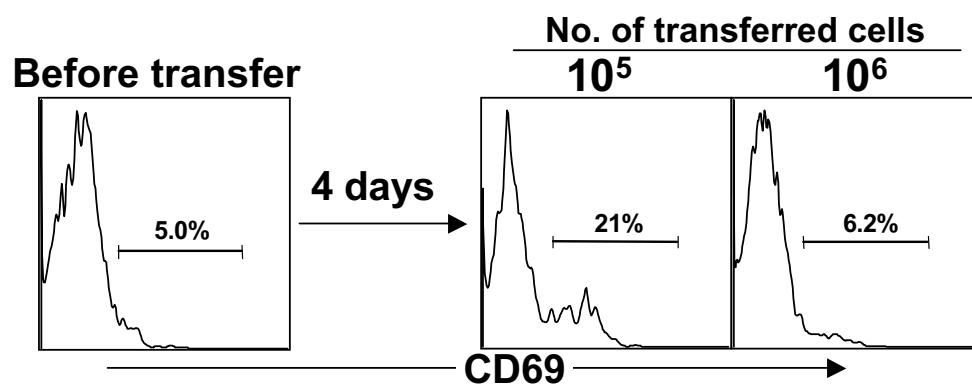
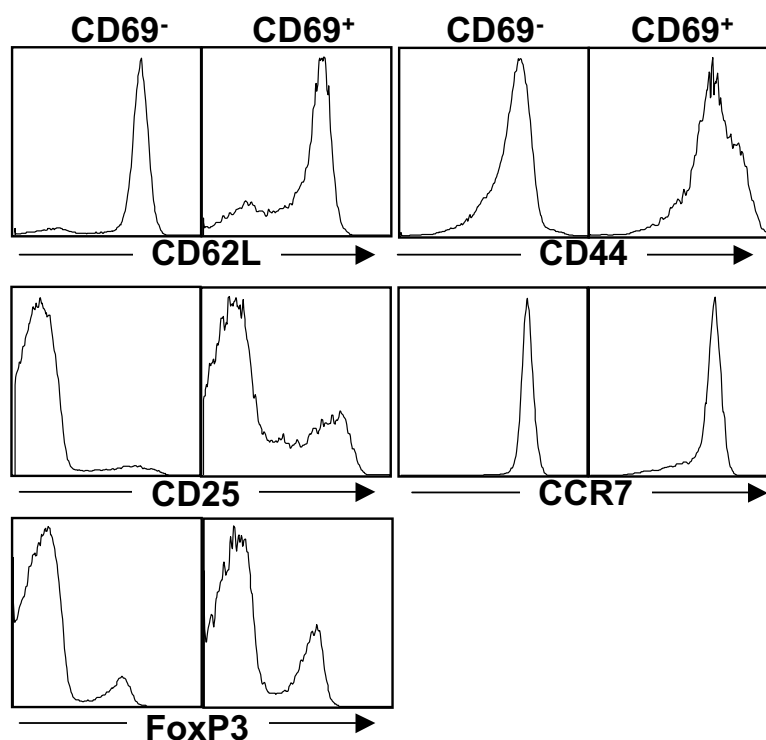


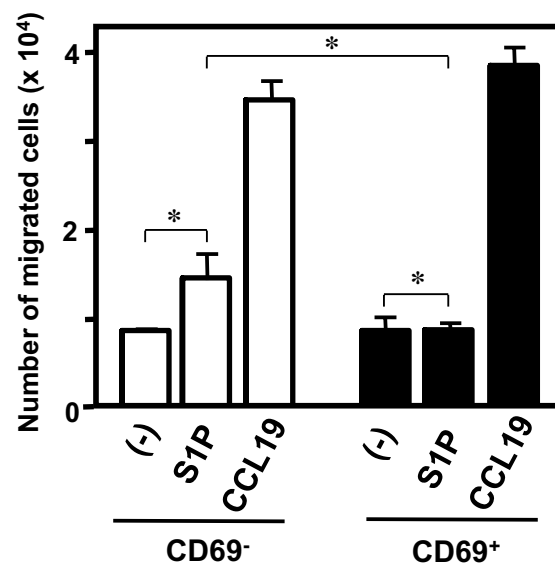
**Figure S1 CD62L and CD44 expression of DO11.10 T cells from DO11.10 TCR-Tg Rag2<sup>-/-</sup> mouse.** LN cells from DO11.10 TCR-Tg Rag2<sup>-/-</sup> mouse were stained with anti-CD44, anti-CD62L, anti-CD4, and KJ1-26 mAbs as described in Methods and subjected to flow cytometry. The staining pattern of cells gated for CD4<sup>+</sup> and KJ1-26<sup>+</sup> is shown.



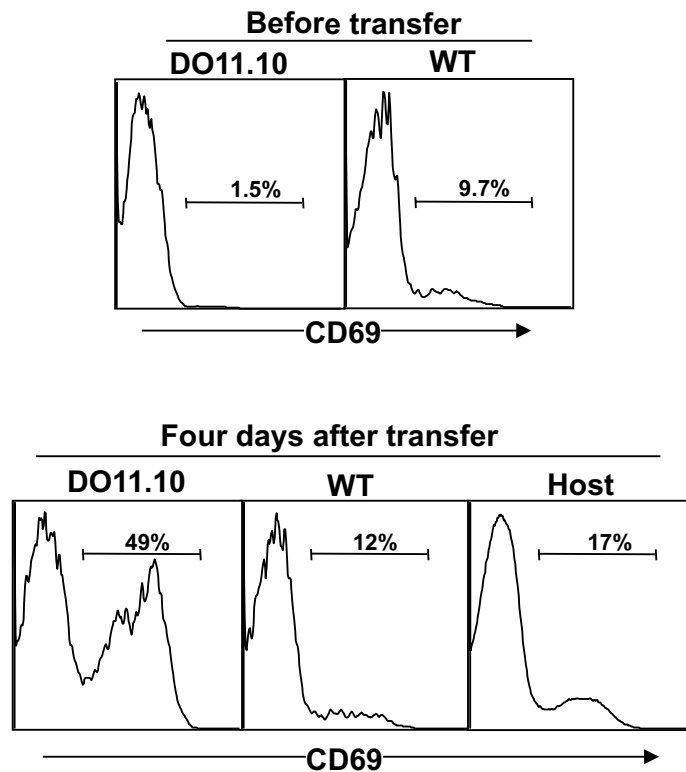
**Figure S2 CD69 expression on 2B4 T cells after transfer.** Indicated numbers of CFSE-labeled transgenic TCR positive T cells from 2B4 TCR transgenic Rag2<sup>-/-</sup> mice were transferred to B10.BR mice intravenously. Four days after the transfer, cells from LNs (pool of cervical, brachial, axillary, inguinal, and popliteal LNs) were stained with anti-CD4, 2B4, and anti-CD69 mAbs and subjected to flow cytometry. CD69 expression of transferred CD4<sup>+</sup>2B4<sup>+</sup> cells is shown. Three animals for each group were independently analyzed with similar results and the results of one representative analysis are shown.



**Figure S3 Phenotype of CD69 negative and positive populations of CD4<sup>+</sup> T cells.** Lymphocytes of peripheral LNs (pool of cervical, brachial, axillary, inguinal, and popliteal LNs) in C57BL/6 wild type mice were stained with anti-CD4 and anti-CD69 mAb and antibodies to indicated molecules as described in Methods. CD4<sup>+</sup> cells were further gated for CD69 positive and negative populations and the expression of CD62L, CD44, CD25, CCR7, and Foxp3 was analyzed for each population.



**Figure S4 Impaired migration of CD69<sup>+</sup> naive CD4<sup>+</sup> T cells to S1P.** CD69<sup>-</sup> and CD69<sup>+</sup> naive CD4<sup>+</sup> T cell were purified as the same manner in Figure 7 and washed in DMEM with 0.5% fatty acid free BSA for several times and resensitized for 30 min at 37 °C. CD69<sup>-</sup> and CD69<sup>+</sup> naive CD4<sup>+</sup> T cell (5 x 10<sup>4</sup>cell/50μl) were added to 5-μm-pore size polycarbonate 96-well tissue culture inserts (Costar), with 150μl of medium (-), S1P (10nM, Sigma) and mCCL19 (100μg/ml, R&D) in lower well, and incubated for 3h at 37 °C. Cell number in lower chamber was counted for evaluation of migrated cell. Data are representative of two individual experiment and data indicate mean±S.D (n=3). The migration of CD69<sup>-</sup> naive CD4<sup>+</sup> T cells to S1P versus medium was statistically significant (\*:  $p < 0.05$ , unpaired t test ). The impaired migration of CD69<sup>+</sup> naive CD4<sup>+</sup> T cells to S1P versus medium was statistically significant (\*:  $p < 0.05$ , unpaired t test ). The migration of CD69<sup>-</sup> naive CD4<sup>+</sup> T cells versus CD69<sup>+</sup> naive CD4<sup>+</sup> T cells to S1P was statistically significant (\*:  $p < 0.05$ , unpaired t test ).



**Figure S5 Induction of CD69 expression on transferred DO11.10 T cells in germfree mice.** Mixture of CFSE-labeled DO11.10 T cells ( $1 \times 10^6$  cells) from DO11.10 TCR transgenic Rag2<sup>-/-</sup> mice and CD4<sup>+</sup> T cells ( $1 \times 10^6$  cells) of BALB/c wild type mice were transferred into germ-free BALB/c wild type mice intravenously. Four days after transfer, CD69 expression of transferred DO11.10 T cells, CD4<sup>+</sup> T cells from wild type mice and host CD4<sup>+</sup> T cells in peripheral LNs was analyzed for the expression of CD69. Two animals were independently analyzed for each group with similar results and the results of one representative analysis are shown.

**Table S1 Number of CD69<sup>+</sup> DO11.10 T cells in mice**

Total number of DO11.10 T cells in mice (n)	% of CD69 <sup>+</sup> cells <sup>c</sup>	Number of CD69 <sup>+</sup> cells
1 x 10 <sup>6</sup> cells (n=5) <sup>a</sup>	70.0 ± 3.0%	0.70 ± 0.03 x 10 <sup>6</sup> <sup>d</sup>
1 x 10 <sup>7</sup> cells (n=3) <sup>a</sup>	20.2 ± 1.3%	2.0 ± 0.13 x 10 <sup>6</sup> <sup>d</sup>
1.88 ± 0.30 x 10 <sup>7</sup> cells (n=5) <sup>b</sup>	1.86 ± 0.27%	0.36 ± 0.09 x 10 <sup>6</sup> <sup>e</sup>

<sup>a</sup>Number of transferred DO11.10 T cells from DO11.10 TCR transgenic Rag2-deficient mice to BALB/c wild type mice.

<sup>b</sup>Number of DO11.10 T cells in DO11.10 TCR transgenic Rag2-deficient mice. Cells of lymph nodes (cervical, brachial, axillary, inguinal, popliteal, and mesenteric lymph nodes) and spleen were pooled, counted, stained with anti-CD4 and KJ1-26 mAbs, and subjected to flow cytometry. The number of DO11.10 T cells in mice was by multiplying the total number of lymph node and spleen cells by the percentage of CD4<sup>+</sup>KJ1-26<sup>+</sup> cells. Means ± S.E. are shown.

<sup>c</sup>Cells of lymph nodes (cervical, brachial, axillary, inguinal, popliteal, and mesenteric lymph nodes) and spleen were stained with anti-CD4, KJ1-26 and anti-CD69 mAbs then subjected to flow cytometry. Percent of CD69 expression of CD4<sup>+</sup>KJ1-26<sup>+</sup> cells (Mean ± S.E.) are shown.

<sup>d</sup>Number of CD69<sup>+</sup> cells was calculated by multiplying the total number of DO11.10 T cells in mice by the percentage of CD69<sup>+</sup> cells. Means ± S.E. are shown.

<sup>e</sup>Number of CD69<sup>+</sup> cells in each mouse was calculated by multiplying the total number of DO11.10 T cells in mouse by the percentage of CD69<sup>+</sup> cells. Means ± S.E. are shown.