SUPPLEMENTAL MATERIAL

Pöllinger et al., http://www.jem.org/cgi/content/full/jem.20090299/DC1

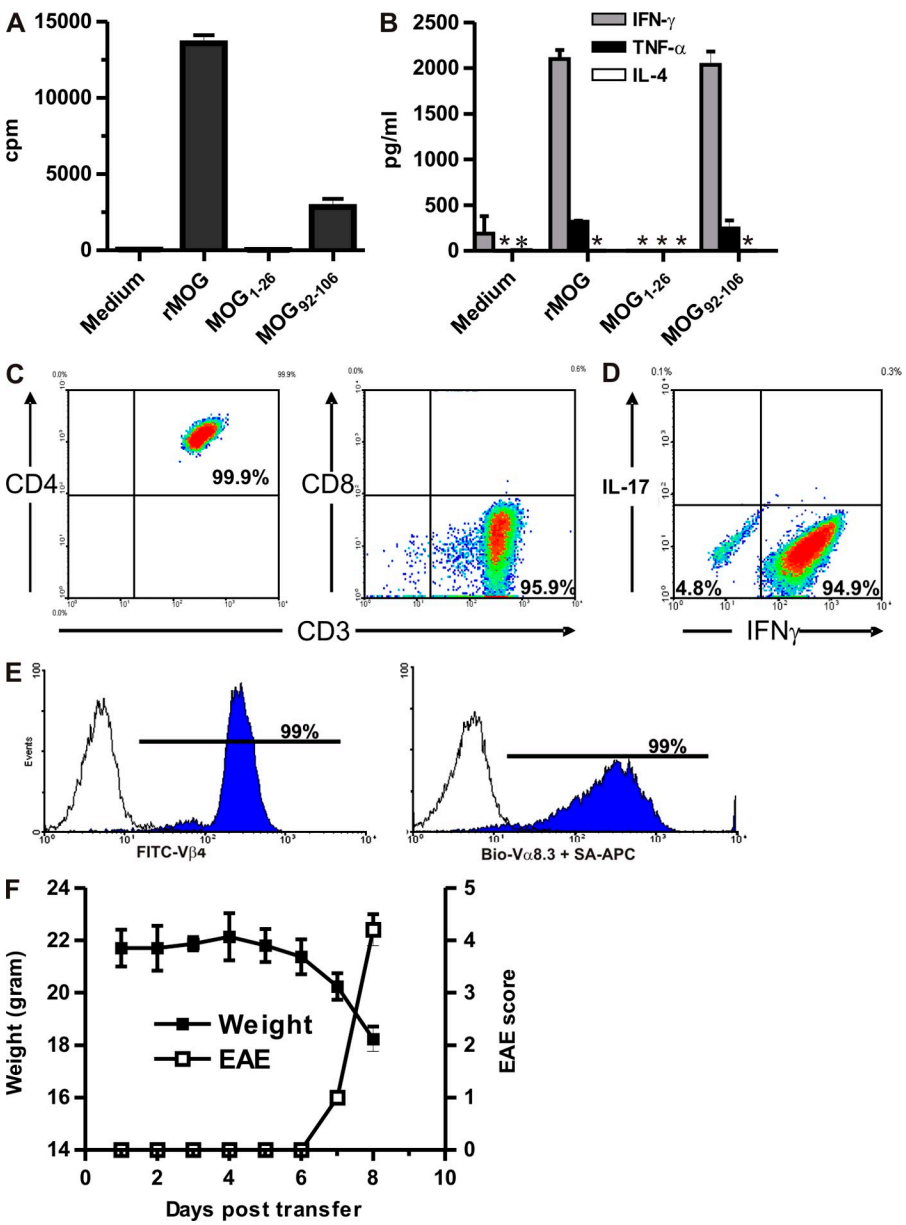


Figure S1. Encephalitogenic TCR donor clone C3. (A and B) Proliferative (A) and cytokine release (B) responses to rMOG and MOG peptides 1–26 and 92–106. Proliferation was measured by ^{3}H-thymidine incorporation and cytokine release by ELISA. C3 T cell clone was cultured without antigen (medium) or with 20 µg/ml rMOG, MOG_{1-26}, or MOG_{92-106}. C3 T cells produce IFN-γ and TNF-α, but not IL-4, in response to rMOG and MOG_{92-106}. IFN-γ, TNF-α, and IL-4 were measured by ELISA on supernatants harvested from proliferation assays 48 h after activation. *, not detectable. Mean values of two independent experiments and SEM are shown. (C) C3 is a $CD4^{+}$ T cell clone. Surface expression of CD3, CD4, and CD8 on C3 T cells was determined by flow cytometry. (D) Th1 nature of clone C3 demonstrated by intracellular flow cytometry. Intracellular cytokine staining for IFN-γ and IL-17 was performed after stimulation with PMA/ionomycin/brefeldin A for 4 h. (E) TCR V chain composition determined by flow cytometry. Histograms show expression of TCR-Vα8.3 and TCR-Vβ4 on C3 cells. Identity of TCR-Vα and -Vβ chain of C3 T cell clone was revealed using an anti-TCR antibody panel (only positives shown). (F) Encephalitogenic potential in transfer EAE. C3 T cells were activated with rMOG for 48 h and 10×10^{6} cells were injected i.p. into SJL/J WT females. Clinical score (right y axis, empty squares) and weight (left y axis, filled squares) of injected animals were monitored daily after transfer of cells. Data from one of two experiments with three animals are shown. Error bars indicate SD.

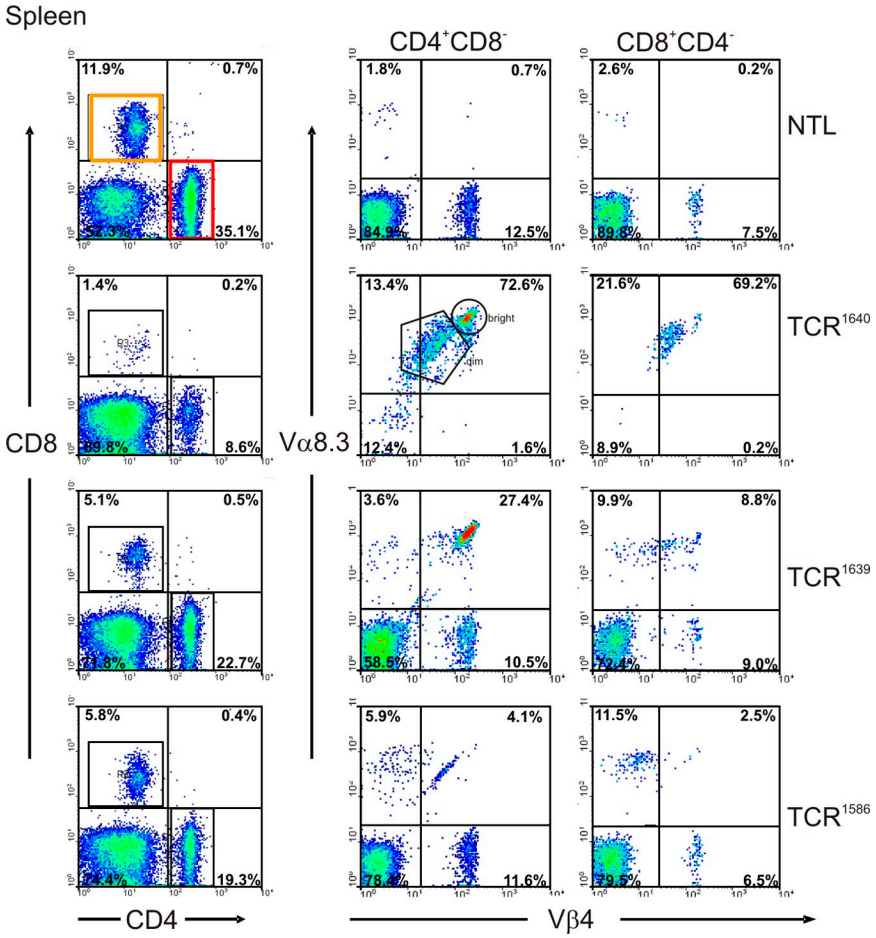


Figure S2. TCR expression in transgenic splenocytes. Splenocytes from 8–10-wk-old TCR transgenic mice or NTLs were stained with antibodies to CD4, CD8, TCR-Vβ4, and TCR-Vα8.3, and cells were analyzed by flow cytometry. Density plots show four-color analysis from NTL, TCR^{1640} (representative of $n = 7$), TCR^{1639} (representative of $n = 3$), and TCR^{1586} (representative of $n = 4$). Transgenic Vα8.3 and Vβ4 are shown on cells gated as indicated, either $CD4^+/CD8^-$ or $CD4^-/CD8^+$. Regions drawn in of $CD4^+/CD8^-$ cells from TCR^{1640} mice indicate cell population with bright or dim expression of the transgenic TCR. Flow cytometry data are representative of at least three independent experiments.

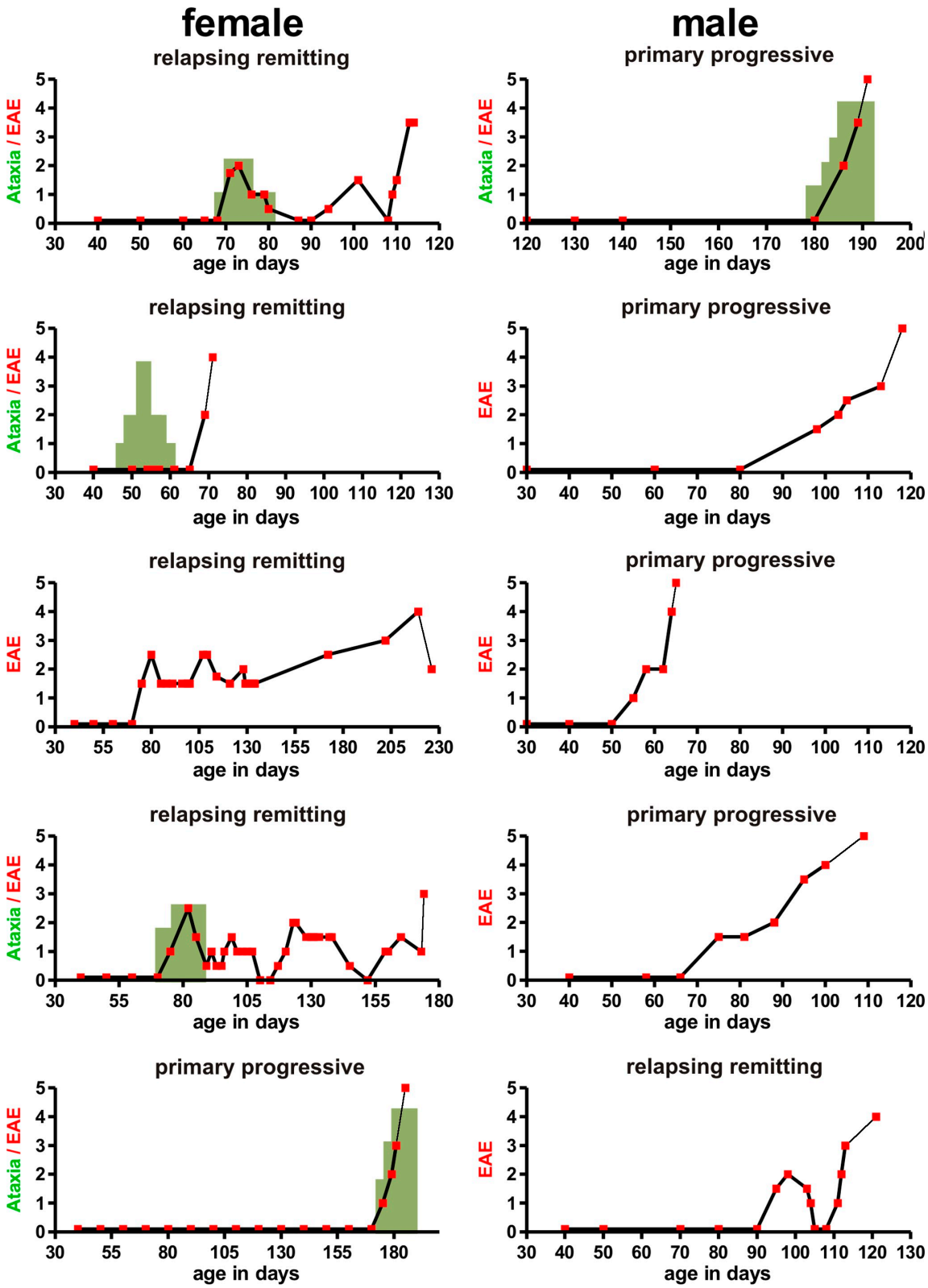


Figure S3. Spontaneous disease course of individual transgenic TCR^{1640} animals. Female mice mostly present with RR-EAE, whereas males present with primary progressive EAE. Green fields indicate ataxic bouts, and black lines with red symbols indicate the course of paralytic EAE. The scale from 1–5 is indicative for both ataxic and paralytic EAE (described in Materials and methods).

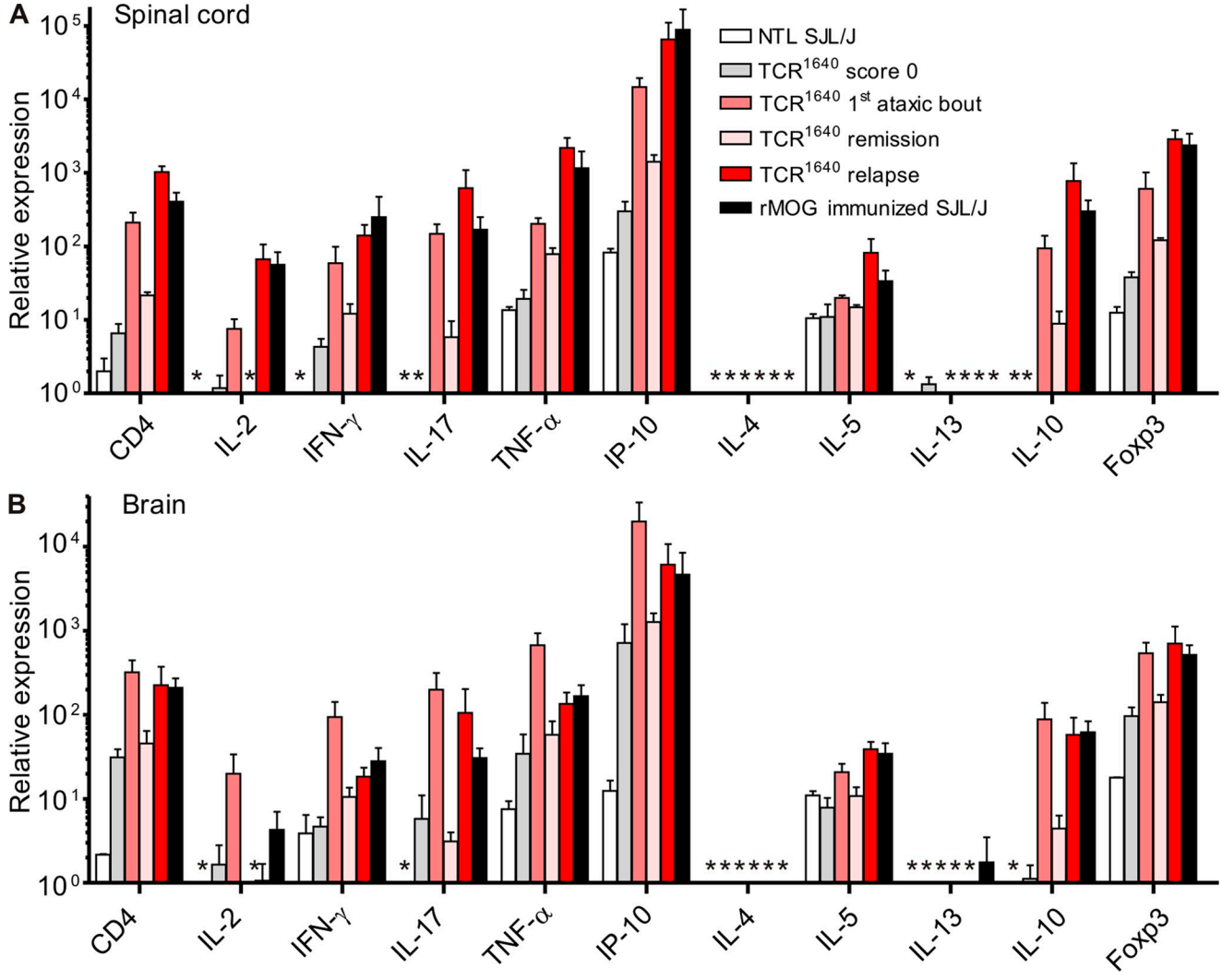


Figure S4. CNS mRNA expression analysis. Expression was analyzed in TCR^{1640} mice, both healthy and in different disease statuses (all three to four mice per group), and NTL mice, either healthy (n = 2) or with EAE induced with rMOG (n = 4). Mice in remission did not show clinical signs and relapsed mice had paralytic EAE of score 3–4. (A and B) Spinal cord (A) and brain (B) cDNA was analyzed by quantitative PCR for expression of the indicated genes. Shown is the relative expression level of the individual gene compared to the control housekeeping gene GAPDH multiplied by 10,000. Values show the mean values of pooled data from individual mice with the SEM. *, not detectable. Data were pooled from two independent experiments.

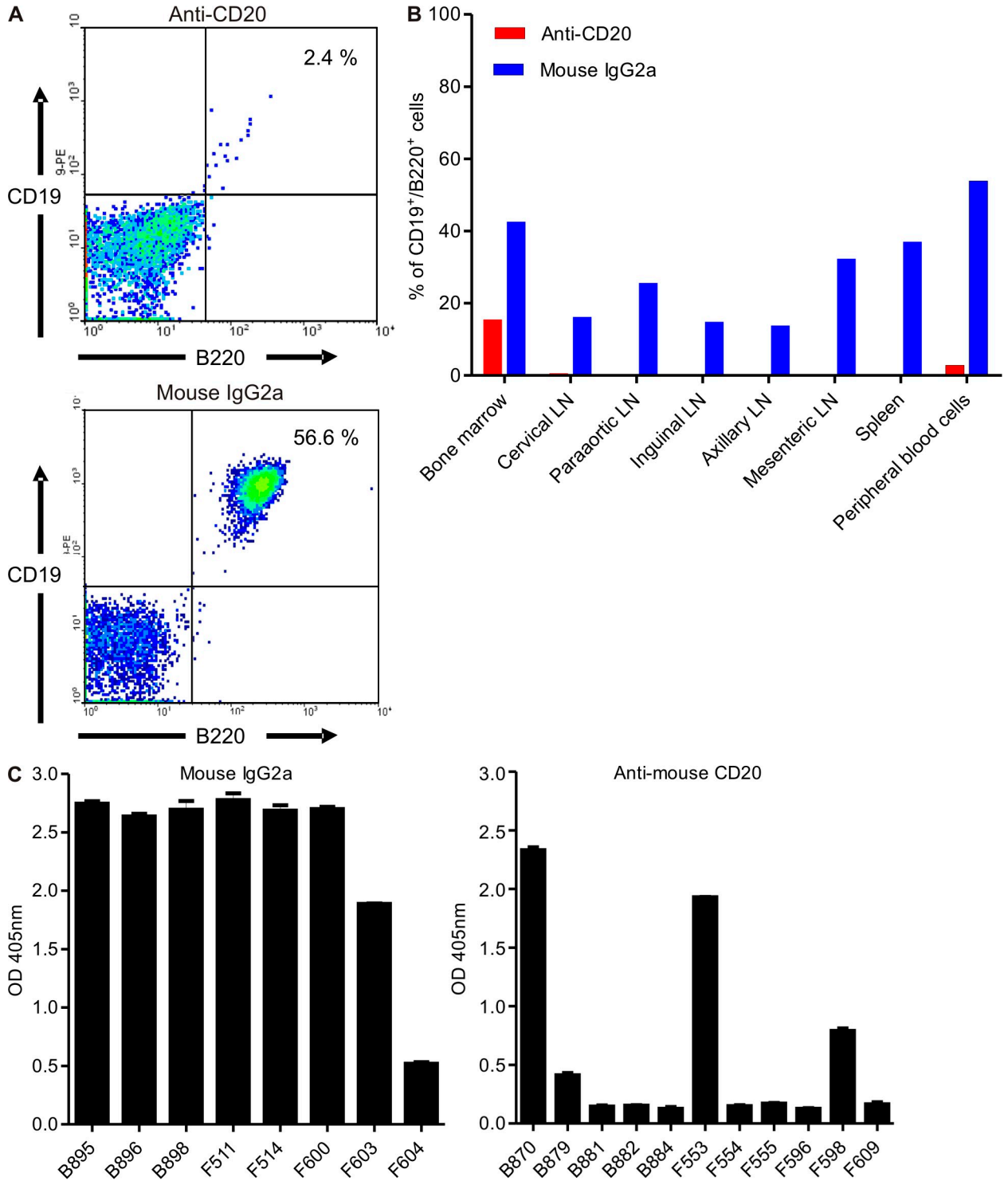
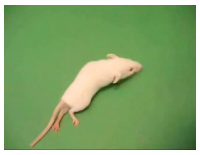


Figure S5. Efficiency of B cell depletion in TCR^{1640} mice. (A) Quantification of B cell depletion in peripheral blood cells of TCR^{1640} mice. Depletion efficiency was analyzed in PBLs of TCR^{1640} mice treated with either CD20 or isotype control antibodies. Representative (seven to nine mice) FACS plot showing B cells stained with CD19 and B220. (B) Analysis of various tissues showed that treatment with anti-CD20 led to reduction of B cells in the bone marrow, whereas lymph nodes, as well as spleen, were almost devoid of B cells. Data are representative of three different mice per group. (C) Serum MOG-specific IgG1^{b} antibodies in B cell–depleted mice. MOG-specific antibodies from serum of individual mice that were treated either with mouse IgG2a (left) or CD20 (right) antibodies were measured by ELISA. Error bars indicate SEM. Note that high titers of anti-MOG antibodies were found in almost all the isotype control antibody-treated mice, whereas most of the CD20 treated group showed negligible amounts of autoantibodies. Data were pooled from two independent experiments.



Video 1. Spontaneous EAE. The video displays one diseased $TCR^{1640} \times IgH^{MOG}$ double-transgenic mouse (#6490) in different disease states. The first state shows ataxia at the age of 9 wk. A few days later, disease is fully remitted. After 1 wk, strong paralytic EAE (score 3) in the same mouse is observed. 1 wk later, the mouse comes down with partial remission showing residual paralysis of score 2 (limp tail and hind limb weakness).

Table S1. Spontaneous EAE in TCR transgenic SJL/J mice: incidence, onset, severity, and gender influence

Transgenic strain (gender)	Incidence (*n*)	Onset	Mean maximal clinical score (*n*)
	%	*wk*	
TCR^{1640} (f)	91.6 (11/12)	14.9 ± 1.5	4.0 ± 0.3 (11)
TCR^{1640} (m)	74.1 (20/27)	15.0 ± 3.45	4.2 ± 1.1 (16)
TCR^{1640} x *Mog*$^{-/-}$ (f/m)	0 (0/6)	-	-
TCR^{1639} (f)	0 (0/22)	-	-
TCR^{1639} (m)	0 (0/15)	-	-
TCR^{1586} (f)	5.5 (1/18)	18.3	4 (1)
TCR^{1586} (m)	30.0 (5/15)	26.6 ± 5.5	4.4 ± 0.5 (5)
TCR^{1640} x IgH^{MOG} (f)	100 (20/20)	12.5 ± 0.7	3.5 ± 0.2 (19)
TCR^{1640} x IgH^{MOG} (m)	73.1 (19/26)	19.7 ± 2.4	3.7 ± 0.3 (19)
TCR^{1639} x IgH^{MOG} (f)	14.2 (1/7)	11.0	3 (1)
TCR^{1639} x IgH^{MOG} (m)	0 (0/10)	-	-
TCR^{1586} x IgH^{MOG} (f)	0 (0/8)	-	-
TCR^{1586} x IgH^{MOG} (m)	0 (0/3)	-	-

Onset: mean week of disease-onset (ataxia or paralytic EAE) ± SEM. Clinical score: Mean maximal score of paralytic EAE of diseased mice ± SEM. f, female; m, male.

Table S2. Summary of histological analysis of representative individual mice with spontaneous EAE

Mouse	EAE Course	Spinal cord		Optical nerve		Brain	
		Inf.[a]	Dem.	Inf.	Dem.	Inf.	Dem.
TCR^{1640} #8222 (f)	RR complete remission paralysis and ataxia	2.4	Confluent plaques	Individual TC	0	Men., medulla, mesenc., periventr. cerbellum	Extreme cerebellum; medulla, periventr.
TCR^{1640} #8287 (m)	Progressive with paralysis and ataxia	3.9	Confluent plaques	Diffuse TC	PV	Medulla, mesenc., cerebellum men.,	Medulla
TCR^{1640} #8290 (f)	Progressive with paralysis and ataxia	2.8	Confluent plaques	n.a.	n.a	Medulla	Periventr., medulla
$TCR^{1640} \times IgH^{MOG}$ #8502 (m)	Progressive with paralysis	2.9	Confluent plaques	Diffuse TC	PV	Men., medulla	n.a.
$TCR^{1640} \times IgH^{MOG}$ #8575 (f)	Progressive with paralysis	2.5	Confluent plaques	Diffuse TC	PV	Men., medulla	Periventr., medulla
$TCR^{1640} \times IgH^{MOG}$ #8589 (f)	RR partial remission with paralysis	3.3	Confluent plaques	Diffuse TC	0	Medulla	n.a.

Abbreviations: Dem, Demyelination; f, female; Inf, infiltration; men, meninges; mesenc, mesencephalon; m, male; n.a.: not analyzed; PV, perivascular; periventr., periventricular; RR, relapsing remitting; TC, T cell infiltration.
[a]The infiltration in the spinal cord is quantified as averaged number of infiltrates per spinal cord section.

Table S3. Spontaneous EAE incidence and mortality in TCR^{1640} mice treated with anti-CD20 and control isotype antibodies

Treatment	Incidence (%)	Female	Male	Mean EAE onset	Mortality
				wk ± SEM	
CD20	1/9 (11.11)	5	4	28.00	0
IgG2a	6/7 (85.71)	5	2	11.00 ± 0.62	2