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Supporting Information for
T cell deletional tolerance restricts AQP4 but not MOG CNS autoimmunity

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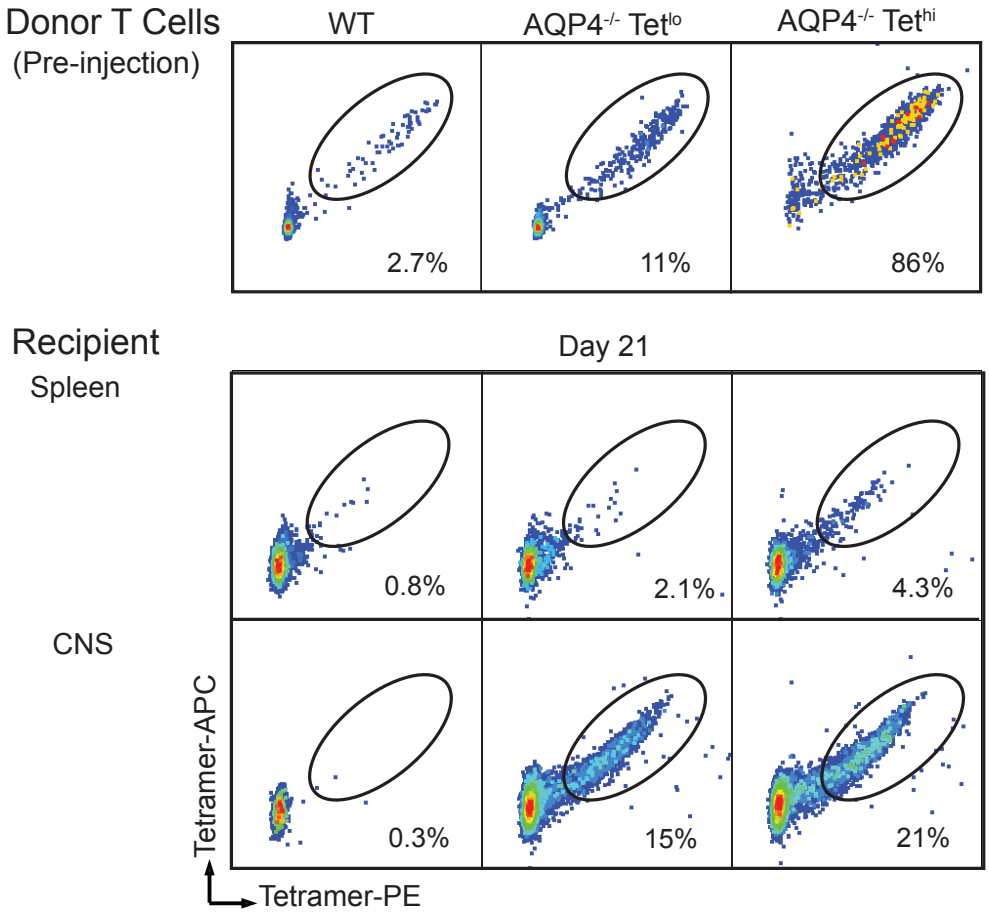

Fig. S1. AQP4$^{-/-}$ tet^{hi} and tet^{lo} AQP4-specific T cells can induce CNS autoimmunity. Lymph node cells from AQP4 p133-149-immunized WT or AQP4$^{-/-}$ mice were cultured with p133-149 for 10 days. CD4^{+} T cells were stained with I-A^{b}:p133-149 tetramers. T cells from AQP4$^{-/-}$ mice were sorted by flow cytometry into tet+ and tet^{-} subpopulations, which were expanded separately by restimulation with irradiated splenic APC and p133-149, then monitored for tetramer binding. WT AQP4 p133-149-primed T cells were expanded in parallel. These separate AQP4 p133-149 T cell lines were restimulated under Th17-polarizing conditions for three days prior to adoptive transfer into TCR$\alpha^{-/-}$ recipient mice. CD4^{+} cells were negatively sorted using magnetic beads (Miltenyi). 11% of the original tet^{-} population stained positive (tet ^{lo}) and 86% of the original tet^{+} sorted population stained positive (tet^{hi}) on the day of adoptive transfer. 5 x 10^{6} donor T cells were transferred to a TCR$\alpha^{-/-}$ recipient. 200 ng Ptx was given on days 0 and 2. On day 21, recipients of tet^{lo} and tet^{hi} T cells had developed clinical CNS autoimmunity (score 2.5 in both groups). WT AQP4 p133-149-expanded T cells did not cause clinical disease. Splenocytes and CNS infiltrating were isolated and measured for AQP4 p133-149 tetramer binding by flow cytometry. Results are representative three experiments (3 recipient mice/group/experiment).

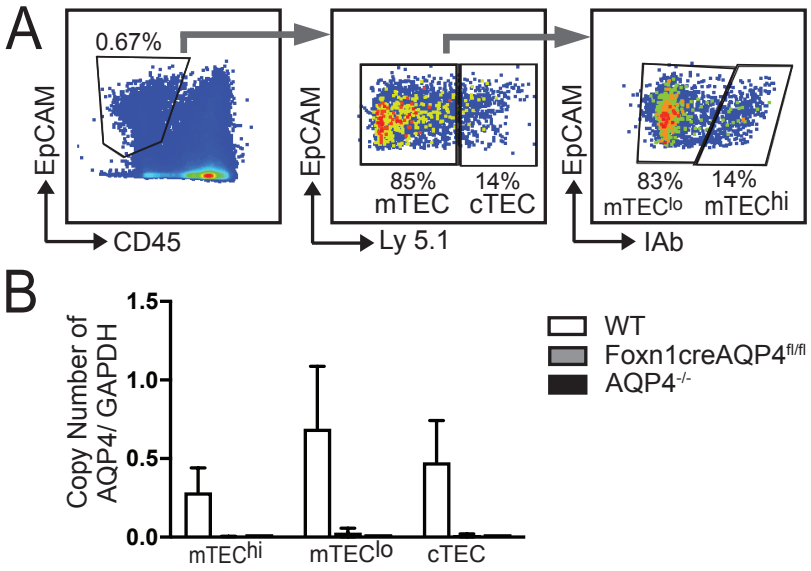

Fig. S2. mTECs in Foxn1cre-AQP4$^{fl/fl}$ mice lack AQP4 expression. Thymi from AQP4$^{-/-,}$ WT and Foxn1cre-AQP4$^{fl/fl}$ mice (1) were isolated, digested and stained with anti-CD45, anti-EpCAM, anti-Ly5.1, and IA^{b} as described (2). mTEC^{hi}, mTEC^{lo} and cTEC (A) were sorted by FACS. (B) mRNA was purified and tested for AQP4 and GAPDH mRNA expression by droplet digital PCR . AQP4 copy number (mean ± SEM) is expressed relative to GAPDH. The mean of three experiments are shown.

Table S1. AQP4 thymic deletion alone does not promote AQP4 targeted CNS disease.

Disease Model and Antigen	genotype	Incidence	Mean maximal clinical score[§]
Direct immunization[*†]			
AQP4 p133-149	WT	0/5	0
	Foxn1cre-AQP4$^{fl/fl}$	0/10	0
	Aire$^{-/-}$	0/10	0
MOG p35-55	WT	5/5	3.1 (±0.6)
Adoptive Transfer[‡] (AQP4 p133-149)	recipients		
AQP4$^{-/-}$ donor	WT	4/4	2.1 (±0.5)
Foxn1cre-AQP4$^{fl/fl}$ donor	WT	0/10	0
AQP4$^{-/-}$ donor	TCR$\alpha^{-/-}$	4/4	2.9 (±0.7)
Foxn1cre-AQP4$^{fl/fl}$ donor	TCR$\alpha^{-/-}$	0/10	0

[*]Direct immunization with 100µg Ag in CFA.
[†]Mice received Ptx on day 0 and 2.
[‡]2 x10^7 donor T cells, polarized to Th17 using 20ng/ml IL-23 and 10 ng/ml IL-6.
[§]All values are shown as mean (± SEM).

Table S2. WT recipient mice recover from CNS inflammation induced by donor AQP4-reactive T cells.

						Mean number of foci (± SEM)[†]		
Donor T Cells*	Recipient	Analysis day	Incidence	Onset day[†]	Mean Maximal Clinical Score[†]	Meninges	Parenchymal	Total
p133-149	WT	9	8/8	7.8 (±0.2)	2.3 (±0.20)	199 (±28[‡]	122 (±22)[‡]	321 (±49)[‡]
		21				4 (±1)	0.2 (±0.2)	4 (±1)
	TCRα$^{-/-}$	21	4/4	12.2 (±0.6)	2.7 (±0.10)	329 (±37)[§]	364 (±23)[§]	693 (±45)[§]
		30	6/6			105 (±28)	87 (±34)	192 (±58)
	RAG1$^{-/-}$	21	4/4	14.0 (±1.2)	2.8 (±0.10)	384 (±7)[¶]	319 (±6)[¶]	703 (±10)[¶]
p202-218	TCRα$^{-/-}$	21		11.3 (±0.5)	2.8 (±0.06)	357 (±19)	328 (±41)	685 (±60)
	RAG1$^{-/-}$	21	4/4	14.0 (±1.2)	2.9 (±0.07)	357 (±25)	302 (±31)	659 (±45)
MOG p35-55	WT	21	5/5	9.0 (±0.6)	4.0 (±0)	223 (±22)	218 (±21)	441 (±42)
		30	5/5			105 (±24)	130 (±45)	225 (±61)

[*]Donor T cells (2 x 10^7), polarized to Th17, were administered i.v. to naïve recipient mice.
[†]All values are shown as mean (± SEM); n = 4 per group unless otherwise indicated.
[‡]Comparison of AQP4 p133-149 WT recipient mice at days 9 and 21. p=0.0016 (Mann-Whitney U test).
[§]Comparison of AQP4 p133-149 WT and TCRα$^{-/-}$ recipient mice at day 21. p=0.0159.
[¶]Comparison of AQP4 p133-149 WT and RAG1$^{-/-}$ recipient mice at day 21. p=0.0159.

Dataset S1 (Accession PRJNA989238)

https://www.ncbi.nlm.nih.gov/bioproject/989238

Single cell TCR sequencing: AQP4$^{-/-}$ and WT mice were immunized with AQP4 p133-149. T cells were harvested from draining lymph nodes and cultured with cognate antigen, receiving fresh cognate antigen and irradiated splenocytes every 10 days for two stimulations. AQP4$^{-/-}$ T cells were sorted into tetramer positive or negative cells were sorted into live CD4^{+} cells by flow cytometry. A single cell library of the T cell V(D)J regions targeting 10,000 cells from each group was constructed using the 10x Genomics Chromium Single Cell 5' Library and Gel Bead Kit, and sequenced using the Illumina NovaSeq. All datasets were analyzed using the Cell Ranger (v5.0.1) variable diversity joining (VDJ) function, which aligned reads to the GRCm38 Alts Ensembl reference (v5.0.0) using STAR (v2.5.1). TCR α and B chain contigs per cell, outputted from CellRanger, were further analyzed using custom bioinformatic programming scripts written in R and perl.

SI References

1. B. J. Hindson *et al.*, High-throughput droplet digital PCR system for absolute quantitation of DNA copy number. *Anal Chem* **83**, 8604-8610 (2011).
2. Y. Xing, K. A. Hogquist, Isolation, identification, and purification of murine thymic epithelial cells. *J Vis Exp* 10.3791/51780, e51780 (2014).