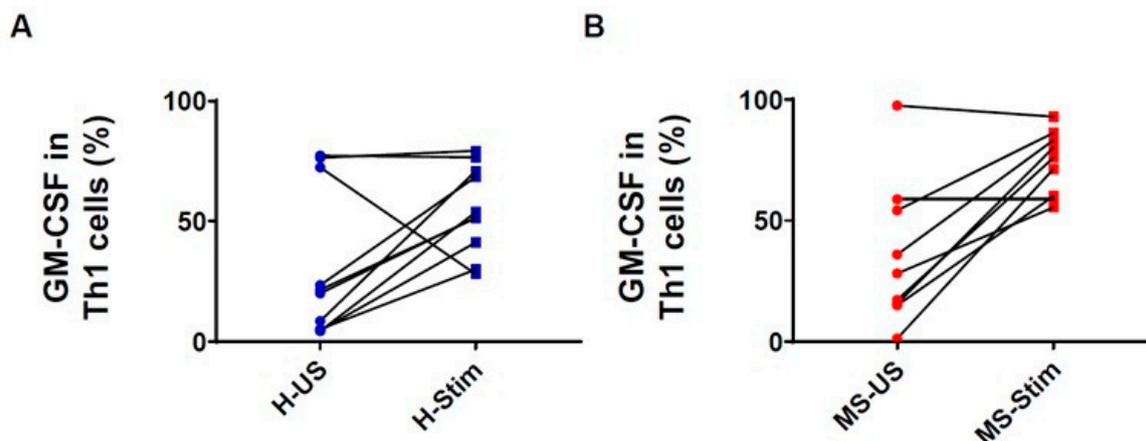
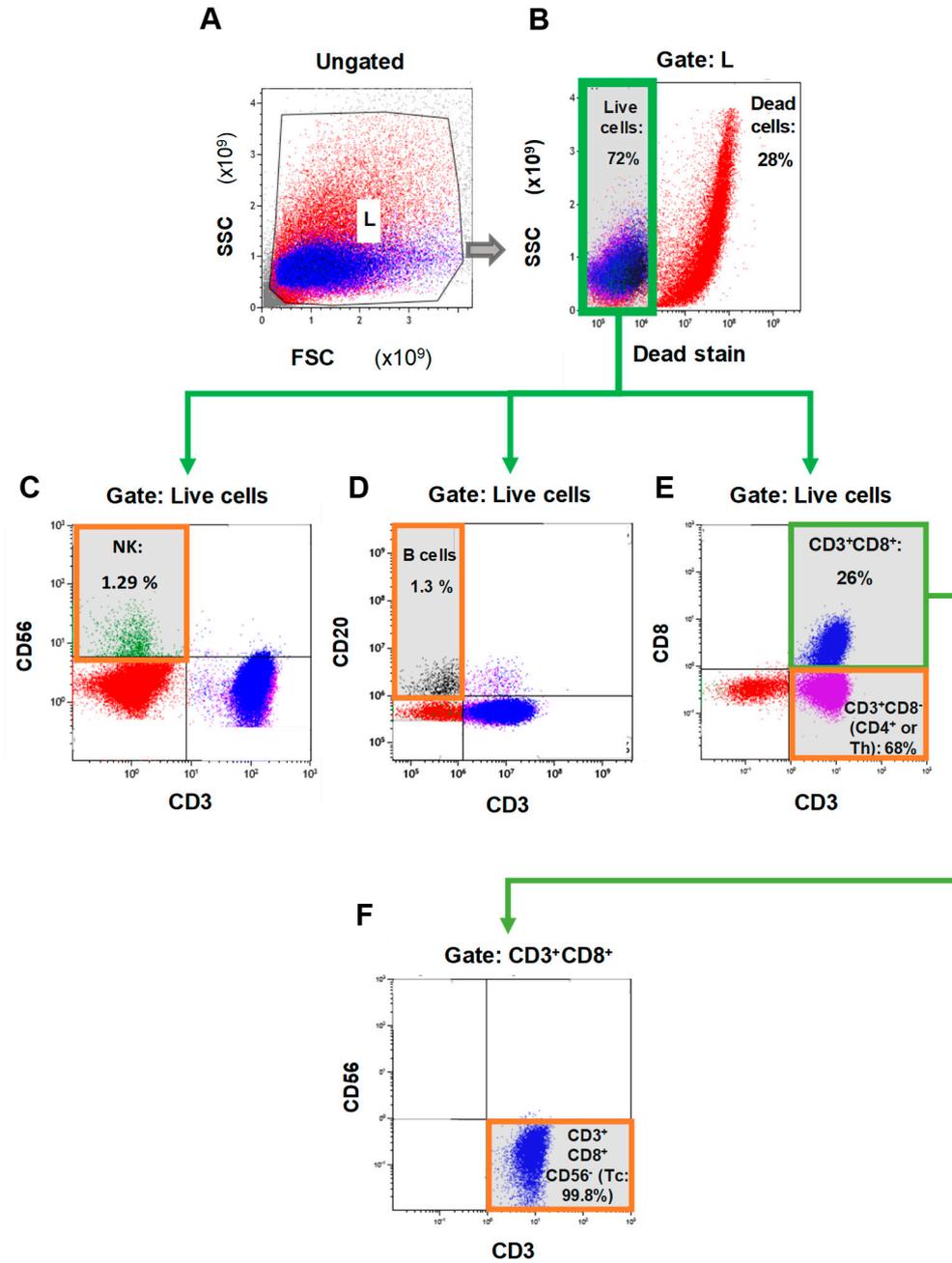




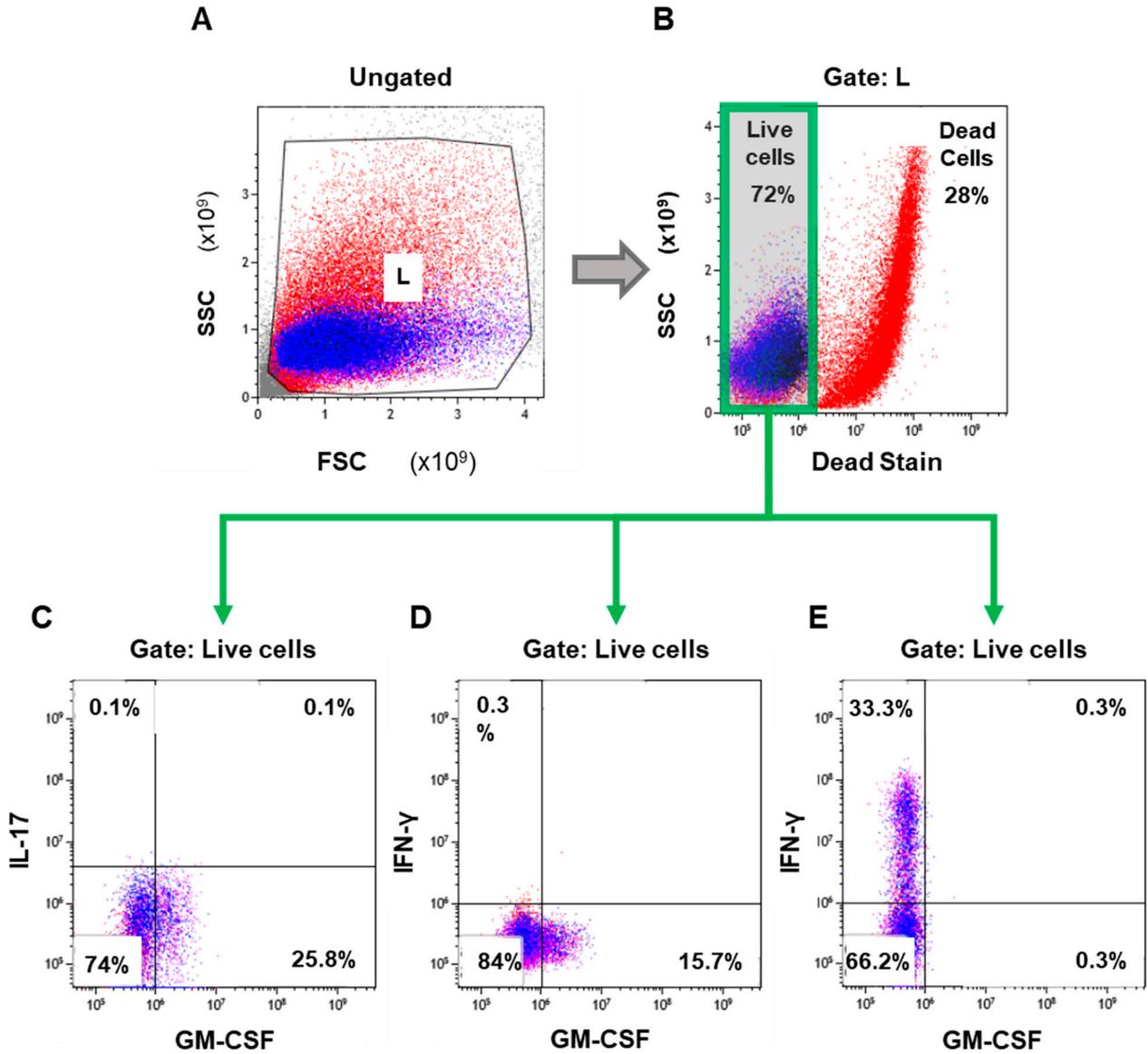
Supplementary figures for manuscript: "Increased IL-2 and reduced TGF- $\beta$  upon T-cell stimulation are associated with of GM-CSF Up-regulation in Multiple Immune Cell Types in Multiple Sclerosis" by Jehan Aram et al.



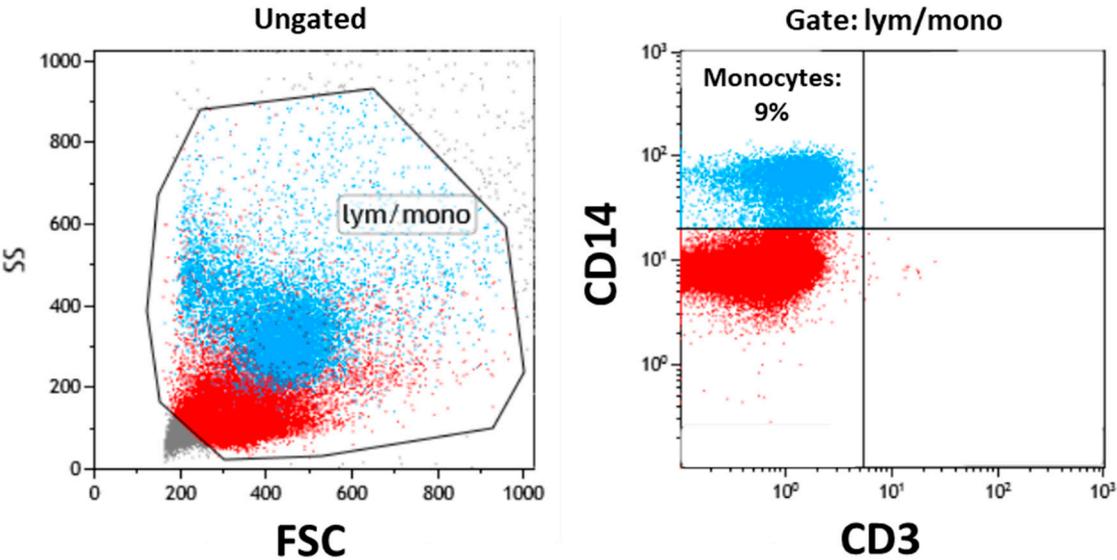
**Figure S1.** GM-CSF expression in Th1 cells. Figure shows the paired US/S data for each healthy control and MS sample. (A) Percentage of Th1 GM-CSF producing cells in healthy control, before and after stimulation. (B) Percentage of Th1 GM-CSF producing cells in MS before and after stimulation.



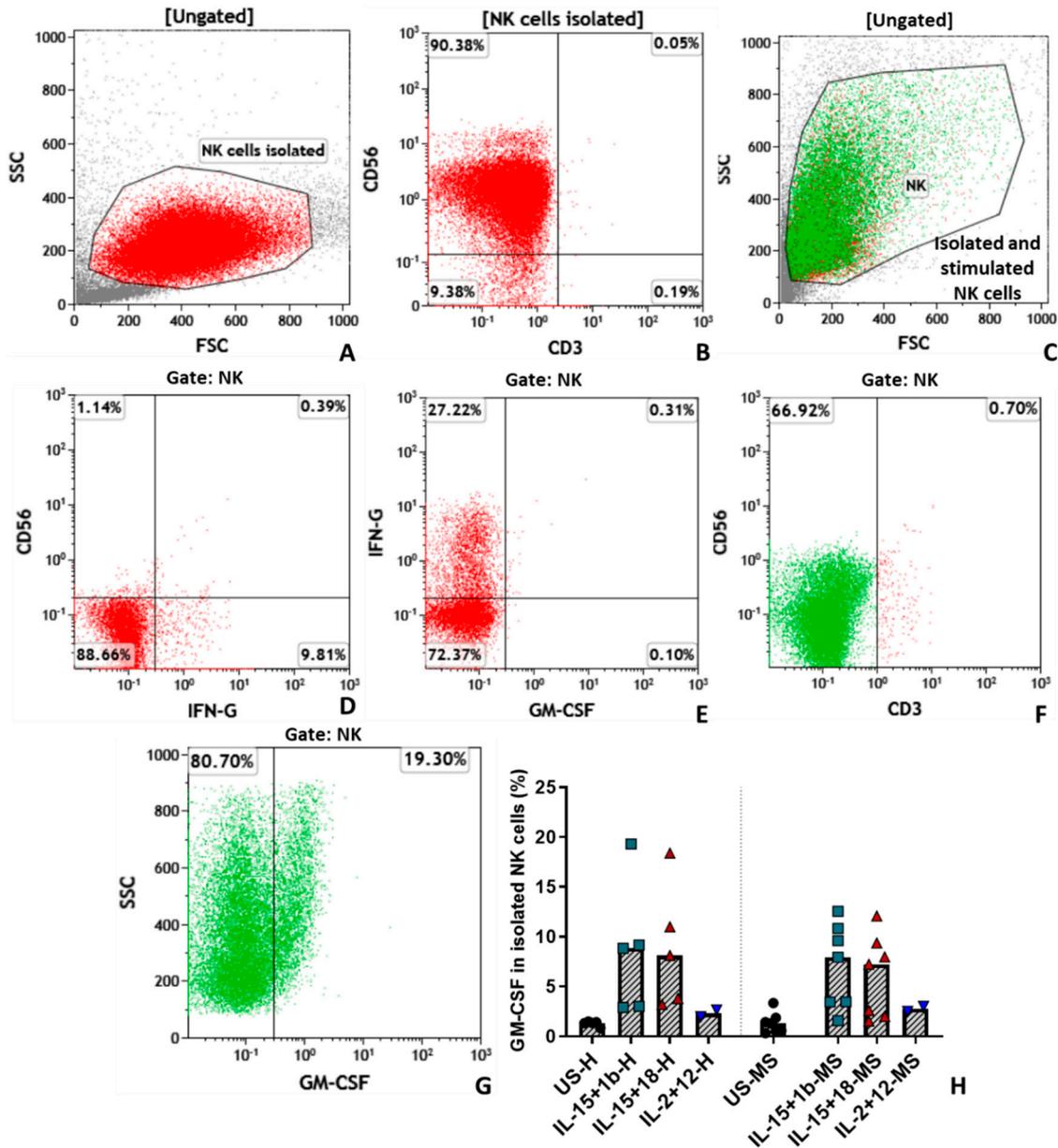
**Figure S2.** Cell gating, selection and identification in flow cytometry. (A) selecting potential cells (L); **B:** selecting live cells and using it as a gate for later plots; (C) NK cells were identified as CD3-CD56<sup>+</sup> cells (green-coloured population); (D) B cells were identified as CD3-CD20<sup>+</sup> cells (black-coloured population); (E) Th cells were identified as CD3<sup>+</sup>CD8<sup>-</sup> cells (purple-coloured population); CD3<sup>+</sup>CD8<sup>+</sup> gate was use in (F) Tc cells were identified as CD3<sup>+</sup>CD8<sup>+</sup>CD56<sup>-</sup> cells (blue-coloured population). Representative plots are shown.



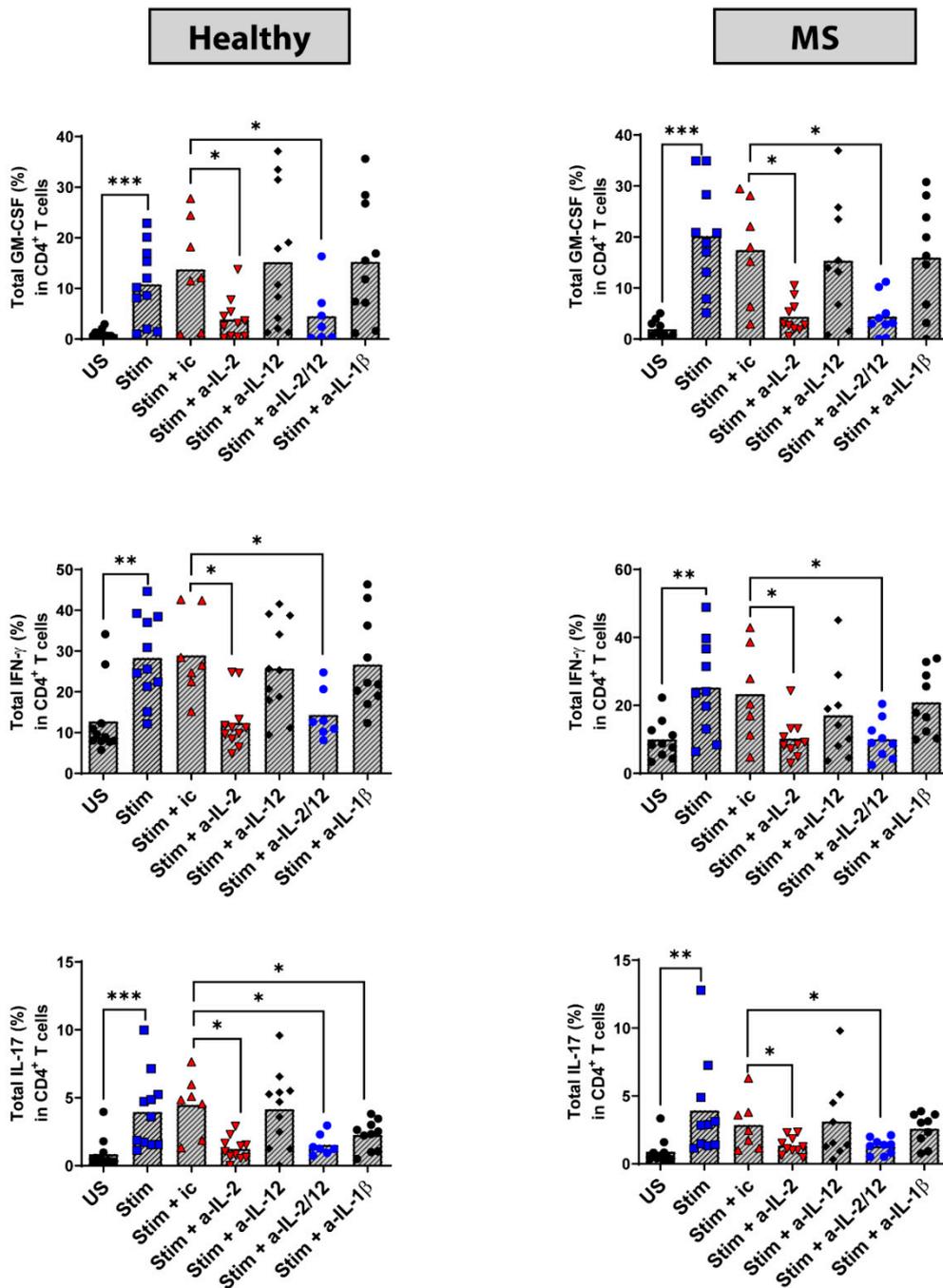
**Figure S3.** Setting flow cytometry quadrants using fluorescence minus one (FMO) controls. (A) selecting potential cells (L); (B) selecting live cells and using it as a gate for later plots; (C–E) representative FMO control plots for IL-17, IFN- $\gamma$ , and GM-CSF, respectively.



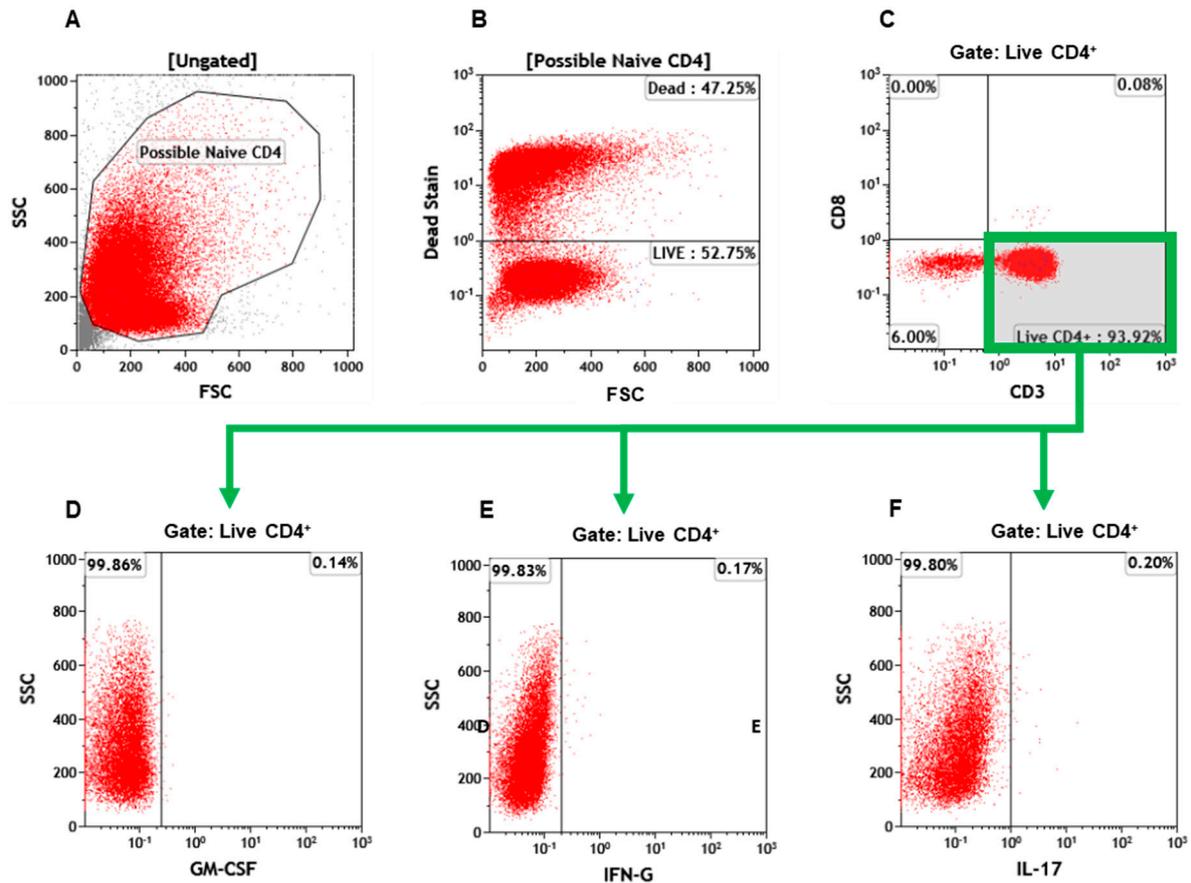
**Figure S4.** Selection and gating for monocytes. After selection of potential cells (lym/mono gate), monocytes were identified as CD3<sup>-</sup>CD14<sup>+</sup> cells (light blue colored population).



**Figure S5.** GM-CSF expression in isolated NK cells. After magnetic isolation of NK cells from freshly isolated PBMC, they were checked for purity. (A) forward and side scatter (FSC and SSC) plot for the freshly isolated NK cells. (B) purity check of freshly isolated NK cells gated by the cells selected in plot A, showing  $\geq 90\%$  CD3<sup>+</sup>CD56<sup>+</sup> cells. After purity check, NK cells were either left unstimulated (US) or stimulated with either IL-15+IL-1 $\beta$ , IL-15+IL-18, or IL-2+IL-12 for 3 days, then re-stimulated for the last 5 hrs. with PMA/I in presence of brefeldin A. (C) the FSC and SSC plot to gate the possible NK cells after stimulation. (D) FMO control of CD56, (E) FMO control of GM-CSF. (F) gating cells to select CD3<sup>+</sup> NK cells (green-colored population) (note that CD56 expression was down-regulated in purity-validated NK cells after being cultured and stimulated). (G) GM-CSF expression by NK cells. (H) collective results from healthy controls (H) and MS patients illustrating the frequency of GM-CSF<sup>+</sup> cells in isolated NK cells (GM-CSF<sup>+</sup> cells in plot (G)), horizontal lines are medians. Each experiment was independently performed for at least three times. Plots from A-G are representative and could be taken from different samples and are not necessarily from the same sample.



**Figure S6.** Effects of anti-cytokine antibodies on CD4<sup>+</sup> T cells expressing either GM-CSF, IFN- $\gamma$  or IL-17. Frozen/thawed cultured PBMC from healthy controls and MS patients were cultured either unstimulated (US) or stimulated (Stim) with anti-CD3/anti-CD28 for 5 days. Stimulated cells were left with or without adding one of the antibodies to block the following cytokine (a-cytokine: cytokine antibody): IL-2, IL-12, IL-2 + IL-12 (IL-2/IL-12) and IL-1 $\beta$ . In addition, stimulated cells were cultured with antibody isotype control (ic) as controls. Left panel represents healthy control results, right panel represents MS patient results \*  $p < 0.05$  \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ . Bars represent medians.



**Figure S7.** Naive CD4 cell gating, selection, and FMO controls. After magnetic isolation of naive CD4 cells from freshly isolated PBMC, they were checked for purity ( $\geq 90\%$  naive CD4 T cells ( $CD4^+CD45RA^+$ ) were included). (A) FSC and SSC plots to select possible Naive CD4 T cells. (B) from the cells selected in A, live cells were identified and selected as a gate for the next plot. (C) Live CD4<sup>+</sup> ( $CD3^+CD8$ ) T cells were identified and selected as a gate for the following plots. (D–F): fluorescence minus one (FMO) controls for GM-CSF, IFN- $\gamma$ , and IL-17, respectively. They were used to set the quadrants for the next plots. Representative plots were shown in this figure.

**Table S1.** Demographic and clinical characteristics of the patients.

ID #	Gender	Age (years)	MS type	EDSS	Prior DMT	Reason off	MS duration (Years)	Exp. Figure
1	M	36	RR	5	IFN	Switch	2	5
2	M	34	RR	6	IFN	Switch	2	5
3	F	33	RR	6	Nil	Pt choice	10	5
4	F	30	RR	2	Nil	Early MS	1	5
5	F	67	SP	6.5	Nil	SPMS	15	5

6	F	45	RR	2	Nil	Pt choice	7	5
7	F	32	RR	4	Nil	Early MS	2	5
8	F	67	RR	1	Nil	Benign MS	16	5
9	F	49	RR	1.5	Nil	Pt choice	14	5
10	M	58	SP	6	Nil	Side effects	6	5
11	F	53	RR	2	Nil	Benign MS	8	5
12	F	50	SP	6.5	Nil	SPMS	14	5
13	M	40	SP	5	Nil	SPMS	10	5
14	M	64	SP	5	Nil	SPMS	3	5
15	M	50	SP	6.5	Nil	SPMS	12	5
16	F	39	RR	1	Nil	Early MS	<1	5
17	F	49	SP	6	IFN	Stop	4	5
18	F	33	RR	2	Daclizumab	Stop	4	2,3,6
19	M	43	SP	2	GA	SPMS	6	2,3,6
20	M	43	RR	2	Nil	Pt choice	5	2,3,6
21	F	33	RR	3	GA	Switch	7	2,3,6
22	F	38	RR	3	Nil	Pt choice	8	2,3,6,8
23	F	56	RR	3.5	IFN	Switch	7	2,3,6
24	F	49	RR	4	IFN	Switch	27	2,3
25	F	51	SP	6	Nil	SPMS	11	2,3
26	F	38	RR	3	Nil	Early MS	1	2,3,6
27	M	18	RR	2.5	Nil	Early MS	2	S5, 6
28	F	52	RR	3	Nil	Pt choice	7	S5, 6
28	F	43	RR	2	Nil	Pt choice	9	S5, 6
30	M	51	SP	5.5	Azathioprine	Stop	11	S5, 6
31	F	58	RR	6	IFN	Stop	10	S5, 6
32	F	31	RR	3	IFN	Stop	6	S5, 6
33	F	41	RR	2	GA	Pt choice	14	S5, 6
34	F	34	RR	2	GA	Switch	4	1,4,7
35	F	27	RR	1.5	IFN	Switch	2	1,4,7
36	F	23	RR	1	Nil	Early MS	2	1,4,7
37	F	55	RR	1.5	Nil	Benign MS	14	1,4,7
38	F	33	RR	2.5	Nil	Pt choice	1	1,4,7
39	F	42	RR	1	Nil	Pt choice	1	1,4,7
40	F	30	RR	2	Nil	Trial entry	10	1,4,7
41	F	34	RR	3	Fingolimod	Pregnancy	8	1,4,7
42	F	55	RR	1	Nil	Early MS	<1	1,4,7
43	M	57	RR	2	Nil	Not criteria	8	1,4,7
44	F	67	RR	5.5	Nil	Not criteria	4	8
45	M	21	RR	1	Nil	Early MS	<1	8

46	F	38	RR	5.5	GA	Side effects	10	8
47	F	42	RR	4	IFN	Switch	8	8

RR, relapsing remitting; SP, secondary progressive; EDSS, Expanded Disability Status Scale; DMT, disease modifying treatment; IFN, interferon beta; GA, Glatiramer acetate; Reason off: reason the patient was off DMT at the time of sample collection; Exp. Figure: sample contribution to the experiments shown in figure with the number given in the table.