

Natalizumab treatment of relapsing remitting multiple sclerosis has no long-term effects on the proportion of circulating regulatory T cells

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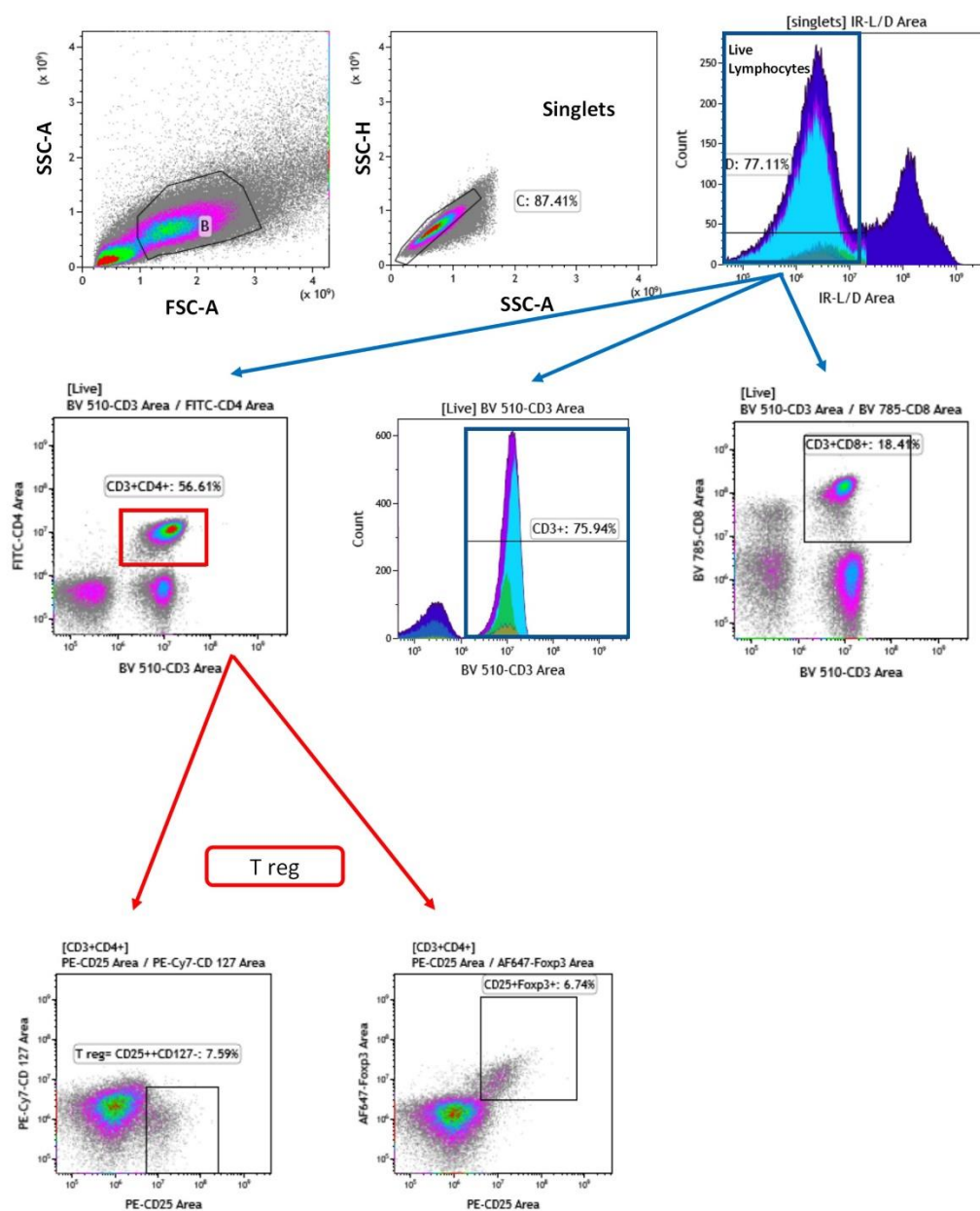
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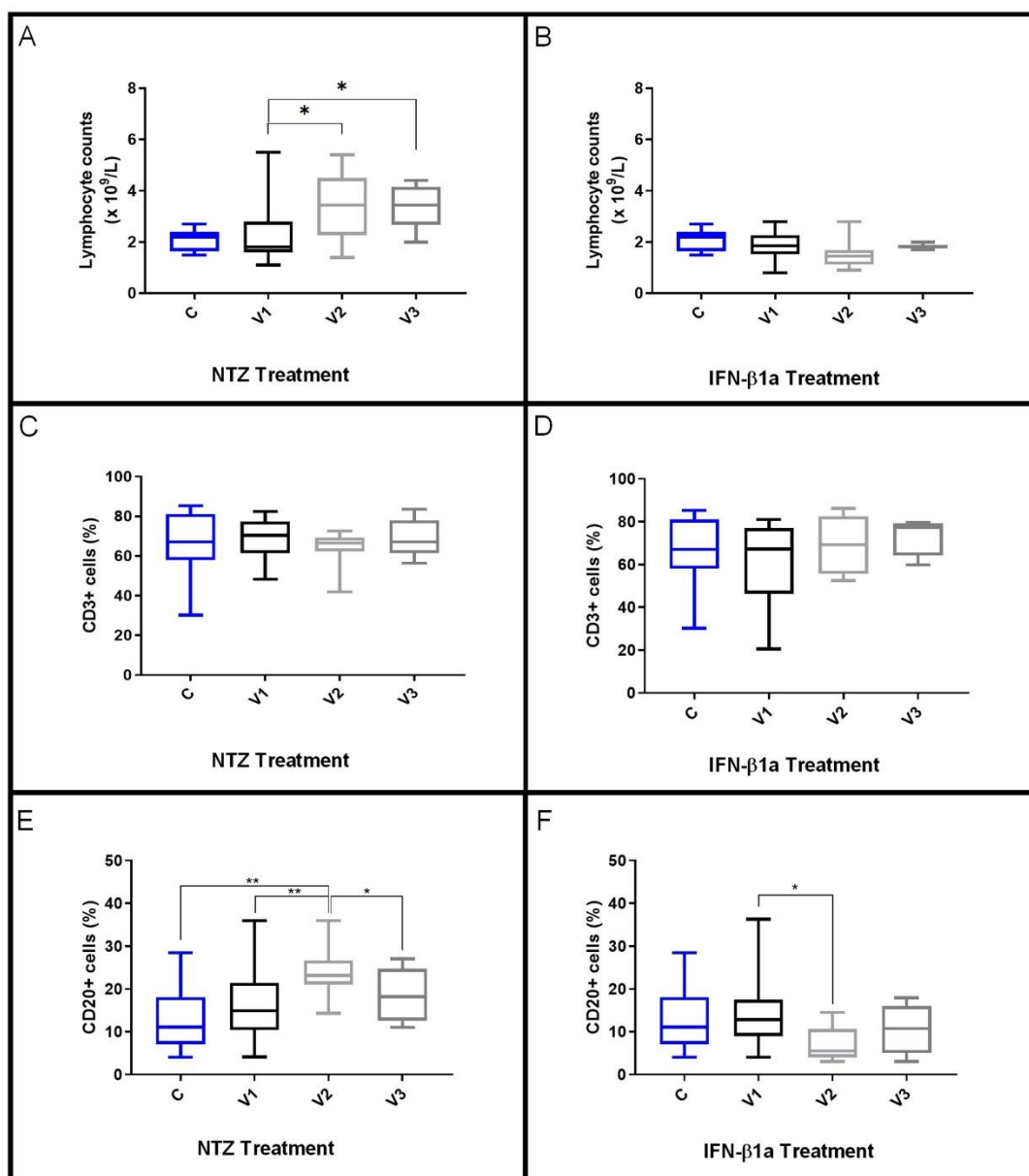
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Supplementary figure 1



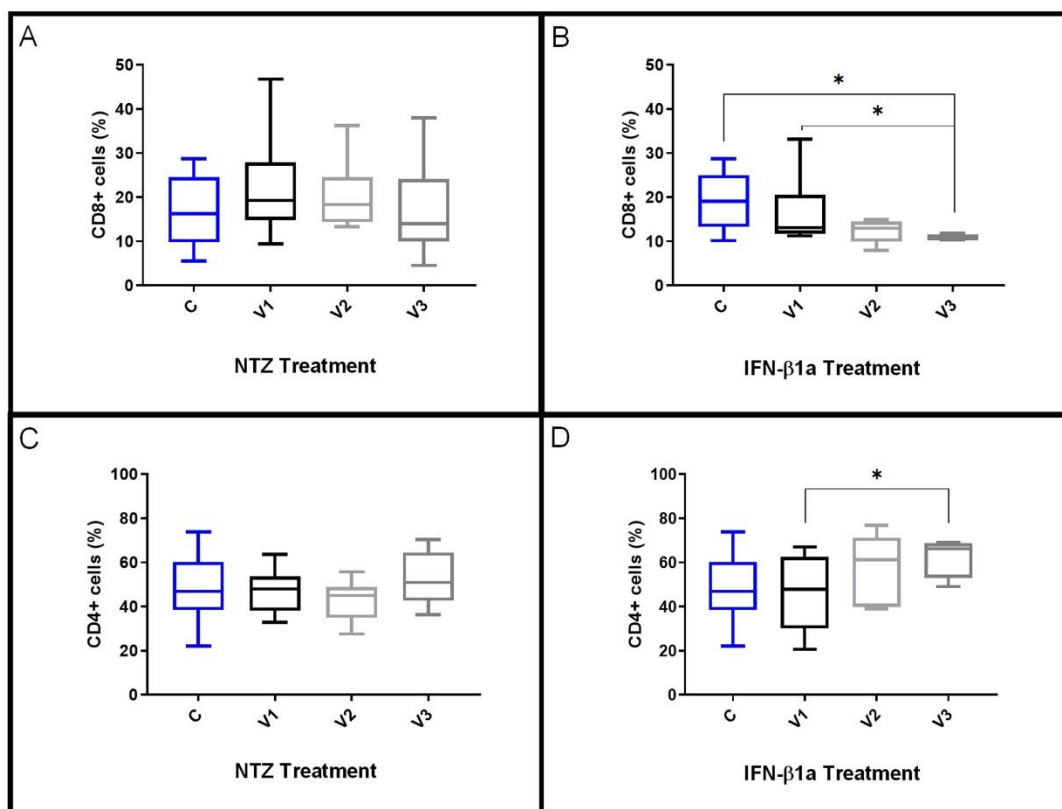
Supplementary Figure 1: Gating strategy used for identification of T regulatory (Treg) cell populations. Peripheral blood mononuclear cells (PBMCs) were first gated for lymphocytes based on size and granularity using forward scatter (FSC) and side scatter (SSC) parameters. Live lymphocytes were then identified using the live/dead stain. T cells were identified by the expression of CD3 and CD4 or CD3 and CD8. Treg cells were then identified as either CD4+, CD25high, CD127low or CD4+, CD25high, FoxP3+.

Supplementary figure 2.1



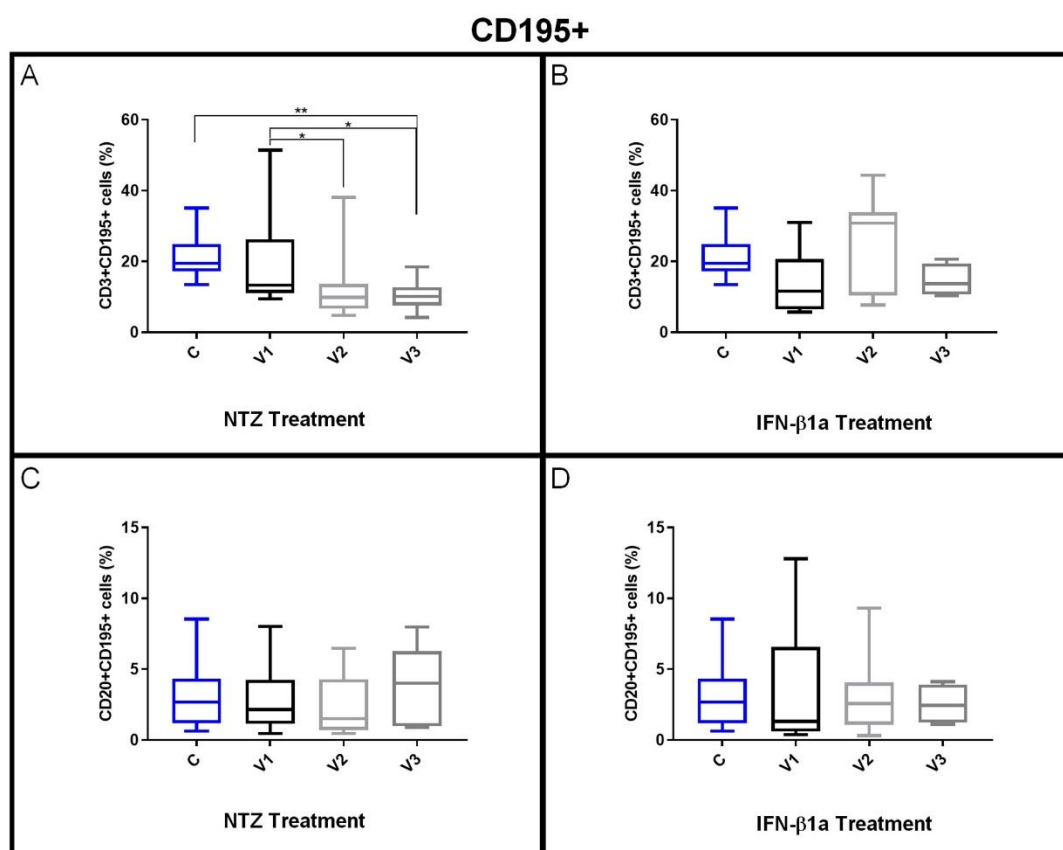
Supplementary figure 2.1: Assessment of lymphocytes counts and CD3+/ CD20+ cell populations proportions in MS patients treated either with NTZ or IFN-β1a. The figure shows the evaluation of absolute lymphocytes counts (A and B); CD3+ cells percentages (C and D) and CD20+ cell percentages (E and F) from MS patients treated either with NTZ (left panels) or IFN-β1a (right panels). C Controls (non-MS), V1 baseline, V2 6-months visit, V3 12-months visit. Boxes represent interquartile range, median value is indicated as the central box line, and whiskers represent min to max range. * p < 0.05; ** p < 0.01.

Supplementary figure 2.2



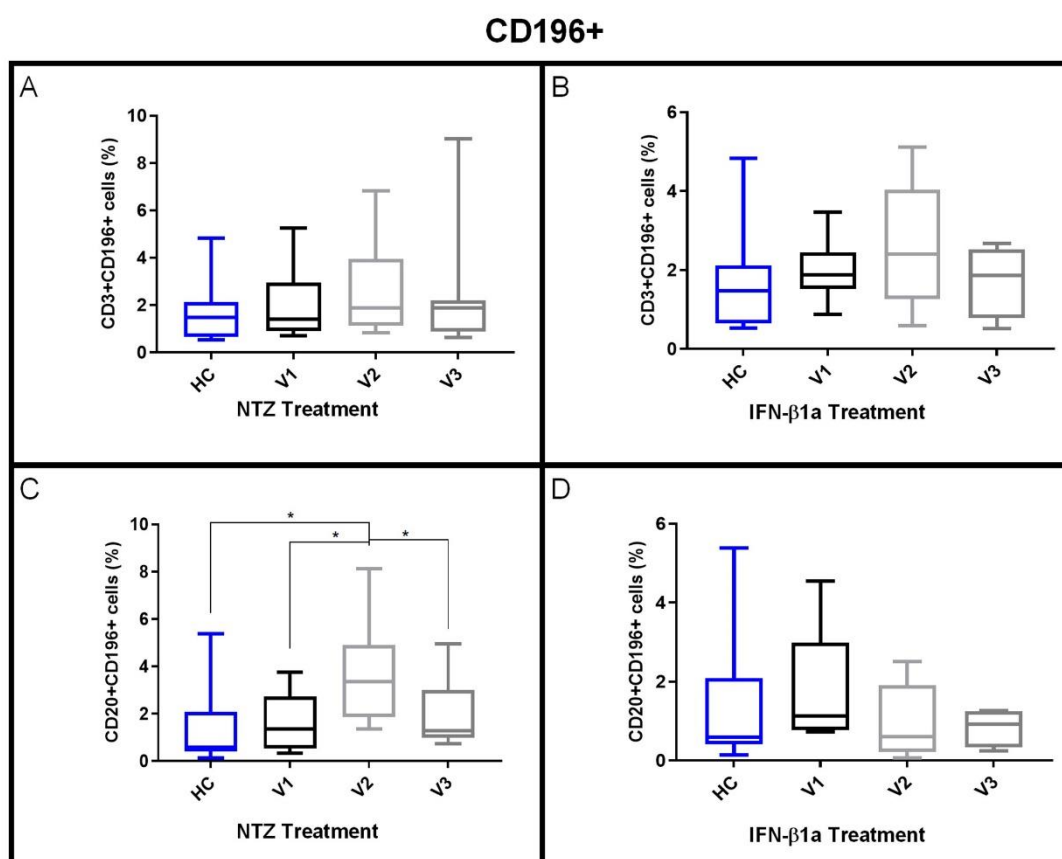
Supplementary figure 2.2: Effect of NTZ and IFN-β1a treatment on the CD8+ and CD4+ populations from MS patients. A) Percentage of CD8+ cells in patients treated with NTZ; B) Percentage of CD8+ cells in patients treated with IFN-β1a; C) Percentage of CD4+ cells in patients treated with NTZ; D) Percentage of CD4+ cells in patients treated with IFN-β1a. C Controls (non-MS), V1 baseline, V2 visit at 6months, V3 visit at 12 months. * $p < 0.05$.

Supplementary figure 2.3



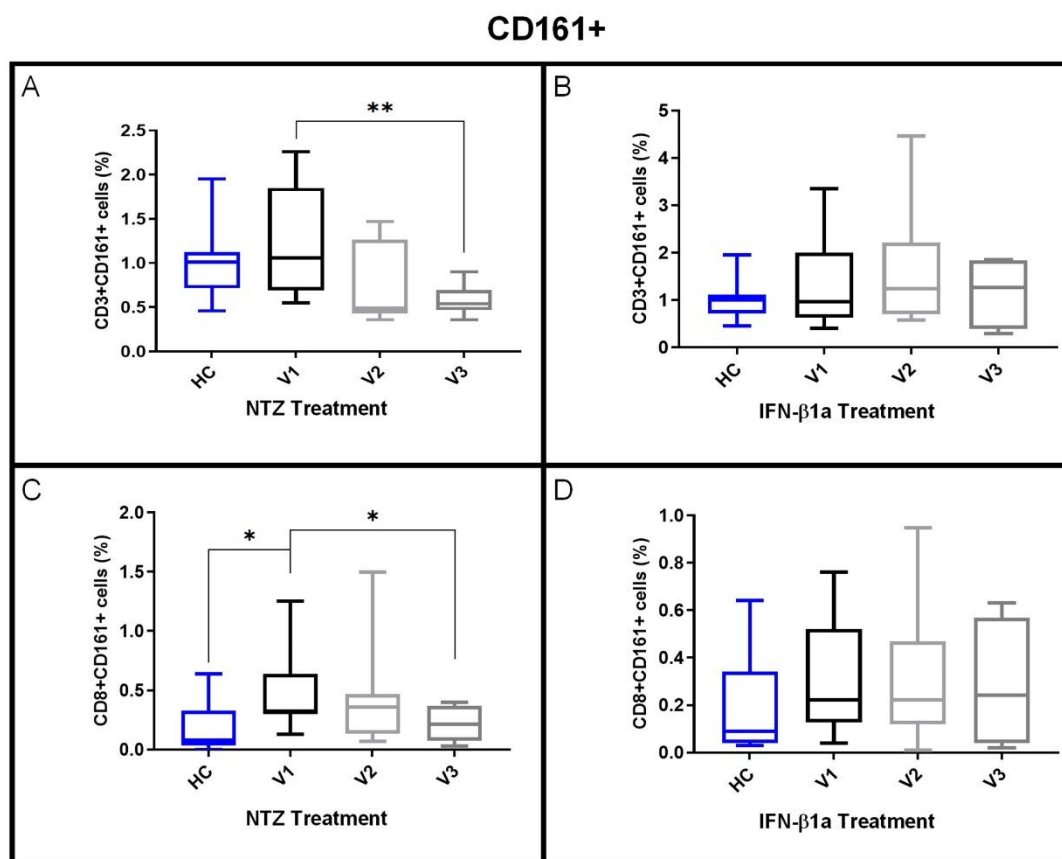
Supplementary figure 2.3: Effect of NTZ treatment on the expression of CD 195 surface marker in lymphocytes from MS patients. A) Percentage of CD3+ CD195+ cells in patients treated with NTZ; B) Percentage of CD3+ CD195+ cells in patients treated with IFN-β1a; C) Percentage of CD20+CD195+ cells in patients treated with NTZ; D) Percentage of CD20+CD195+ cells in patients treated with IFN-β-1a. C Controls (non-MS), V1 baseline, V2 visit at 6months, V3 visit at 12 months. * p < 0.05; ** p < 0.01.

Supplementary figure 2.4

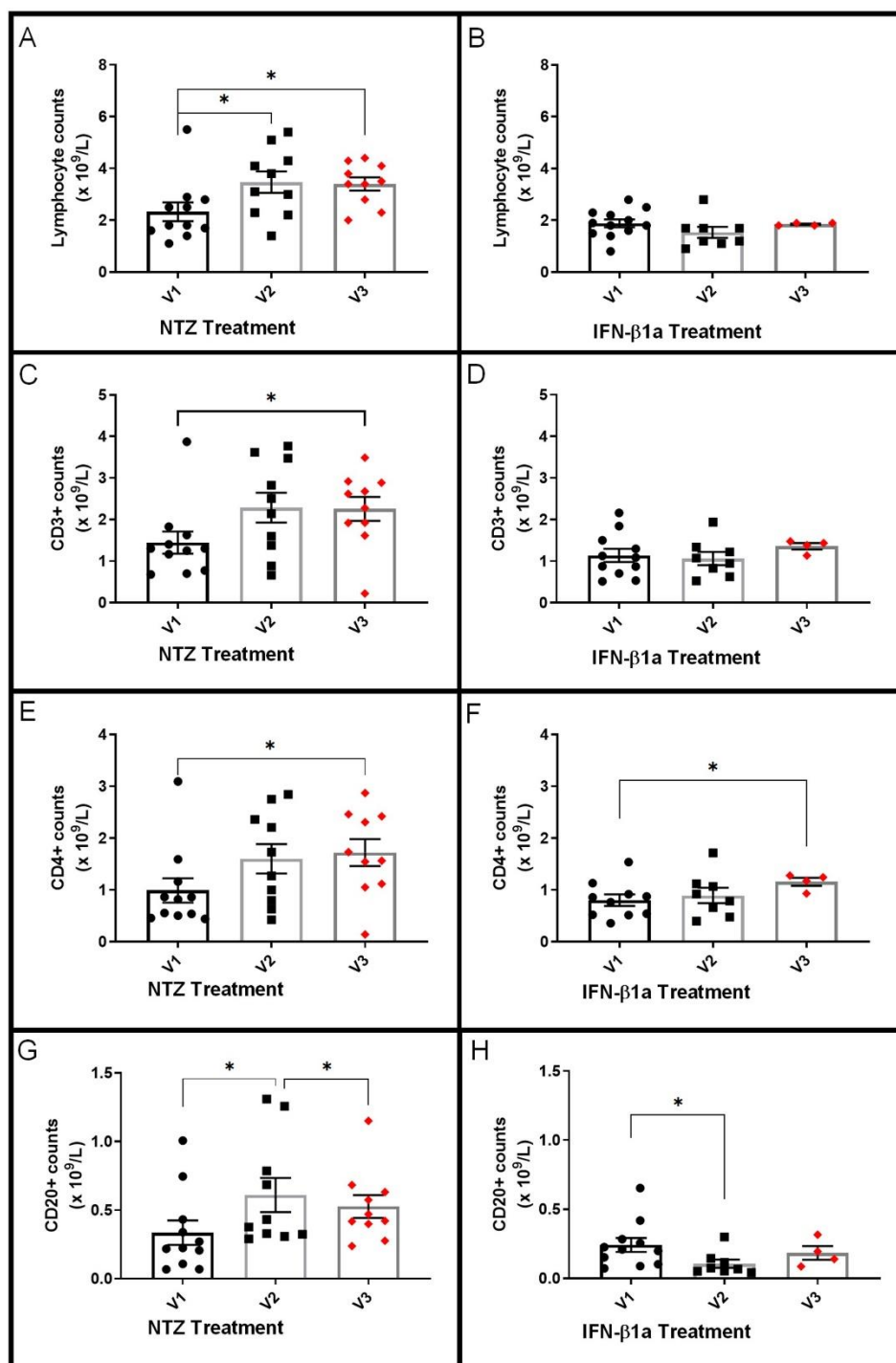


Supplementary figure 2.4: Effect of NTZ treatment on the expression of CD 196 surface marker in lymphocytes from MS patients. A) Percentage of CD3+ CD196+ cells in patients treated with NTZ; B) Percentage of CD3+ CD196+ cells in patients treated with IFN-β1a; C) Percentage of CD20+ CD196+ cells in patients treated with NTZ; D) Percentage of CD20+ CD196+ cells in patients treated with IFN-β1a. C Controls (non-MS), V1 baseline, V2 visit at 6months, V3 visit at 12 months. * p < 0.05.

Supplementary figure 2.5



Supplementary figure 2.5: Effect of NTZ treatment on the expression of CD 161 surface marker in lymphocytes from MS patients: A) Percentage of CD3+ CD161+ cells in patients treated with NTZ; B) Percentage of CD3+ CD161+ cells in patients treated with IFN-β1a; C) Percentage of CD8+ CD161+ cells in patients treated with NTZ; D) Percentage of CD8+ CD161+ cells in patients treated with IFN-β-1a. C Controls (non-MS), V1 baseline, V2 visit at 6 months, V3 visit at 12 months. * p < 0.05; ** p < 0.01.



Supplementary figure 3: Effect of NTZ treatment on absolute number of different populations of T cells in MS patients. A, C, E, and G: Absolute number of total lymphocytes, CD3+ cells, CD4+ cells, and CD20+ cells, respectively, from NTZ treated patients. B, D, F, and H: Absolute number of total lymphocytes, CD3+ cells, CD4+ cells, and CD20+ cells, respectively, from IFN-β1a treated patients. V1 baseline, V2 visit at 6 months, V3 visit at 12 months. * p < 0.05.