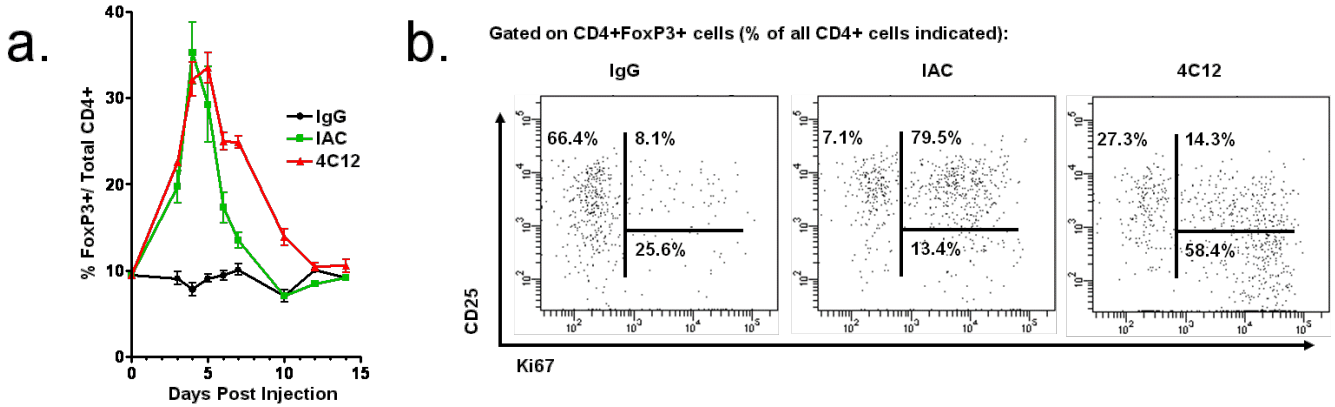
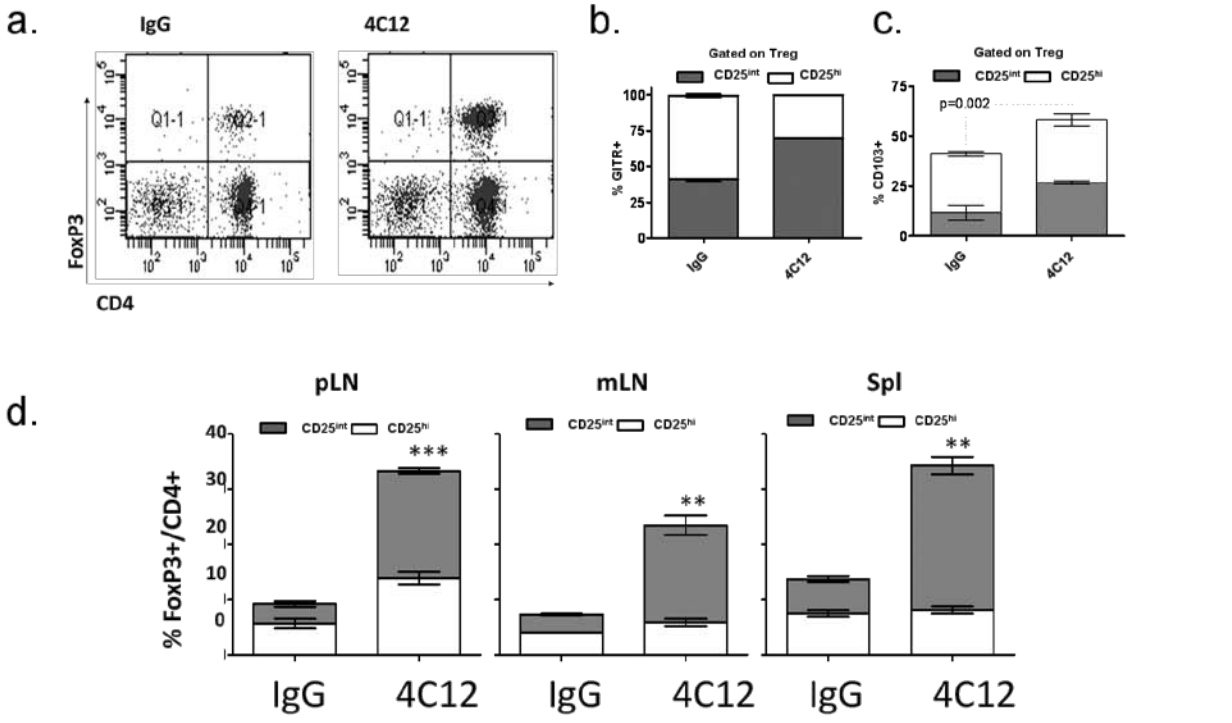
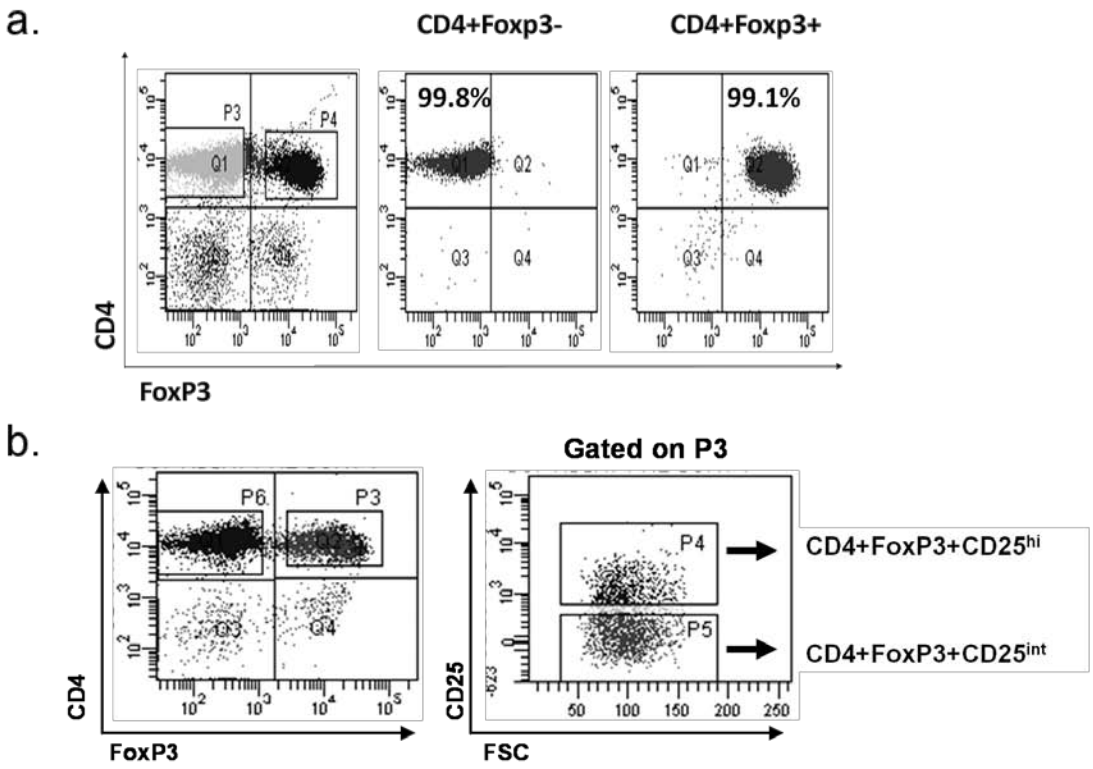


Supplemental Figure 1: Comparison of Treg expanded by treatment with 4C12 or recombinant IL-2/anti-IL-2 antibody complex (IAC). **a)** FIR mice were treated with 4C12 (10 µg) on day 0 or with a series of three injections with IAC on days 0-2. The proportion of FoxP3+ cells within the CD4+ T cell population was measured in the peripheral blood daily by flow cytometry.
b) Splenocytes were isolated from FIR mice 4 on day 4 after treatment with IAC, 4C12 or isotype control IgG. The proportion of CD4+FoxP3+ cells expressing CD25 and the proliferation marker Ki67 are shown.



Supplemental Figure 2: 4C12 treatment induces Treg expansion in all tissues analyzed. **a**) An example of a typical flow cytometry dot plot staining for CD4 and FoxP3 (RFP). CD4+FoxP3+ cells from quadrant Q2-1 were gated for subsequent analysis of $CD25^{hi}$ and $CD25^{int}$ cells as shown in **(b-d)**. **(b)** The ratio of GITR and **c)** CD103 expression among $CD25^{hi}$ versus $CD25^{int}$ Treg in splenocytes 4 days after the indicated treatment. **(d)** Data are represented as mean ± SEM from over 8 independent experiments with at least 3 mice per group per experiment. Paired analysis was performed using the students T-test. ** indicates $p < 0.01$ and *** indicates $p < 0.001$.



Supplemental Figure 3: Example of sorting strategy and results. **a)** Splenocytes were harvested from FIR mice, enriched for CD4+ T cells and sorted on the basis of CD4+ and FoxP3+ (RFP). The left panel illustrates a typical CD4-enriched population of splenocytes. The middle and right panels illustrate representative post-sort analysis for CD4+FoxP3- (P3 gate) and CD4+FoxP3+ (P4 gate) populations. **b)** For some experiments CD4+FoxP3+ cells (gate P3) were sorted based on CD25 expression. Representative plots are shown demonstrating the gating strategy for $CD25^{hi}$ and $CD25^{int}$ sorting.

Condition	Splenocytes	CD4+	LN
1. Unstim.	X	X	X
2. Unstim. + 4C12	X	X	X
3. Unstim + 4C12 crossl.	X		
4. α-CD3	X	X	X
5. α-CD3 + 4C12	X	X	X
6. α-CD3 + 4C12 crossl.	X		
7. α-CD3 + TGF-β	X	X	X
8. α-CD3 + TGF-β + 4C12	X	X	X
9. α-CD3 + RA	X		
10. α-CD3 + RA + 4C12	X		
11. α-CD3 + RA + 4C12 crossl.	X		
12. α-CD3 + α-CD28	X	X	X
13. α-CD3 + α-CD28 + 4C12	X	X	X
14. α-CD3 + IL-2	X	X	X
15. α-CD3 + IL-2 + 4C12	X	X	x
16. α-CD3 + α-CD28 + IL-2	X		
17. α-CD3 + α-CD28 + IL-2 + 4C12	X		
18. α-CD3 + α-CD28 + TGF-β	X		
19. α-CD3 + α-CD28 + TGF-β + 4C12	X		
20. α-CD3 + IL-2 + TGF-β	X		
21. α-CD3 + IL-2 + TGF-β + 4C12	X		
22. α-CD3 + α-CD28 + IL-2 + TGF-β	X		
23. α-CD3 + α-CD28 + IL-2 + TGF-β + 4C12	X		
24. One day in vivo + 4 days in vitro: IL-2 + 4C12 titration	x		

Supplemental Table 1: Conditions tested *in vitro* using various purified lymphocyte populations (indicated) to examine requirements for TNFR25 induced Treg proliferation.