allogeneic thymic tissue. Transplanted thymus explanted at 8.5 month after transplantation showed positive cytokeratin staining as well as T cell staining similar to native thymus. Original magnification ×400.

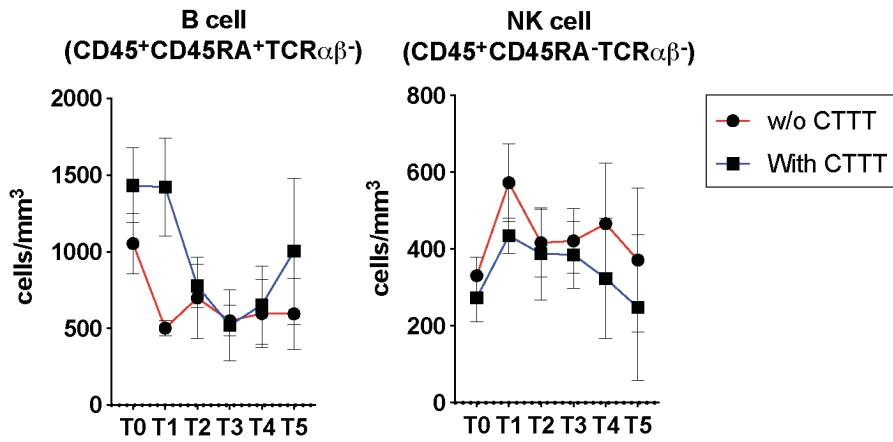**Fig. S4**. Circulating B and NK cell repopulations after T cell depletion and thymus and heart transplantation. B cell (CD45$^+$TCR$\alpha\beta^-$ CD45RA$^+$) and NK cell (CD45$^+$ TCR$\alpha\beta^-$ CD45RA$^-$NKR-P1A$^+$) population showed similar patterns in both animals with CTTT (blue line) and without CTTT (red line).
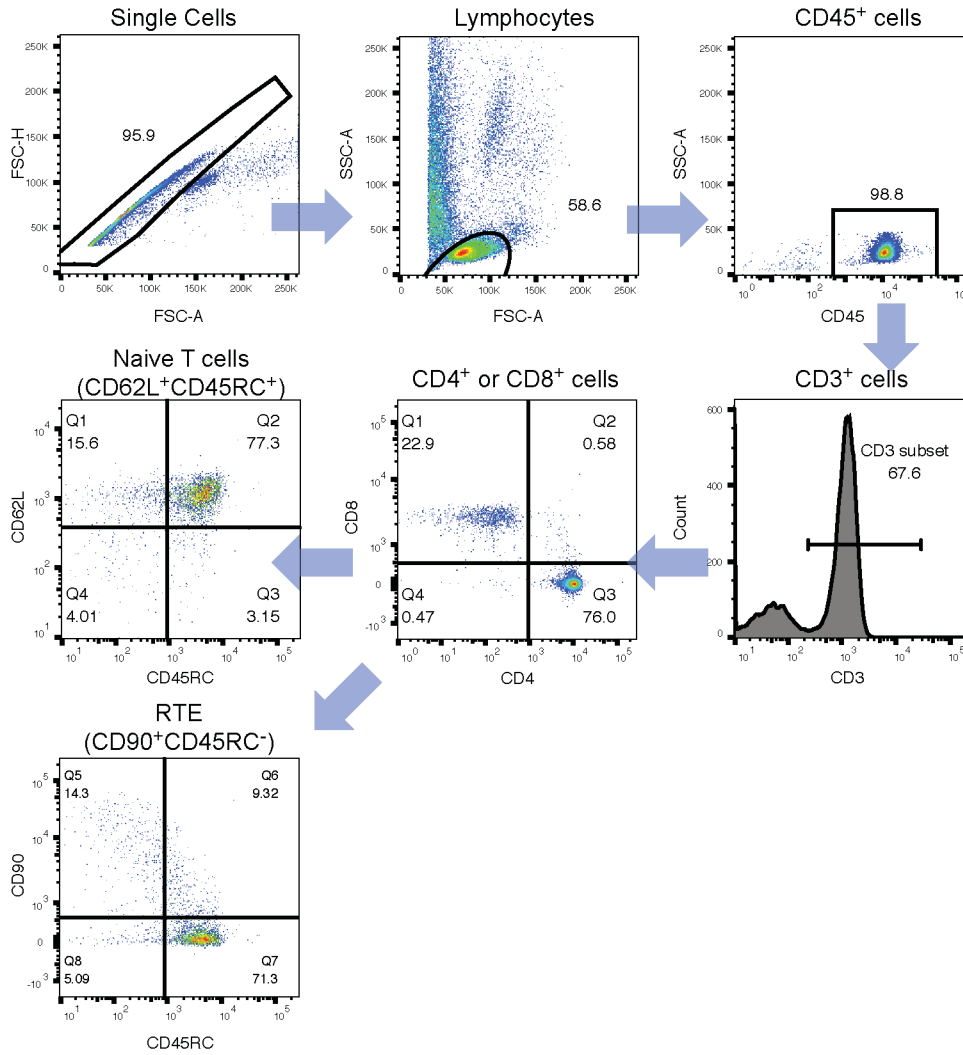
Fig. S5. Gating strategy for T cell subpopulations. T cells were visualized via singlet, lymphocyte gate, common lymphocyte antigen CD45, and Pan-T cells antigen CD3. Naïve T cells (CD62L$^+$CD45RC$^+$) and recent thymic emigrants (RTE; CD90$^+$CD45RC$^-$) were identified with CD4 and CD8 T cells.
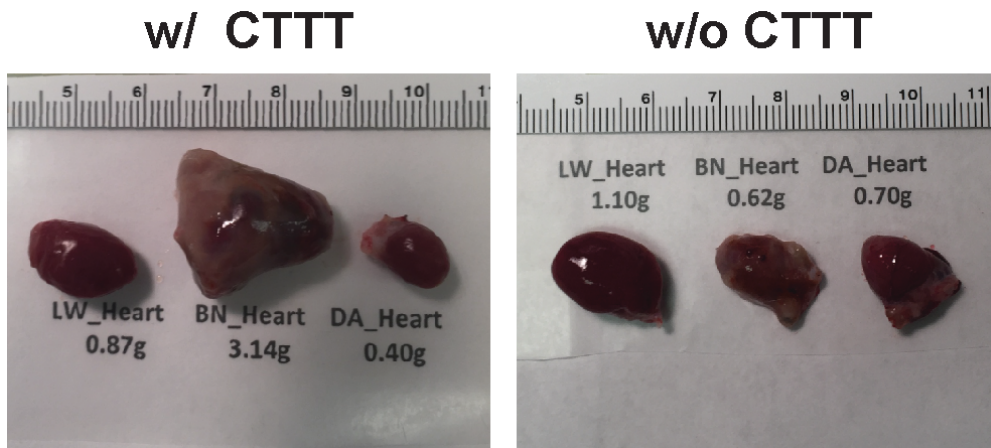
Fig. S6. Gross appearance of native, BN (cervical, secondary), and DA (abdominal, primary) hearts. Animals with CTTT showed enlarged BN and atropic DA hearts while control animals (w/o CTTT) showed similar size hearts for all.
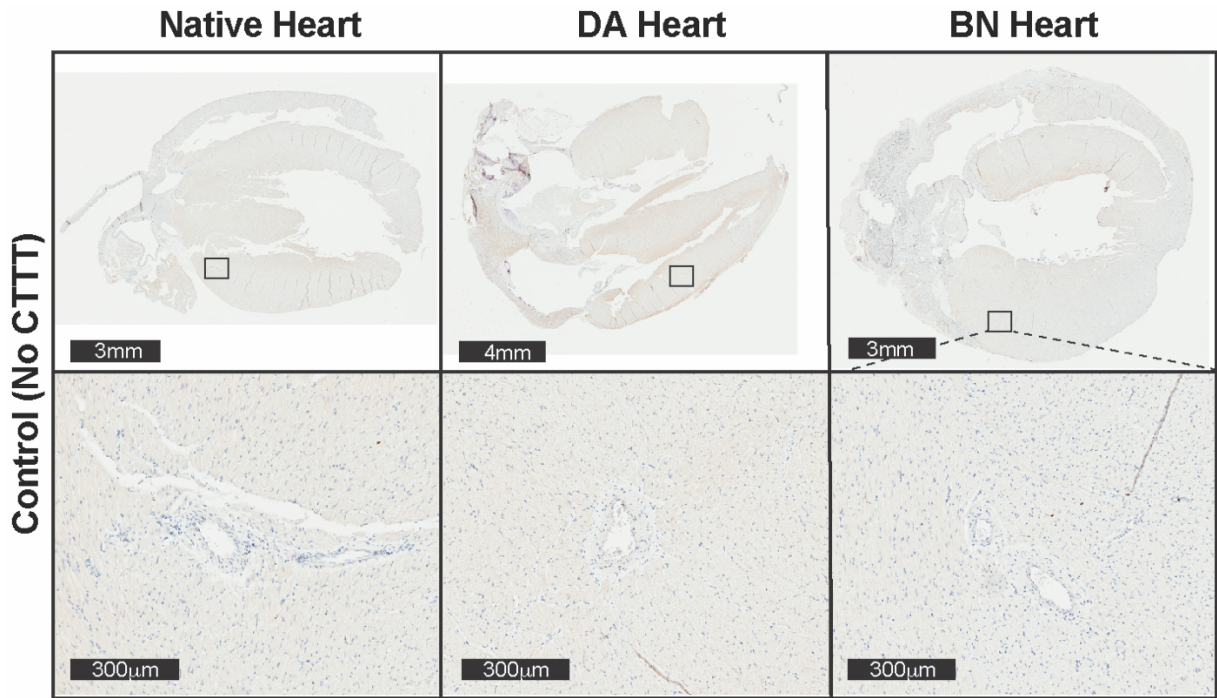
Fig. S7. Animals without CTTT (CD3 IHC). T cell infiltration in the LW, DA, and BN hearts from control animals without CTTT. Allografts from DA and BN were harvested with native heart from control animals (time matched to BN heart rejection of animals with CTTT). Grossly, all explanted hearts (native, DA, and BN) showed no sign of T cell infiltration. Images were adapted from whole slide scan.