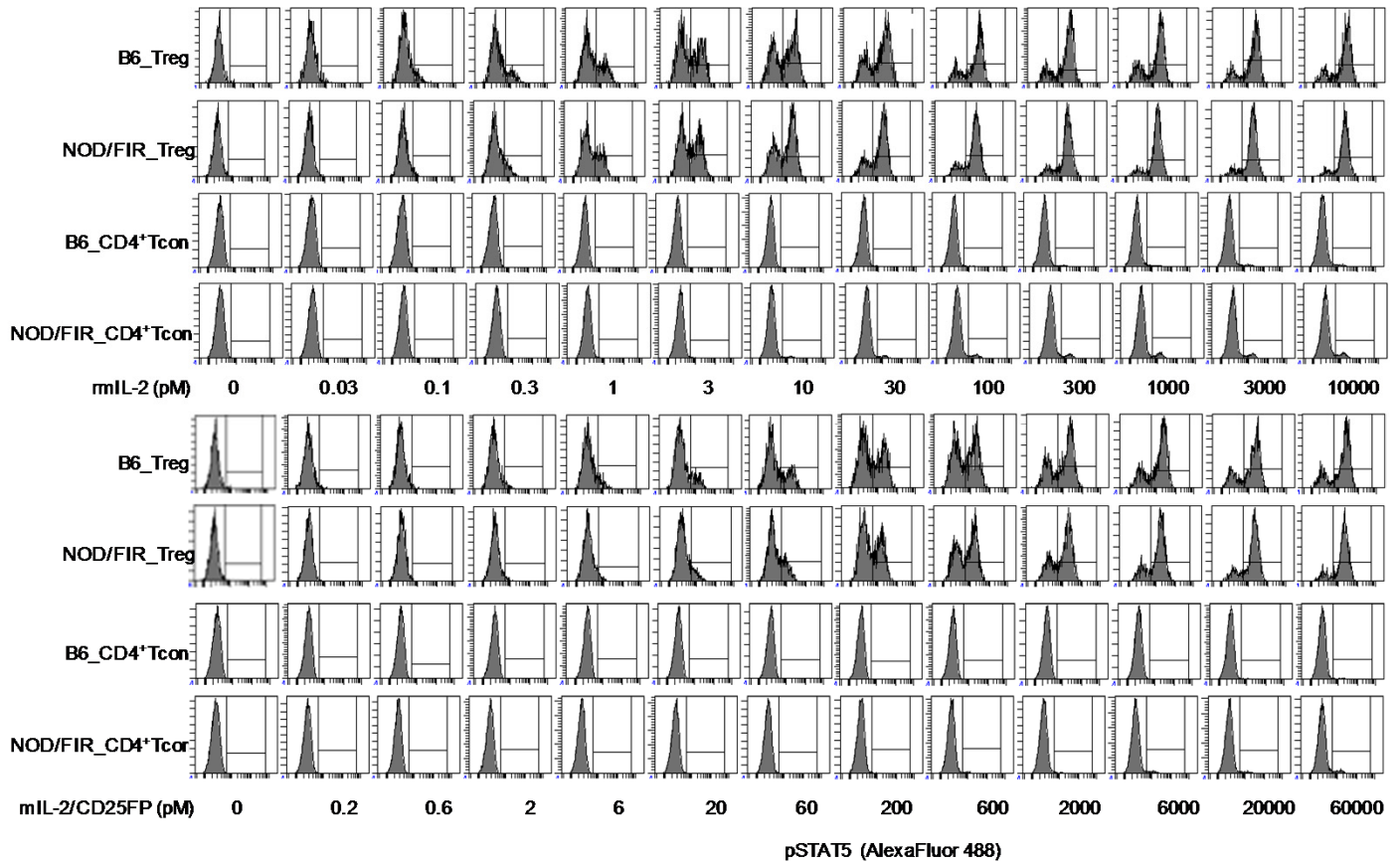


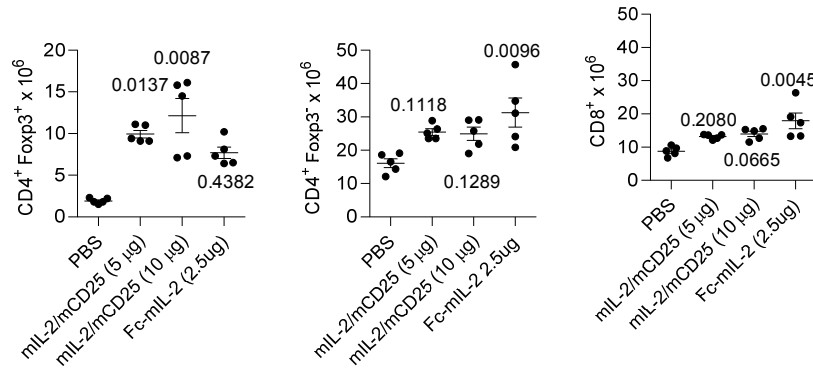
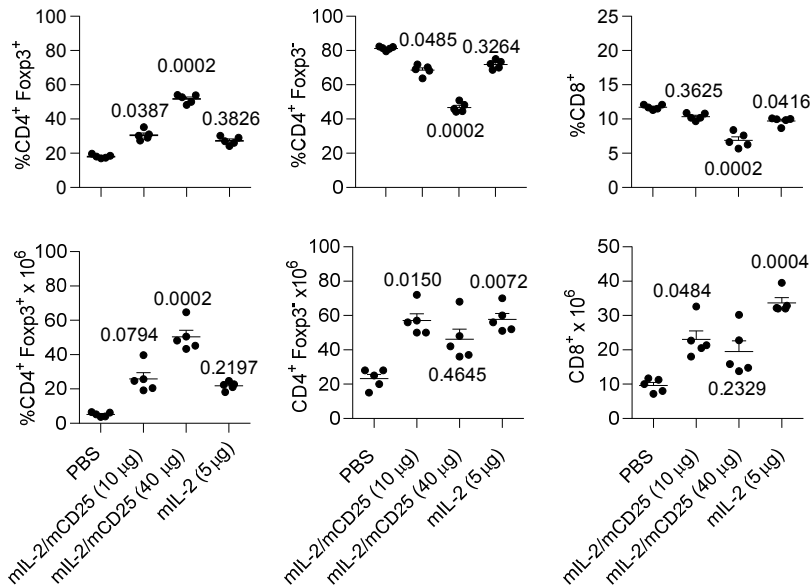
Supplemental Table 1: PCR primers used to generate DNA fragments required to assemble IL-2/CD25 FPs

Primer	Description	Orientation	Primer	Purpose
1	pCIneo/Xho/koz/r	Reverse	5' ccatggcctcgcctcaggctagcctatagtg	Amplify and modify pCIneo vector
2	pCIneo/Not/Ink2/6xhis/stop/f	Forward	5' ggtggacatcacccatcacccatcactaataagcgccgctcccttag	
3	pCI/Koz/mL2/f	Forward	5'aatacgactcactataggctagcctcaggccaccatggacagatgcagctcgcaccc	Amplify mL-2 fragments
4	mL2/Ink7/IL2ra/r	Reverse	cagacacagttcacctccacctccacctcaggcctgttgagatgatg	
5	mL2/(G3S) <sub>3</sub> /r	Reverse	5' cgaacctccacctgaacctccaccagaacctccaccttgaggcct	
6	mL2/(G4S) <sub>4</sub> /r	Reverse	5' tccacctccacctgatccacctccaccagatccacctccaccttgaggcct	
7	mL2/(G4S) <sub>5</sub> /r	Reverse	5' ccacctgatccacctccaccagatccacctccacctgatccacctccaccttgaggcct	
8	(G3S) <sub>3</sub> /mCD25/f	Forward	5' ggtggaggttctggtggaggtcagggtggaggttcggaactgtgtctgtatgacct	Amplify mCD25 fragments with various linkers
9	(G3S) <sub>4</sub> /mCD25/f	Forward	5' tctggtggaggtcagggtggaggttcgggtggaggttctgaactgtgtctgtatgacct	
10	(G4S) <sub>4</sub> /mCD25/f	Forward	5' ggtggatcagggtggaggtggatccggtggaggtggatctgaactgtgtctgtatgacct	
11	(G4S) <sub>5</sub> /mCD25/f	Forward	5' aggtggatcagggtggaggtggatccggtggaggtggatctgaactgtgtctgtatgacct	
12	mCD25/Ink/6xHis/stop/r	Reverse	5' ttattagtgtggtgatggtgatgccaccaactgaggcctgttgagatgatg	
13	(G3S) <sub>3</sub> /hCD25/Ink12/f	Forward	5' ggtggaggttctggtggaggtcagggtggaggttcggagctctgtgacgatgacct	Amplify hCD25 fragment with 12 amino acid linker
14	hCD25/Ink/6xHis/r	Reverse	5' gtgatggtgatggtgatgccacctggtactctgtttaaataatgga	

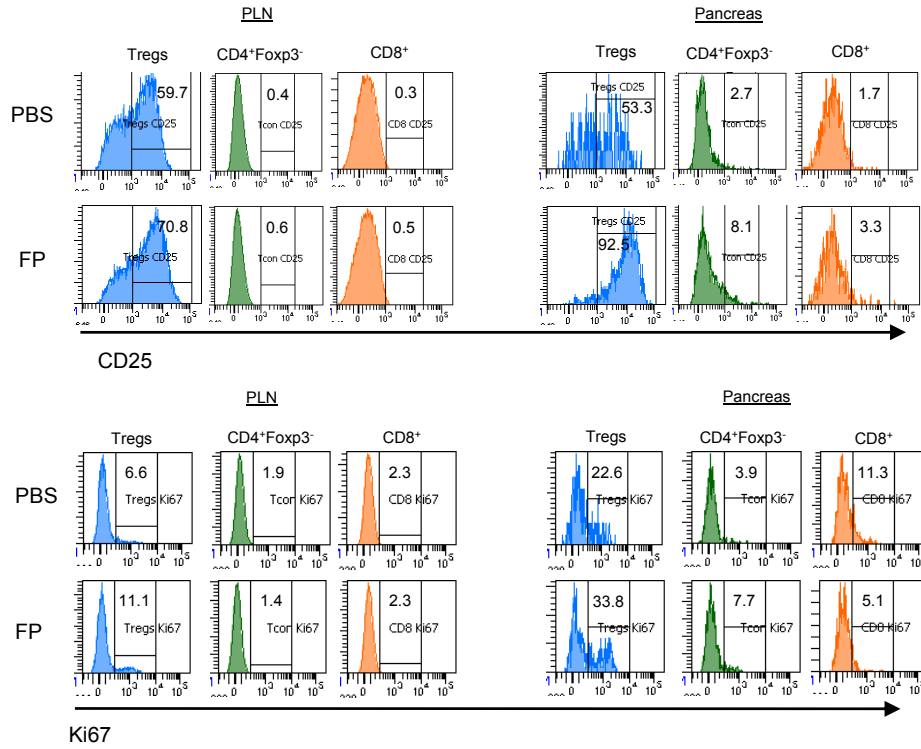
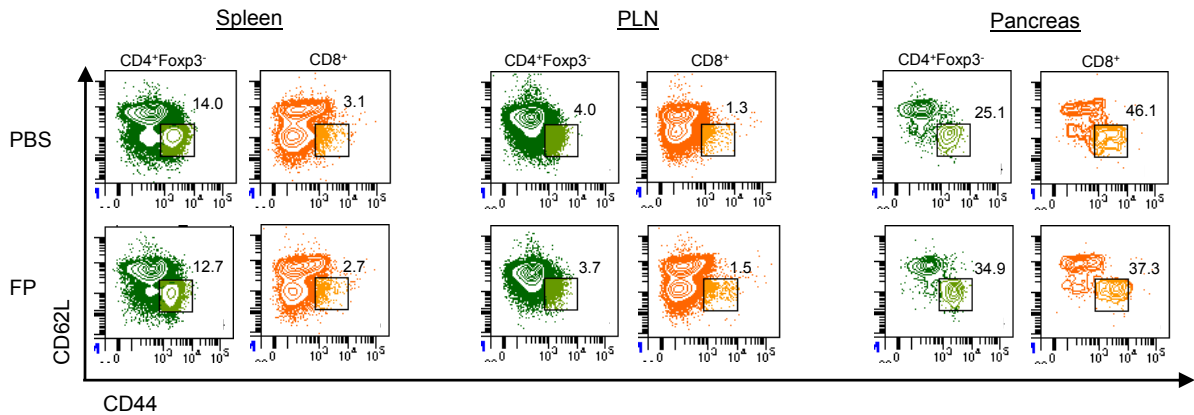
Note: Fragment of mCD25 from primers 8/12 was used with the fragments of mL-2 from primers 3/5 and 3/6 to generate mL-2/mCD25 with 12 and 16 amino acid (G3S)<sub>3</sub> and (G3S)<sub>4</sub> linkers.



**Supplemental Figure 1.** Representative FACS histograms of CD4<sup>+</sup> Foxp3<sup>+</sup> and CD4<sup>+</sup> Foxp3<sup>-</sup> T cells from C57BL/6 and NOD-Foxp3/RFP (NOD/FIR) spleen cells after in vitro stimulation with the indicated concentrations of mIL-2 (top histograms) or mIL-2/mCD25 (bottom histograms).

**A****B**

**Supplemental Figure 2.** Effect of mIL-2/mCD25 on T cells in male BALB/c mice. A) Mice (n=5/group) were treated with FC-mIL-2 on days 0, 2, 4, and 6 or with PBS or mIL-2/mCD25 on days 0, 2, and 6. 24 hr after the last injection, the numbers of the indicated T cell populations in the spleen were enumerated by FACS analysis. B) Mice (n=5/group) were treated with PBS or mIL-2 daily for 9 days or with mIL-2/mCD25 on days 0, 3, 6, and 9. 24 hr after the last injection, the percentage and numbers of the indicated T cell populations in the spleen were enumerated by FACS analysis. Data are shown as the mean  $\pm$  SEM. Numbers within the graphs represent the p values, which were determined by the Kruskal-Wallis one way ANOVA using the Dunn's multiple comparison test in relationship to the PBS control treated group.

**A****B**

**Supplemental Figure 3.** Representative FACS plots for expression of CD25 and Ki67 (A) and CD44 vs CD62L (B) for the indicated cell populations 2-3 days after NOD mice received twice weekly injections of mIL-2/mCD25 FP (4  $\mu$ g) for 5 weeks. The numbers on each plot represent the percent positive cells within the indicated gate.