SUPPLEMENTAL MATERIAL

Lathrop et al., http://www.jem.org/cgi/content/full/jem.20081359/DC1

A

			Lymph node				
Expt.	Phenotype	Spleen	Mes	Cerv	Axil	Ing	Total
1	Foxp3$^+$	322	298	329	285	357	1,591
	CD44^{hi}	295	330	246	305	291	1,467
	CD44^{lo}	315	306	307	335	274	1,537
2	Foxp3$^+$	330	298	301	333	304	1,566
	CD44^{hi}	268	321	321	321	298	1,529
	CD44^{lo}	333	338	344	344	333	1,692
3	Foxp3$^+$	283	361	284	298	280	1,506
	CD44^{hi}	283	276	365	311	377	1,612
	CD44^{lo}	278	306	278	330	453	1,645
4	Foxp3$^+$	146	161	179	*	*	486
mouse 1	CD44^{hi}	154	198	139	*	*	491
	CD44^{lo}	110	123	167	*	*	400
4	Foxp3$^+$	141	120	164	*	*	425
mouse 2	CD44^{hi}	136	143	116	*	*	395
	CD44^{lo}	113	120	115	*	*	348
4	Foxp3$^+$	122	153	124	*	*	399
mouse 3	CD44^{hi}	161	149	143	*	*	453
	CD44^{lo}	114	152	115	*	*	381
	Total:	3,870	4,124	4,003	2,959	2,967	17,923

B

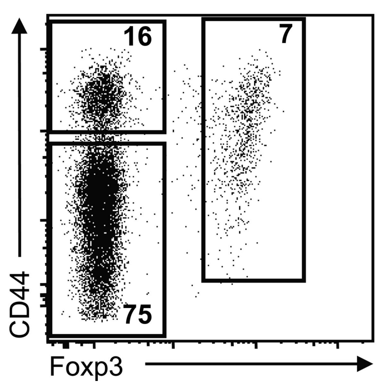


Figure S1. Generation of the peripheral TCR database. (A) Sequence datasets from normal mice. The number of TRAV14 ($V\alpha 2$) TCRα chain sequences from T cell subsets at each location are shown. Experiment numbers 1–3 consisted of cells pooled from three to five mice, whereas experiment 4 consisted of three independently sequenced mice. Cells from axillary and inguinal LNs were not isolated in experiment 4, as indicated by asterisks. Axil, axillary; Cerv, cervical; Ing, inguinal; Mes, mesenteric. (B) FACS purification strategy. Representative flow cytometric plot of CD4$^+$ splenocytes from TCli TCRβ transgenic × *Foxp3^{gfp}* × *Tcra$^{+/-}$* mice illustrate the three sorted populations: CD44^{hi}, CD44^{lo}, and Foxp3$^+$. Numbers indicate percentages in the gates.

# of times TCR is found	Foxp3+					Foxp3−CD44lo					Foxp3−CD44hi				
	Spl	Mes	Cerv	Axil	Ing	Spl	Mes	Cerv	Axil	Ing	Spl	Mes	Cerv	Axil	Ing
1	425	494	452	282	246	322	305	315	273	269	347	330	322	218	213
2	108	121	117	61	55	62	60	56	42	59	78	93	66	46	54
3	31	46	40	24	18	19	27	21	27	17	39	38	33	21	30
4	21	22	24	14	15	10	12	14	11	7	18	19	20	17	15
5	10	10	12	12	7	10	15	14	7	15	16	11	17	7	9
6	6	9	15	3	7	4	5	4	2	6	5	7	10	7	4
7	6	4	3	2	5	1	4	8	5	3	6	11	8	1	4
8	5	5	7	7	4	4	1	2	2	1	4	4	5	3	1
9	2	1	3	1	5	2	2	4	3	1	3	5	3	4	1
10	6	2	3	2	4	3	3	1	1		3	3	1	1	1
11	2	1		2	1	3	1	3		2	7	1	5		1
12	2	1	3	1			2	1	2		2		1	1	1
13		3	1	1	2	1		1	1	1		1	1		3
14			1	1	2	1	2	2			2		1		
15	3				2	1	2	1	2	2		5	2		1
16		2		2	1	2	2	3	1	1	1	1	1		
17	2			1		1	1			1	1	1			
18		1				1			2			1		1	
19			1	1	1	1	2		2			1	1		
20					1				1	2	1		1		
21						1		1	3	1	1				
22	1	1		1		2	3	1							
23						1							1	2	1
24		1	1			1		1		1	1				
25			1					1			1			1	
26			1		1	1	1	1							
27							1						1		
28										1		1			
29						1		1	1		1				
30								1		1		1			
31								1	1						
32	1						2			1					
33		1							1	1					
34							2			1					
36								1		1					
37		1				1		1							
38			1												
39							2						1		2
40	1						1	1							
41						1									
42						1									
43								1				1			
44													1		
47													1		
50												1			
53						1	1					1			
54															1
55								1							
56				1										1	
57												1			
58						1									
59									1						
60													1		
61	1														
66					1										
69										1					
83											1				
139															1
185														1	
Total Unique	633	726	686	419	378	460	459	463	391	396	538	538	504	332	343
Total Seq	1,344	1,391	1,381	916	941	1,263	1,345	1,326	1,009	1,060	1,297	1,417	1,330	937	966
Est Diversity	1,791	1,934	1,766	1,163	1,032	1,546	1,290	1,388	1,237	1,095	1,362	1,256	1,231	855	757

Figure S2. Characterization of TRAV14 TCRα datasets from normal mice. The numbers of unique CDR3 amino acid sequences that were found the indicated numbers of times in the pooled TCRα sequence data (Table I) from the indicated populations are shown. Estimated (Est) diversity is calculated as described in Materials and methods. LNs are shown as in Fig. S1.

	CDR3 a.a. sequence	location p-value	Foxp3+ Spl	Foxp3+ Mes	Foxp3+ Crv	Foxp3+ Axil	Foxp3+ Ing	Foxp3–CD44lo Spl	Foxp3–CD44lo Mes	Foxp3–CD44lo Crv	Foxp3–CD44lo Axil	Foxp3–CD44lo Ing	Foxp3–CD44hi Spl	Foxp3–CD44hi Mes	Foxp3–CD44hi Crv	Foxp3–CD44hi Axil	Foxp3–CD44hi Ing	Number of Instances
Top 15 Foxp3+ TCRs	AAEPNYNVLY	< 0.01	0.6	0.1	1.9	6.1	7.0					0.1						158
	AARPSGSFNKLT	< 0.01	0.3	1.7	2.8	0.2							0.1					69
	AASDGNNRIF	< 0.01	4.5	0.1	0.1		0.1											64
	AARTASLGKLQ	< 0.01	3.0	0.7	0.4	0.3	0.4											63
	AASADYSNNRLT	0.92	0.7	0.4	1.8	0.8	1.6								0.3			67
	AARNYNQGKLI	0.84	0.7	0.9	0.6	1.9	1.5	0.2	0.1	0.5	0.4	0.5	0.2	0.1	0.1	0.4		89
	AASNYQGGRALI	< 0.01	0.1	0.1	0.7	2.4	2.8			0.1								61
	AANSGTYQR	1.00	0.9	0.9	0.9	0.9	0.5		0.1		0.1		0.2		0.1	0.1	0.3	61
	AASELYQGGRALI	< 0.01	0.8	2.7		0.1	0.1							0.1				51
	AASDYGSSGNKLI	< 0.01	0.6	0.2	0.6	1.7	1.6				0.2			0.1				53
	AASGTGSWQLI	< 0.01	2.4	0.6	0.1		0.3											45
	AASRGGNYKYV	< 0.01	0.2	0.1	0.6	2.1	1.1											41
	AASYNTNTGKLT	0.04	0.3	0.3	0.5	0.9	1.7											39
	AASSGTYQR	0.05	0.2	0.5	1.7	0.2	0.2			0.1	0.1				0.1			41
	AARNNYAQGLT	1.00	0.8	1.3	0.1	0.5	0.2						0.1	0.6				48
Top 15 CD44hi TCRs	AARGVTNSAGNKLT	< 0.01				0.4					0.1		2.2	0.1	3.3	19.7	14.4	404
	AASAVNNYQLI	< 0.01											1.1	0.2	4.5	6.0	5.6	187
	AARDHNYAQGLT	< 0.01	1.3	0.2	0.1	0.4	0.2						6.4		0.3		0.5	120
	AAWGYQGGRALI	< 0.01	0.1							0.1			1.9	0.3	3.5	0.5	0.2	85
	AAIPSGNMGYKLT	< 0.01											1.9	0.3	2.9	1.0	0.6	82
	AASANSGTYQR	1.00	0.5	0.4	0.4	0.9	0.1	1.9	2.4	1.1	1.3	0.7	1.2	1.3	2.0	0.6	0.6	193
	AASDGGSGNKLI	< 0.01		0.1									0.6	4.0	0.3		0.1	71
	AASAGTGGYKVV	< 0.01	0.1	0.4	0.4	0.4	0.4	0.7	0.9	0.8	0.7	0.4	0.2	0.3	1.2	2.5	2.4	133
	AATSSSFSKLV	< 0.01											0.2		0.1	2.5	4.0	66
	AASPRLGKLQ	< 0.01					0.2		0.1				0.2		0.8	1.3	4.0	67
	AAIDLPGTGSNRLT	< 0.01		0.1									0.5	3.7				62
	AASMASGSFNKLT	< 0.01		0.1									0.5	3.5				57
	AASDTNTGKLT	1.00											1.3	0.5	1.7	0.2	0.3	52
	AARPGPEDSNYQLI	< 0.01											0.5	3.0				49
	AARDPTTGGNNKLT	1.00						0.1					0.8	0.2	1.5	1.0	0.6	50
Top 15 CD44lo TCRs	AASATGANTGKLT	< 0.01	0.1	0.1		0.1	0.5	4.6	3.9	4.1	5.8	6.5	0.7	0.4	0.8	0.5	0.7	294
	AASEDNNNAPR	1.00		0.1	0.1	0.3	0.1	4.2	3.0	3.0	3.1	3.0		0.1		0.3	0.3	196
	AASETGANTGKLT	0.74		0.2	0.1		0.6	3.3	2.9	2.0	3.3	3.2	0.1	0.1	0.2	0.1	0.4	174
	AASVTGANTGKLT	0.07				0.2	0.1	1.7	2.5	2.8	2.9	3.4		0.2		0.3	0.3	158
	AASQGGSAKLI	1.00	0.1	0.1	0.2	0.1		3.2	2.5	3.2	1.9	1.5	0.1		0.2	0.3	0.1	153
	AASGYGSSGNKLI	1.00	0.1		0.1	0.4	0.6	2.3	2.9	2.3	1.9	2.3		0.2		0.1	0.3	142
	AAANTGANTGKLT	1.00		0.1	0.1	0.1	0.3	2.9	2.4	1.9	1.8	2.6		0.1	0.3		0.5	140
	AASRTGANTGKLT	1.00	0.1		0.1	0.2	1.1	1.8	2.0	2.3	2.1	3.1		0.1	0.3	0.1		134
	AASLTGANTGKLT	1.00		0.1	0.1			2.1	1.6	1.8	2.1	2.8		0.1		0.1		123
	AASGGTGGYKVV	1.00		0.1	0.1	0.3		1.5	1.6	2.7	2.0	1.4	0.3	0.4	0.4	0.3	1.1	112
	AARRNNNNRIF	1.00			0.1	0.1	0.3	1.3	1.6	1.6	1.8	1.9	0.2	0.1	0.2	0.2	0.3	97
	AARGQGGRALI	1.00		0.1			0.1	1.3	1.0	2.2	1.5	1.9		0.1		0.2	0.3	95
	AASKTGANTGKLT	0.87						1.4	1.1	1.2	2.1	2.0			0.1		0.1	91
	AASANSGTYQR	1.00	0.5	0.4	0.4	0.9	0.1	1.9	2.4	1.1	1.3	0.7	1.2	1.3	2.0	0.6	0.6	91
	AASADNNNAPR	1.00	0.1	0.1	0.1	0.2	0.5	1.7	1.9	1.1	1.2	1.6		0.2	0.2		0.1	90

Figure S3. Distribution of top 15 TCRs from each phenotype. The 15 most prevalent TCRα sequences found in the T reg, CD44^{hi}, and CD44^{lo} T cell populations in the data pooled from all four experiments (Table I) are shown in order of decreasing counts within each phenotype. The number of instances is the total count for each TCR in the pooled dataset. Red TCR sequences showed statistically significant differences in their prevalence at different locations based on the generalized linear model testing described in Materials and methods. We considered the Bonferroni adjusted p-value (second column from the left) significant if it was <0.05. Of note, cell purification typically results in the contamination of minor populations (T reg and CD44^{hi} cells) by the major population (naive cells), with the reverse occurring to a much lower degree. A 95–98% purity of the Foxp3^{+} population generally means that 1 out of 20–50 cells are naive cells, which creates a small degree of false overlap. For example, a naive TCR found at 2–5% could be found at 0.1% in the T reg TCR dataset because of impurity from FACS purification.

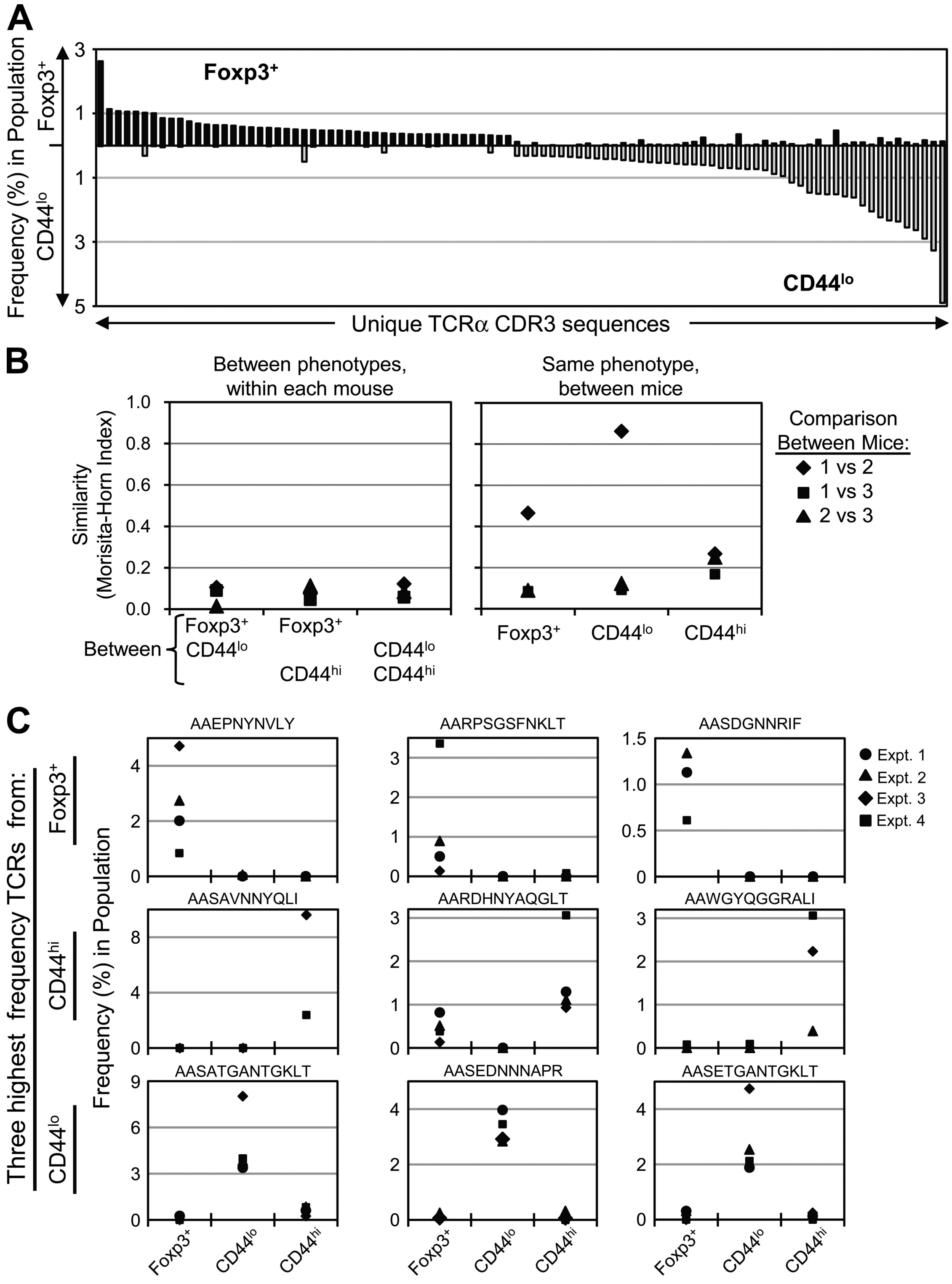


Figure S4. The CD44^{hi}, CD44^{lo}, and Foxp3$^+$ TCR repertoires are distinct. (A) The frequency of the 50 most abundant TCRs in the Foxp3$^+$ and CD44^{lo} subsets are shown as in Fig. 2 A. (B) Analysis of mouse-to-mouse variability. Morisita-Horn similarity indices from individual mice datasets (experiment 4 in Fig. S1) show that the various subsets use distinct TCR repertoires (left), consistent with analysis of experiments using pooled mice (Fig. 2 B). However, there was variability between individual mice (right) that was not obvious in those experiments using pooled mice (Fig. 2 B). (C) The three most frequent TCRα sequences from each phenotype were selected, and their frequency within each phenotype in each experiment was plotted. Different symbols represent each of the four experiments. Each TCR tends to be prominent in only one of the three phenotypes, as would be expected from the overall dissimilarity.

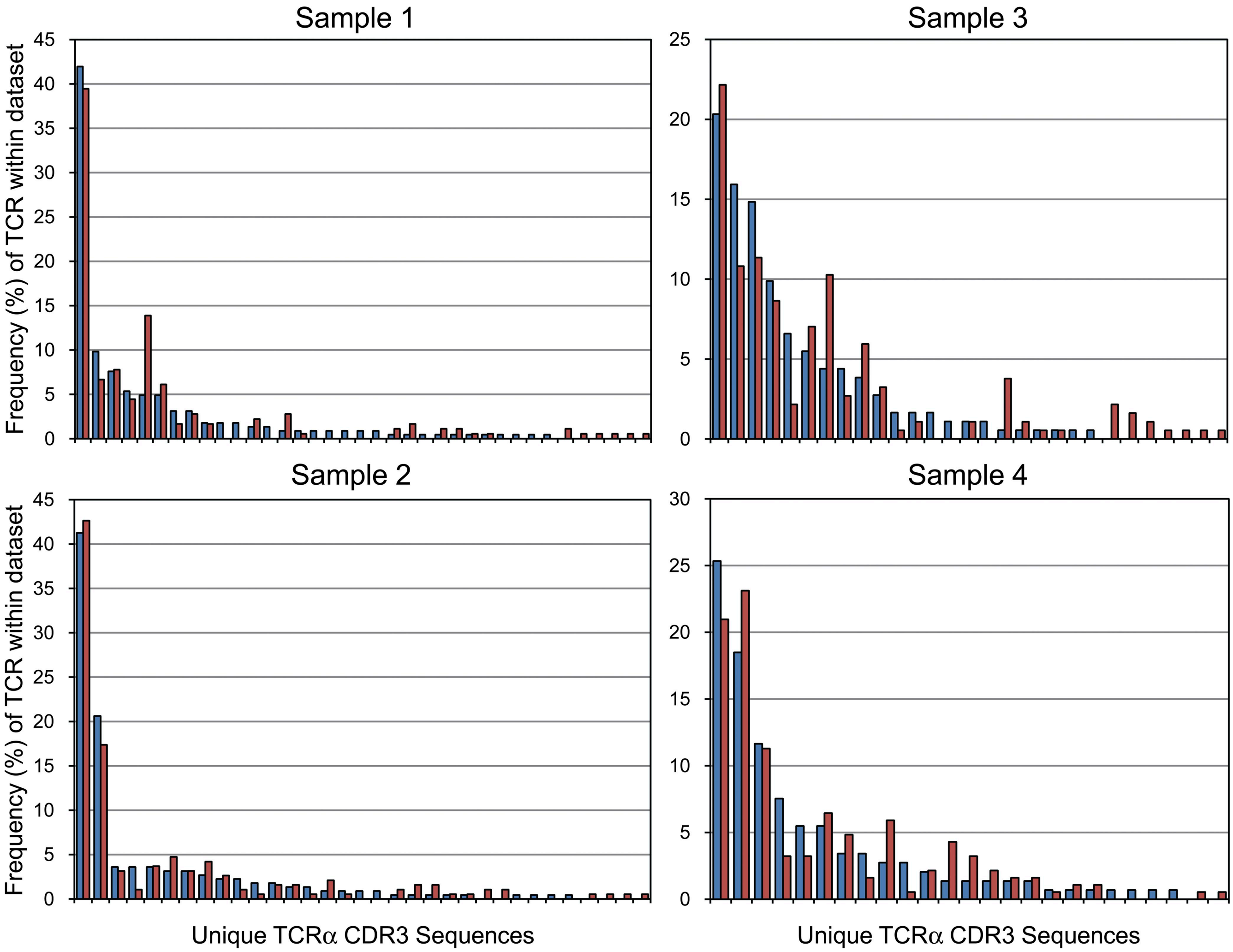
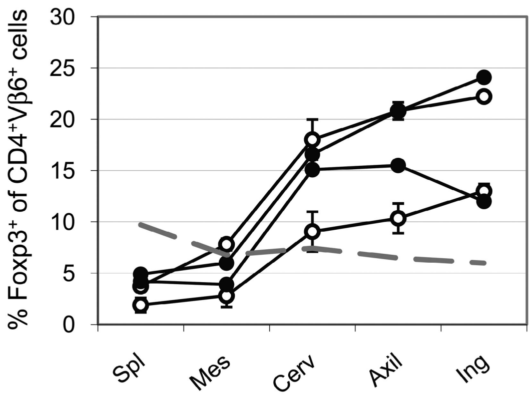


Figure S5. The effect of random sampling on observed TCR frequency. Two independent TCR sequence datasets from each of four TRAV14 cDNA libraries were obtained by random sampling, with 150–200 sequences obtained per dataset. Shown for each sample is the frequency with which each unique TCRα CDR3 amino acid sequence was found in the first (blue) and second (red) dataset.

A



B Number of TCR-α sequences obtained after lymphopenia-induced proliferation and conversion

Expt.	Phenotype	Spleen	Lymph node: Mes	Lymph node: Crv	Total
1, mouse 1	Foxp3+	159	171	194	524
	Foxp3–	151	147	172	470
1, mouse 2	Foxp3+	162	150	185	497
	Foxp3–	184	180	209	573
1, mouse 3	Foxp3+	176	123	175	474
	Foxp3–	162	170	166	498
1, mouse 4	Foxp3+	184	174	174	532
	Foxp3–	155	200	174	529
2, mouse 1	Foxp3+	404	413	159	976
	Foxp3–	201	154	193	548
2, mouse 1	Foxp3+	367	332	215	914
	Foxp3–	150	248	190	588
					7,123

C TCR-α distribution after lymphopenia-induced proliferation and conversion

			Foxp3+												Foxp3–											
			mouse 1			mouse 2			mouse 3			mouse 4			mouse 1			mouse 2			mouse 3			mouse 4		
	CDR3 a.a. sequence	Precursor	Spl	Mes	Crv	Spl	Mes	Crv	Spl	Mes	Crv	Spl	Mes	Crv	Spl	Mes	Crv	Spl	Mes	Crv	Spl	Mes	Crv	Spl	Mes	Crv
Top 14 Foxp3+ TCRs	AASANSGTYQR	1.1	7.5	12.3	5.2	46.9	13.3	8.1	2.8	2.4	0.6	9.2	6.3	2.9	2.0	0.7	0.6		2.2	0.5		0.6		3.2	0.5	1.7
	AARNYNQGKLI*	0.2	2.5	4.7	15.5	3.7	19.3	30.8	6.3	8.1	8.6	1.6	2.3		0.7		0.6			0.5		1.2	4.8	0.6	1.0	5.2
	AARHNTNTGKLT											25.5	27.0	47.1										0.6		1.7
	AASWAQVVGQLT					11.1	25.3	30.3																	2.0	
	AASASGSFNKLT		18.9	15.2	20.1	0.6																				
	AASEENYNQGKLI											17.9	20.1	13.8				1.6						2.6	0.5	1.7
	AATGNYKYV	0.2	3.8	6.4	2.6	8.6	9.3	4.3	2.3	6.5	0.6	1.6	7.5	0.6				0.5			0.6	0.6				
	AASLSGSFNKLT	0.2		1.8	3.6	0.6			9.1	22.8	17.7							0.5					0.6			
	AASSAQVVGQLT								18.8	8.1	12.0							1.1								
	AASTGTYQR											20.7	7.5	2.3												
	AASADNAGAKLT	0.2	5.7	5.3	2.6				2.8	4.1	2.3							0.5	0.6							2.3
	AARGTNAYKVI								5.7	8.1	9.7															
	AASGETGGLSGKLT								7.4	3.3	10.9							0.5			0.6	0.6	1.2			
	AASPSGTGSNRLT		11.9	2.9	4.1																					
Top 14 Foxp3– TCRs	AASRNSNYQLI											3.3			0.7	1.4	2.9	0.5		0.5	48.8	10.0	7.8	15.5	2.0	6.3
	AASRSNYAQGLT					0.6		0.5							0.7			34.2	23.9	22.5						
	AASDETGNTGKLI								3.4		1.7				0.7		15.7	0.5		10.5			3.0			5.7
	AASAYRAGNKLT														10.6	0.7	25.6									
	AATSNNNNAPR					3.7		2.2	0.6		0.6	1.1	0.6	1.7				1.1		23.9		0.6	1.8			1.7
	AASGYGSSGNKLI*	4.5	1.3			7.4	7.3	2.2	1.1					0.6	2.0	0.7	2.3	3.3	1.7	5.7	0.6		1.2	1.9	3.5	5.2
	AASPRNSGGSNYKLT		6.3	0.6	0.5				0.6						5.3	1.4	1.7	0.5			8.6	2.4	4.8	1.3		
	AASASGNTGKLI	0.2		0.6									0.6		2.0	4.1		0.5	1.7		1.2	1.8		5.2	7.0	
	AASAGGGNYKPT														9.9	10.9	0.6				0.6	1.8	0.6	0.6	1.0	
	AASARATGGLSGKLT														1.3	6.8	0.6				2.5	5.3			6.0	
	AASAHHTGGLSGKLT																				3.7	9.4		0.6	7.5	
	AASASQVVGQLT					0.6	2.0											0.5	17.2							
	AGNYGSSGNKLI	0.7													0.7									10.3	2.0	6.3
	AASGGTGGYKVV	1.9															0.6		0.6		0.6	9.4	3.6	1.3		1.1

Figure S6. Analysis of the immune response caused by Foxp3– cells in lymphopenic hosts. (A) The frequencies of Foxp3+ cells arising from the transferred Foxp3– T cells are summarized, with each line representing an independent experiment. The open circles represent the mean values (±SEM) from three mice within an independent experiment; the closed circles are values from experiments that consisted of pooled cells. The dashed line is the percentage of Foxp3+ cells found in a normal animal (for comparison). (B) Description of TCR sequence datasets obtained 2.5 wk after adoptive transfer of Foxp3– cells into *Tcrb*–/– hosts. The total number of sequences by experiment, location (Crv, cervical; Mes, mesenteric; Spl, spleen) and phenotype are shown. (C) The 14 most prevalent TCR sequences pooled from the four mice in experiment 1 are listed. The 15th most frequent TCR in the Foxp3+ and Foxp3– TCRs are each also found in the opposite dataset, as indicated by asterisks. We did not observe a strong skewing of T reg TCR usage based on anatomical location in these mice, unlike normal mice (Fig. 2), possibly as a result of the empty T reg cell niche in lymphopenic animals. Precursor frequency (%) is calculated using 534 TCRα sequences from the sorted CD4+Foxp3– input cell population (note that 0.2% represents a TCR found once in this dataset).

Foxp3⁺

# of times TCR is found	Expt. 1 mouse 1 S	M	C	mouse 2 S	M	C	mouse 3 S	M	C	mouse 4 S	M	C	Expt. 2 mouse 1 S	M	C	mouse 2 S	M	C
1	6	12	7	8	7	4	14	10	12	12	13	12	10	8	6	6	6	7
2	7	2	2	6		1	2		6	3	3	5	8	6	3	5	1	
3	4	5	3	2	3	2		3	2	2	3	2	5	2	2	3	2	1
4	4	2	4	1	1	2	5	3	2	2	2	1	3	3	1	3		1
5	2	2	3				3	2	2	1		3		1	1	1	3	
6	2	3		2		1	2	1		2				2			1	1
7		1	1			1	1						3	2	2		1	1
8		1	2			1	1	1			1					1	2	
9	2	1			2				1									
10	1		2				1	4					1	2	1		1	1
11		3			1		1				2				1	1		1
12	1			1									1					
13							2				2			1		1		
14			1	1	1									2			2	
15						1			2					1			1	
16						1	1							1		1		
17			1						1	1							1	
18				1											1	1		
19	1								1									
20					1								1				1	
21		1							1			1						
22													1					
23																1		
24												1						
26		1																
27																1		
28								1										
29					1													
30	1		1															
31									1				1					
33							1			1								
34													1			1		
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57						1												
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63																		
70																	1	
76				1													1	
78																1		
79														1	1			
82												1						
107																		1
165													1					
173														1				
Unique	31	34	28	23	18	16	34	25	31	26	28	26	37	33	19	29	25	15
Total	159	171	194	162	150	185	176	123	175	184	174	174	404	413	159	367	332	215
Est Div	35	45	33	31	29	19	49	35	46	41	50	39	46	40	25	33	29	26

Foxp3⁻

# of times TCR is found	Expt. 1 mouse 1 S	M	C	mouse 2 S	M	C	mouse 3 S	M	C	mouse 4 S	M	C	Expt. 2 mouse 1 S	M	C	mouse 2 S	M	C
1	35	34	39	41	16	25	36	32	46	47	50	31	48	23	24	24	26	13
2	17	9	6	10	7	7	7	9	11	10	11	13	6	8	4	8	8	5
3	7	4	5	7	8	3	3	6	4	4	5	6	3	4	1	8	3	5
4	1	3	1	2	5	1	1	9	2	2	9	2	3	2	2	1	4	
5	1	3	2	1	1				4	4	1	1	1		3		4	2
6	1	1		2			1		2					2	1		1	3
7	1	1		2	1				1		2	1	3		1	3	1	
8	1	1	1			2		1	2	1	1		2					
9		1			1			1			1	3				1		2
10		1				1			1			3		1		2		
11					1							2			1			
12						1					1				1			
13			1						1								1	
14							1				1			1				
15	1										1						1	
16	1	1						2		1						2	1	
17								1									1	
18																		
19														1				
20														2			1	
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22						1												
23																	2	
24										1								
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60																		
63				1														
70																		
76																		
78																		
79							1											
82																		
107																		
165																		
173																		
Unique	66	59	57	66	42	43	50	61	74	70	83	62	68	44	40	49	55	33
Total	151	147	172	184	180	209	162	170	166	155	200	174	201	154	193	150	248	190
Est Div	117	124	163	142	57	111	146	107	174	180	180	120	215	87	90	89	89	48

Figure S7. TCR dataset after adoptive transfer of Foxp3⁻ cells into T cell–deficient hosts. Data shown are the number of unique TRAV14 TCRα sequences that were found the indicated numbers of times in each population (similar to Fig. S2). Estimated diversity (Est Div) is typically lower in the converted than in the nonconverted TCR datasets. C, cervical LN; M, mesenteric LN; S, spleen.

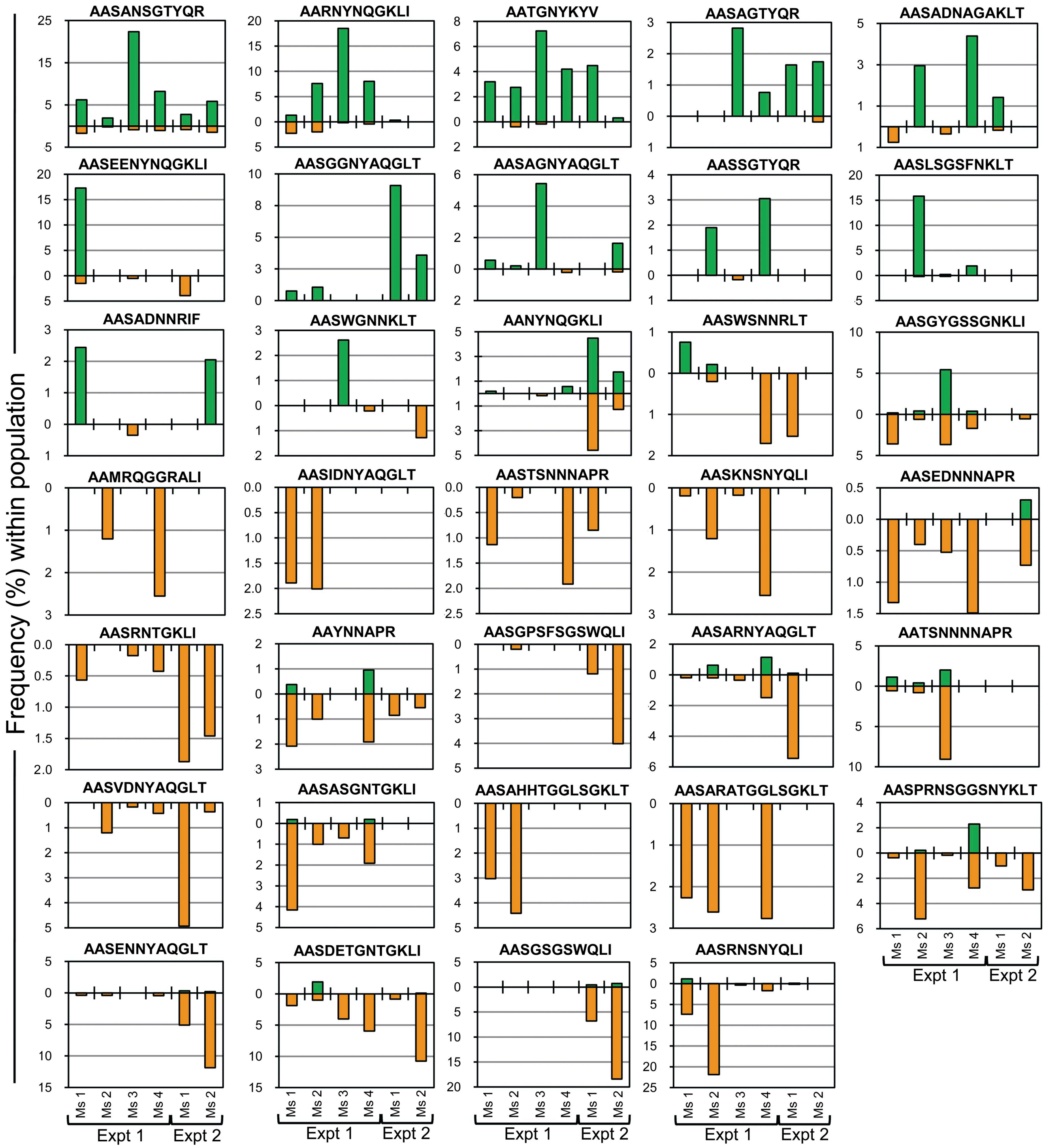

Figure S8. Reproducibility of TCR-dependent conversion. Foxp3⁻ CD4⁺ cells were transferred into αβ T cell–deficient hosts and recovered 2.5 wk later for TCR sequencing (see Fig. 4 and Figs. S4–S6). TCRs that were identified at a frequency of >1% in two or more recipients were selected, and the frequencies of the indicated TCR in each of the six individual mice are shown. The frequencies within the Foxp3⁺ (converted) population (green) are shown above the axis, and the frequencies within the Foxp3⁻ (nonconverted) population (orange) are shown below the axis. Ms, mouse.

TCRα CDR3 Sequence	Conv %	Post-transfer		Normal peripheral TCRs			Thymic data set	
		Foxp3+	Foxp3–	Foxp3+	CD44lo	CD44hi	Foxp3+	Foxp3–
AASDYGSSGNKLI	100	263		50	2	1	4	1
AASRPSNNRIF	100	192		2				
AAKGNTGGLSGKLT	100	187						
AARTNYAQGLT	100	185						
AASASGSFNKLT	100	96		4	1	1		1
AASTGTYQR	100	55		4				
AASKDNYAQGLT	100	51						
AARDYGGSGNKLI	100	43						
AARGTNAYKVI	100	37		10			3	5
AASPSGTGSNRLT	100	32						
AAPYQGGRALI	100	30						
AASDGTYQR	100	21		5			4	
AASAPSNNRIF	100	20		1				
AASGGNYAQGLT	100	127		1	4	2	2	1
AAKGNTGGLSDIQN	100	26						
AASAGTYQR	97.6	50	1	16		1	3	1
AATGNYKYV	97.3	132	3	3	10		1	3
AASLSGSFNKLT	97.2	86	2	12	10		1	1
AARHNTNTGKLT	96.6	176	5					
AASSAQVVGQLT	96.3	64	2					
AASWAQVVGQLT	95.8	112	4			1		
AASADAQGLT	95.8	28	1	2	1			
AASPAGTGSNRLT	95.7	27	1					
AASSGTYQR	95.3	25	1	38	2	1	3	
AASAGNYAQGLT	95.1	47	2	5	8			4
AASEDAQGLT	93.9	56	3		1			
AASGSQVVGQLT	93.3	34	2					
AASADNNRIF	93.1	33	2	29	2			
AASEYNYGNEKIT	88.9	205	21					
AASDEGNYKYV	87.6	26	3	1	2			
AASANSGTYQR	87.3	278	33	28	91	74	3	23
AARNYNQGKLI	85.5	180	25	61	19	9	19	13
AASGETGGLSGKLT	85.5	36	5	2				
AASADNAGAKLT	85.4	50	7	21	2	8		1
AASSNSGTYQR	85.1	21	3	7	8	29	1	4
AASEENYNQGKLI	68.9	92	34	2				
AAMGNMGYKLT	66.0	19	8			2		1
AANYNQGKLI	59.2	62	35	7	19	13		3
AASWGNNKLT	57.1	13	8					
AANSNSAGNKLT	43.9	22	23					
AASGYGSSGNKLI	32.7	32	54	13	142	7		2
AASTNTGKLT	30.6	7	13	4	23	17		
AASAGNSNNRIF	26.2	10	23					
AASGAQVVGQLT	23.2	7	19	13				1

TCRα CDR3 Sequence	Conv %	Post-transfer		Normal peripheral TCRs			Thymic data set	
		Foxp3+	Foxp3–	Foxp3+	CD44lo	CD44hi	Foxp3+	Foxp3–
AATSNNNNAPR	20.0	18	59	3		4		1
AASWSNNRLT	18.5	5	18		1			
AASGNNAGAKLT	17.6	6	23		7			1
AASARNYAQGLT	16.0	10	43					
AAYNNAPR	14.8	7	33	33	1	14		
AASPRNSGGSNYKLT	14.3	13	64		3	12		
AASGGNYKPT	11.4	3	19	1	5	1		2
AGNYGSSGNKLI	10.3	6	43	2	11	2		
AASEDNNNAPR	9.6	3	23	7	196	7		13
AASASQVVGQLT	9.3	4	32	1				
AASWVVGQLT	7.2	2	21					
AASGSGSWQLI	6.0	11	141	15		2	3	3
AASDETGNTGKLI	5.9	10	130	11	1	1		
AASVNTGKLT	4.9	4	63	14		39	1	
AASQGGSAKLI	4.6	1	17	6	153	7		4
AASASGNTGKLI	3.9	2	40					
AASDPGYNKLT	3.9	1	20		1	6		
AASENNYAQGLT	3.9	5	101	4	53	5		5
AASPTASLGKLQ	3.6	1	22	2	22	1		4
AASRNSNYQLI	3.5	7	159	1		2		
AASGGTGGYKVV	2.7	1	29	6	112	28		14
AASRSNYAQGLT	1.1	2	154	2				
AASAYRAGNKLT	0.0		61					
AASAGGGNYKPT	0.0		41		4	19		
AASVDNYAQGLT	0.0		40		5	6		2
AASARATGGLSGKLT	0.0		38					
AASAHHTGGLSGKLT	0.0		38					
AANNYAQGLT	0.0		31	2		9		
AASEGGSNAKLT	0.0		31					
AASGPSFSGSWQLI	0.0		30					
AASRNTGKLI	0.0		25	1	4	12		1
AASEPGYNKLT	0.0		22	2		1		
AASADNYAQGLT	0.0		21	9	46	14	3	2
AASTSNNNAPR	0.0		21	2		15		
AASIDNYAQGLT	0.0		20		3			
AASKNSNYQLI	0.0		20		3	3		
AAMNQGGSAKLI	0.0		19	2	42	1		3
AAGDTNAYKVI	0.0		19		5	2		3
AANANNNAGAKLT	0.0		18					
AAMRQGGRALI	0.0		18					
AASQDQVVGQLT	0.0		16					
	Sum:	**3417**	**2126**	**467**	**1025**	**379**	**51**	**123**
	Total in data set:	**3917**	**3206**	**5973**	**6003**	**5947**	**955**	**1149**

Figure S9. Comparison of the normal dataset with TCRs found after adoptive transfer of Foxp3⁻ cells into T cell–deficient hosts. TCRs isolated after adoptive transfer are listed by their ability to facilitate peripheral conversion (Conv %) as in Fig. 4 B. Rare TCRs found at <0.5% in the posttransfer Foxp3+ and Foxp3– subsets were excluded. The number of times the TCR was found in the posttransfer and normal peripheral and thymic TCR datasets is shown. These more abundant TCRs found in our peripherally converted dataset (Conv >80%) account for ~5% (302 out of 5,973) of the total sequences in the normal T reg TCR repertoire. However, the appearance of a number of these TCRs in the thymic T reg cell subset suggests that the actual contribution of peripheral conversion may be considerably lower under normal conditions.

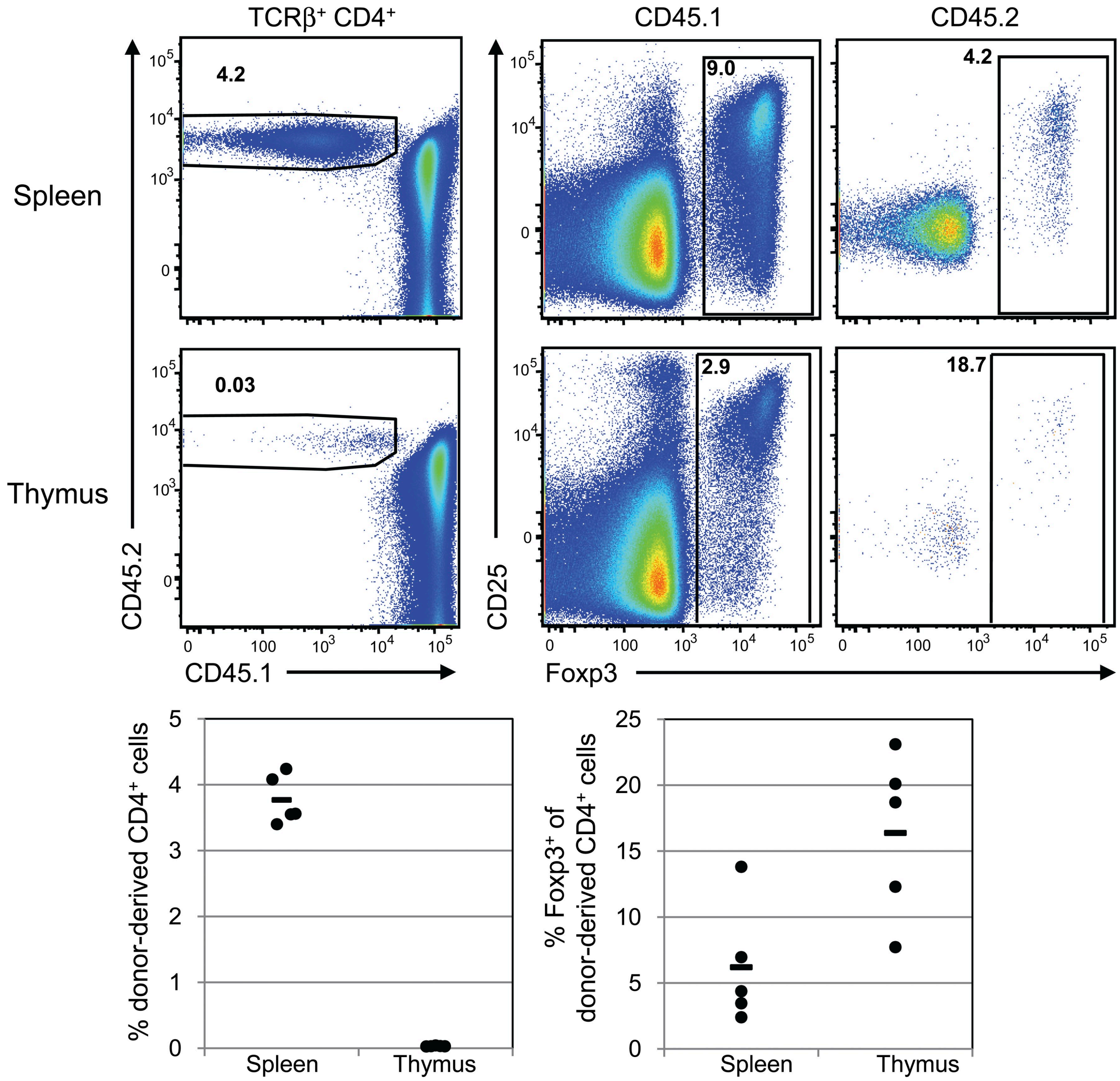

Figure S10. Recirculation of peripheral CD4+ T cells to the thymus. 20 million CD4+ T cell–enriched spleen and lymph node cells were injected into normal congenic recipients. 1 wk later, the spleen and thymus were harvested and analyzed by flow cytometry to determine the percentage of CD4+ T cells derived from the donor (left), and the percentage of those that are Foxp3+ (right). Data are pooled from two independent experiments (*n* = 5 mice) and are summarized in the bottom graphs (closed circle, individual mouse; horizontal line, mean). To estimate the percentage of thymic T reg cells that come from recirculating peripheral T cells, we assumed that the behavior of our adoptively transferred CD4+ cells represented that of the normal peripheral T cell population. Therefore, recirculating cells comprise ~0.8% of the CD4SP population (0.03% of donor T cells in the CD4SP thymus multiplied by 26.3, the ratio of host to donor cells in the spleen [1:0.038]). Because 16.4% of recirculating cells are Foxp3+, this suggests that recirculating T reg cells comprise 0.13% of the CD4SP population. Thus, ~4% of the normal thymic T reg cell subset may arise from recirculation (0.13% of ~3%, the normal thymic T reg cell frequency; not depicted). If we assume that T reg cells that develop in the periphery recirculate to the thymus with the same frequency as estimated, then the extent of thymic recirculation is not sufficient to account for the presence of thymic T reg cells with TCRs that are also found in the converted population. For example, two of the most frequent TCRs found within the converted Foxp3+ population, represented by the CDR3 amino acid sequences AASDYGSSGNKLI and AASANSGTYQR (see Fig. S7), are found in the normal T reg cell population at a frequency of 0.8 and 0.4%, respectively. Therefore, we could expect that they would account for 0.032% (0.8 x 4%) and 0.016% (0.4 x 4%) of thymic T reg cells. However, their frequencies within the normal thymic Foxp3+ population are far greater (0.4 and 0.3%, respectively).