

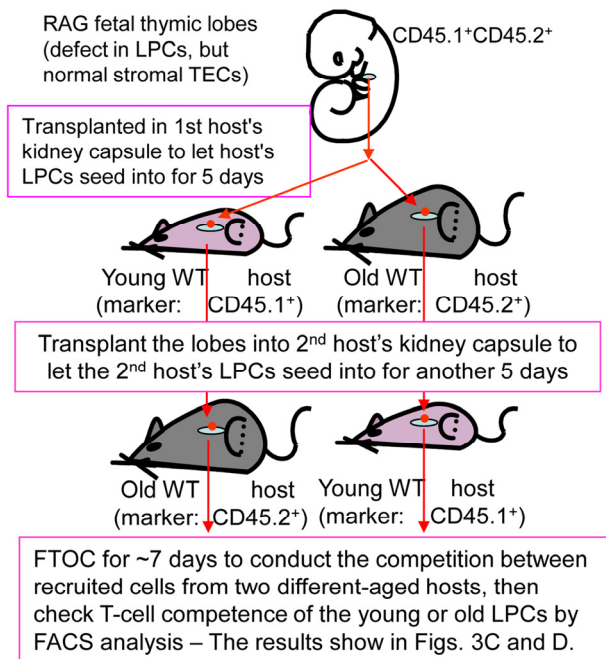
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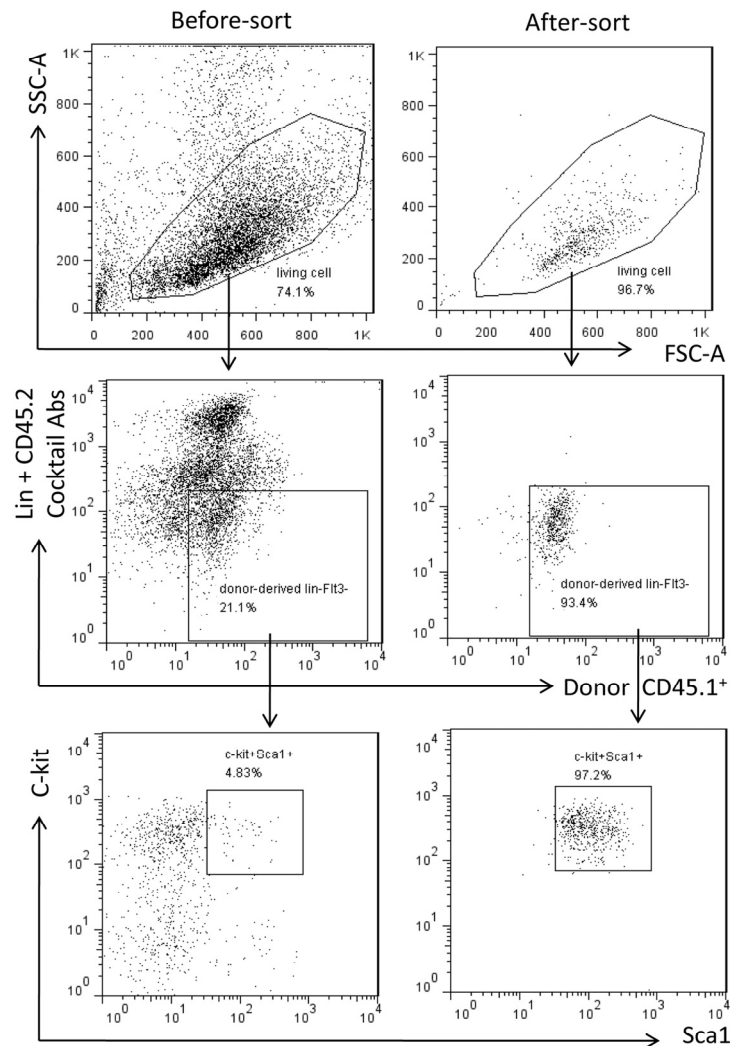
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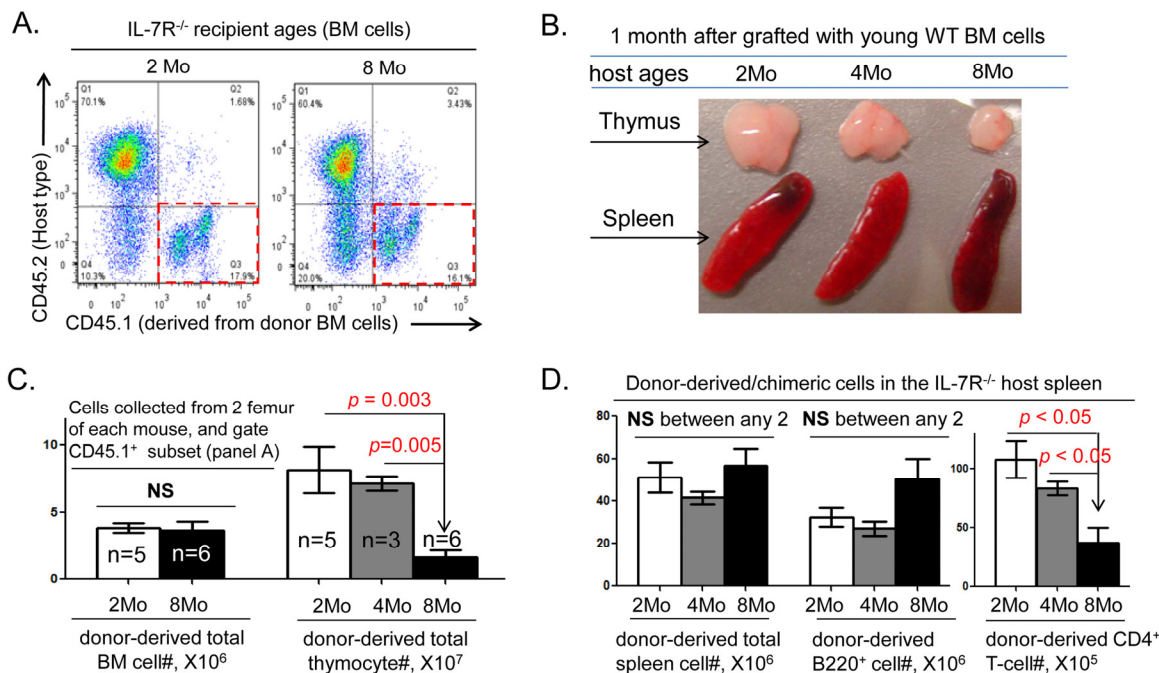


Supplementary Fig. S2. Schematic workflow of the *in vivo* competitive repopulation assay via a cross kidney capsule transplantation setting. Schematic representation of the *in vivo* competitive cross kidney capsule transplantation (cKCT) assay to accumulate both young and old natural thymus-seeding cells in the same grafted fetal thymic lobes *in vivo* and to conduct the competitive *in vitro* assay in a fetal thymic organ culture (FTOC) setting.

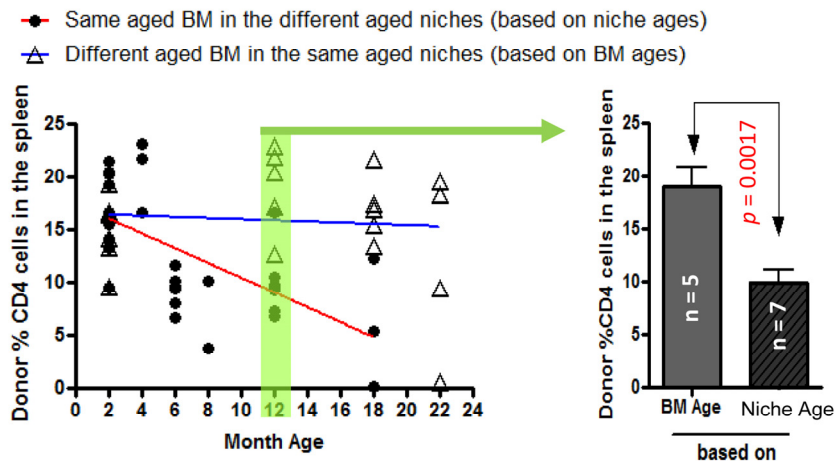
SUPPLEMENTARY FIGURES



Supplementary Fig. S1. Purification of LSK cells before and after FACS-sort. Top panels show live gates; middle panels show lineage and host-CD45.2 (cocktail antibodies) negative and donor-CD45.1⁺ gate; Bottom panels show Sca-1⁺ and c-Kit⁺ gate in Lin⁻ and host-CD45.2⁻, and Donor-CD45.1⁺ population. Left panels show BM cells before sorting; Right panels show BM cell after sorting.



Supplementary Fig. S3. Integrated analysis of CD45.1/ CD45.2 chimerism pre- and post-thymus. One month after BMT of CD45.1⁺ WT young BM progenitors into CD45.2⁺ IL7R^{-/-} recipients of a range of ages, donor-type BM cells, thymocytes, splenic B and T cells were analyzed. **(A)** Representative result of donor-derived CD45.1⁺ BM cells (red boxes) in CD45.2⁺ IL7R^{-/-} hosts of a range of ages; **(B)** Image shows size of the thymus and spleen from IL7R^{-/-} grafted with same WT young BM progenitors; **(C)** Left: donor-derived total BM cell #, cell counting from 2 femur, multiplying panel A % in red boxes; Right: a summary of donor-derived total thymocyte #. "NS = no significant." **(D)** Donor-derived total spleen cell#, B cell# and CD4⁺ T-cell# in the recipients' spleens of a range of ages. (In this supplemental experiment, recipient IL7R^{-/-} mouse number: 2Mo = 5; 4Mo = 3; 8Mo = 6).



Supplementary Fig. S4. Comparing engraftment capacity to repopulate periphery based on LPCs' age or niche cells' age in a CD45 subtype-mismatched BMT model with an unirradiated IL7R^{-/-} mouse as a host.

Left Panel: A linear regression assay of % CD4⁺ T cells in the spleen, derived from donor WT BM cells of different ages in young IL7R^{-/-} host niches (based on donor BM progenitor ages, blue line) and derived from young donor WT BM cells in IL7R^{-/-} host niches of different ages (based on recipient niche ages, red line). Test for equal slopes for the blue and red lines a (2-sided) p-value of 0.009 (significantly different). Each triangle and round represents one recipient animal. Right panel: Donor-derived % CD4⁺ T cells in the spleen of 12-month-old donor WT BM cells in young IL7R^{-/-} host niches (left bar) and young donor WT BM cells in 12-month-old IL7R^{-/-} host niches (right bar). Data show mean ± SEM in bar graph, n = IL7R^{-/-} host animal number.