Phoenix-Eco was maintained in Dulbecco’s modification of Eagle medium (Mediatech Inc.) and supplemented with 10% FBS (22). The IL-7 dependent mouse thymocyte cell line D1 was maintained in RPMI 1640 (Mediatech Inc.), supplemented with 10% fetal bovine serum (FBS; HyClone), 50µM β-mercaptoethanol (Invirogen), 100U/mL of penicillin, 100µg/mL of streptomycin (Mediatech Inc.), and 50ng/mL of mouse IL-7 (Pepro Tech).

**DNA Constructs**

The full length murine IL-7 receptor (mIL-7R) was cloned into the retroviral vector pMIG.2 Mutations were selectively introduced into the mIL-7R via PCR to inhibit STAT 5 and p85 binding. Chimeric receptors containing fused extracellular hIL-4R and transmembrane and intracellular domains of mIL-7R were cloned into the pMIG retroviral vector. All constructs were verified by DNA sequencing.²

**Retroviral Infection**

Constructs were transfected into the Phoenix-Eco packaging cell line using FuGene 6 reagent (Roche). The replication incompetent but infectious retroviral supernatant was collected after 48hr and plated onto a RetroNectin (TaKaRa) coated plate. D1 cells were added and infected overnight, after one week GFP positive cells were sorted. The stable cell lines expressing different chimeric receptors were maintained in mIL-7. Bone marrow cells were cultured for 48hr and infected by plating on RetroNectin coated plates with the different retroviral supernatants. The infection was repeated after 72hr.

**MTT assay**

At a density of 5 x 10⁴ cells/well, D1 cells were plated in 96-well plates and incubated for 24 and 48 hr. 8µl of MTT was added (5mg/ml; Sigma) and cells were incubated for 4 to 6hr. 100µl of solubilization solution (Promega) was added, and cells were incubated overnight at 37 °C. Plates were read by spectrophotometer at wavelengths of 570 and 620nm.

**Phospho-STAT5 intracellular staining**

Single cell thymocyte suspensions were prepared from normal C57BL/6 mice. Thymocyte populations were stimulated 20 min with mIL-7, and cells were prepared for intracellular staining of phospho-STAT 5. Permeabilization of the cells was performed in the presence of PE-conjugated anti-phosphor-STAT 5 (Tyr694) (BD Pharmingen). Cells (2 x 10⁶) were incubated for 1 hr in the dark, washed twice in PBS plus 1% FCS, and analyzed on a FACScan.

**Immunoprecipitation and immunoblotting**

Cell lysates were prepared from 1 x 10⁸ D1 cells cultured for 4 hr supplemented with 50ng/mL IL-7 or starved of IL-7. Phospho-stop (Roche) phosphatase inhibitor was added to the cell lysis buffer, calyculin was used to inhibit PTEN. Anti-phosphotyrosine, clone 4G10, was used for immunoprecipitation (Millipore). Protein supernatants were separated by SDS-PAGE and transferred to nitrocellulose. Western blot analysis was performed using anti-phosphotyrosine 449 (Rockland) (1:10,000 dilution in BSA) and anti-phospho Akt (ser473)