

Figure S1. WT mice and FoxP3-GFP reporter have similar Treg numbers and develop similar EAU scores in vivo.

B10.RIII WT and B10.RIII FoxP3-GFP reporter mice (GFP) were immunized with 2.5 or 10 µg of IRBP161-180. Eyes were harvested 21 days later. A) CD4⁺FoxP3⁺ eye cells were detected by flow cytometry. Each symbol represents the combined data of one eye from 2 mice. B) Histology. Each symbol represents one eye of each of the 12 mice per immunization dose (For A and B: Shown is mean +/- SEM. Unpaired t-test= ns for WT vs GFP-reporter mice at all doses).

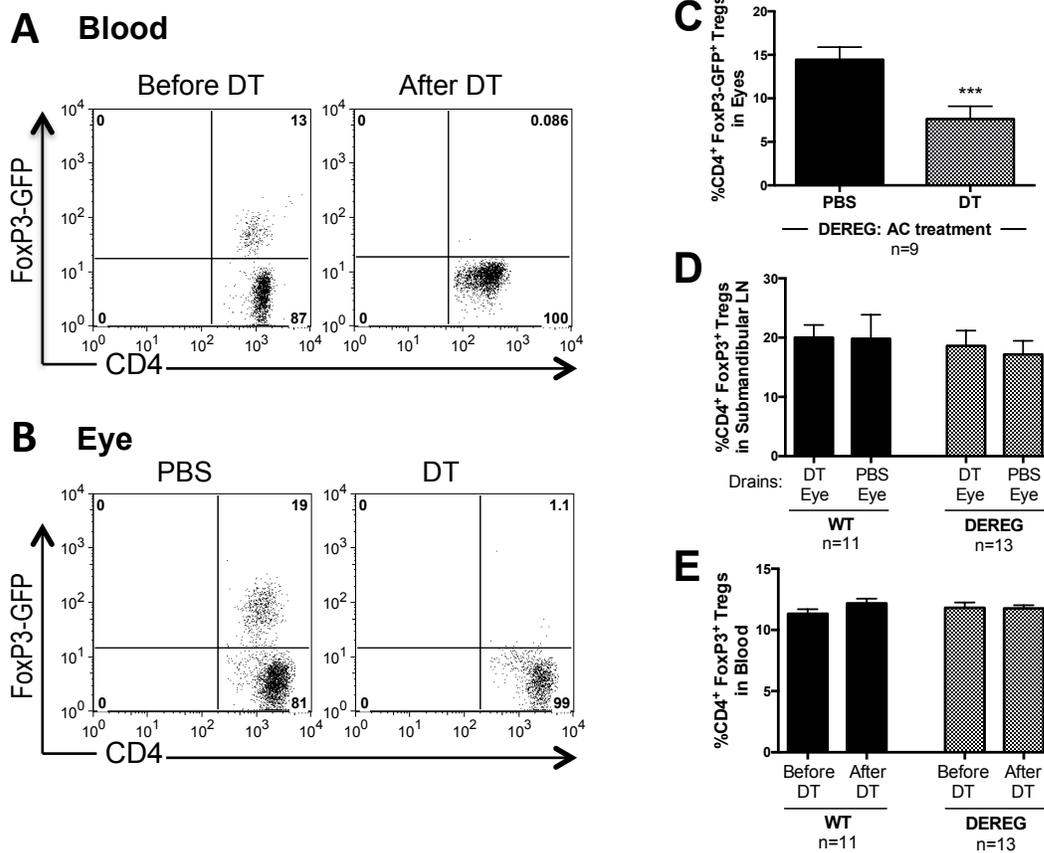


Figure S2. Systemic DT injection depletes Tregs in the periphery and in the eye, whereas AC injection of DT depletes Tregs only in the eye.

The indicated mice (DEREG or WT) were challenged for EAU. **A-B:** DERE mice were treated with DT systemically. (A) Tregs in blood. DT was injected i.p. at peak disease on 2 successive days. CD4⁺FoxP3-GFP Tregs were assayed from tail bleeds by flow cytometry before EAU challenge and DT treatment, and again 48 h after the last DT treatment. Shown is one representative mouse. Verification of Treg depletion in blood samples was completed on all experimental mice. (B) Tregs in the eye. CD4⁺FoxP3-GFP Tregs were assayed (in different mice) 48h after administration of either PBS or DT. Shown is 1 representative experiment of 3. **C-E:** WT or DERE mice injected with DT into the AC. Contralateral eye received PBS. (C) Eyes. AC delivery of DT depletes Tregs from the eyes of EAU-challenged DERE mice. CD4⁺FoxP3-GFP Tregs assayed 36 h after the last treatment (paired t-test: $p < 0.0002$ for mouse's PBS-injected eye vs. its own DT-injected eye). (D) Submandibular LNs. The CD4⁺FoxP3⁺ Tregs from the submandibular LN draining the DT-injected eye of each DERE and WT mouse was compared to that of its own submandibular LN draining the PBS-injected eye 36 h after the last AC treatment (paired t-test=ns). (E) Blood. The CD4⁺FoxP3⁺ Tregs from the blood of each DERE and each WT mouse 24 h prior to AC treatments was compared to its own blood 36 h after the last DT treatment. (paired t-test=ns). The average value +/- SEM for each group are shown for C, D, and E.

Table S1 Flow cytometric analysis of cells in eyes of individual mice¹

C57BL/6

Naive		21 days			3 months		
FoxP3+	FoxP3-	FoxP3+	% ²	FoxP3-	FoxP3+	%	FoxP3-
2	23	5185	10.5	44077	1283	21.6	4666
8	77	4885	12.1	35427	426	18	1936
1	9	6579	10.1	58245	521	22.4	1807
1	1	15044	7.1	197436	727	19.9	2926
1	1	5218	10	46760	6	7.6	73
10	8	7688	12.5	54044	1523	20.3	5512
4	6	7854	12.3	55819			4281
4	18	13641	19.6	55819			
3	48						
4	21	8,262	12	68,453	748	18	3,029

AV

B10.RIII

Naive		17 days			2 months		
FoxP3+	FoxP3-	FoxP3+	%	FoxP3-	FoxP3+	%	FoxP3-
6	38	2962	15.8	15798	21	14.9	121
4	10	2983	16.6	15032	843	31.7	1818
3	18	3252	15.3	18020	309	29.7	730
18	66	4419	14.1	26843	28	26.7	77
7	12	5860	15.2	32613	191	25.7	553
6	23	4385	18.6	19202	806	37.8	1328
		3929	18.5	17344			
		2586	17	12609			
		3004	15.2	16736			
		2345	15.5	12796			
7	28	3,573	16	18,699	366	28	771

AV

¹ Both eyes are combined

²percent of total CD4⁺ cells (Foxp3⁺ and Foxp3⁻)

AV = average

Table S2 Infiltrating cells in eyes of individual mice¹

DT and PBS administered at Peak disease				DT and PBS administered after Resolution			
DEREG MICE		WT Mice		DEREG MICE		WT Mice	
DT	PBS	DT	PBS	DT	PBS	DT	PBS
183	115.5	122	286	385.5	38.5	196.5	114
124	305.5	210.5	205.5	927	77	24	23.5
308.5	372	392	163.5	134.5	115.5	35.5	43
158.5	390	40.5	241.5	147.5	175.5	152.5	316.5
50	19	281	260	375.5	136	221	34.5
364	191.7	153	48	205.5	174.5	20	119.5
452	600	98	161	587	216.5	94.5	23.5
95.5	472.7	169	255.5	365	62.5	130.5	24.5
455.5	187.5	52	92.5	214	26.5	347.5	124.3
374.5	20	80.5	96	472	282.5	123	24
233.5	145.5	293.5	336.5	528	72.5	148	67
403	23	216.5	266.5	299.5	28.5	286.5	267.5
915	724.5	108	86.5	79.5	15.5	212	240
231.5	160	182.5	275.5	727.5	67	202.5	285.5
434	161.5	57.5	35	316	5.5	117	130
278.5	96	409	68	560	412.5	29	25.5
250.5	20.5	480.5	184.5	298.5	42.5	25.5	47.5
783.5	318	96	103.5	487	126	11.5	6.5
479	152.5	387.5	173.5	13	1.5	184.5	105
324.5	242	210	116.5	104	72.5	27	34.5
147.5	123	248.5	240	136.5	59.5	33.5	1
475	134	381.5	250.5	43.5	20	157.7	148.5
168	97.5	303	350	23.5	119.5	121	313.5
981.5	233.5	185	202.5	392.5	41.5	296.5	367.5
50	0	101.5	159.5			37.5	118
260.5	193.5	355.5	301.5			56	226
183.5	123.5	107	109			91.5	194
97	71	175.5	496.5	Sum	7823	2390	3382
45.5	27	152	206.5	Mean	325.9	99.56	125.3
99	223.5	94.5	646				3425
485.5	326	252	417.5				
211	12	504	280.5				
106.5	102	165.5	355				
114.5	31	217.5	247				
266.5	144.5	37	12				
323	97.5	174	193				
263.5	325	371.5	669.5				
136	127.5	415	249.5				
276.5	163.7	34	21				
164	187	44.5	44.5				
525.5	353.5	526.5	356.5				
482.5	352	184	127.3				
		42.5	58				
		299	160				
		213.5	135.5				
		41.5	79.5				
		418.3	110.5				
		421	159				
		231	81.5				
Sum	12761	8166	10736				
Mean	303.8	194.4	219.1				

¹Infiltrating leukocytes in hematoxylin-eosin stained histological sections cut through the pupillary-optic nerve plane were counted under the microscope in a masked fashion. Counts from entire histological sections from each eye were averaged.