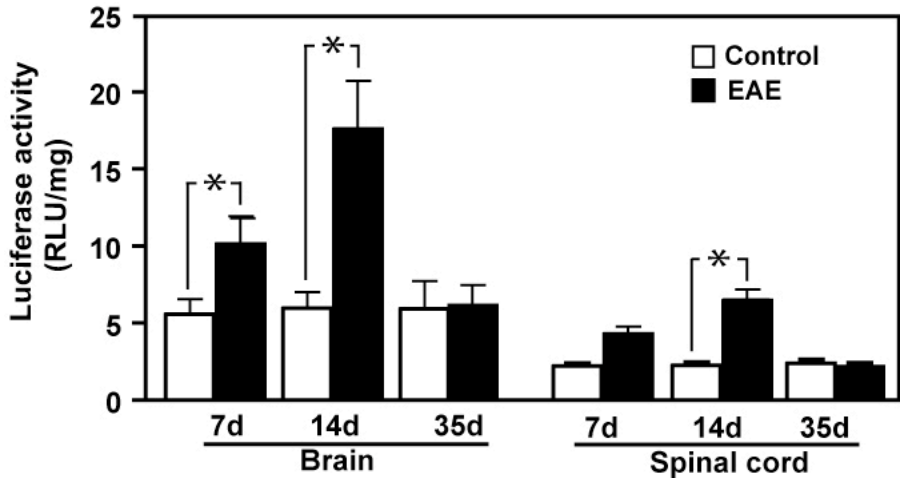
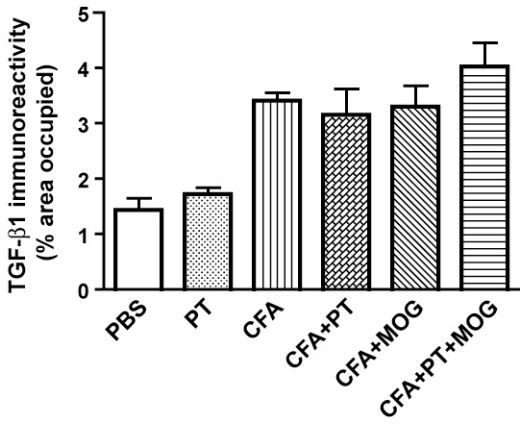
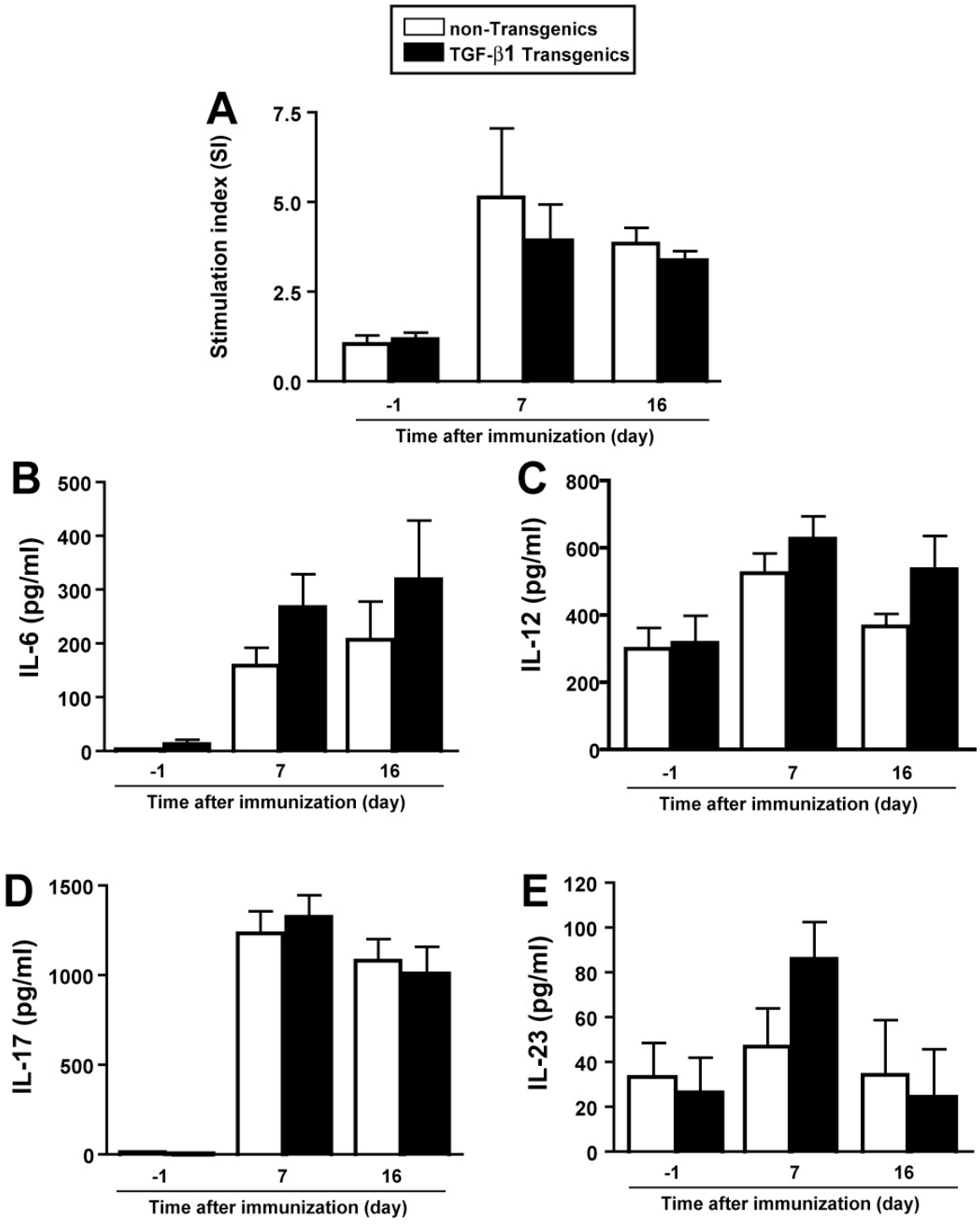


Supplement Figure S1. Early activation of TGF-β signaling in the CNS of SBE-luc mice. Mice immunized with MOG_{35-55} emulsified in complete Freund's adjuvant plus pertussis toxin (EAE) or injected with PBS (control) were sacrificed at 7, 14 and 35 dpi, brains (entire hemibrain) and spinal cords were removed and luciferase activity was measured in tissue homogenates. Bars are mean ± SEM (n = 3-5 mice per group). * $P <$ 0.01 compared with control by unpaired *t*-test.



Supplement Figure S2. Increased TGF-β1 production in the CNS in response to CFA. SBE-lucRT mice were injected with PBS, CFA, PT or combinations thereof with MOG_{35-55} peptide and sacrificed at 7 dpi. Sagittal brain sections were immunostained for TGF-β1 and relative immunoreactivity was quantified in the cerebellum as percentage of area occupied. Bars are mean ± SEM (n = 3-4).



Supplement Figure S3. Overexpression of TGF-β1 in the brain does not significantly alter T cell priming. Splenocytes from TGF-β1 transgenic mice or non-transgenic littermates with (day 7 and 14) or without (day -1) EAE were cultured and stimulated with MOG_{35-55} peptide (10 μg/ml) or medium only (n = 3-4 mice per group). (**A**) Cell proliferation, assessed by thymidine incorporation, was expressed as stimulation index.

(**B**-**E**) Cell culture supernatants were collected 48 h later and levels of IL-6 (**B**), IL-12 (**C**), IL-17 (**D**), and IL-23 (**E**) were measured by ELISA. No significant differences were observed in any of the measurements (**A**-**E**) between the two groups of mice as evaluated by ANOVA and Tukey's post-hoc test. Similar results were obtained with cells exposed to 20 μg/ml of MOG_{35-55} peptide.

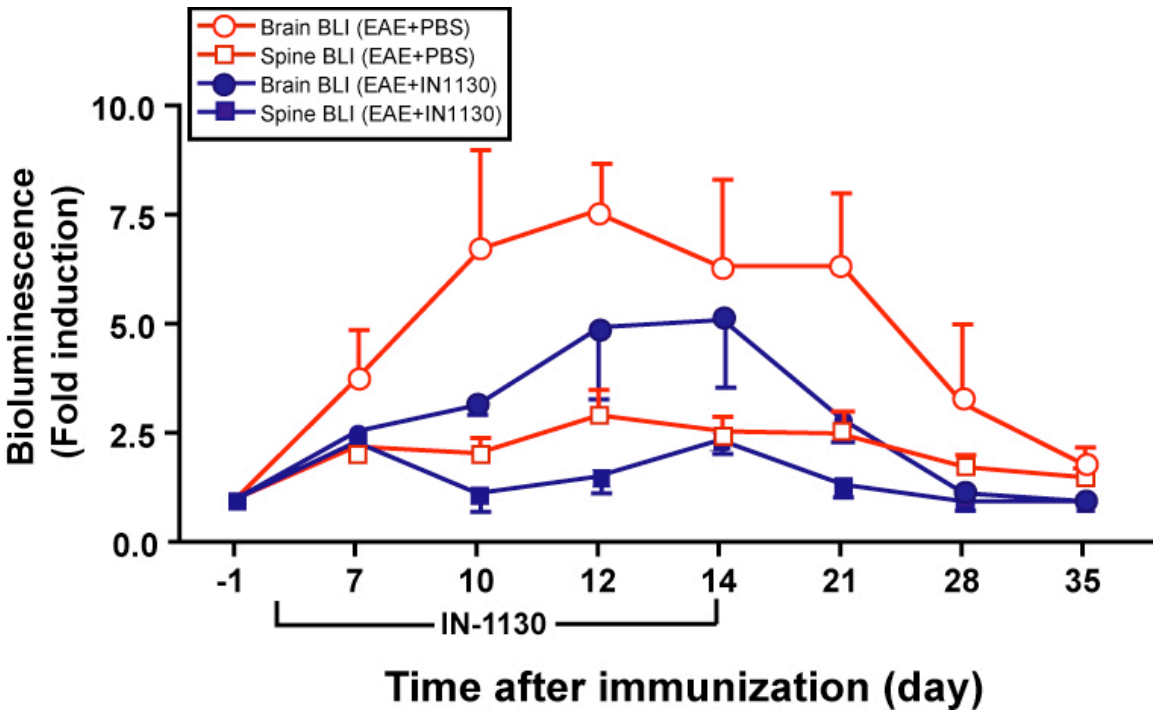
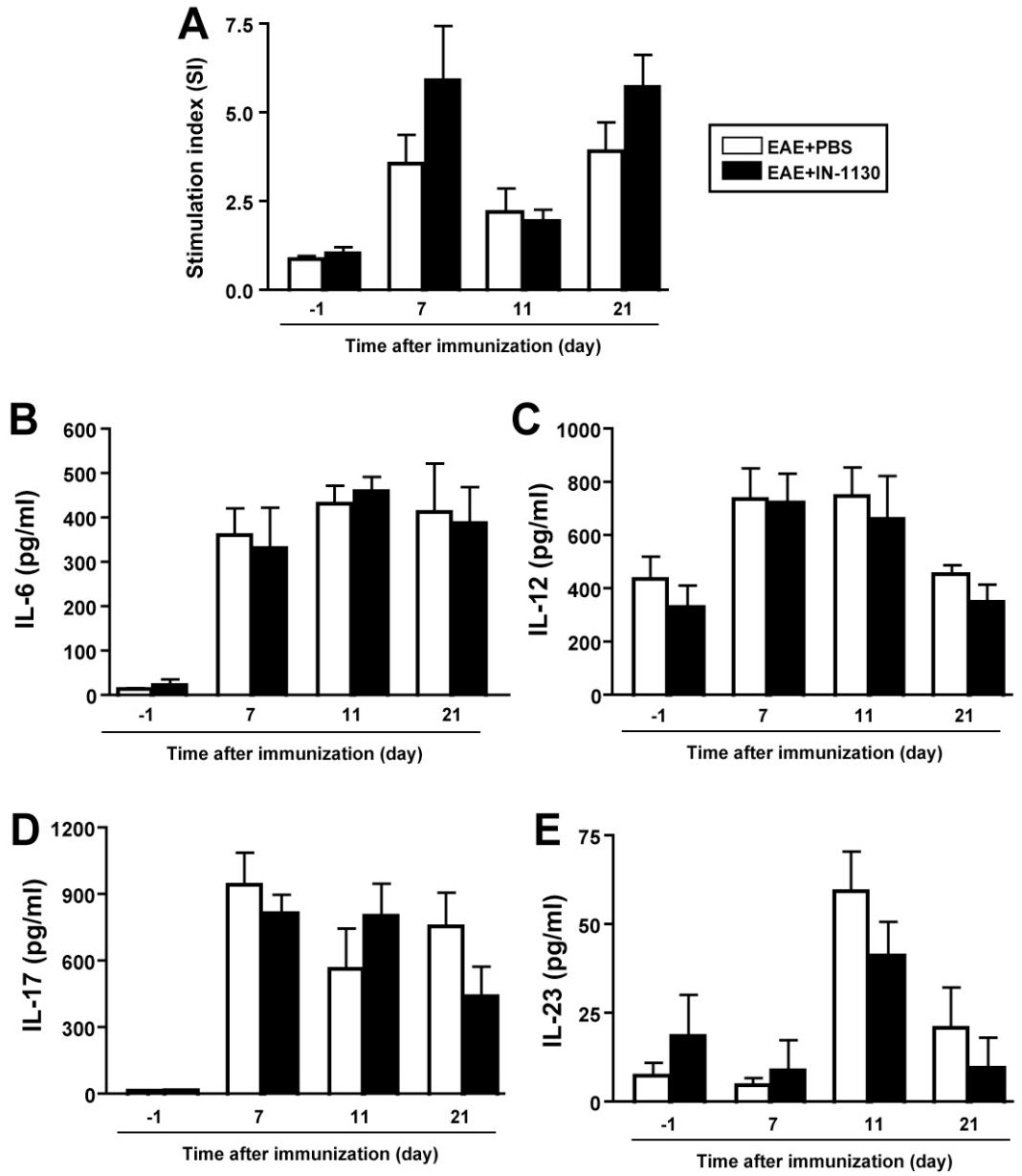


Figure S4. Pharmacological inhibition of TGF-β signaling by IN-1130. SBE-luc mice (n = 10-12 mice per group) were immunized with MOG_{35-55} emulsified in CFA and treated with the TGF-β receptor kinase inhibitor IN-1130 (closed symbols) or PBS (open symbols) from 1-14 dpi. Daily bioluminescence was recorded and bioluminescence was shown as mean ± SEM of each group.



Supplement Figure S5. Treatment of IN-1130 does not significantly affect T cell priming. Splenocytes from EAE (day 7, 11 and 21) or non-immunized (day -1) mice treated with PBS or IN-1130 (n = 3-4 mice per group) were cultured and stimulated with MOG_{35-55} (10 μg/ml) or medium only. (**A**) Cell proliferation analysis, assessed by thymidine incorporation, was expressed as stimulation index. (**B**-**E**) Cell culture supernatants were collected 48 h later and assayed for IL-6 (**B**), IL-12 (**C**), IL-17 (**D**), and IL-23 (**E**) by ELISA. No significant differences were observed in any of the measurements (**A**-**E**) between IN-1130 treated and control mice as evaluated by ANOVA

and Tukey's post-hoc test. Similar results were obtained with cells exposed to 20 μg/ml of MOG_{35-55} peptide.

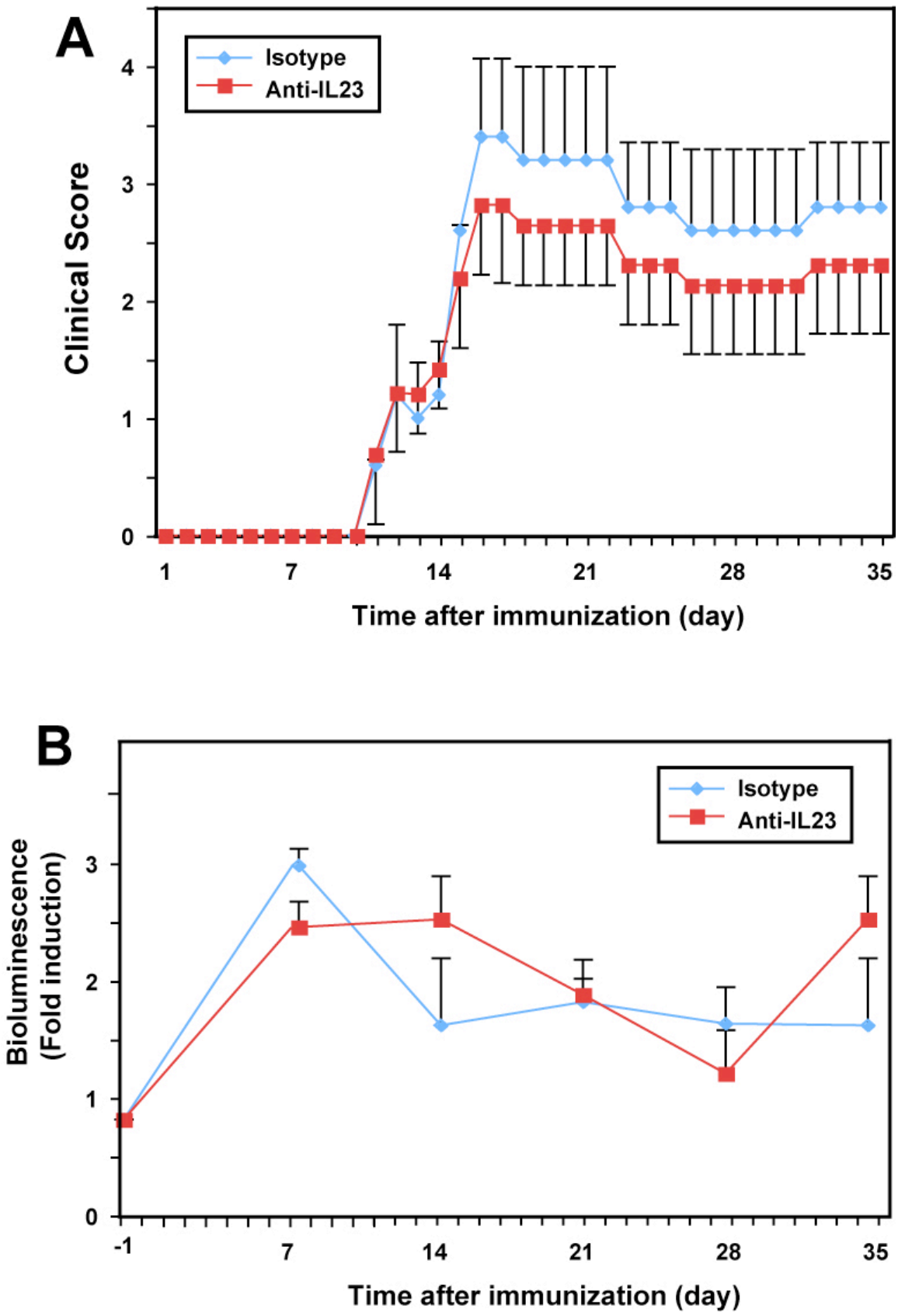


Figure S6. Anti-IL-23 treatment does not significantly inhibit TGF-β signaling. SBE-lucRT mice (n = 5 per group) were immunized with MOG_{35-55} emulsified in CFA and

treated with the anti-IL-23 (red symbols) or isotype control (blue symbols) injected at 10, 17, 24 and 31 dpi. Clinical score (**A**) and brain bioluminescence (**B**) were recorded and were shown as mean ± SEM. Brain bioluminescence was expressed as fold induction over pre-immunization level (day -1). No significant difference was observed between anti-IL-23 and isotype controls.

Table S1. TGF-β1 transgenic mice exhibit more severe EAE

Group	Incidence (No./total, %)			Day of onset	Clinical score				Weight loss
	day 8	day 10	day 12	(range)	day 8	day 10	day 12	day13	% of pre-injury
TGF-β1 transgenic	4/9 (44.4)	9/9 (100.0)	9/9 (100.0)	8.8 ± 0.6 (8-10)*	0.7 ± 1.1	1.6 ± 2. 0	3.8 ± 0.8*	4.1 ± 0.6**	78.8 ± 0 (day13)*
Non-transgenic	1/7 (14.3)	5/7 (71.4)	7/7 (100.0)	9.7 ± 1.7(8-12)	0.1± 0.1	1.0 ± 1.6	3.1 ± 0.2	3.2 ± 0.2	87.5 ± 0

*: $P < 0.05$; **: $P < 0.01$ vs non-transgenics

Supplement Table S1. Astrocyte-targeted overexpression of TGF-β1 results in more severe EAE**.** EAE was induced in TGF-β1 transgenic female mice or non-transgenic littermates. Daily clinical evaluation was performed in a blinded manner. Data are expressed as mean ± SEM. * $P < 0.05$, ** $P < 0.01$ vs non-transgenic.