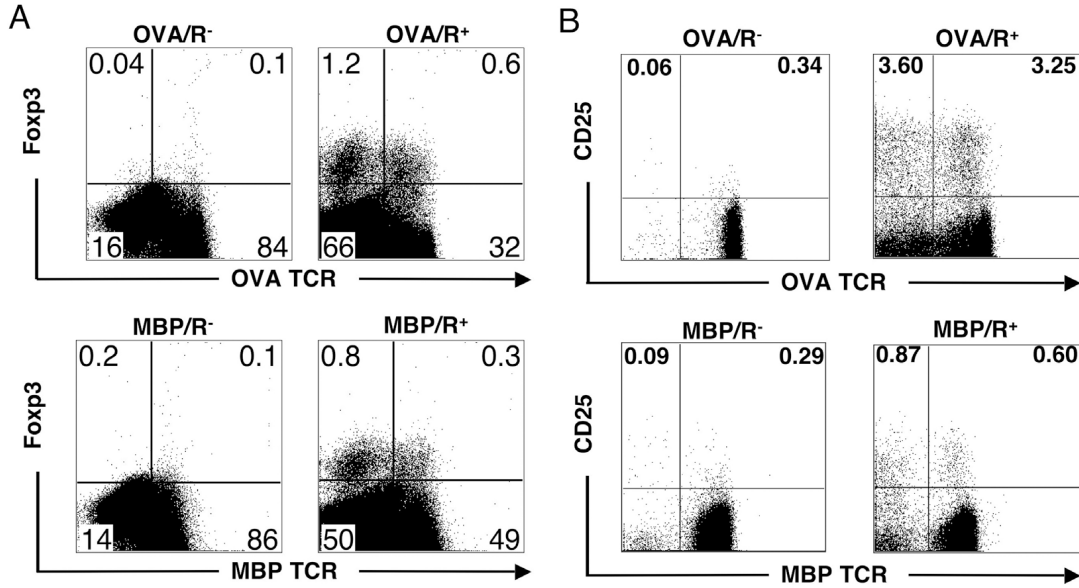


Supplementary Figures and Figure Legends

Supplementary Figure 1

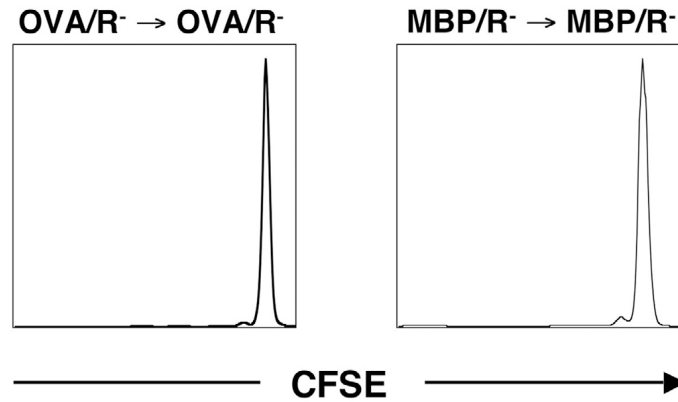


Supplementary Figure 1. Presence of naturally occurring T_R in OVA/R⁺ and MBP/R⁺ mice but not in OVA/R⁻ and MBP/R⁻ mice.

A. Lymph node cells from 4-7 week old OVA/R⁻, OVA/R⁺, MBP/R⁻, and MBP/R⁺ mice were surface-stained for KJ1-26 (clonotype antibody for OVA-specific TCR) or 3H12 (clonotype antibody for MBP-specific TCR), permeabilized and stained for anti-Foxp3 reactivity. Gated on live lymphocytes. Numbers indicate frequency among total live lymphocytes. Representative of ten mice.

B. Lymph node cells from 4-7 week old OVA/R⁻, OVA/R⁺, MBP/R⁻, and MBP/R⁺ mice were stained for KJ1-26 (clonotype antibody for OVA-specific TCR) or 3H12 (clonotype antibody for MBP-specific TCR), and for anti-CD4 reactivity. Gated on CD4⁺ cells. Numbers indicate frequency among total CD4⁺ cells. Representative of ten mice.

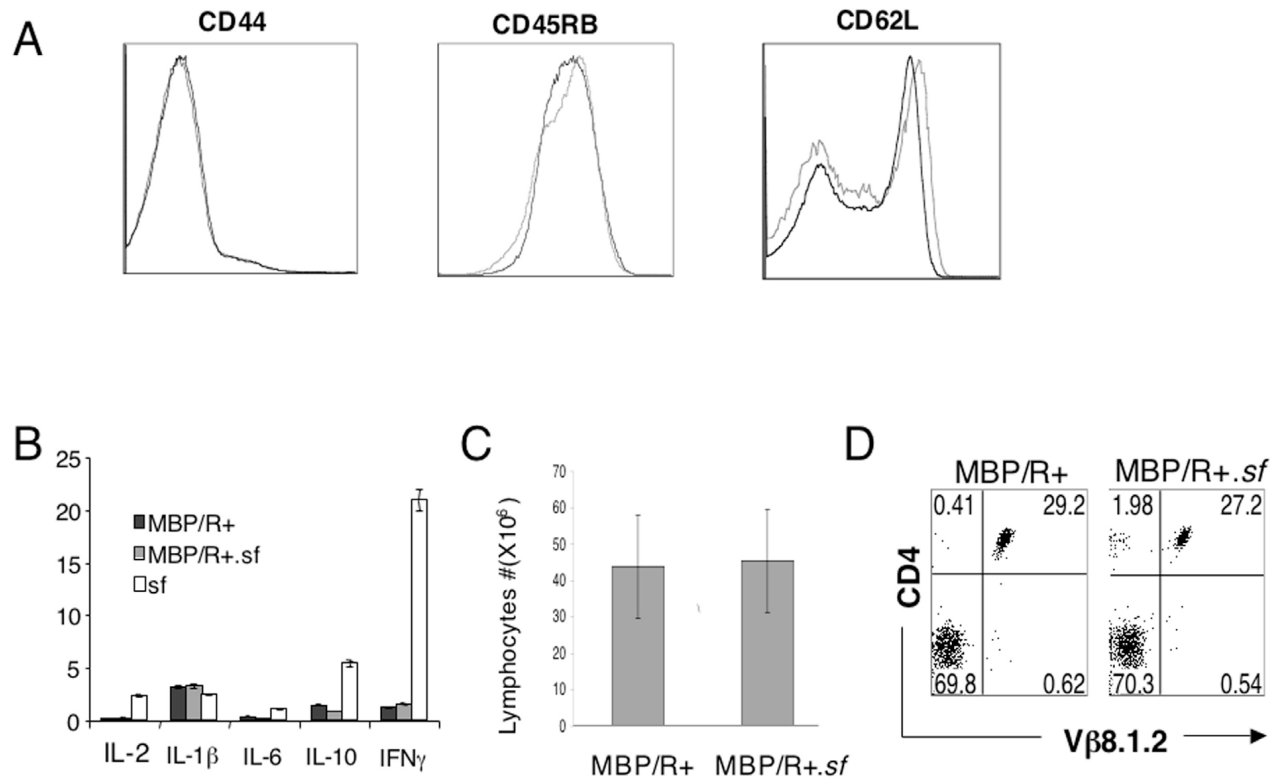
Supplementary Figure 2



Supplementary Figure 2. Absence of homeostatic proliferation due to intraclonal competition.

1×10^7 CFSE-labeled splenocytes from H-2^{d/u} Thy1.1 OVA/R⁻ donor mice were transferred into H-2^{d/u} Thy1.2 OVA/R⁻ mice (left), or 1×10^7 CFSE-labeled splenocytes from H-2^{d/u} Thy1.1 MBP/R⁻ donor mice were transferred into H-2^{d/u} Thy1.2 MBP/R⁻ mice (right) recipients; 12 days after transfer, recipients were sacrificed and single cell suspension was made from brachial, inguinal, axillary lymph nodes. Gated on Thy1.1⁺ CD4⁺ cells. Representative of four mice.

Supplementary Figure 3



Supplementary Figure 3. Characterization of MBP/R⁺.sf mice at the age of termination of the experiments shown in Figures 4-7.

A. No up-regulation of activation/memory cell makers in MBP/R⁺.sf mice compared with MBP/R⁺ mice. Single cell suspensions of lymph nodes from 4 week old MBP/R⁺ and MBP/R⁺.sf mice were stained with anti-CD4 and the indicated T cell activation/memory markers. Black: MBP/R⁺, grey: MBP/R⁺.sf.

B. No up-regulation of inflammatory cytokine RNA in MBP/R⁺.sf mice compared with MBP/R⁺ mice. Compare to the massive upregulation of inflammatory cytokines in non-TCR transgenic scurfy mice at 3 weeks of age. Splenic RNA from 4 week old MBP/R⁺.sf and MBP/R⁺ mice, and 23 day old non-transgenic scurfy mice, were transcribed into

cdNA, and real-time PCR was used to compare the relative mRNA level indicated cytokines. Among these cytokines, IL-1 β is not significantly different among three groups, and all other cytokines are up-regulated in non-transgenic scurfy mice, while comparable between MBP/R⁺ mice and MBP/R⁺.*sf* mice.

C. Comparable number of lymphocytes in 4 week-old MBP/R⁺ and MBP/R⁺.*sf* mice.

Spleen and LN (superficial cervical, axillary, brachial and inguinal) were made into single cell suspension and total cell number was counted. Lymphocyte number was calculated by the percentage of lymphocytes in the total cell population, as determined by FACS analysis. (n=5 in each group)

D. Comparable percentage of T cells expressing TCR encoded by endogenous TCR receptor genes in 4 week old MBP/R⁺ and MBP/R⁺.*sf* mice. Peripheral blood samples were stained with anti-CD4 and anti-TCR V β 8.1.2(transgenic β chain). The numbers in each quadrant represent the percentage of cells in the lymphocyte gate (FSC \times SSC) from red blood cell-lysed samples. Representative of four mice in each group.