Supplemental Material and Methods

Thoracic duct canulation

Differentially labeled lymphocytes were injected at –18h and –2.5h. At –1h C57BL/6 mice were fed with 700 µl of olive oil by gavage. Mice were subsequently anaesthetized by i.p. injection of ketamine (50 mg/kg) and xylazine (10 mg/kg), the peritoneal cavity was opened by an infradiaphragmatic incision and the abdominal contents where retracted into the right side of the peritoneal cavity. Using a stereomicroscope, the cysterna chili, located dorsal to the left renal lymph node, was identified and further exposed by dissection. A PE-10 polyethylene catheter connected to a 1ml syringe (both heparinized) was inserted into the cysterna chyli and lymph was slowly aspirated over 10-20 min. Lymph cells were washed once with PBS, stained with TCRβ-APC and B220-PerCP and analyzed by flow cytometry.

Assessment of homed lymphocyte dwell time in LNs

To analyze the effect of S1PR agonists on T cell emigration from SLO, GFP^+ T cells were adoptively transferred into BALB/c mice at time = 0h. 4h later, mice were injected i.v. with mAb Mel-14 (150 µg) to neutralize L-selectin, an essential receptor for lymphocyte homing to all SLO other than the spleen. The effect of Mel-14 was assessed by injecting a second, TRITC-labeled population 30 min after Mel-14 administration. mAb-induced blockade of homing was virtually complete in PLN and pronounced, but not complete in MLN and PP (where α4β7 integrins partially substitute for L-selectin). Thus, GFP^+ T cells could only home to PLN during the initial 4h window before Mel-14 treatment, and any subsequent decrease in intranodal numbers should be a consequence of T cell exit. To assess the effect of FTY-P on the retention of GFP^+ T cells, mice were treated with FTY-P (1 mg/kg i.p.) or saline 5h and 20h after injection and sacrificed after 24h. PBL and SLO were subsequently analyzed by FACS. To study dwell times of $S1P_1^{-/-}$ or $S1P_1^{+/}$ thymocytes, differentially labeled cells were adoptively transferred and recipients were treated with Mel-14, followed by tissue harvest as described above. Single-cell suspensions were stained with anti-CD4-PerCP and anti-CD8α-APC and analyzed by FACS.