

Figure S1. Representative flow cytometry gating

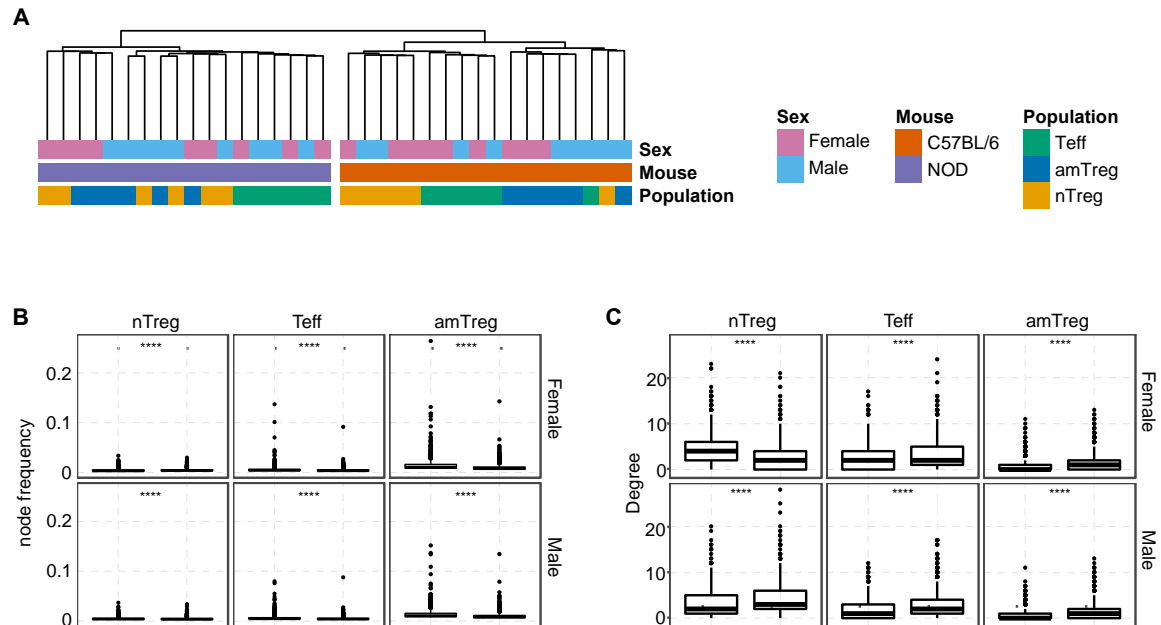


Figure S2: No sex-biased differences between B6 and NOD samples. (A) A hierarchical clustering was performed using Ward's method on the pairwise Jaccard distances calculated at the clonotype level. Colors represent the sex, mouse background and cell population. (B) For each cell population, the top most frequent 1000 CDR3s across same sex mice were identified based on their mean frequency and used to construct networks based on the Levenshtein distance. CDR3 frequency comparison between B6 and NOD

mice for each cell subset and sex. (C) Comparison of the degree of connectivity, which represent the number of connections of each node in a network, by sex within each cell subset. (B, C) Each dot represents a CDR3 and boxplots represent the median, first and third quartiles. Ns $P > 0.05$, *** $P \leq 0.001$, **** $P \leq 0.0001$, Wilcoxon test with Holm's correction.

Mouse background	Sample	Proportion in CD4+ T cells (%)		Proportion in Tregs (%)	
		Tefts	Tregs	nTregs	amTregs
C57BL/6	MM1	81.3	17.0	54.7	40.9
	MM2	79.2	19.3	44.6	49.4
	MM3	82.8	15.7	46.3	47.1
	MM4	81.0	17.7	38.4	55.3
	MM5	85.3	13.3	49.5	44.3
	MM6	82.1	16.4	36.0	58.8
NOD	MM7	86.0	12.8	62.1	17.3
	MM8	85.0	13.3	63.7	31.8
	MM9	88.0	11.1	50.6	32.6
	MM10	85.5	13.5	55.1	38.4
	MM11	86.5	12.4	42.8	38.6
	MM12	85.3	13.6	54.3	38.8

Table S1. Cell proportions in B6 and NOD mice

Mouse background	Sex	Cell population	Sample	No of sorted cells	No of raw reads	No of productive sequences	No of amino acid clonotypes
C57BL/6	Female	nTreg	MM1	1,323,341	2,127,673	1,015,715	233,660
			MM2	1,071,840	2,004,480	733,190	218,263
			MM3	1,246,936	2,817,720	446,115	135,535
		CD4 Teff	MM1	5,364,460	3,761,403	1,349,423	223,224
			MM2	4,234,206	2,581,178	714,488	120,433
			MM3	4,185,207	4,060,009	807,256	172,964
		amTreg	MM1	1,500,745	2,398,861	1,023,589	96,799
			MM2	1,358,814	1,631,017	589,358	101,868
			MM3	1,036,280	2,955,847	1,028,115	68,919
	Male	nTreg	MM4	1,131,312	1,279,418	500,408	199,423
			MM5	1,345,776	1,688,914	649,218	195,734
			MM6	1,071,282	1,533,625	451,775	54,575
		CD4 Teff	MM4	5,345,742	2,383,211	1,029,539	300,505
			MM5	3,975,052	2,946,289	794,384	192,373
			MM6	4,594,811	663,005	222,483	70,411
		amTreg	MM4	1,153,056	2,509,275	857,770	106,989
			MM5	1,201,394	2,551,643	823,937	125,671
			MM6	818,961	2,309,942	550,789	40,864
NOD	Female	nTreg	MM7	1,262,693	4,479,584	1,125,054	173,355
			MM8	663,051	2,429,643	592,815	71,037
			MM9	910,987	2,601,781	822,592	86,159
		CD4 Teff	MM7	3,560,531	5,999,296	1,212,278	207,209
			MM8	4,083,330	4,051,115	742,367	180,372
			MM9	4,794,056	3,286,016	734,086	112,917
		amTreg	MM7	1,015,255	3,069,939	685,274	97,993
			MM8	525,617	3,459,893	644,604	77,448
			MM9	1,044,756	4,664,421	912,418	134,700
	Male	nTreg	MM10	2,829,659	44,533,470	924,356	228,347
			MM11	1,187,673	4,842,671	1,152,406	171,911
			MM12	1,417,099	3,743,743	905,722	162,334
		CD4 Teff	MM10	1,828,793	8,402,032	1,440,109	293,362
			MM11	4,069,621	4,026,173	812,452	137,904
			MM12	5,129,815	5,104,745	1,242,181	172,096
		amTreg	MM10	1,439,570	4,483,668	1,118,172	140,069
			MM11	954,098	2,936,788	551,128	82,532
			MM12	1,073,035	5,172,199	926,015	116,134
NOD AAV-IL2	Female	nTreg	M1	260,496	561,066	180,251	24,024
			M2	298,114	1,722,615	578,756	34,327
			M3	279,845	1,903,257	563,135	30,487
			M4	221,511	2,582,384	817,599	45,441
			M5	160,748	3,020,522	884,592	45,018
			M6	178,671	3,628,737	943,936	51,778
		CD4 Teff	M1	4,202,771	3,634,559	706,155	165,175
			M2	4,683,268	2,885,576	689,779	165,703
			M3	3,922,300	4,688,586	1,170,572	182,343
			M4	4,226,220	3,575,702	1,003,248	115,356
			M5	3,814,764	4,931,386	1,054,540	181,844
			M6	3,763,450	3,252,391	644,635	139,630
		amTreg	M1	734,338	1,055,355	346,526	15,037
			M2	690,255	1,241,344	526,005	8,224
			M3	556,780	1,393,314	398,156	22,360
			M4	741,106	1,888,828	494,156	19,905
			M5	465,508	1,906,114	497,337	21,903
			M6	507,546	1,762,516	455,426	19,596

Table S2. Sample properties. MM: non-treated pools of mice, M: IL-2 treated NOD mice; Number of FACS-ARIA sorted cells, sequencing read counts, number of productive TR sequences, number of unique amino acid clonotypes.

Mouse background	Sample	Proportion in CD4+ T cells (%)		Proportion in Tregs (%)	
		Tefts	Tregs	nTregs	amTregs
NOD AAV-IL2	M1	74.2	24.1	8.1	74.7
	M2	76.8	22.0	11.3	70.4
	M3	74.8	23.7	11.2	73.8
	M4	77.8	21.0	13.7	71.5
	M5	81.0	17.8	13.2	69.6
	M6	79.8	18.9	12.7	70.9

Table S3. Cell proportions in AAV-IL-2–treated NOD mice

Mouse background	Cell population	Sample	Expanded clonotypes	
			Number	Percentage
C57BL/6	nTreg	MM1	8,480	3.63
		MM2	4,863	2.23
		MM3	2,878	2.12
	CD4 Teff	MM1	7,015	3.14
		MM2	3,324	2.76
		MM3	5,921	3.42
	amTreg	MM1	3,883	4.01
		MM2	3,352	3.29
		MM3	2,490	3.61
	nTreg	MM4	3,961	1.99
		MM5	4,230	2.16
		MM6	1,799	3.30
	CD4 Teff	MM4	6,393	2.13
		MM5	3,910	2.03
		MM6	1,336	1.90
	amTreg	MM4	4,269	3.99
		MM5	4,001	3.18
		MM6	1,625	3.98
NOD	nTreg	MM7	5,980	3.45
		MM8	3,148	4.43
		MM9	3,460	4.02
	CD4 Teff	MM7	8,085	3.90
		MM8	6,061	3.36
		MM9	4,921	4.36
	amTreg	MM7	3,821	3.90
		MM8	2,950	3.81
		MM9	4,797	3.56
	nTreg	MM10	8,023	3.51
		MM11	6,924	4.03
		MM12	6,410	3.95
	CD4 Teff	MM10	8,617	2.94
		MM11	5,445	3.95
		MM12	6,082	3.53
	amTreg	MM10	6,043	4.31
		MM11	3,007	3.64
		MM12	4,440	3.82
NOD AAV-IL2 Female	nTreg	M1	761	3.17
		M2	1,525	4.44
		M3	1,407	4.62
		M4	1,951	4.29
		M5	2,158	4.79
		M6	2,335	4.51
	CD4 Teff	M1	3,795	2.30
		M2	5,565	3.36
		M3	6,667	3.66
		M4	4,127	3.58
		M5	7,735	4.25
		M6	4,100	2.94
	amTreg	M1	765	5.09
		M2	365	4.44
		M3	1,043	4.66
		M4	925	4.65
		M5	1,121	5.12
		M6	937	4.78

Table S4. Expanded clonotypes. MM/M: mouse identifier; Number of expanded clonotypes, Percentage of expanded clonotypes: # of expanded clones divided by the total number of clonotypes

Population	Pathology	TRBV	CDR3	TRBJ	Sharing
amTreg	Type-1 diabetes	13-1	CASSDDNIEQYF	2-7	2/6
		5	CASSDRYEQYF	2-7	1/6
		16	CASSLGQQDTQYF	2-5	1/6
		5	CASSQRGANTEVFF	1-1	1/6
		16, 5	CASSRDSSYEQYF	2-7	3/6
		17	CASSRDWGYEQYF	2-7	1/6
	Systemic lupus erythematosus	13-1	CASSDSQNTLYF	2-4	1/6
		16	CASSPRLGGNQDTQYF	2-5	1/6
nTreg	Type-1 diabetes	1	CTCSGGLGGREQYF	2-7	1/6
		13-2	CASGGYEQYF	2-7	3/6
		13-3	CASSDDNIEQYF	2-7	1/6
		13-3	CASSDRYEQYF	2-7	1/6
		13-1	CASSDSQNTLYF	2-4	2/6
		15	CASSLGGNTEVFF	1-1	1/6
		16	CASSLGGYAEQFF	2-1	1/6
		14	CASSLGQQDTQYF	2-5	1/6
		12-1, 16	CASSLGTGGYAEQFF	2-1	2/6
		12-2	CASSLGWGDEQYF	2-7	1/6
		5	CASSQRGANTEVFF	1-1	1/6
	Systemic lupus erythematosus	19, 29, 5	CASSRDSSYEQYF	2-7	4/6
		5	CASSRDWGYEQYF	2-7	5/6
		19	CASSILGNIAEQFF	2-1	1/6
		5	CASSPRLGGNQDTQYF	2-5	1/6
	Cancer	1	CTCSGGLGGREQYF	2-7	1/6
		13-1	CASSGTGQDTQYF	2-5	1/6
		13-2	CASGDAGTGYEQYF	2-7	1/6

Table S5. List of sequences identified in both the annotated database and nTreg and amTreg samples from AAV-IL-2–treated NOD mice.