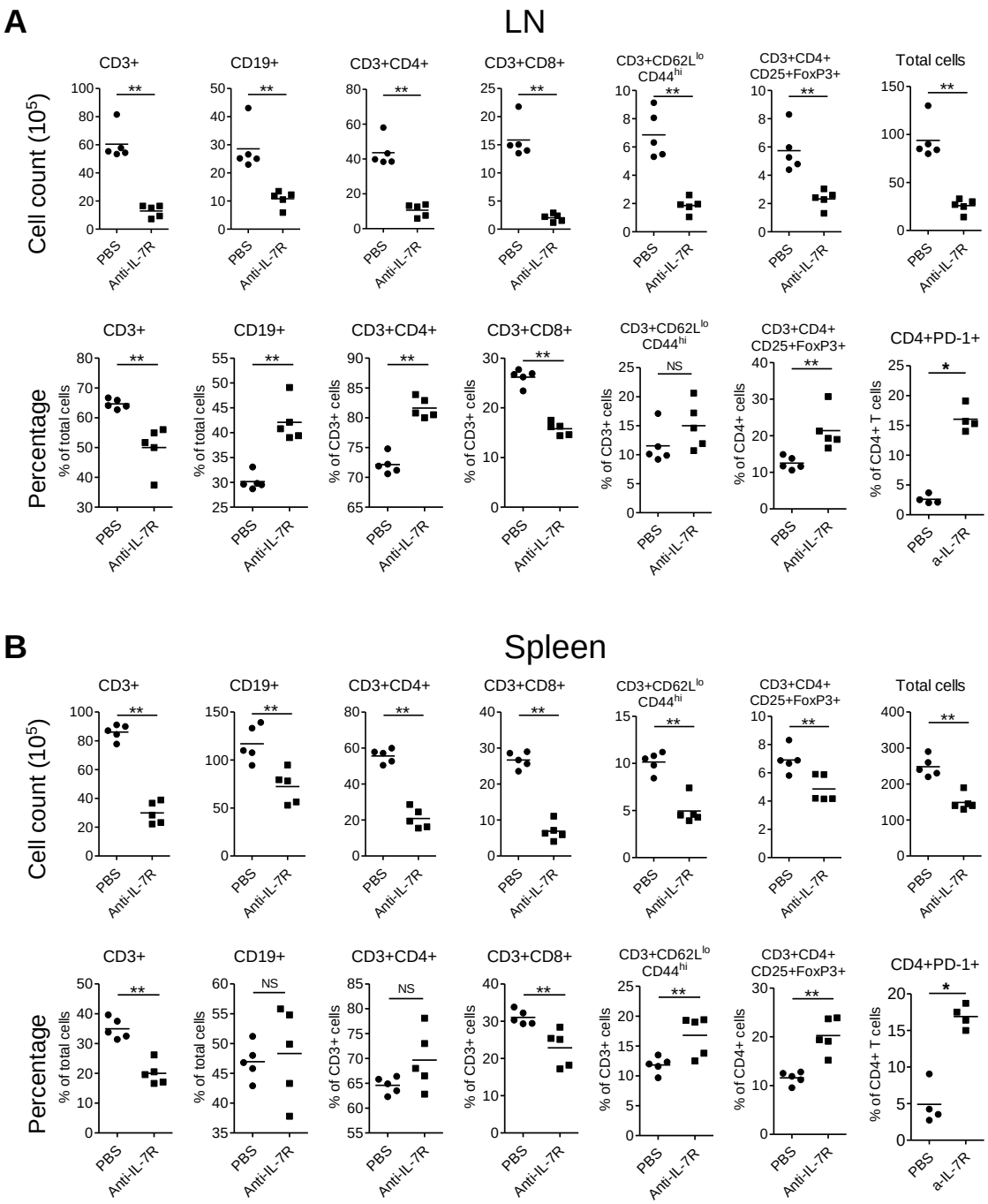
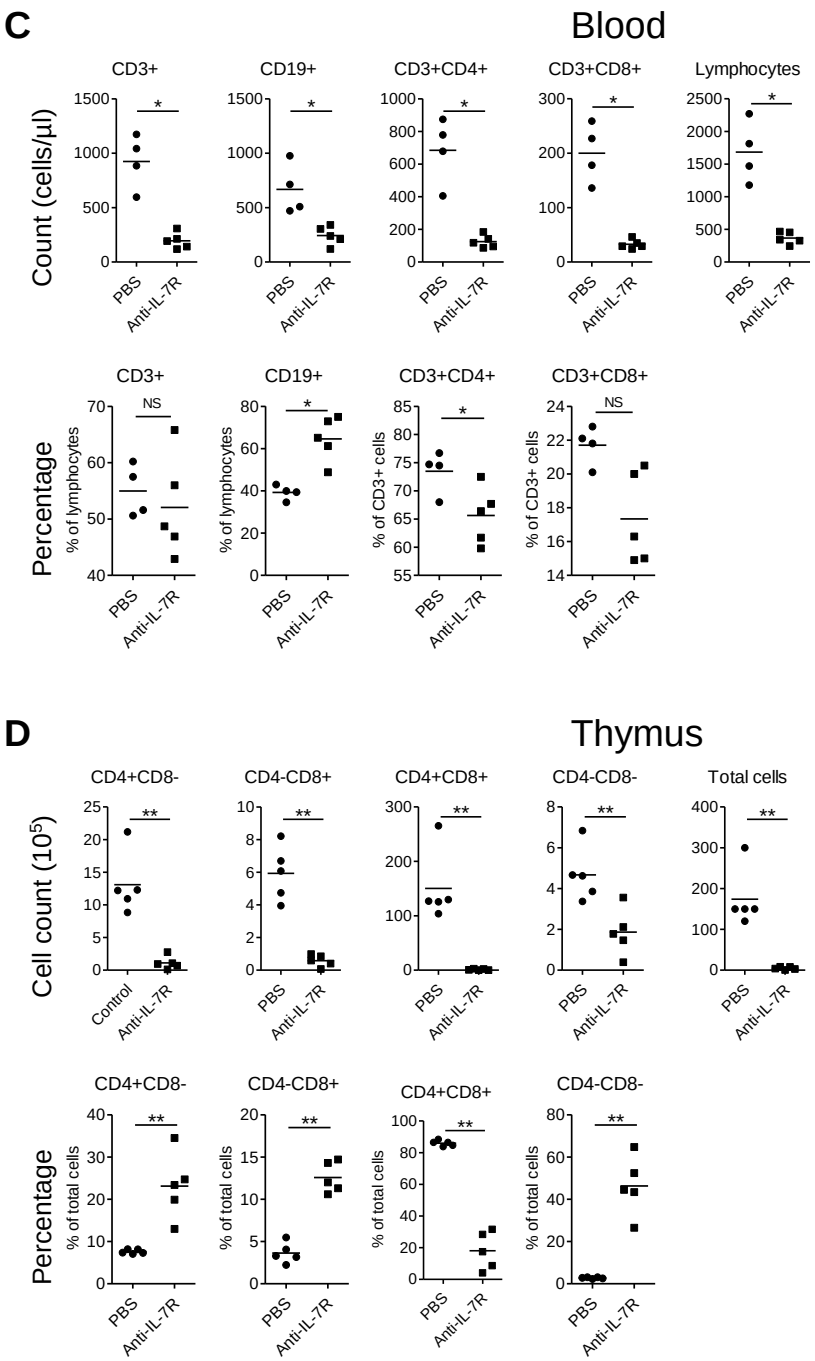
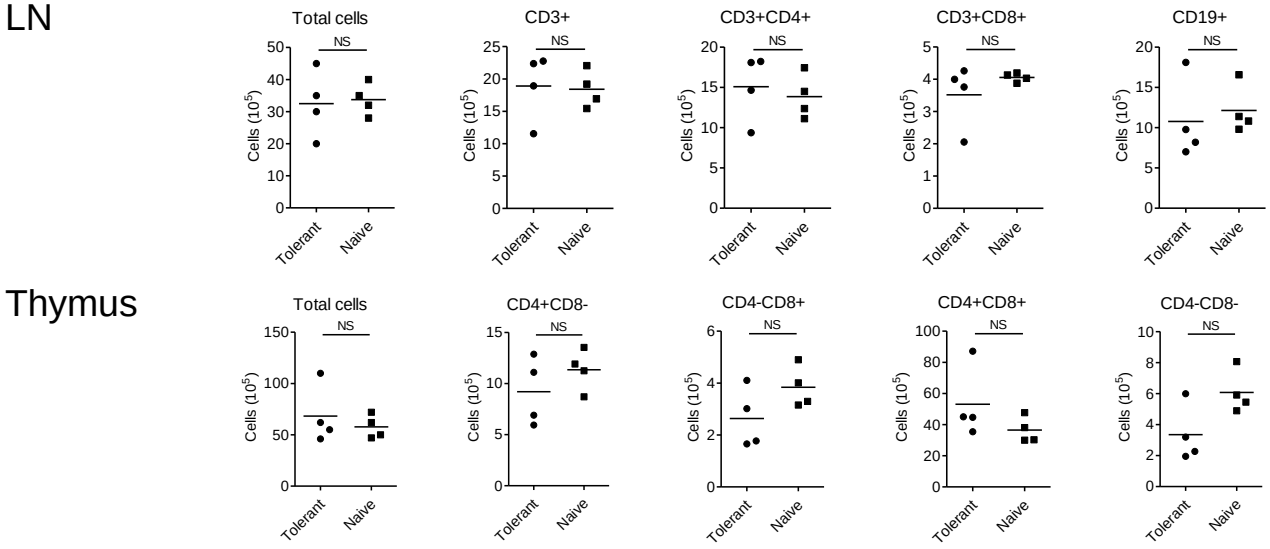
SUPPLEMENTARY FIGURE 1





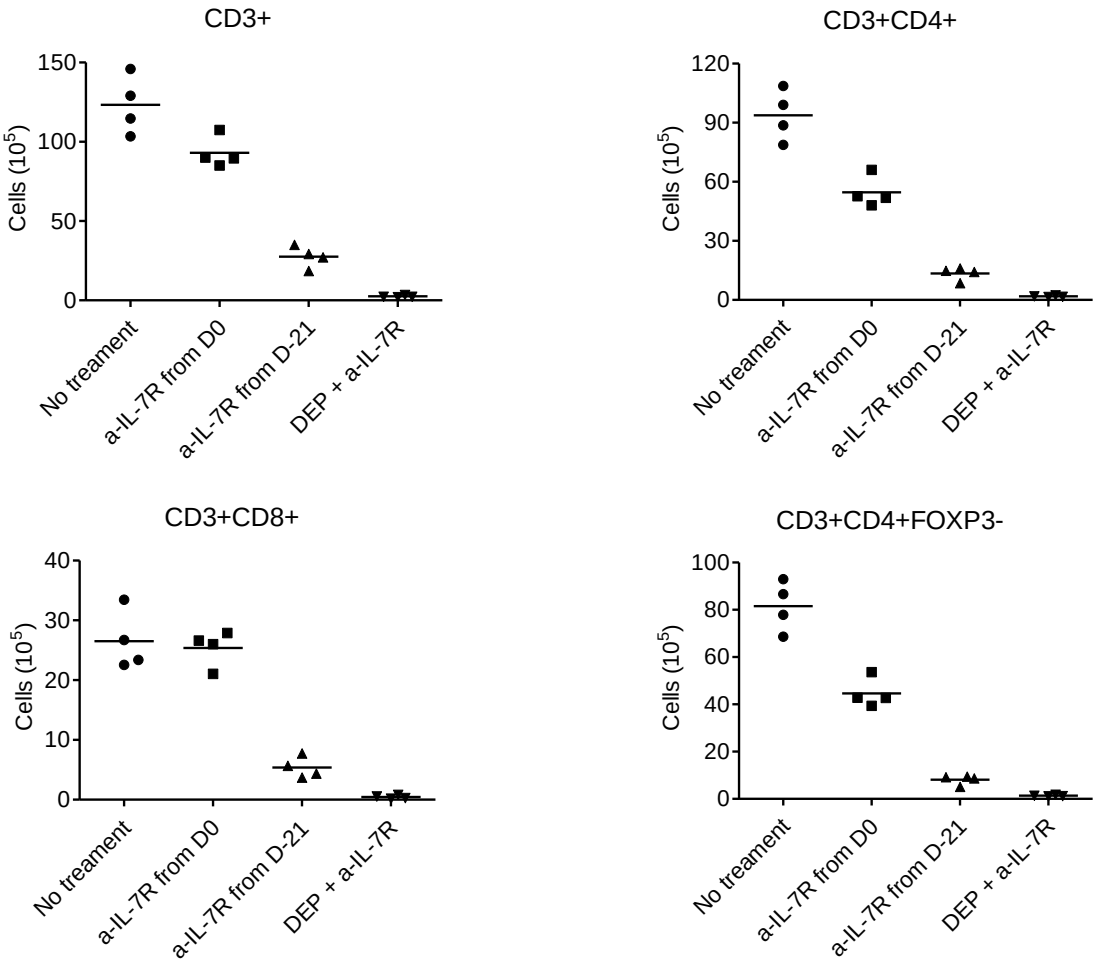
Supplementary Figure 1:
IL-7R blockade reduces almost all lymphocyte subset numbers and increases Treg frequency. Naïve male BALB/c mice 8 weeks of age were given either PBS or anti-IL-7Rα mAb 400 µg qod for 3 weeks and sacrificed. Shown are the absolute numbers and the percentages of different lymphocyte subpopulations in the lymph nodes (**A**), spleen (**B**), blood (**C**), and thymus (**D**). *: $p<0.05$, **: $p<0.01$.

SUPPLEMENTARY FIGURE 2



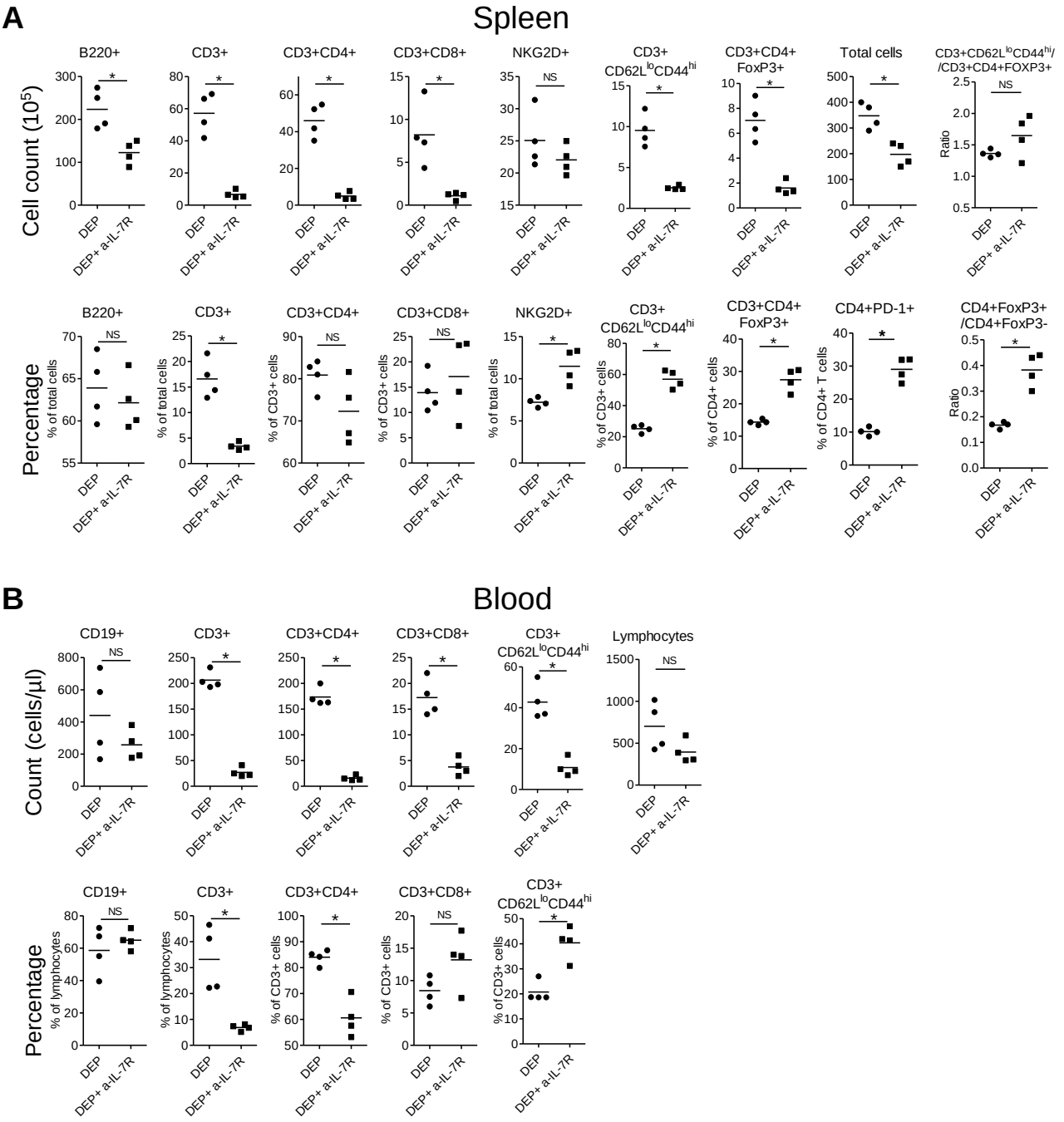
Supplementary Figure 2:
Lymphocyte reconstitution following the cessation of anti-IL-7Rα mAb treatment in mice tolerant to islet allograft. BALB/c mice received islet graft from C57BL/6 donors. Anti-IL-7Rα mAb was given to BALB/c recipients from D-21 before graft to PTD30, inducing islet graft tolerance. Tolerant mice sacrificed at PTD180 and naïve mice of similar ages had comparable numbers of total lymphocytes, T cells, and B cells in their lymphoid organs, demonstrating that lymphocyte reconstitution had occurred after the end of anti-IL-7R treatment.

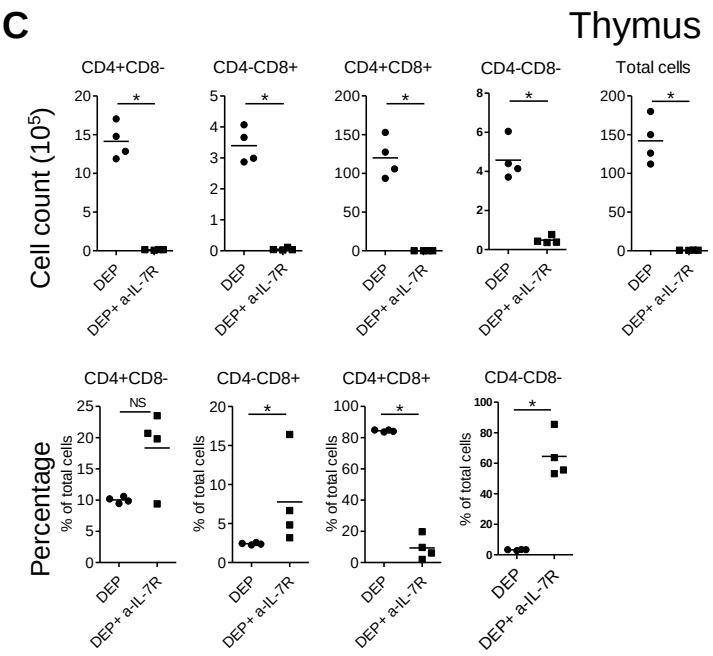
SUPPLEMENTARY FIGURE 3

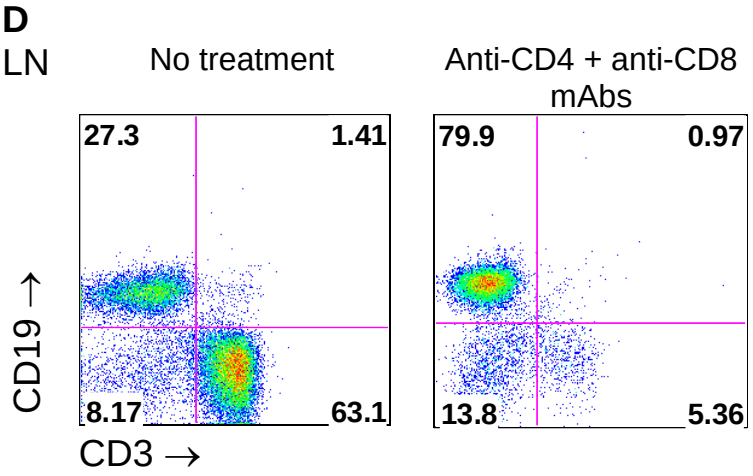


Supplementary Figure 3:
Levels of peripheral T cell depletion in different treatment protocols using IL-7R blockade. BALB/c mice received skin graft from C57BL/6 donors and were treated as indicated. The median graft survivals associated with no treatment, anti-IL-7R alone from D0, anti-IL-7R alone from D-21, and T cell depletion by anti-CD4-8 followed by anti-IL-7R (DEP + a-IL-7R) were 9.5, 10, 12, and 58 days, respectively (see also Figure 2). Shown here are the absolute numbers of T cells and T cell subsets in the spleen of skin graft recipients under these treatment protocols at about 2 weeks post-graft. We observed that a profound reduction of total T cells and T cells subsets, including CD4+FOXP3- effector T cells such as achieved with the “DEP + a-IL-7R” treatment protocol was necessary for the prolongation graft survival.

SUPPLEMENTARY FIGURE 4

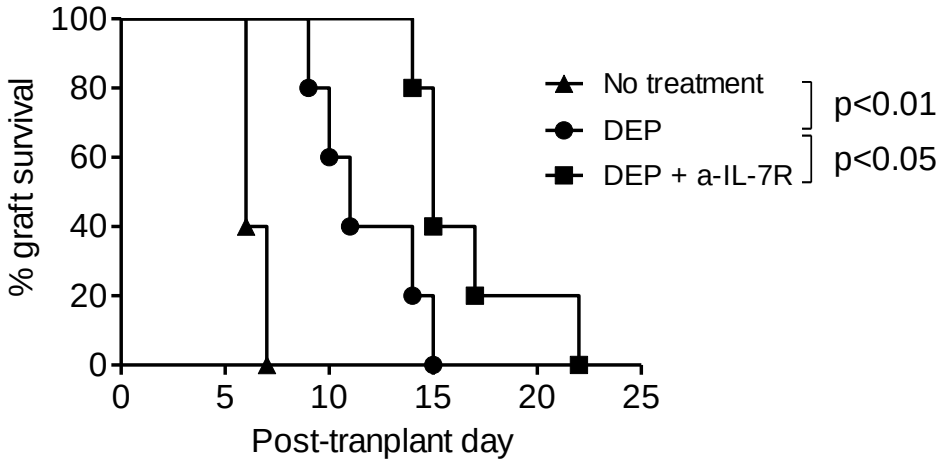






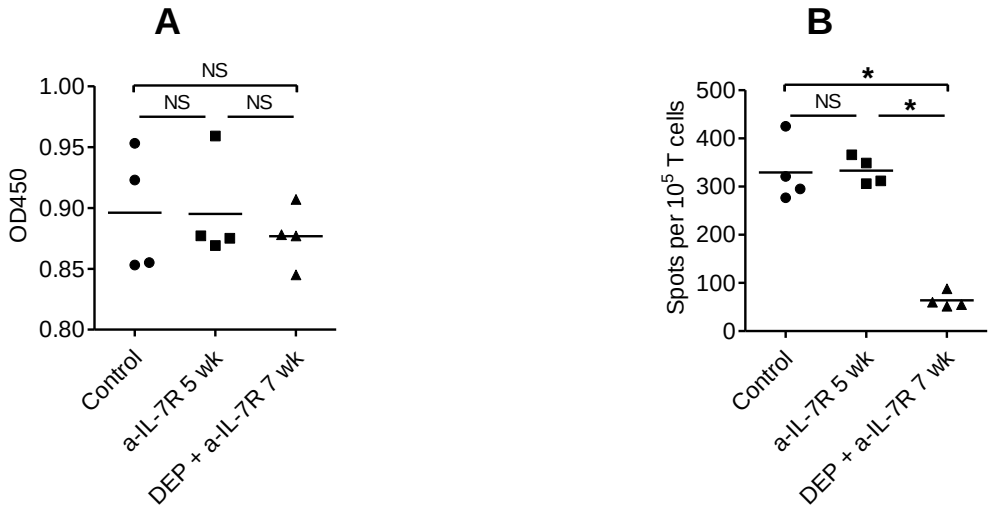
Supplementary Figure 4:
IL-7R blockade following T cell depletion inhibits lymphocyte reconstitution. (**A–C**) C57BL/6 skin was transplanted to BALB/c recipients which were treated with T cell depletion by a combination of anti-CD4 and anti-CD8 mAbs, followed by either isotype Ig (DEP) or anti-IL-7Rα mAb (DEP + a-IL-7R). These two groups of mice were then sacrificed on PTD35 for lymphocyte phenotyping. The absolute numbers and the percentages of different lymphocyte subpopulations in the spleen (**A**), peripheral blood (**B**), and thymus (**C**) are compared between the two groups. See also Figure 3A and B for the LN. (**D**) Representative FACS plot of the LN of a mouse having received an injection of anti-CD4 and anti-CD8 mAbs 100 μg each and sacrificed 4 days later shows that the combination of anti-CD4 and anti-CD8 effectively depletes T cells but not B cells. *: $p<0.05$.

SUPPLEMENTARY FIGURE 5



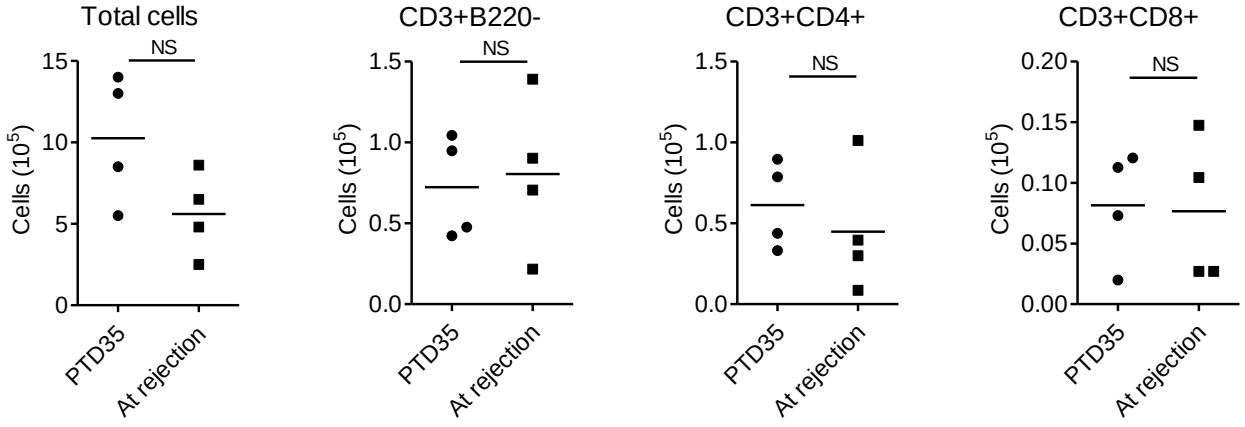
Supplementary Figure 5:
IL-7R blockade following T cell depletion prolongs skin graft survival in alloimmunized mice. C57BL/6 skin was transplanted to BALB/c recipients without treatment, grafts were rejected within 10 d. One month after first graft, these alloimmunized mice received a second skin graft from C57BL/6 mice and were divided into 3 groups: no treatment, T cell depletion by anti-CD4 and anti-CD8 mAbs (DEP), and T cell depletion by anti-CD4 and anti-CD8 mAbs followed by anti-IL-7Rα mAb (DEP + a-IL-7R). Skin graft median survival time (MST) of these 3 groups were 6, 11, and 15 d, respectively.

SUPPLEMENTARY FIGURE 6



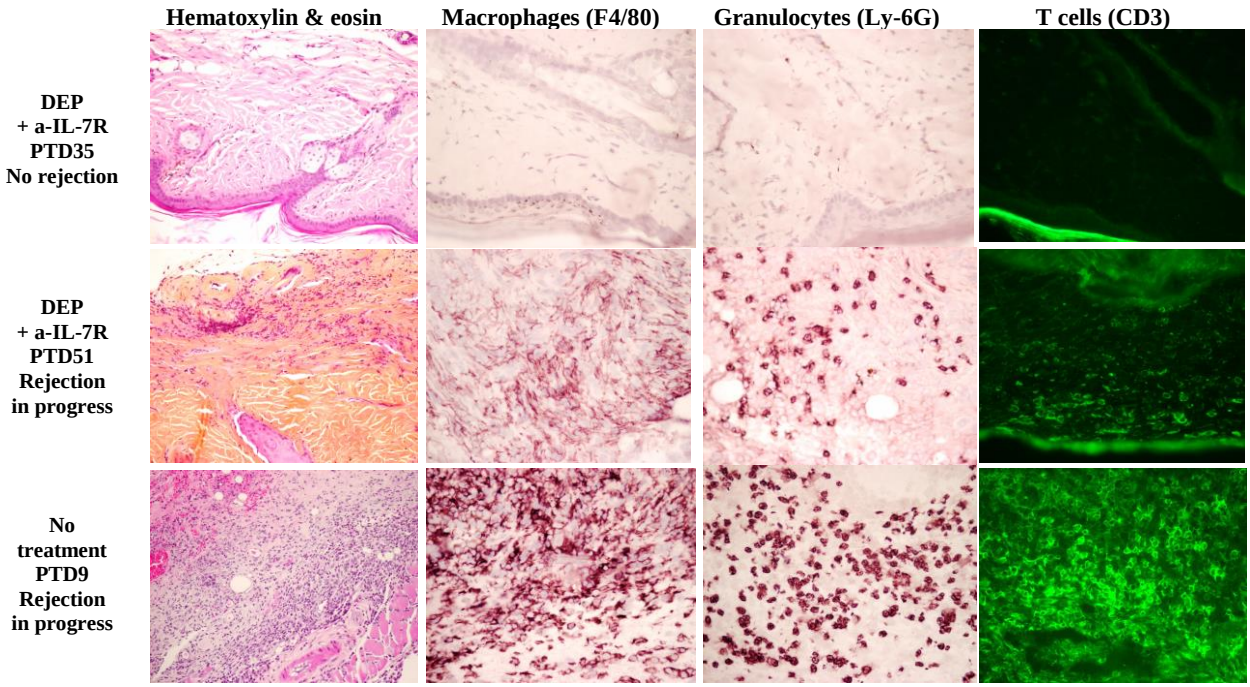
Supplementary Figure 6:
Effects of anti-IL-7R mAb alone or in combination with T cell depletion on previously acquired antiviral immunity. Naïve BALB/c mice received 2 injections of an Adenovirus vector serotype 5 (AdV5) at 4 weeks apart. Two weeks after the second injection, mice received PBS for 5 weeks (control), anti-IL-7Rα mAb alone 400 µg qod for 5 weeks, or T cell depletion by a combination of anti-CD4 and anti-CD8 mAb at 200 µg for 2 injections at D0 and D2 and then anti-IL-7Rα mAb 400 µg qod for 7 weeks (DEP + a-IL-7R). (**A**) Anti-IL-7R treatment, either alone or in combination with T cell depletion, did not affect anti-adenovirus antibody concentrations as measured by ELISA. (**B**) IFN-γ ELISPOT showed that anti-IL-7Rα mAb given alone did not alter antiadenoviral memory T cell frequency, but the combined use of anti-IL-7R and T cell depletion for a more prolonged period of time significantly reduced antiadenoviral memory T cell frequency. *: $p<0.05$.

SUPPLEMENTARY FIGURE 7



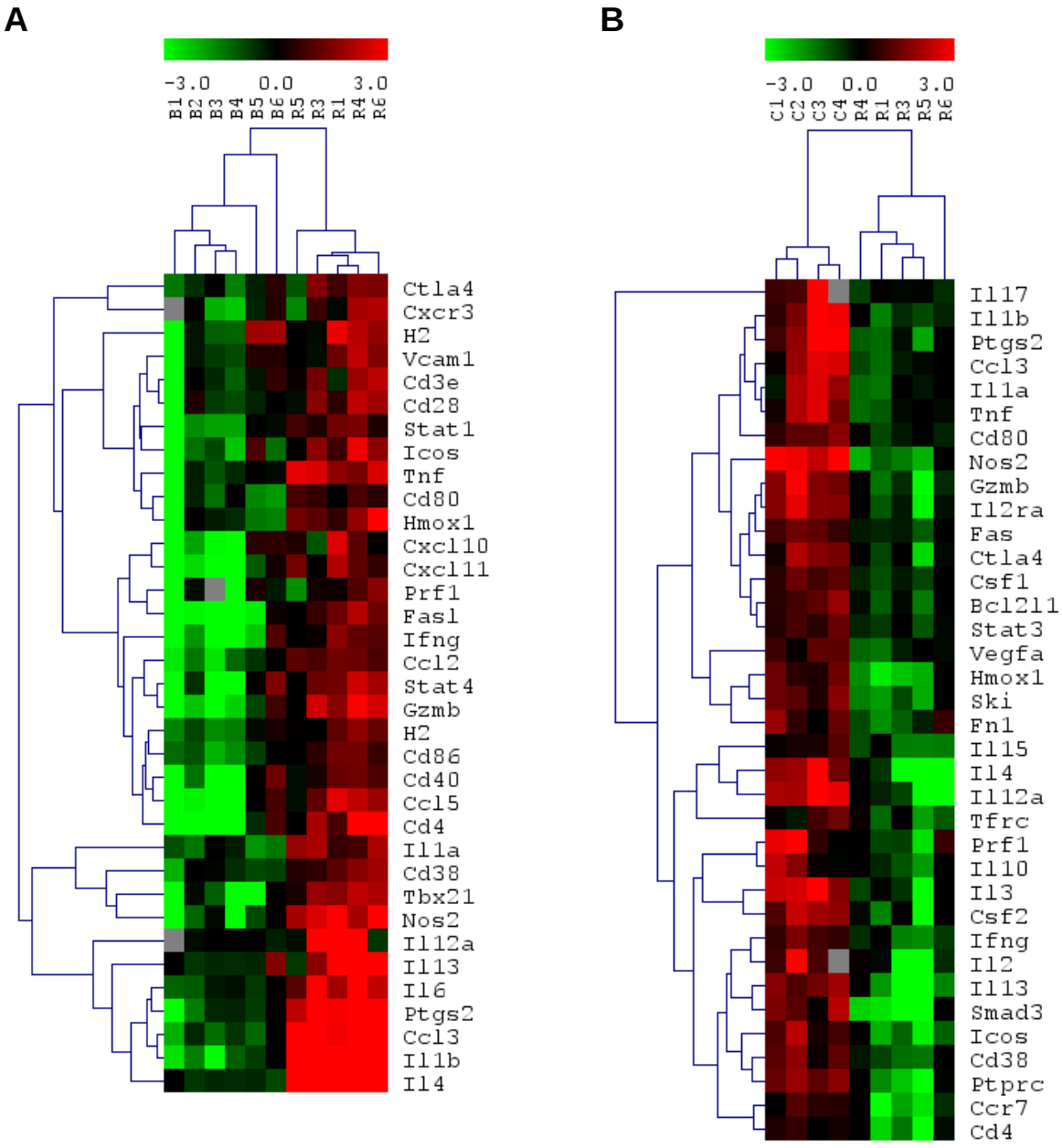
Supplementary Figure 7:
Absence of lymphocyte reconstitution at the time of skin graft rejection. BALB/c mice received skin graft from C57BL/6 donors and were treated with IL-7R blockade following T cell depletion by anti-CD4 and anti-CD8 mAbs. This treatment strongly prolonged graft survival, however skin grafts were finally rejected. At the time of rejection, lymphocyte and lymphocyte subset numbers were still very low, comparable to those observed earlier on PTD35, when graft were still accepted. Shown are data from the LN, similar results were obtained in other lymphoid organs.

SUPPLEMENTARY FIGURE 8



Supplementary Figure 8:
IL-7R blockade attenuates leukocyte infiltration in rejecting skin grafts. BALB/c mice received skin graft from C57BL/6 donors and were either treated with IL-7R blockade following T cell depletion by anti-CD4 and anti-CD8 (DEP + a-IL-7R) or left untreated. For the treated group, skin grafts were harvested either on PTD35, when they were still accepted or at the time of rejection, usually from PTD50 to PTD65. Rejecting skin grafts from untreated groups were harvested on PTD9. Skin grafts were stained with H&E, F4/80 for macrophages, anti-Ly-6G for granulocytes, and anti-CD3 for T cells; representative photomicrographs are shown here. Accepted skin grafts of treated mice on PTD35 had little leukocyte infiltration (top row). When rejection occurred, skin grafts of treated mice had an influx of macrophages, granulocytes, and T cells (middle row), but the inflammatory infiltration was less intense than that observed in rejecting skin grafts of untreated control mice (bottom row). Original magnification: x200 for H&E stain and x400 for other stains.

SUPPLEMENTARY FIGURE 9



Supplementary Figure 9:
IL-7R blockade attenuates inflammatory gene expression in rejecting skin grafts. BALB/c mice received skin graft from C57BL/6 donor and were either treated with IL-7R blockade following T cell depletion by anti-CD4 and anti-CD8 or left untreated. Accepted skin grafts of treated mice on PTD35 (B1-6), rejecting skin grafts of treated mice at later time points (R1-6), and rejecting skin grafts of untreated mice at PTD9 (C1-4) were harvested and cryopreserved. Total RNA was extracted from these skin graft samples and reverse transcribed. Shown are unsupervised clusterings of differentially expressed genes in skin grafts using mouse immune panel TLDA. The heat maps represent normalized and color-coded relative expression values ($2^{-\Delta\Delta Cq}$) in which red values indicate over-expression and green values indicate under-expression. (**A**) In treated mice, skin grafts undergoing rejection displayed an increased expression of inflammatory genes compared to accepted skin grafts on PTD35. (**B**) However, the levels of inflammatory gene expression in rejecting skin graft of treated mice were still significantly lower than those observed in rejecting skin grafts of untreated control mice.