

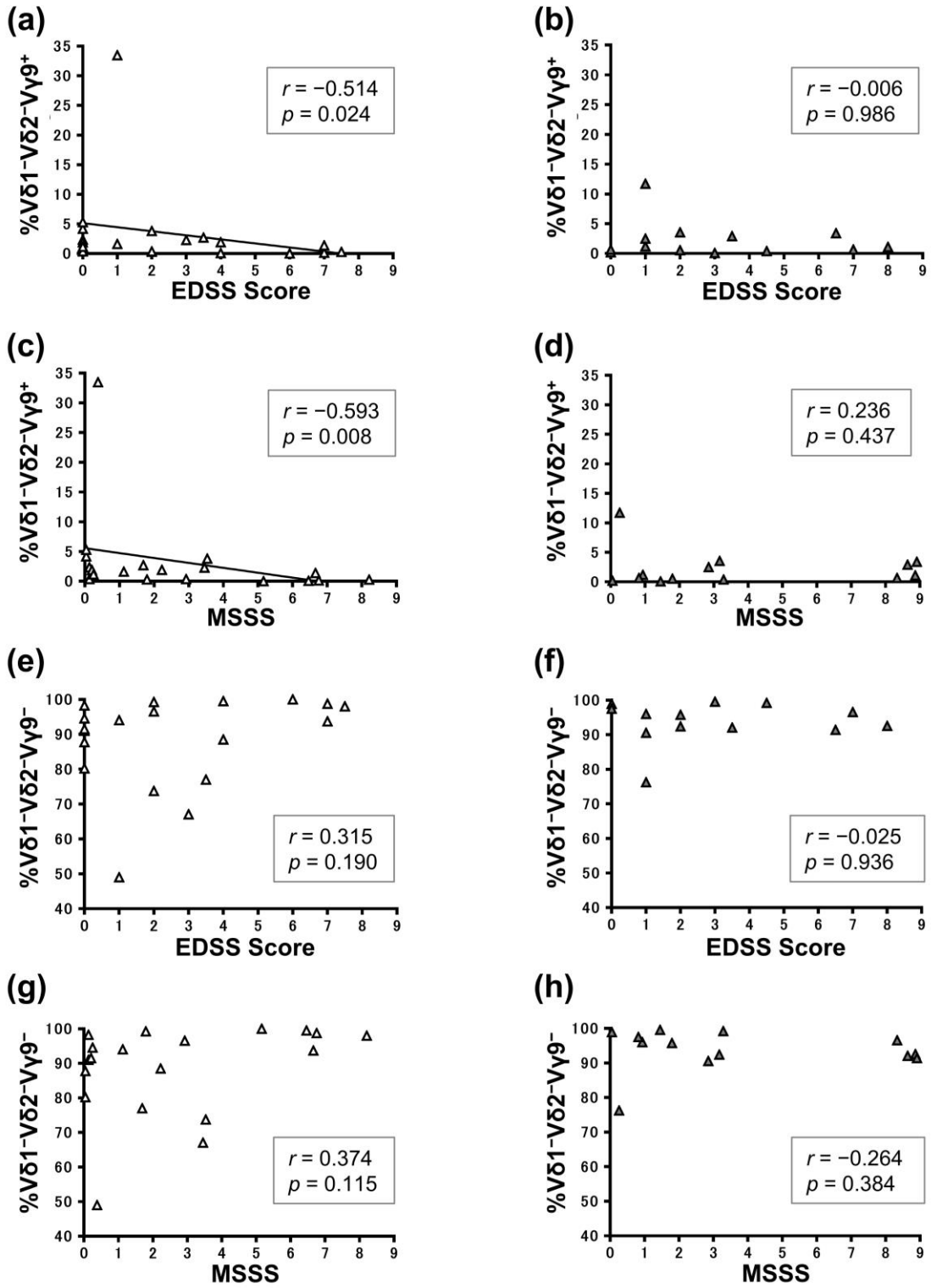
**Figure S1** Immunophenotyping gating strategy for  $\gamma\delta$  T cells and their comparison between HCs and patients with MS.

**(a)** Gating strategies for the flow cytometric analysis of  $\gamma\delta$  T cells are shown. Peripheral blood mononuclear cells (PBMCs) were initially gated on singlet cells by forward-scatter (FSC)-area (FSC-A) and FSC-height (FSC-H), and then lymphocytes were gated by FSC-A and side-scatter (SSC)-area (SSC-A).  $\gamma\delta$  T cells were gated from CD3<sup>+</sup> T lymphocytes by TCR $\gamma\delta$  and TCR $\alpha\beta$ , and classified by the presence of V $\delta$ 1 and V $\delta$ 2 into V $\delta$ 1<sup>+</sup>V $\delta$ 2<sup>-</sup>, V $\delta$ 1<sup>-</sup>V $\delta$ 2<sup>+</sup> and V $\delta$ 1<sup>-</sup>V $\delta$ 2<sup>-</sup>  $\gamma\delta$  T cells, which were then examined for the expression of V $\gamma$ 9. Representative data of HCs, untreated MS patients and IFN- $\beta$ -treated MS patients are shown. **(b)** Comparison of the percentages of V $\delta$ 1<sup>+</sup>, V $\delta$ 2<sup>+</sup> and V $\delta$ 1<sup>-</sup>V $\delta$ 2<sup>-</sup> cells in  $\gamma\delta$  T cells between HCs, untreated MS patients and IFN- $\beta$ -treated MS patients. Boxes depict the median and IQR, and upper/lower whiskers extend from the hinge toward the largest/smallest values.  $p^{adj}$  values were obtained by multivariate linear regression analyses adjusting for age and sex (\*  $p^{adj} < 0.05$ , \*\*  $p^{adj} < 0.01$  and \*\*\*  $p^{adj} < 0.001$ ). Abbreviations: IFN- $\beta$  = interferon- $\beta$ ; MS = multiple sclerosis; TCR = T cell receptor.

In untreated MS

NEDA

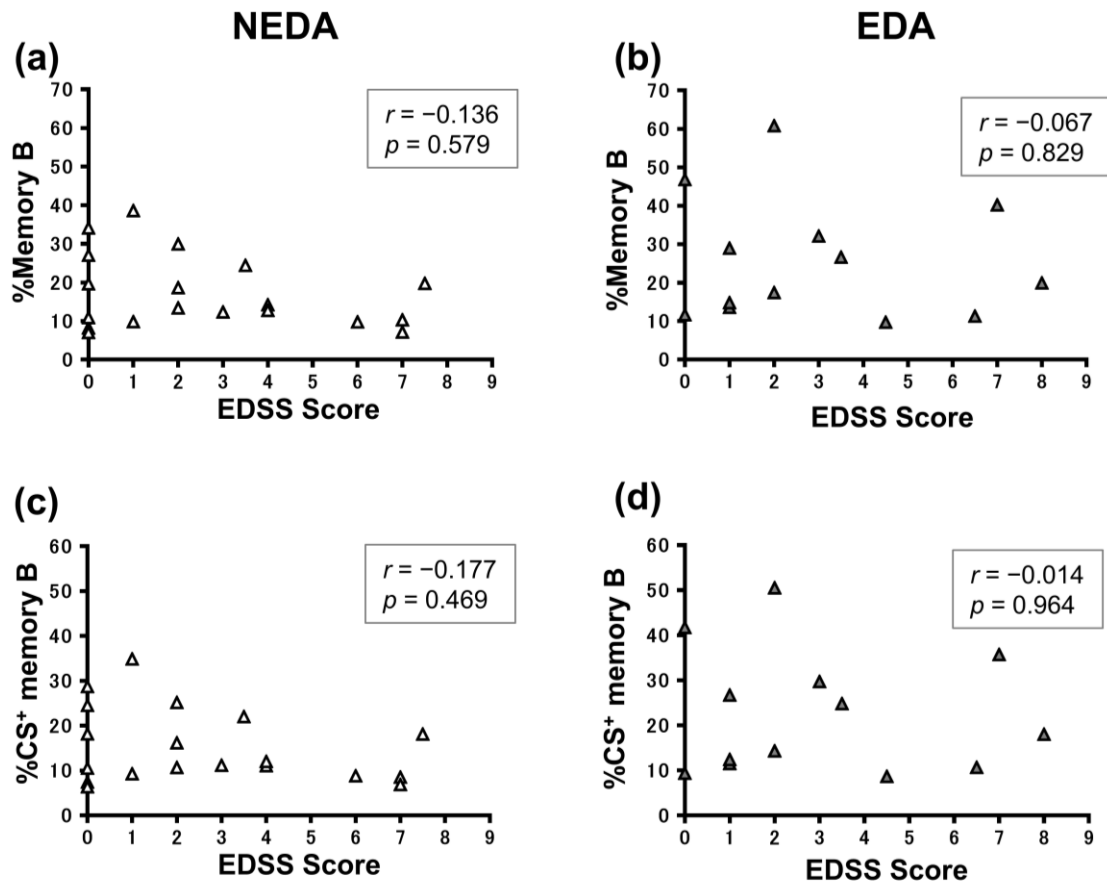
EDA



**Figure S2** Correlations between the percentages of  $\gamma\delta$  T cell subsets and disability in untreated MS patients.

**(a-d)** Correlation between the percentage of  $V\delta 1^{-}V\delta 2^{-}V\gamma 9^{+}$  cells in  $\gamma\delta$  T cells and EDSS scores (**a, b**) or MSSS (**c, d**) at examination in untreated MS patients in the NEDA (**a, c**) and EDA (**e, f**) groups. **(e-h)** Correlation between the percentage of  $V\delta 1^{-}V\delta 2^{-}V\gamma 9^{-}$  cells in  $V\delta 1^{-}V\delta 2^{-}$   $\gamma\delta$  T cells and EDSS scores (**e, f**) or MSSS (**g, h**) at examination in untreated MS patients in the NEDA (**e, g**) and EDA (**f, h**) groups. Correlations were calculated using Spearman's rank correlation coefficient. Abbreviations: EDA = evidence of disease activity; EDSS = Expanded Disability Status Scale; MS = multiple sclerosis; MSSS = multiple sclerosis severity score; NEDA = no-evidence of disease activity.

## In untreated MS



**Figure S3** Correlations of memory B cell proportions with disability in untreated MS patients.

(a, b) Correlation between the percentage of memory B cells and EDSS scores in untreated MS patients in the NEDA (a) and EDA (b) groups.

(c, d) Correlation between the percentage of class-switched memory B cells and EDSS scores in untreated MS patients in the NEDA (c) and EDA (d) groups. Correlations were calculated using Spearman's rank correlation coefficient.

Abbreviations: CS<sup>+</sup> = class-switched; EDA = evidence of disease activity; MS = multiple sclerosis; NEDA = no-evidence of disease activity.