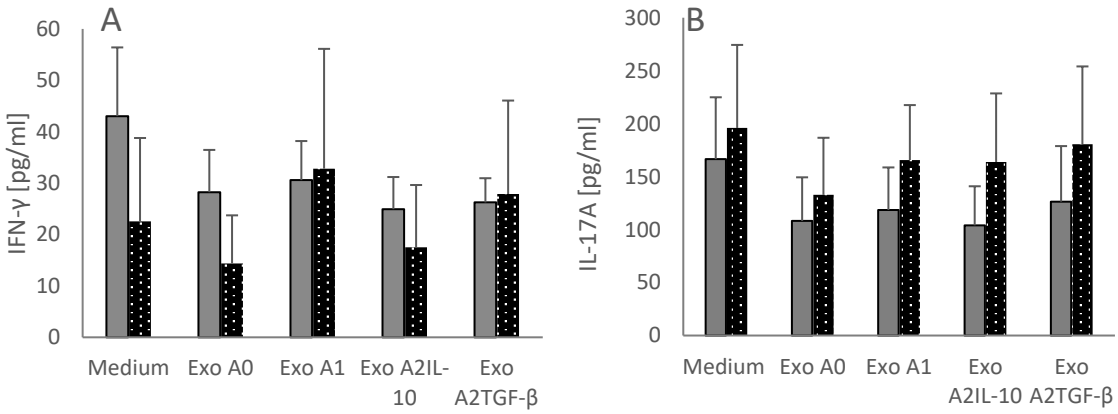
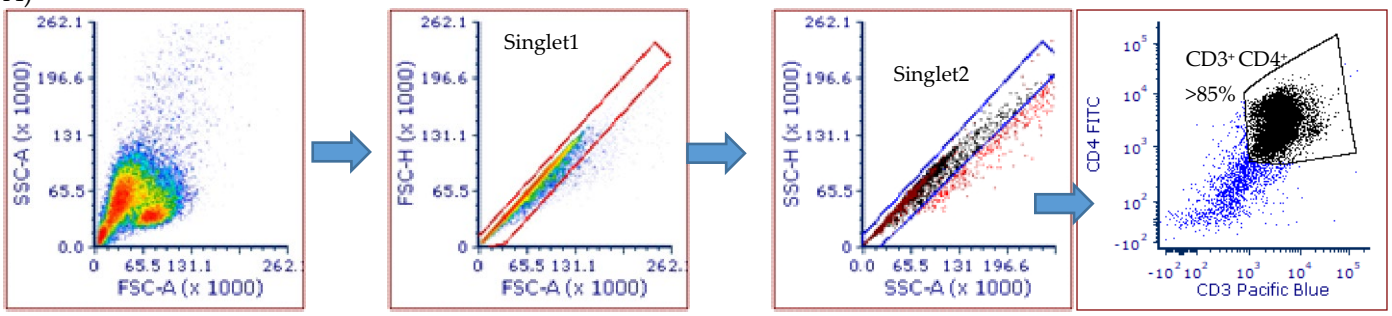
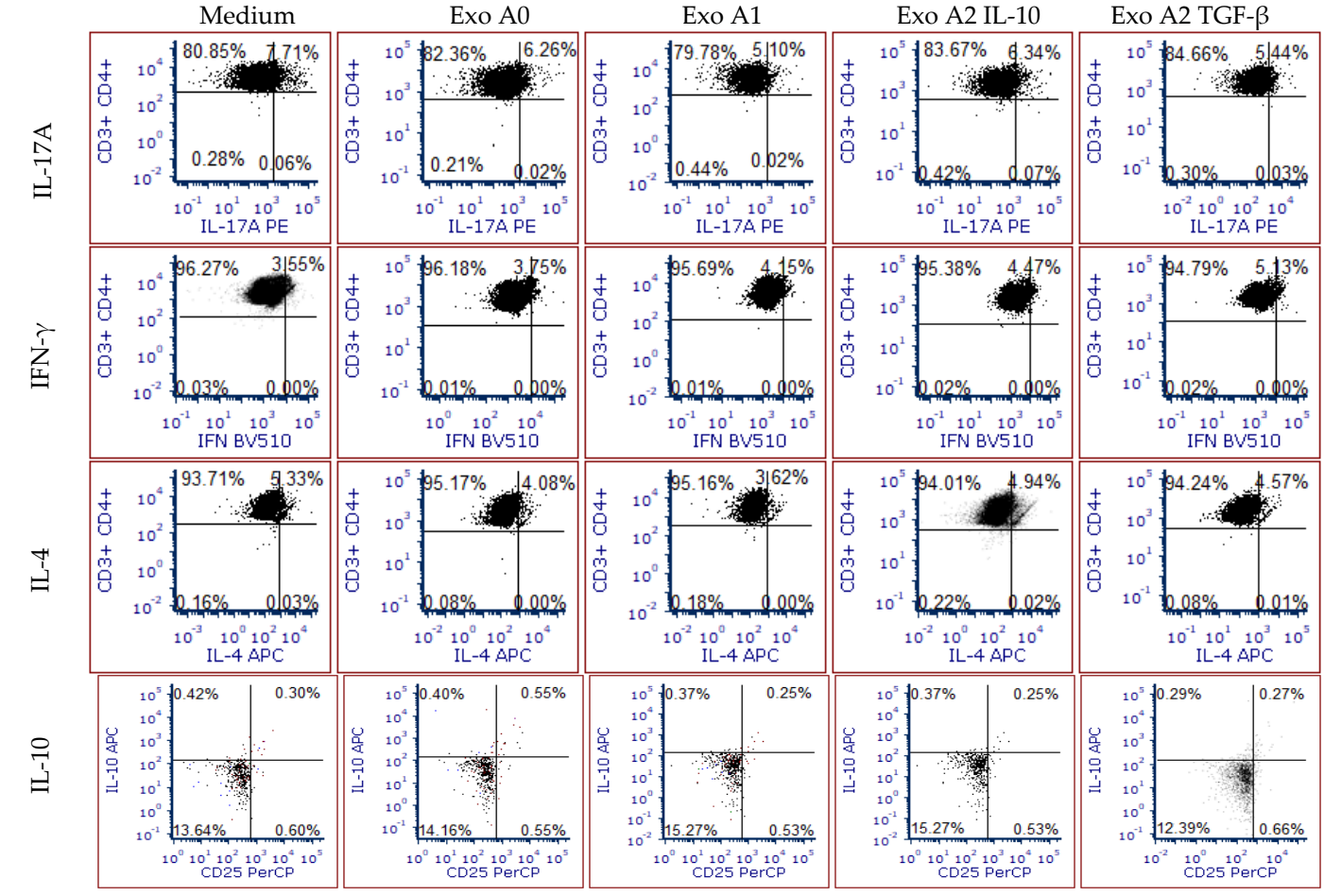


Supplement Figure S1. Comparison of IFN-γ (A) and IL-17A (B) production in CD4+ T-cell cultures from multiple sclerosis-affected patients (filled bars) and healthy donors with excluded autoimmune disease (white dotted bars). After separation from PBMC, CD4+ T-cells from every donor were isolated by magnetic sorting, and cultured on 48-well culture plates (10^6 cells/well) with exosomes isolated from non-stimulated (Exo A0), pro-inflammatory cytokines' stimulated (Exo A1) and anti-inflammatory cytokines' (IL-10, TGF-β) stimulated (respectively Exo A2IL-10, Exo A2TGF-β) human astrocytes. Data shown as mean ± SEM. Comparisons of groups were made with U Mann-Whitney rank-sum test.

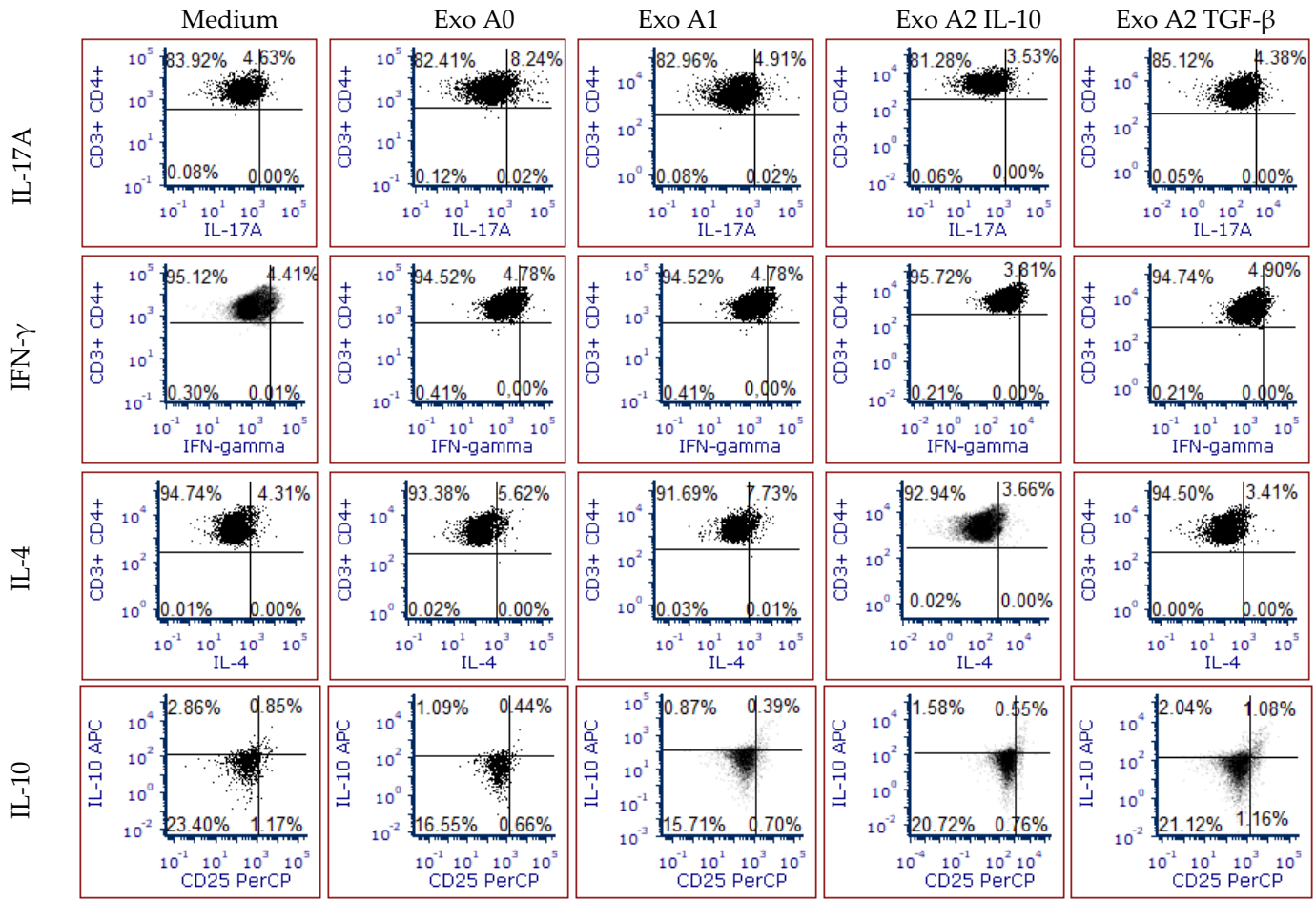
A)


SSC-A (x 1000)
FSC-A (x 1000)
Singlet1
FSC-H (x 1000)
Singlet2
SSC-H (x 1000)
CD3+ CD4+
>85%
CD4 FITC
CD3 Pacific Blue

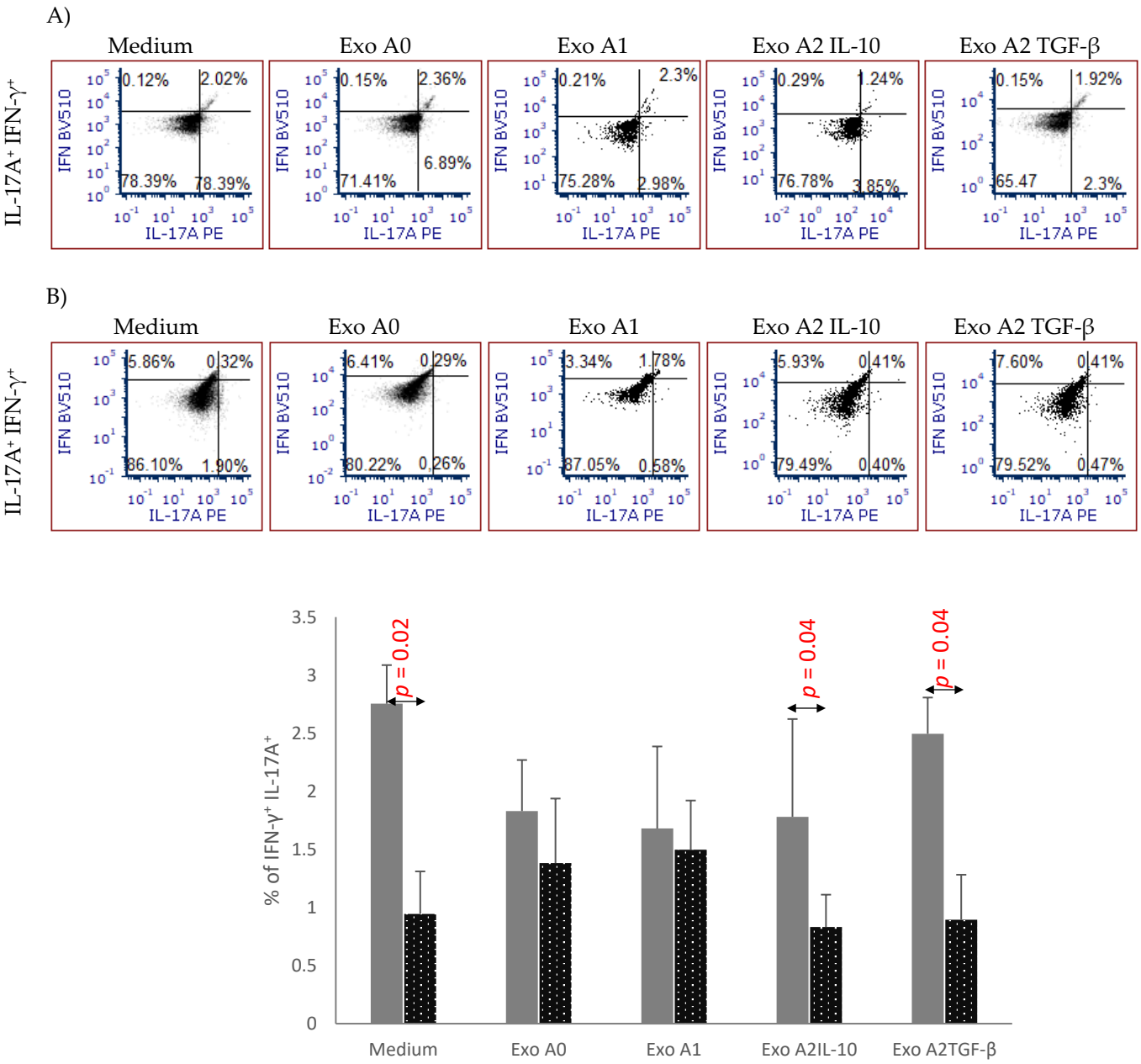
B)


Medium
Exo A0
Exo A1
Exo A2 IL-10
Exo A2 TGF-β
IL-17A
IFN-γ
IL-4
IL-10
CD3+ CD4+
IL-17A PE
IFN BV510
IL-4 APC
IL-10 APC
CD25 PerCP
80.85%
7.71%
0.28%
0.06%
82.36%
6.26%
0.21%
0.02%
79.78%
5.10%
0.44%
0.02%
83.67%
6.34%
0.42%
0.07%
84.66%
5.44%
0.30%
0.03%
96.27%
3.55%
0.03%
0.00%
96.18%
3.75%
0.01%
0.00%
95.69%
4.15%
0.01%
0.00%
95.38%
4.47%
0.02%
0.00%
94.79%
5.13%
0.02%
0.00%
93.71%
5.33%
0.16%
0.03%
95.17%
4.08%
0.08%
0.00%
95.16%
3.62%
0.18%
0.00%
94.01%
4.94%
0.22%
0.02%
94.24%
4.57%
0.08%
0.01%
0.42%
0.30%
13.64%
0.60%
0.40%
0.55%
14.16%
0.55%
0.37%
0.25%
15.27%
0.53%
0.37%
0.25%
15.27%
0.53%
0.29%
0.27%
12.39%
0.66%

C)



Supplement Figure S2. Representative dot plots from flow cytometry analyses of CD4⁺ T-cells. A) Gating strategy and elimination of cell aggregates. Percentage values of IL-17A, IFN-γ, IL-4 and IL-10 -producing T-cells from MS-affected patients (B), and non-MS donors (C), expressed as % of CD3, CD4 -positive T lymphocytes. T cells were separated from PBMC fraction after density gradient centrifugation (FicolPaquePlus), with magnetic sorting (MACS, Miltenyi Biotec) and cultured for 96h in culture medium (RPMI1640/10% FBS/penicillin, streptomycin) alone or with addition of astrocyte-derived extracellular vesicles.



Supplement Figure S3. Results of flow cytometry analysis of double producers (IFN-γ+ IL-17A+). Percentage values of IL-17A/IFN-γ -producing T-cells from MS-affected patients (A), and non-MS donors (B), expressed as % of CD3, CD4 -positive T lymphocytes. Mean percentage of $CD3^+$ $CD4^+$ cells double positive for IFN-γ and IL-17A cytokines were compared between study groups. Data for MS patients are presented with filled bars (7 persons), results for non-MS donors are in white dotted bars (4 persons). After 96h of T-cell cultures in presence of astrocyte-derived exosomes or medium alone, culture media were collected, cells were suspended in new medium containing brefeldin A for 12h, next cells were harvested and stained for flow cytometry. Cells were analysed as a % of $CD3^+$ $CD4^+$ cells double positive for IFN-γ and IL-17A. Data were shown as mean ± SE. Comparisons between variables were made with non-parametric Wilcoxon signed-rank test, no significant differences were observed. Comparisons between groups were made with U Mann-Whitney rank-sum test.