		CD4	CD8	DP	DN	TCRβ	TCRγδ
Day11	WT YS	5.0±1.8	1.1±0.8	2.2±2.7	91.7±4.9	1.4±1.1	4.5±1.7
	WT PSP	3.5±1.7	0.5±0.2	0.7±0.6	95.4±1.9	0.8±0.3	4.1±1.9
	$Ncx1^{-/-}$ YS	1.5±1.8	15.1±10	48.9±34	34.5±33	24.5±29	5.8±5.1
	$Ncx1^{-/-}$ PSP	3.9±5.6	7.12±5.7	0.8±0.6	88.2±5.4	0.4±0.1	1.9±0.4
Day18	WT YS	4.8±1.8	11±2.9	44.2±14	40.0±12	31.3±9.6	18.7±0.4
	WT PSP	4.3±2.5	17.4±4.4	25.6±18	52.6±16	18.0±6.2	29.3±4.3
	$Ncx1^{-/-}$ YS	4.6±0.6	15.7±0.8	49.5±1.4	30.3±1.4	34±1.4	17.3±8.8
	$Ncx1^{-/-}$ PSP	4.3±1.1	10.0±0.3	46.2±6.6	40.0±5.9	33.6±3.6	6±2.1

Table S1. Frequency of each cell fraction in cultured YS and P-Sp cells.

The non-adherent cell supernatant of YS or P-Sp culture was collected and stained with anti-CD4, CD8, TCRβ, and TCRγδ antibodies. Percentage of each cell fraction among the live cell gate was depicted.

		Cell #	Thymus	SPL
WT YS	DP	1.2M	0/2	0/2
	DN	0.9M	2/2	2/2
WT PSP	DP	1.5M	0/2	0/2
	DN	0.5M	1/1	1/1

Table S2. DN cells of YS-and P-Sp derived T progenitor cells engraft in the thymus and spleen.

$CD4^+CD8^+$ double positive (DP) cells or double negative (DN) cells derived from YS or P-Sp were injected into NOG neonates and engraftment in recipient thymus and/or spleen was analyzed 2 weeks after injection

	Only B-1 cell engraftment	Only T cell engraftment	No engraftment	Total number of transplant
WT YS	5	1 (4ee)	9	15
WT P-Sp	6	0	8	14
$Ncx1^{-/-}$YS	0	2 (2ee)	11	13
$Ncx1^{-/-}$P-Sp	0	0	10	10

Table S3. Freshly isolated YS cells engrafted in the recipient NOG neonates with only B or T cell lineage. Freshly isolated E9.5 YS and P-Sp cells were injected into NOG neonates. 1 to 5 ee of each sample was transplanted. Peripheral blood was examined for engraftment of donor cell type.

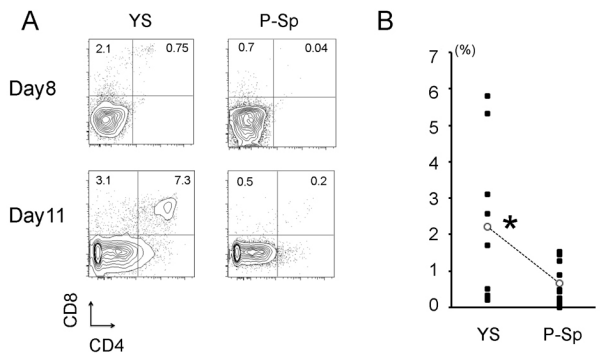

Figure S1. DP cells appear earlier in the YS culture than in the P-Sp culture.

E9.5 YS and P-Sp cells were cultured on OP9-DL1 and the supernatant was analyzed. (A) Time course appearance of cell types in the supernatant of the YS (left) and P-Sp (right) culture. A representative FACS dot plot is depicted. (B) Percentage of DP cells at day 11 culture of YS and P-Sp. YS culture produced more DP cells than P-Sp culture at this time point. (p<0.05, n=11)

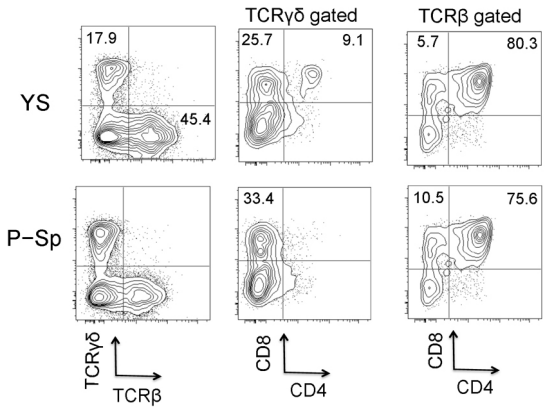

Figure S2. TCR$\alpha\beta^+$ and TCR$\gamma\delta^+$ cell phenotypes on day18 YS and P-Sp culture with OP9-DL1.

E9.5 YS and P-Sp cells were cultured on OP9-DL1 and the supernatant was analyzed on day 18 of co-culture. There were good number of TCR$\alpha\beta^+$ and TCR$\gamma\delta^+$ cells and the expression of CD4 or CD8 was examined in each TCR$^+$ cells. Representative FACS dot plots are depicted from 3 independent experiments for each YS and P-Sp culture.

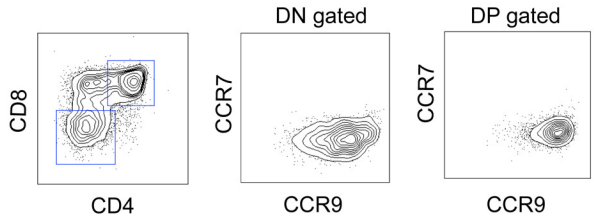

Figure S3. Cultured DP and DN cells derived from YS and P-Sp express CXCR9.
DP and DN cells derived from YS and P-SP on OP9-DL1 culture were stained with monoclonal antibodies for thymic homing receptor, CCR7 and CCR9. Both DP and DN cells expressed CXCR9. One representative FACS dot plot of YS is depicted. P-Sp derived cells showed similar phenotype with YS-derived cells (n=3).

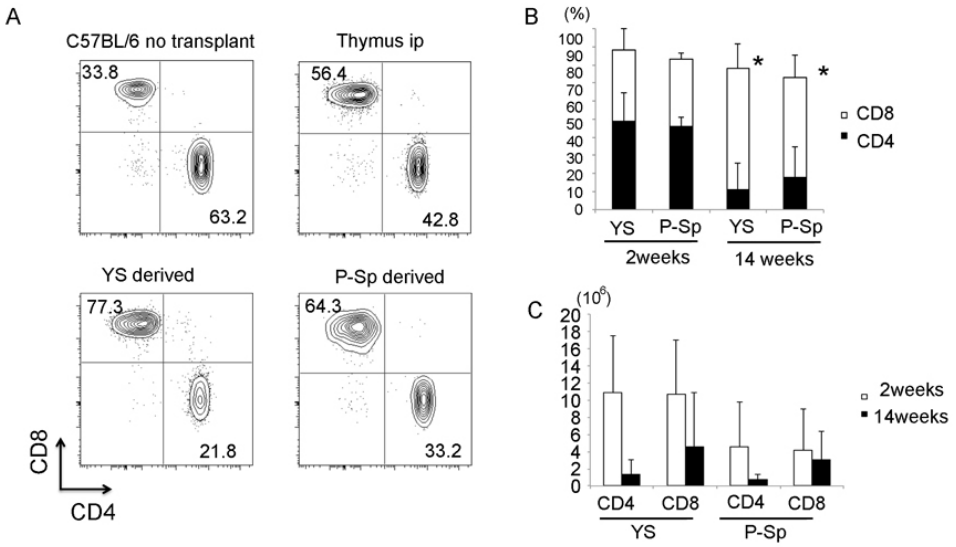

Figure S4. Donor CD4/8 ratio declined over time after transplantation.

(A) CD4 and CD8 ratio in the spleen. Upper left: spleen from normal C57BL/6 mouse. Upper right: spleen from NOG mice transplanted with adult thymic cells, 32weeks after injection. Lower left: spleen from NOG mice transplanted with YS-derived T cells, 14 weeks after injection. Lower right: spleen from NOG mice transplanted with P-Sp-derived T cells, 14 weeks after injection. All dot plots were within TCRβ^+ population. (B) Percentages of CD4 and CD8 expressing cells in donor derived TCRβ^+ cells in the recipient spleen. The percentage of CD4 was significantly decreased at 14 weeks compared at 2 weeks (p<0.05). (C) Total numbers of YS and P-Sp derived CD4$^+$ and CD8$^+$ cells in the recipient spleen.