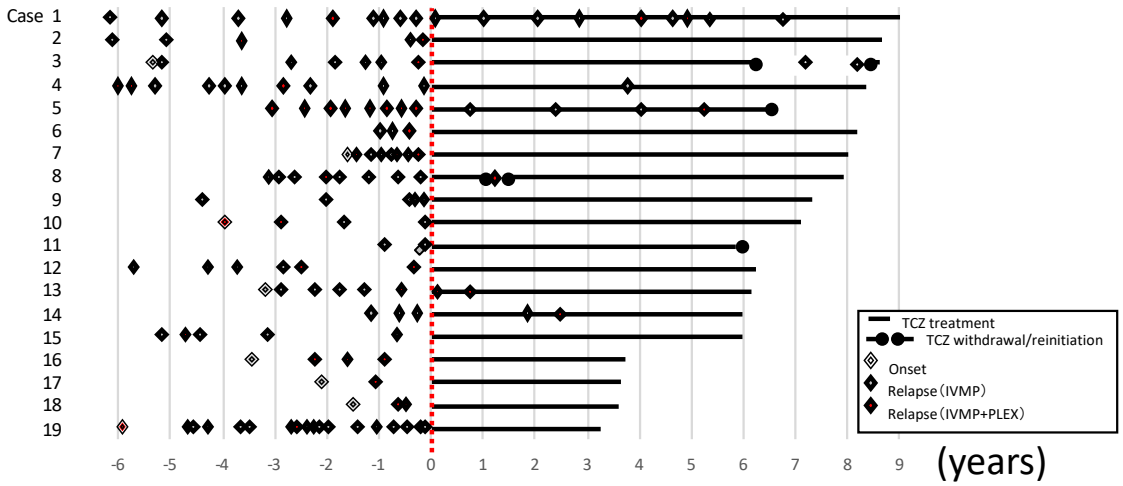
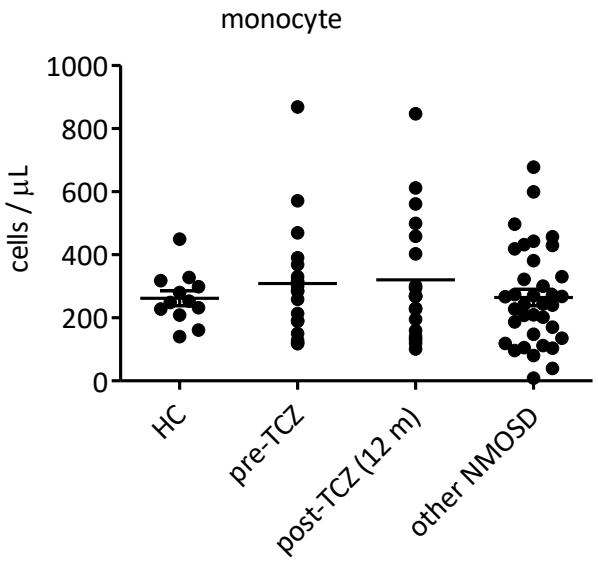


eFigure 1: Clinical course of the 19 participants

For each participant, double-lined diamond shows the onset, and gray color indicates a mild relapse treated with IVMP. Red color indicates a severe relapse requiring IVMP and plasma exchange. Arrowed line shows the duration of the TCZ therapy. Patient 8 wished to withdraw from the clinical trial after 12 months of treatment, because of the long journey to the hospital, but returned back to the trial after having a severe relapse during withdrawal period. IVMP: intravenous methyl prednisolone pulse therapy; PLEX: plasma exchange.

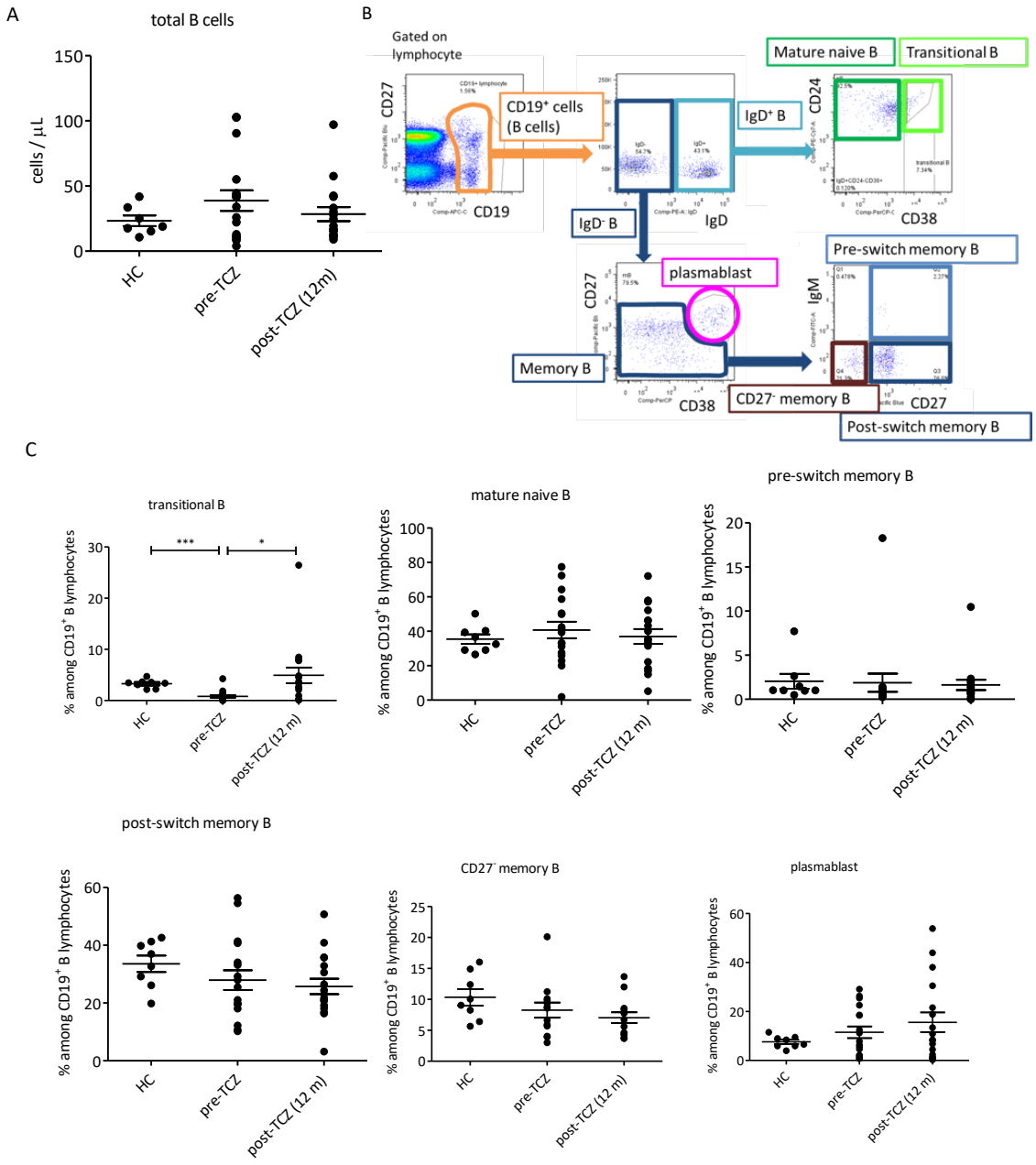


eFigure 2: The number of monocytes in the peripheral blood of HC, NMOSD before and after TCZ treatment for 12 months, and other NMOSD

The number of monocyte was comparable between NMOSD and healthy control subjects (HC).

HC, n = 12; pre and post TCZ, n = 19; other NMOSD, n = 36.

Average ± standard errors are shown



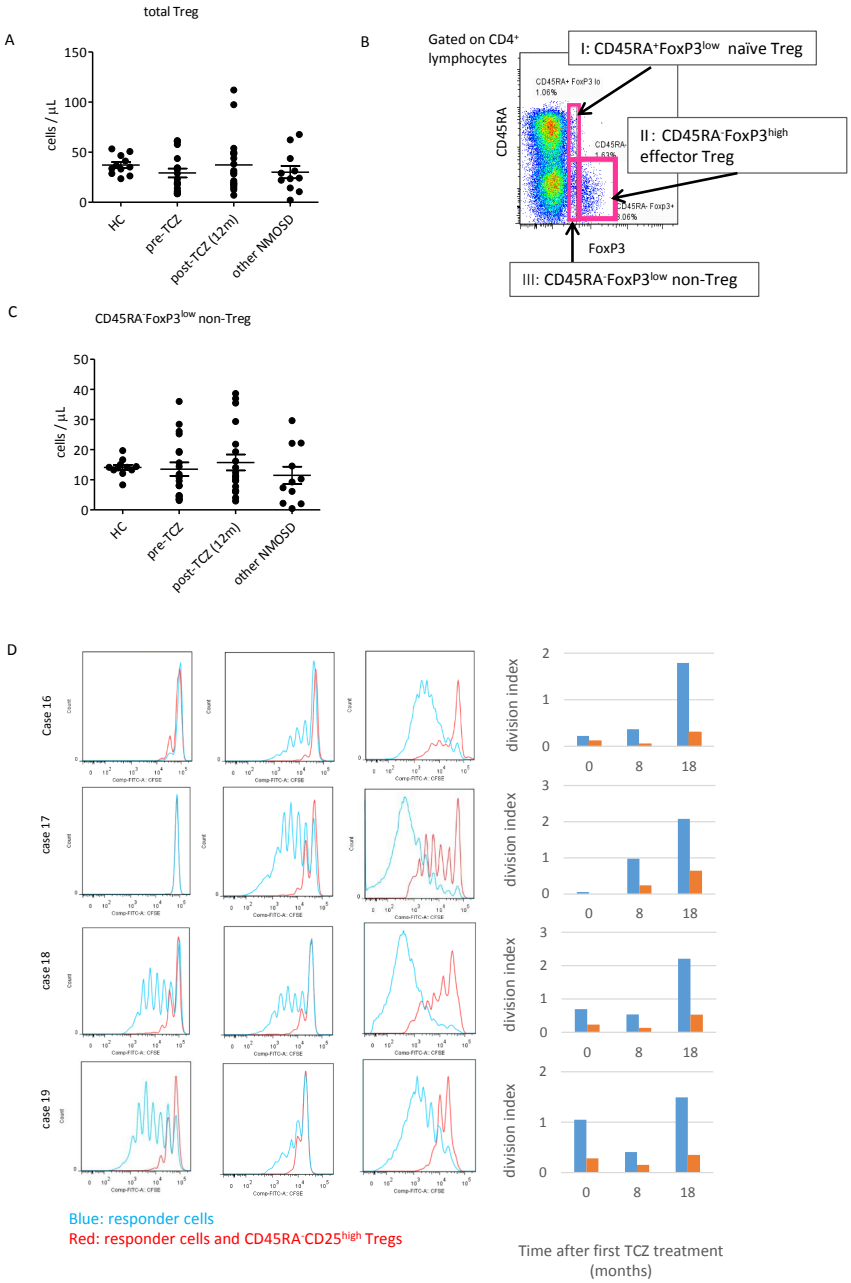
eFigure 3: The B cell subset analysis in HC and NMOSD before and after TCZ treatment for 12 months.

A. The number of total B cells was comparable between NMOSD and healthy control subjects (HC).

B. Gating strategy for the B cell subset analysis.

C. Among B cell subsets, the percentage of transitional B cells was reduced in NMOSD upon entry to this study compared to HC ($p = 0.0004$), but increased after one year therapy with TCZ ($p = 0.0106$). HC, n = 7; pre and post TCZ, n = 17.

A and C, Average ± standard errors are shown.



eFigure 4: Treg cell subset analysis

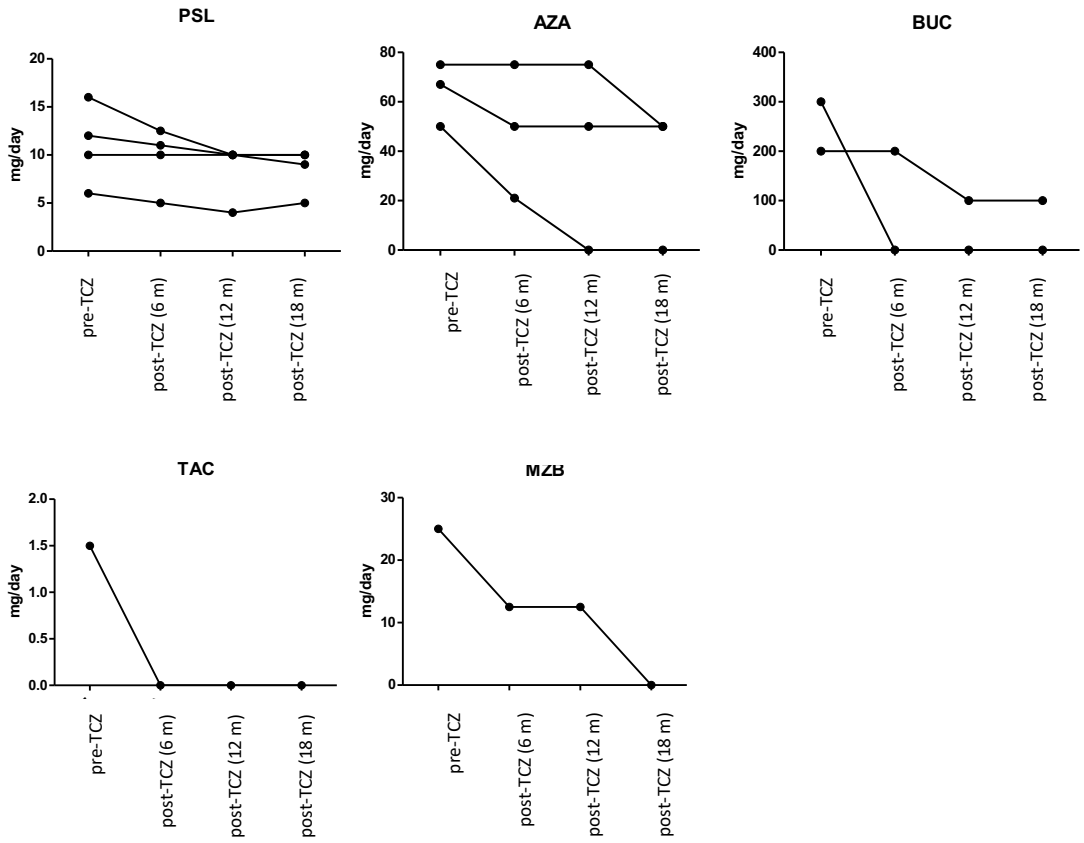
A. The total number of Treg cells. There was no difference between HC and pre-TCZ treatment or between before and after TCZ treatment.

B. The gating strategy of Treg cell subsets using CD45RA and FoxP3 expression.

C. The number of CD45RA^{-}FoxP3^{low} Treg cell subset. No differences between HC, pre-TCZ or pre-TCZ and TCZ-treated groups.

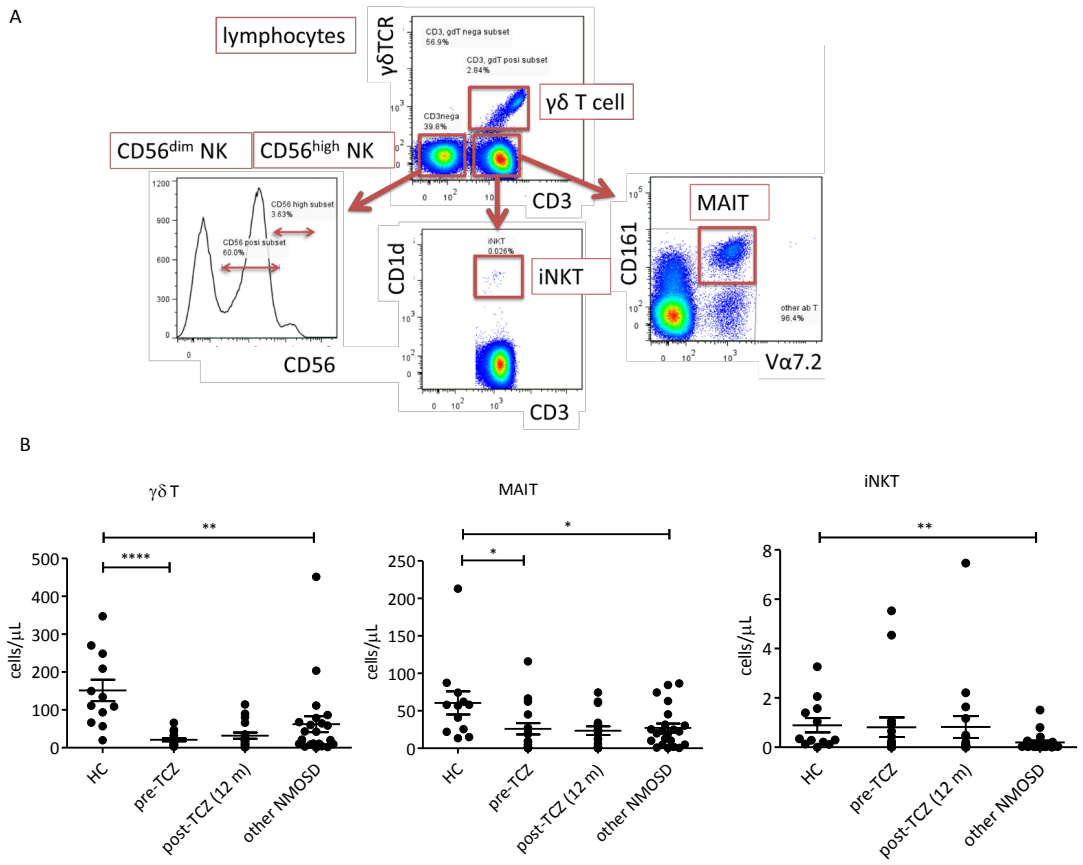
D. T cell co-culture assays of before and after the TCZ treatment from four cases (case 16 -19).

A and C, Average ± standard errors are shown. HC, n = 14; pre and post TCZ, n = 19.



eFigure 5: Changes in doses of concomitant drugs during the 18 months after the initial TCZ treatment. Data present individual cases

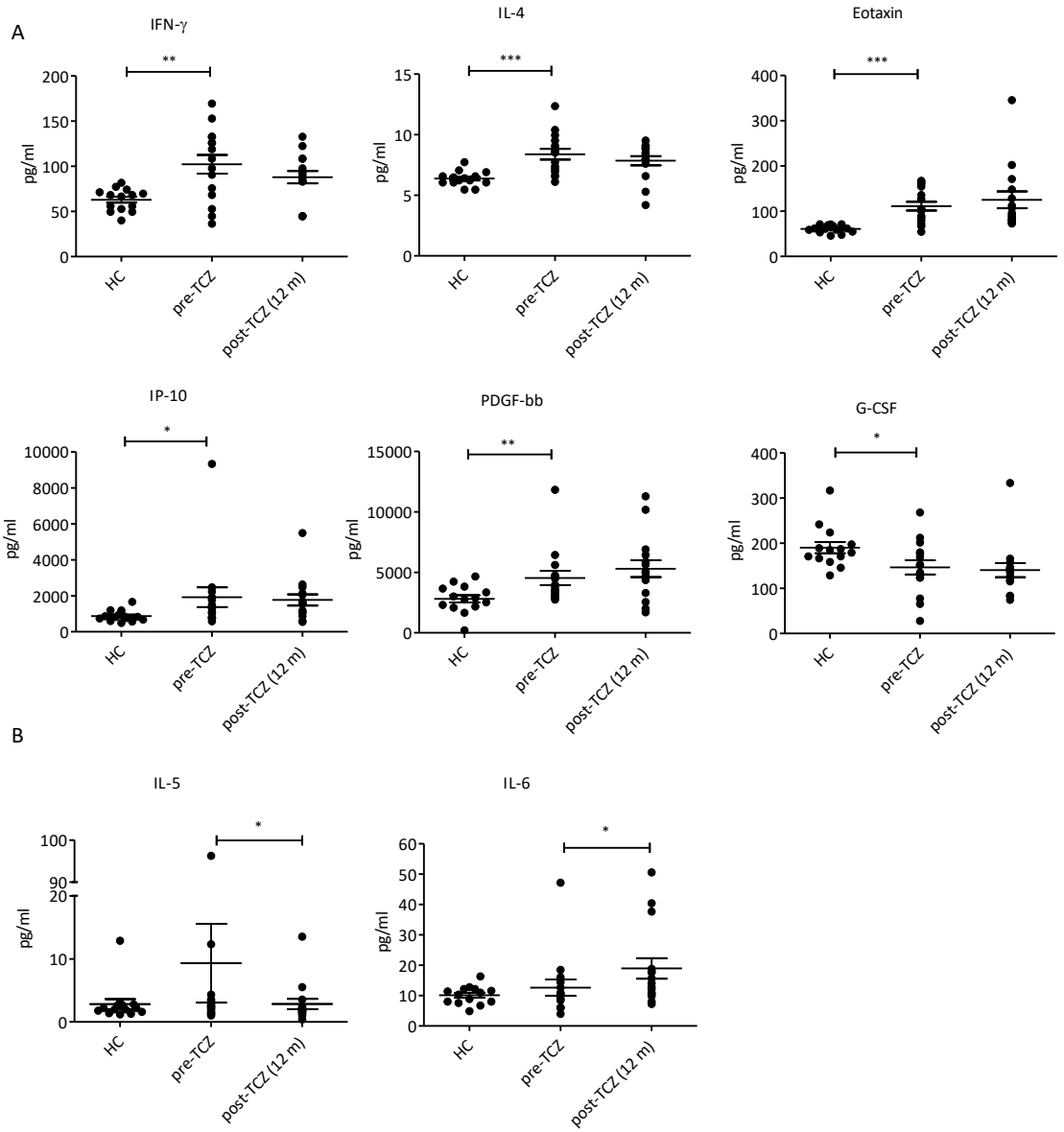
PSL, predonisolone; AZA, azathioprine; BUC, bucillamine; TAC, tacrolimus; MZB, mizoribine.



eFigure 6: The analysis of innate lymphocyte subsets.

A. Gating strategy for γδ T cells, MAIT cells and iNKT cells.

B. The numbers of γδ T cells ($CD3^+\gamma\delta TCR^+$ T cells) and MAIT cells ($CD3^+\gamma\delta TCR^-V\alpha7.2^+CD161^+$ T cells) were reduced in NMOSD patients, including both pre-TCZ (n = 17) and other NMOSD (n = 22), as compared with HC (n = 12). iNKT cells ($CD3^+\gamma\delta TCR^-V\alpha7.2^-CD1d^+$ T cells) were reduced in other NMOSD, but not in pre-TCZ, as compared with HC. Data represent average ±standard errors.



eFigure 7: The serum concentrations of cytokines and chemokines in HC, and NMOSD before and after TCZ treatment for 12 months.

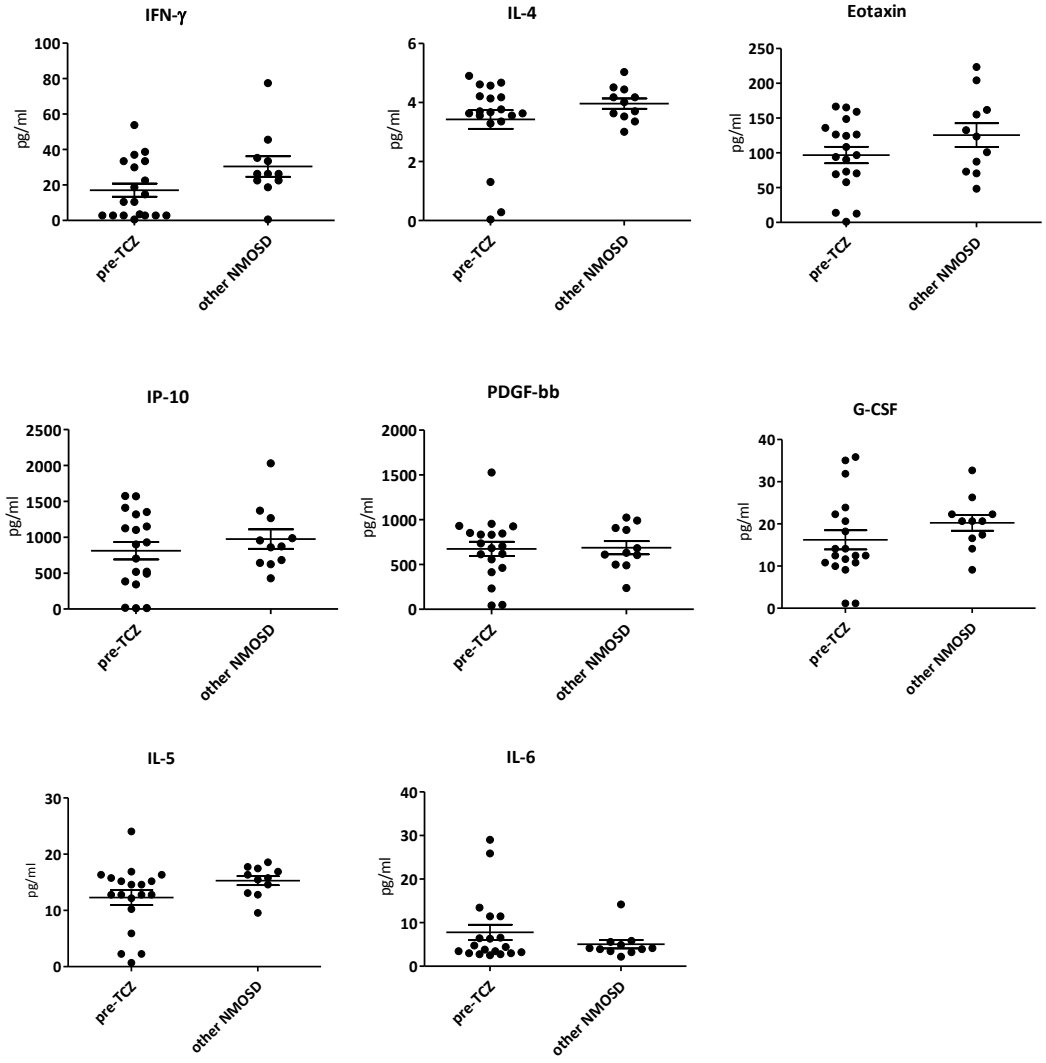
A. The serum concentrations of selected chemokines and cytokines, which were altered in NMOSD patients, but not significantly affected by TCZ treatment.

Comparison between HC and pre-TCZ indicated that increased were IFN-ɤ, p = 0.0094; IL-4, p = 0.0002; eotaxin, p = 0.0001; IP-10, p = 0.0121; and PDGF-bb, p = 0.0056; and decreased was G-CSF, p = 0.0424.

B. The serum concentrations of IL-5 and IL-6. Only these showed significant changes after one year treatment with TCZ.

HC, n = 14; pre and post TCZ, n = 15.

Average ±standard errors are shown.

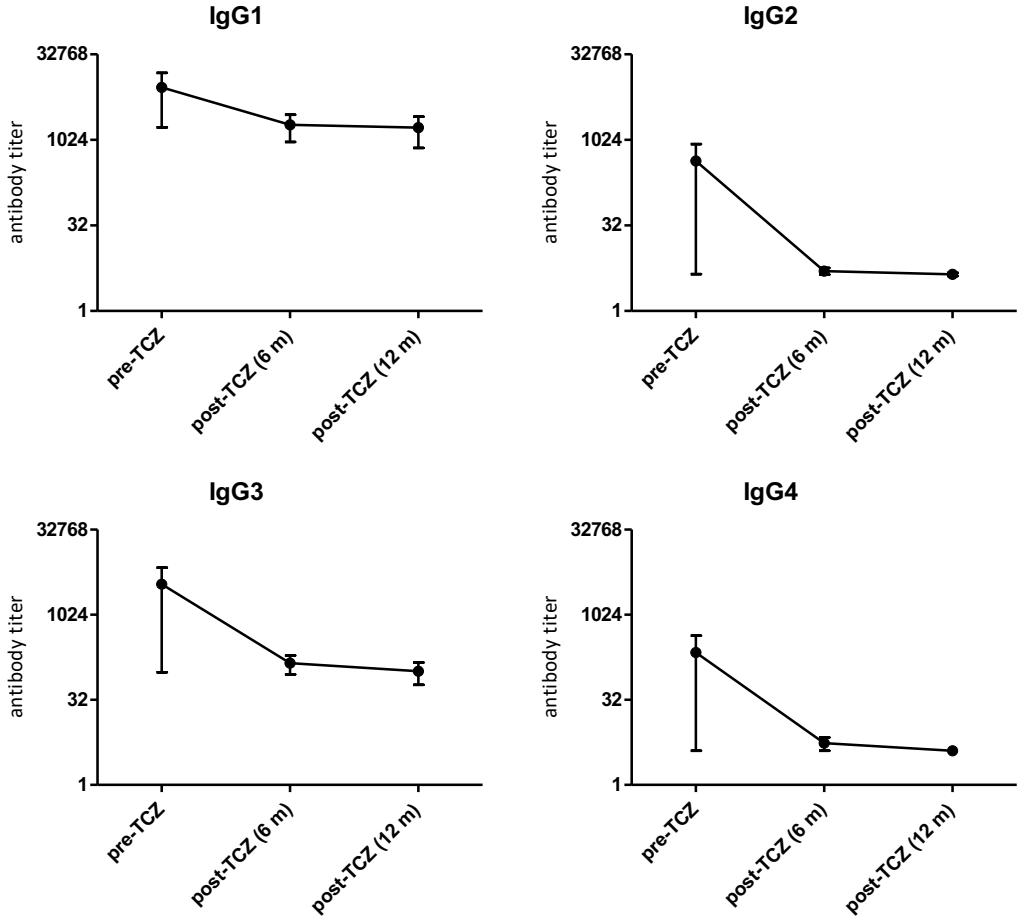


eFigure 8: The serum concentrations of cytokines and chemokines in NMOSD before TCZ and other NMOSD patients

No significant difference was demonstrated between NMOSD before TCZ treatment and other NMOSD patients (not receiving TCZ treatment) in the above shown.

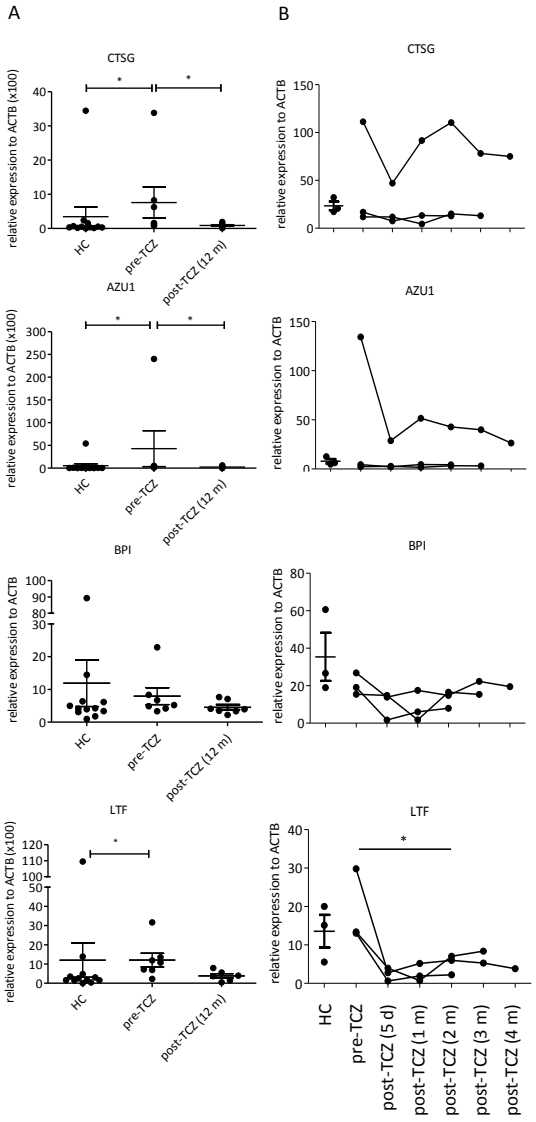
pre-TCZ, n = 19; other NMOSD, n = 11.

Average ±standard errors are shown.



eFigure 9: The serum anti-AQP4 antibody titer at pre-TCZ, 6 and 12 months after TCZ

The mixture of 70% M23-AQP4 and DsRED fused construct expressing CHO cells and 30% non-expressing CHO cells were incubated with heat-inactivated serum to detect the antibody positivity for an 8-fold dilution (volume ratio of 7:1). After incubation and washes in PBS containing 1% BSA, cells were incubated with a secondary antibody, washed and analyzed with a CANTO II flow cytometer. The results were analyzed with the Flowjo software, detecting each median fluorescence intensity (MFI) for IgG in DsRED positive and negative populations to calculate the MFI ratio (IgG MFI of DsRED positive cells / IgG MFI of DsRED negative cells) for each serum. If the MFI ratio was greater than the mean + 3SD of the control serum MFI ratio, the sample was determined as positive. The antibody titer was determined by limited dilutions of the serum. Serum antibody titers smaller than 8 were not analyzed due to high background of the staining. No significant change was observed in the first 12 months of TCZ treatment. n = 19. Average ± standard errors are shown.



eFigure 10: RT-PCR analysis of CTSG, AZU1, BPI and LTF in HC and TCZ-treated patients (before and after the treatment)

A. Confirmation of the microarray results by RT-PCR of the whole blood cDNA. HC, n = 12; pre and post TCZ, n = 7.

B. The early changes of mRNA expression by TCZ treatment was examined by RT-PCR with cDNA from FACS-sorted neutrophils in three cases. HC, n = 3, pre and TCZ, n = 3.