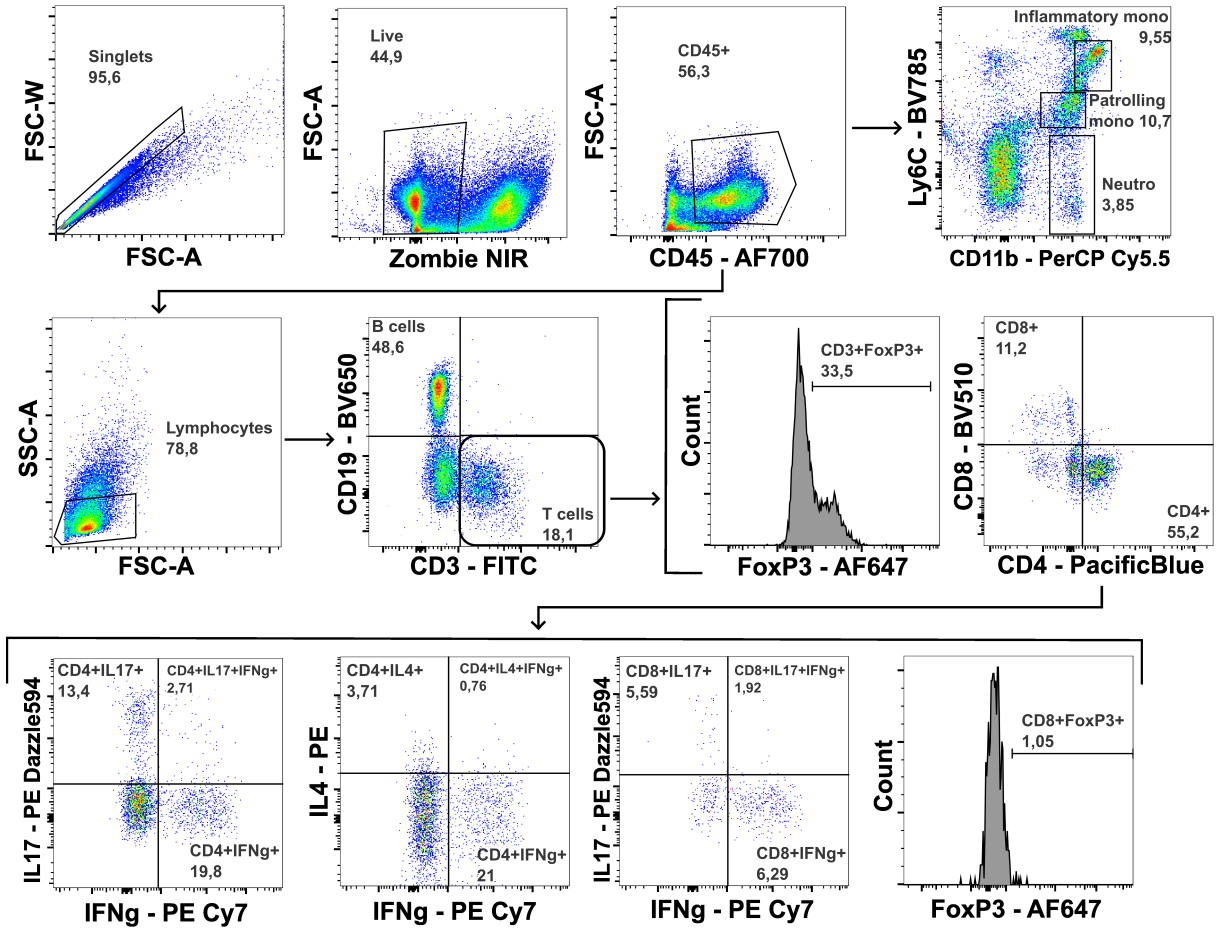

Fig. S1. Example of the gating strategy used to distinguish different immune cell subsets. Representative spleen sample from a PBS-treated hu/m TNFR2-ki mouse sacrificed at 25 days post immunization.

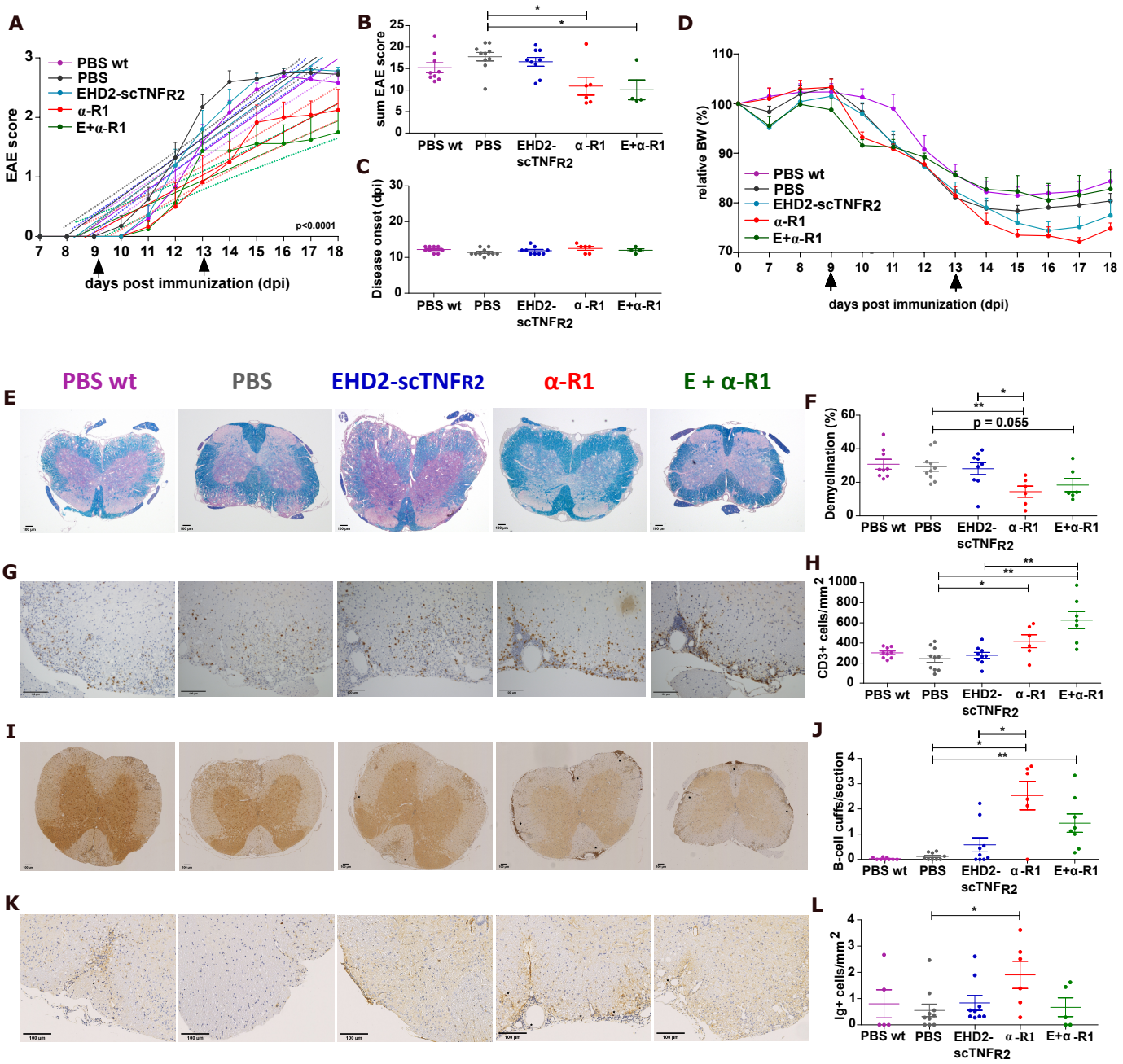

Fig. S2. *Blocking TNFR1 with and without a TNFR2 agonist limits EAE development by decreasing demyelination while increasing lymphocyte numbers in the spinal cord*. Hu/m TNFR2-ki and WT mice were immunized with MOG_{35-55} and treated at 9- and 13-days post immunization (dpi; **black arrows**) either with saline (n= 10/genotype), EHD2-scTNF*R2* (TNFR2 agonist; n= 9; 10 mg/kg), anti-mouse TNFR1 (α-R1; n= 6; 20 mg/kg) or a combination (E+ α-R1; n=4). Disease development (**A**, **B** and **C**) and body weight (BW; **D**) were measured daily from 7 to 18 dpi. Mice were sacrificed at 18 dpi and spinal cord sections were analyzed as follows. Demyelination degree was measured with Klüver Barrera staining (n= 6-10/group; **E** and **F**). The number of T cells present in the sections was measured by counting CD3 positive cells per mm^2 (n= 6-10/group; **G** and **H**). B-cell clustering was assessed by counting the number of B220 positive cuffs (**black stars**) present in the perivascular space (n= 6-9/group; **I** and **J**) while Ig staining revealed the number of plasma cells per mm^2 (**black stars**, n= 5-10/group; **K** and **L**). Data are presented as mean ± SEM and differences between groups were assessed with Mann-Whitney test, except for body weight loss for which two-way ANOVA and Tukey's post-test were used. **p<0.05, **p<0.01*

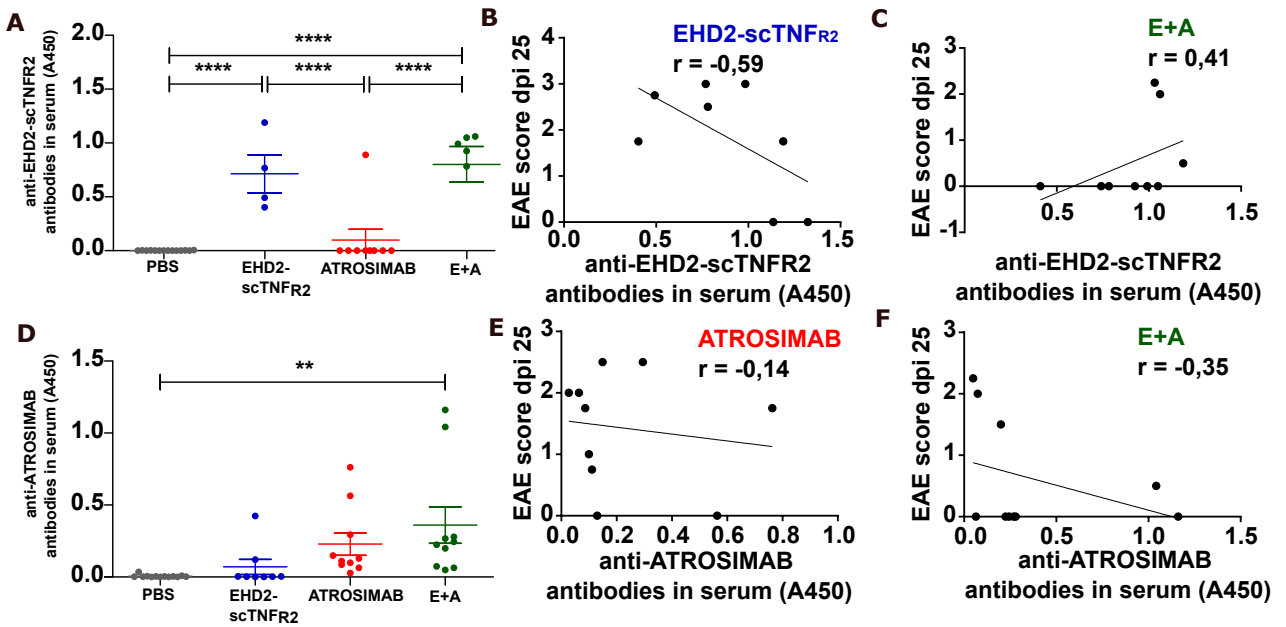

Fig. S3. *EHD2-scTNFR2 and ATROSIMAB treatments increase the level of anti-drug antibodies in serum but do not affect clinical EAE development. Levels of EHD2-scTNFR2-specific antibodies (A) and ATROSIMAB-specific antibodies (D) in serum of hu/m TNFR1-ki x hu/m TNFR2-ki were measured with ELISA and correlated (Pearson coefficient r) with EAE score of animals treated with EHD2-scTNFR2 (TNFR2 agonist; B), ATROSIMAB (TNFR1 antagonist; E) and combination (E+A; C and F) at time of sampling (25 days post immunization, dpi). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001*

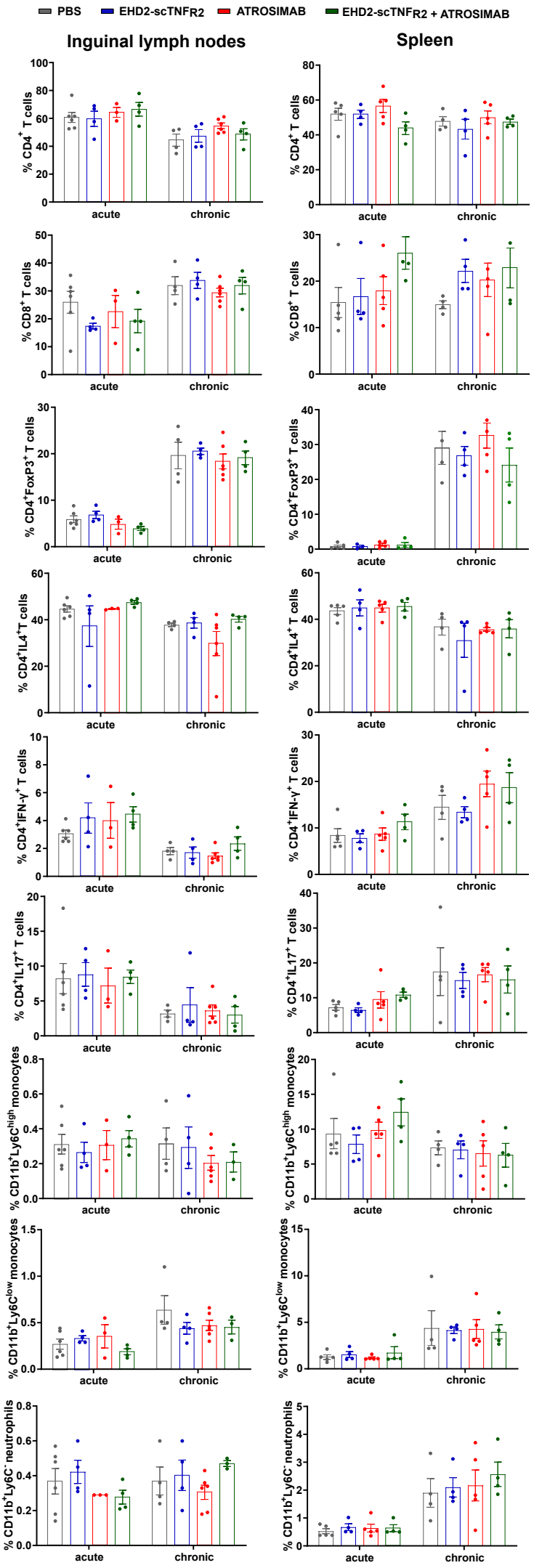

Fig. S4. *Frequency of immune cell subsets in inguinal lymph nodes and spleen.*

Hu/m TNFR1-ki x hu/m TNFR2-ki mice were immunized with MOG_{35-55} and treated either with PBS, EHD2-scTNFR2 (TNFR2 agonist), ATROSIMAB (TNFR1 antagonist) or a combination. Immune cell subsets from spleen and inguinal lymph nodes of mice sacrificed either 18- (acute) or 25-days (chronic) post immunization were analyzed by flow cytometry using the gating strategy described in Figure S3.

CD = cluster of differentiation; IFN-γ = interferon gamma; IL = interleukin.

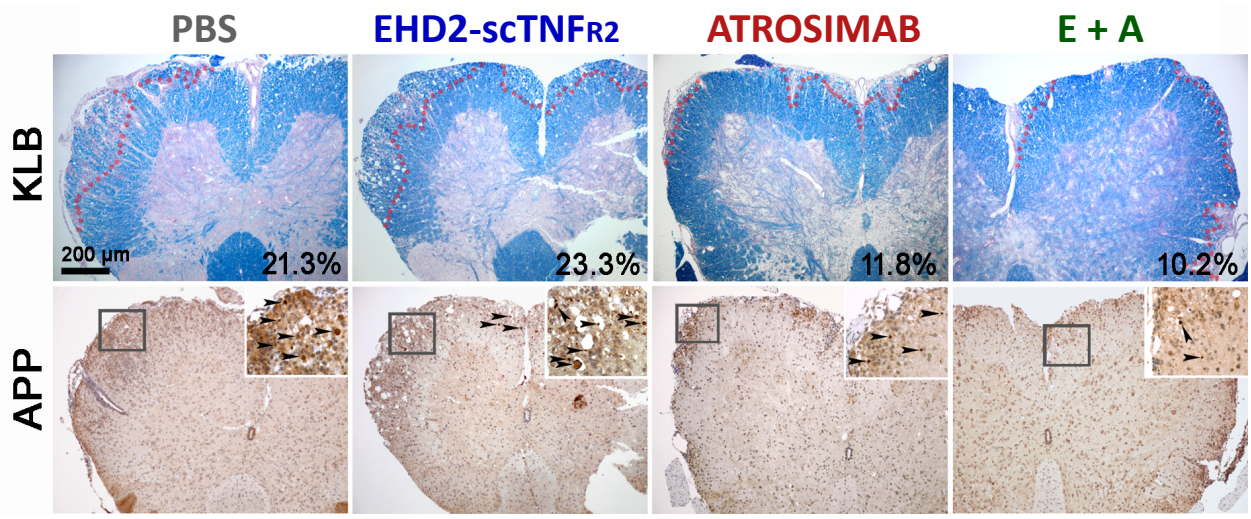

Figure S5*. APP staining of spinal cord sections from representative mice from the acute cohort shows that axonal degeneration increases similarly to demyelination.* Immunohistochemical analysis of Alzheimer precursor protein (APP) staining of hu/m TNFR1-ki x hu/m TNFR2-ki mice immunized with MOG_{35-55} and treated either with saline, EHD2-$scTNF_{R2}$ (TNFR2 agonist), ATROSIMAB (TNFR1 antagonist) or a combination (E+A). Within the same animal, the number of APP+ cells (black arrowheads) were compared with the degree of demyelination (Kluver-Barrera-KLB). The percentage of demyelination of each animal is depicted at the bottom right of each picture. Only spinal cord sections from animals sacrificed 18 days after immunization (acute cohort) were used for this staining. APP antibody (clone 22C11, Millipore, 1:1000). Scale bar is 200 µm.

***Table S1**. Primary antibodies used for immunohistochemistry and flow cytometry*

Primary antibody	**Clone**	**Company**	**Target**	**Assay**
Alzheimer Precursor Protein (APP)	22C11	Millipore	Neurons	Immunohistochemistry
CD3	SP7	Neomarkers	T cells	Immunohistochemistry
FoxP3	PCH101	eBioscience	Regulatory T cells	Immunohistochemistry
CD19	D4V4B	Cell Signaling	B cells	Immunohistochemistry
CD45R/B220	RA3-6B2 (RUO)	BD Pharmingen	B cells	Immunohistochemistry
IgG	-	Jackson	Plasma cells	Immunohistochemistry
CD3-FITC - extracellular	17A2	Biolegend	T cells	Flow cytometry
CD4-PB - extracellular	GK1.5	Biolegend	T helper cells	Flow cytometry
CD45-AF700 - extracellular	30-F11	Biolegend	Leukocytes	Flow cytometry
CD8-BV510 - extracellular	53-6.7	Biolegend	Cytotoxic T cells	Flow cytometry
CD19-BV650 - extracellular	6D5	Biolegend	B cells	Flow cytometry
CD11b-PerCP/Cy5.5 - extracellular	M1/70	Biolegend	Myeloid cells	Flow cytometry
Ly6C-BV785 - extracellular	HK1.4	Biolegend	Myeloid cells	Flow cytometry
IL-4-PE - intracellular	11B11	Biolegend	IL-4 secreting cells	Flow cytometry
IL-17-PE/Dazzle594 - intracellular	TC11-18H10.1	Biolegend	IL-17 secreting cells	Flow cytometry
IFN-γ-PE/Cy7 - intracellular	XMG1.2	Biolegend	IFN-γ secreting cells	Flow cytometry
Foxp3-AF647 - intracellular	MF-14	Biolegend	Regulatory T cells	Flow cytometry

Table S2. *Clinical EAE development in hu/m TNFR1-ki x hu/m TNFR2 -ki mice treated either with PBS, a human TNFR2 agonist (EHD2-scTNF$_{R2}$) and/or a human TNFR1 antagonist (ATROSIMAB).*

Treatment group	Day of disease onset	Sum EAE score	AUC EAE score	Mean EAE score	Median EAE score	Max EAE score	Days with EAE score ≥ 2	EAE disease incidence	Death incidence due to EAE
PBS	12.8 ± 0.7	26.9 ± 3.7	25.9 ± 3.6	1 ± 0.1	0.8 ± 0.2	2.5 ± 0.2	9.5 ± 1.4	13/13 (100%)	1/13 (7.7%)
EHD2-scTNF$_{R2}$	13.5 ± 0.9	25.3 ± 3.0	24.3 ± 2.9	0.9 ± 0.1	0.3 ± 0.2	2.6 ± 0.1	7.5 ± 1.6	8/10 (80%)	1/10 (10%)
ATROSIMAB	13.9 ± 0.9	15.9 ± 3.3	15.1 ± 3.2	0.6 ± 0.1	0.5 ± 0.2	2 ± 0.2 *[a]	3.2 ± 1.3 *[a]	11/12 (91.7%)	0/12 (0%)
EHD2-scTNF$_{R2}$ + ATROSIMAB	13.6 ± 1.4	12.2 ± 3.3 *[a, b]	11.9 ± 3.2 *[a, b]	0.4 ± 0.1 *[a, b]	0.2 ± 0.1 *[a]	1.6 ± 0.2 **[a, b]	2.3 ± 1.2 **[a, b]	9/10 (90%)	0/10 (0%)

Data are presented as mean ± SEM and difference s between groups were assessed with Mann-Whitney test, except for disease onset and days with EAE score ≥ 2 for which one way ANOVA and Tukey's post test were used. a = compared to PBS and b = compared to EHD2-scTNFR2. *p<0.05, **p<0.01