Supplementary materials and methods

Biological sample collection

30 mL of venous blood was collected from each subject into EDTA-coated tubes. Peripheral blood mononuclear cells (PBMCs) were isolated by Histopaque-1077 density gradient centrifugation (Sigma-Aldrich) and stored frozen in liquid nitrogen in 90% heat-inactivated fetal bovine serum (FBS, ThermoFisher), 10% dimethyl sulfoxide (Sigma-Aldrich) until use.

Sorting efficiency and PBMC immunophenotype

To evaluate the sorting efficiency and the immunophenotype of the patients, 100.000 PBMCs and sorted lymphocytes and monocytes were analysed by flow cytometry. Cells were suspended in 100 µL Phosphate-Buffered Saline (PBS, Euroclone) with 0.1% Fixable Dead Cell Stain near-IR-fluorescent reactive dye (Thermo Fisher Scientific), and stained for 15 min at room temperature. After washing with PBS + 1% FBS, monocytes were suspended in 400 µL PBS + 1% FBS to be acquired while PBMCs and lymphocytes were suspended in 100 µL PBS + 1% FBS containing the following antibodies: PerCP mouse anti-human CD3 (clone BW264/56), PE anti-human CD56 (clone REA196), APC anti-human CD4 (clone REA623), PE-Vio770 anti-human CD19 (clone REA675) and FITC anti-human CD8 (clone REA734). All antibodies were purchased from Miltenyi Biotec and used as suggested by the manufacturer. Cells were stained for 30 min on ice, washed, and suspended in 400 µL PBS + 1% FBS. Samples were acquired with the FACSCanto II flow cytometer (BD Biosciences), equipped with two lasers for excitation at 488 and 633 nm. Data were analysed with the Kaluza Analysis 2.1 software (Beckman Coulter).

Co-culture system

DPSCs were seeded in 24-well plates at a density of 50.000 cells/well in alpha-MEM supplemented with 10% heat-inactivated FBS. The following day, media were removed, and sorted lymphocytes or monocytes were added unstimulated or activated: 400.000 lymphocytes or 100.000 monocytes per well in 500 µl RPMI 1640 (catalogue: 21875, Gibco, ThermoFisher) + 10% FBS. Lymphocytes were activated with CD3/CD28 Dynabeads™ for T cell activation (1:1 cell: bead ratio, Gibco, ThermoFisher) or with ODN 2006-G5 at 1 µM concentration (InvivoGen, tlrl-2006g5) for B lymphocyte activation. Monocytes were activated with LPS at 0.1 ng/mL (L4391, Sigma-Aldrich). Unstimulated or activated lymphocytes and monocytes, and DPSCs cultured alone, were also included in the experimental design as reference. Cells were cultured for 48 hours at 37°C, 5% CO_2.

Collection of supernatants

After 48 hours of culture, supernatants were collected and centrifuged at 10.000 x g for 1 minute to obtain cell-free supernatants for cytokine quantification. Supernatants were stored at -80°C until use.

Flow cytometry to evaluate immune checkpoint expression on DPSCs

DPSCs were detached with trypsin/EDTA (Gibco, ThermoFisher) and analysed by flow cytometry to quantify the expression of PD-L1, PD-L2, CD155, Galectin-9, CD80, CD86, and 4-1BBL. Cells were suspended in 100 µL PBS with 0.1% Fixable Dead Cell Stain near-IR-fluorescent reactive dye (Thermo Fisher Scientific), and stained for 15 min at room temperature. After washing with PBS + 1% FBS, cells were suspended in 100 µL PBS + 1% FBS containing the following antibodies combined in two mixes: (I) PerCP anti-human CD45 (clone REA747), PE anti-human CD155 (clone REA1081), PE-Cy7 anti-human PD-L1 (clone 29E.2A3), APC anti-human CD80 (clone REA661) and FITC anti-human CD86 (clone REA968); (II) PerCP anti-human CD45 (clone REA747), PE anti-human 4-1BBL (CD137L) (clone REA254) and APC anti-human PD-L2 (clone REA 985). Cells were stained for 30 min on ice. After washing, cells stained with mix I were suspended in 300 µL PBS + 1% FBS for subsequent acquisition while cells stained with mix II were fixed and permeabilised for 20 minutes at 4°C using 250 µl BD fixation/permeabilisation solution (BD Biosciences). After washing twice with 1 ml BD permeabilisation/washing buffer (BD Biosciences), cells were stained with the FITC anti-human Galectin 9 antibodies (clone 435) in 100 µl BD permeabilisation/washing buffer for 30 minutes at 4°C. After washing with 1 ml BD permeabilisation/washing buffer, cells were suspended in 300 µL PBS + 1% FBS to be acquired.

All cells were acquired with the FACS Canto II flow cytometer (BD Biosciences) equipped with two lasers for excitation at 488 and 633 nm. Data were analysed with the Kaluza Analysis 2.1 software (Beckman Coulter).

Lymphocytes and monocytes were cultured in contact with DPSCs. Part of the cells were in suspension but some were adhered to DPSCs and were collected together with DPSCs. We thus used anti-CD45 antibodies to restrict the analysis to DPSCs only, which are negative for CD45. Moreover, we analysed only viable cells. The gating strategies are shown in Supplementary figures S1, S2 and S3. Median fluorescence intensities (MFI) in the different fluorescence channels of DPSCs stained with the dead cell dye plus anti-CD45 antibodies were used as blank and were subtracted. At least 10.000 cells in the DPSC gate based on forward and side scatters were acquired. All antibodies were purchased from Miltenyi Biotec, except PE-Cy7 anti-human PD-L1 which was purchased from BioLegend Inc, and used as suggested by the manufacturers.

Cytokine quantification in supernatants

Concentrations of: BAFF, sFASL, IFNγ, IL-1β, IL-9, IL-10, IL-17A, IL-18, IL-21, IL-23, IP-10, M-CSF, MIP-1β, sIL-6RA, Perforin, and TNFα were determined in the supernatants by a custom human ProcartaPlex Mix&Match 16-plex (ThermoFisher), following the manufacturer's instructions. Supernatants were thawed, mixed and centrifuged at 1400 rpm for 5 minutes at 4 °C. Eight point standard curves were prepared based on four-fold serial di-

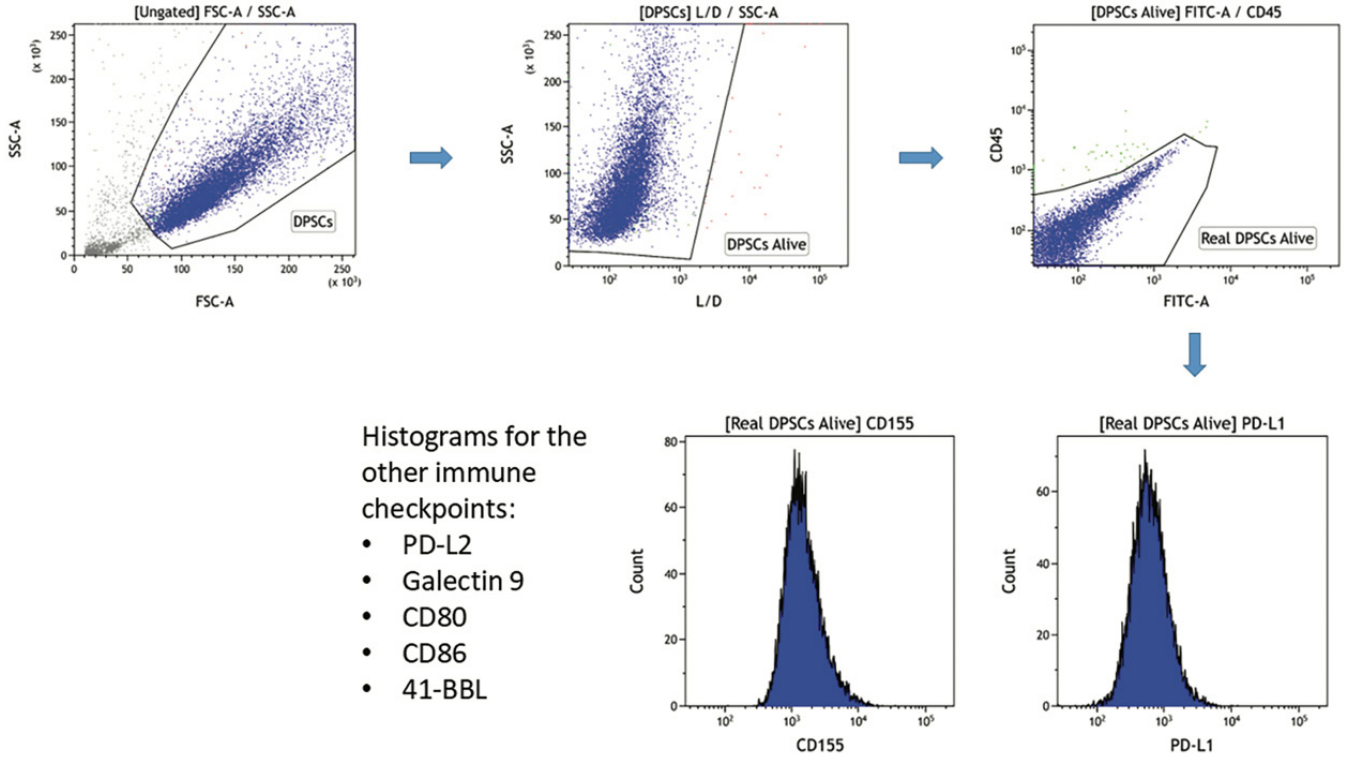
lutions. Data were obtained with the Luminex200™ Multiplex Reader instrument and the ProcartaPlex Analysis App (https://www.thermofisher.com/it/en/home.html). Values extrapolated from the standard curves were considered not reliable and a concentration = ¼ of the lowest detectable standards were arbitrary assigned to be able to draw the graphs. Supernatants of lymphocytes stimulated with CD3/CD28 ± DPSCs and supernatants of monocytes stimulated with LPS ± DPSCs were tested both undiluted and diluted 64-fold in order to have fluorescence intensities for each analyte within the range of those of the respective standards.

Concentrations of IL-6 were determined with the human IL-6 DuoSet ELISA (R&D Systems) following the manufacturer's instructions. Nine-point standard curve was prepared based on two-fold serial dilutions. Supernatants were tested undiluted, diluted 25-fold or 3000-fold so as to have optical densities within the range of those of the standards.

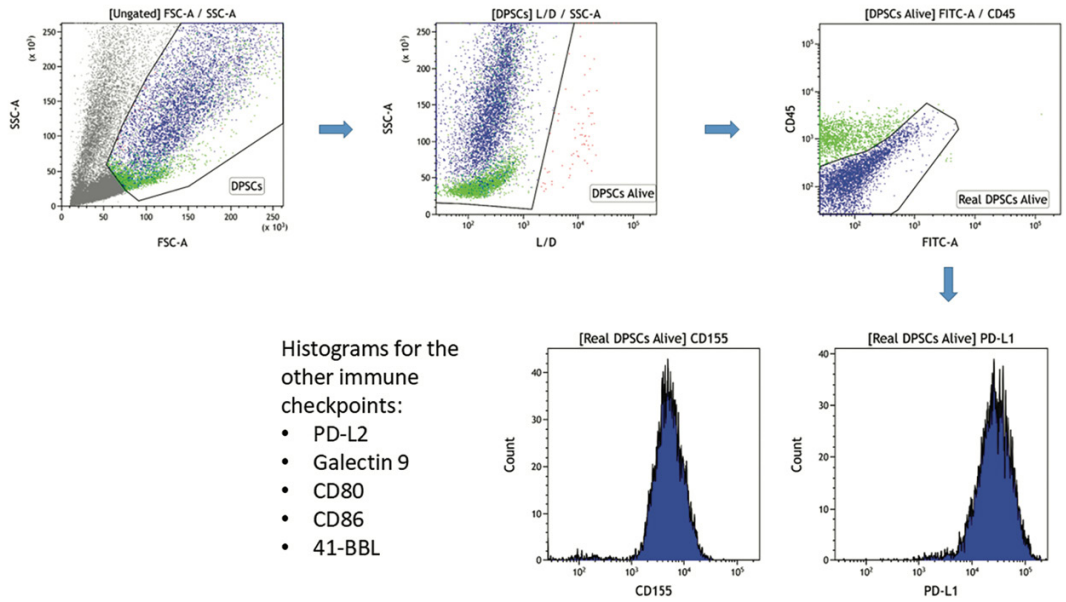
Supplementary Table S1. Characteristics of the patients whose PBMCs were used for the *in vitro* assays. All the patients had clinically active disease with articular manifestations at blood withdrawal.

Patients	Therapy	Age	Gender
1	MTX 20 mg/week	69	F
2	LEF 20 mg/day + PDN 10 mg/day	73	F
3	MTX 10 mg/week	82	F
4	MTX 10 mg/week	59	F
5	MTX 20 mg/week + PDN 5 mg/day	56	F
6	MTX 15 mg/week	63	F
7	HCQ 400 mg/day + LEF 20 mg/day +PDN 7.5 mg/day	56	F
8	MTX 20 mg/week	59	F
9	SSZ 2 g/day	56	F
10	MTX 15 mg/week + PDN 5 mg/day	55	M
11	MTX 12.5 mg/week	71	M
12	MTX 12.5 mg/week	63	F

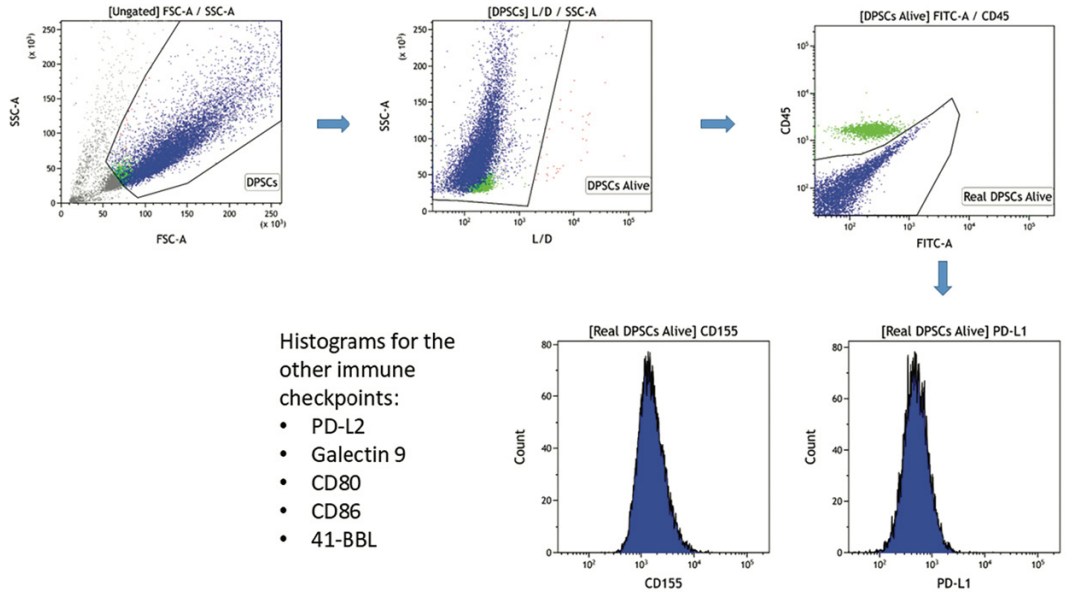
MTX: methotrexate; LEF: leflunomide; PDN: prednisone; HCQ: hydroxychloroquine; SSZ: sulfasalazine; F: female; M: male.



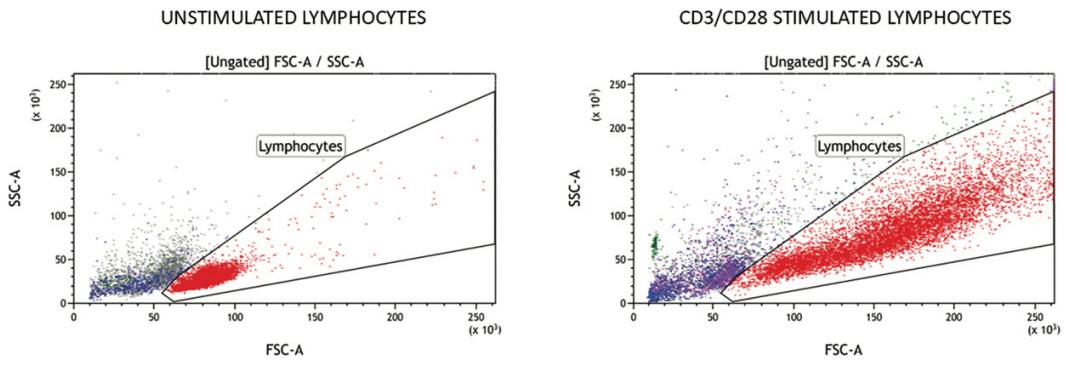
Supplementary Fig. S1. Gating strategy applied on DPSCs cultured alone, DPSCs co-cultured with unstimulated lymphocytes and DPSCs co-cultured with ODN 2006 stimulated lymphocytes. DPSC were gated based on forward- and side-scatter. Live cells were selected by means of a dye which enters into dead cells. DPSCs were then identified for being negative for CD45 and the median fluorescence intensities of the immune checkpoints were quantified (histograms).



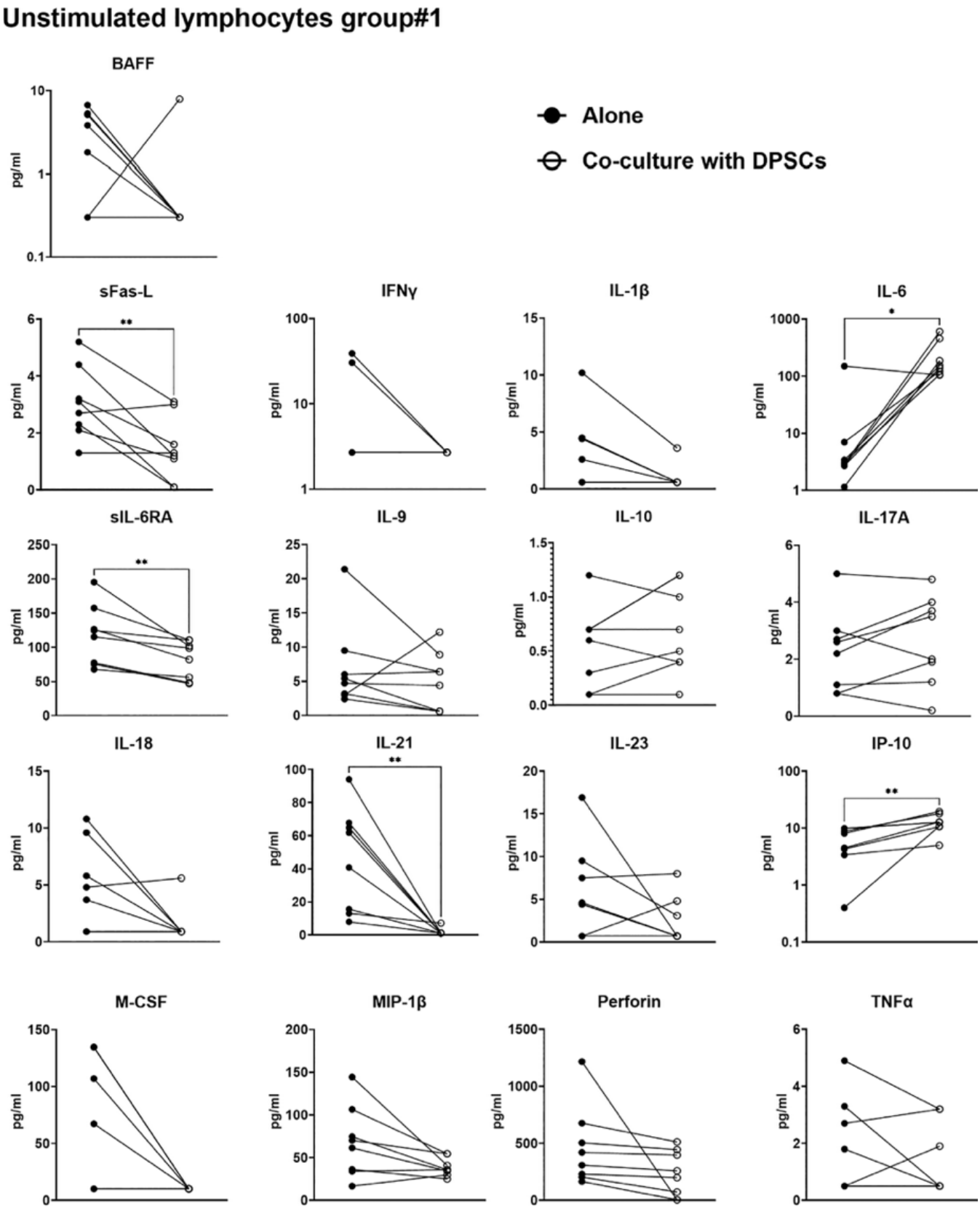
Supplementary Fig. S2. Gating strategy applied on DPSCs co-cultured with CD3/CD28 stimulated lymphocytes. DPSC were gated based on forward- and side-scatter. Live cells were selected by means of a dye which enters into dead cells. DPSCs were then identified for being negative for CD45 and the median fluorescence intensities of the immune checkpoints were quantified (histograms).



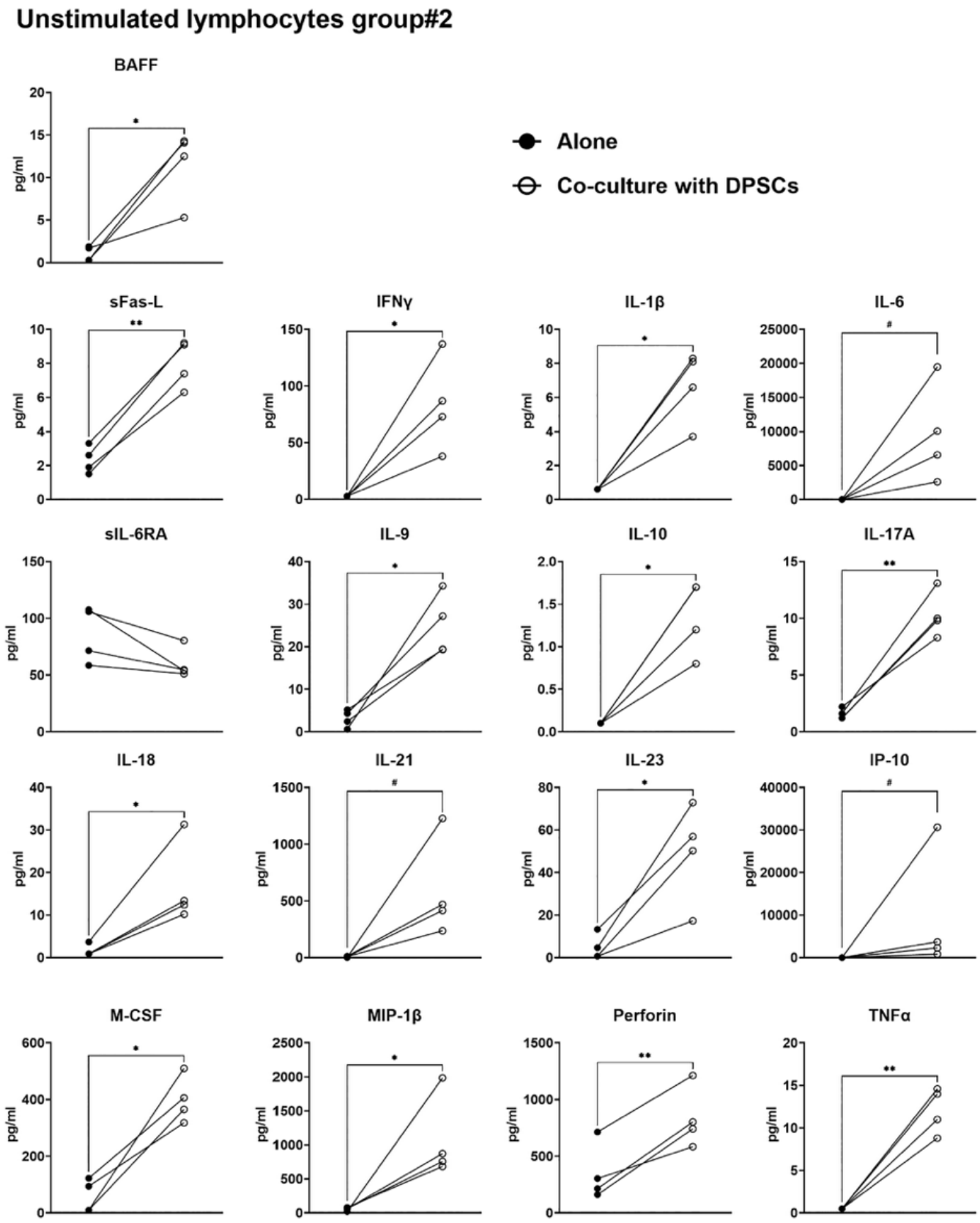
Supplementary Fig. S3. Gating strategy applied on DPSCs co-cultured with unstimulated and LPS stimulated monocytes. DPSC were gated based on forward- and side-scatter. Live cells were selected by means of a dye which enters into dead cells. DPSCs were then identified for being negative for CD45 and the median fluorescence intensities of the immune checkpoints were quantified (histograms).



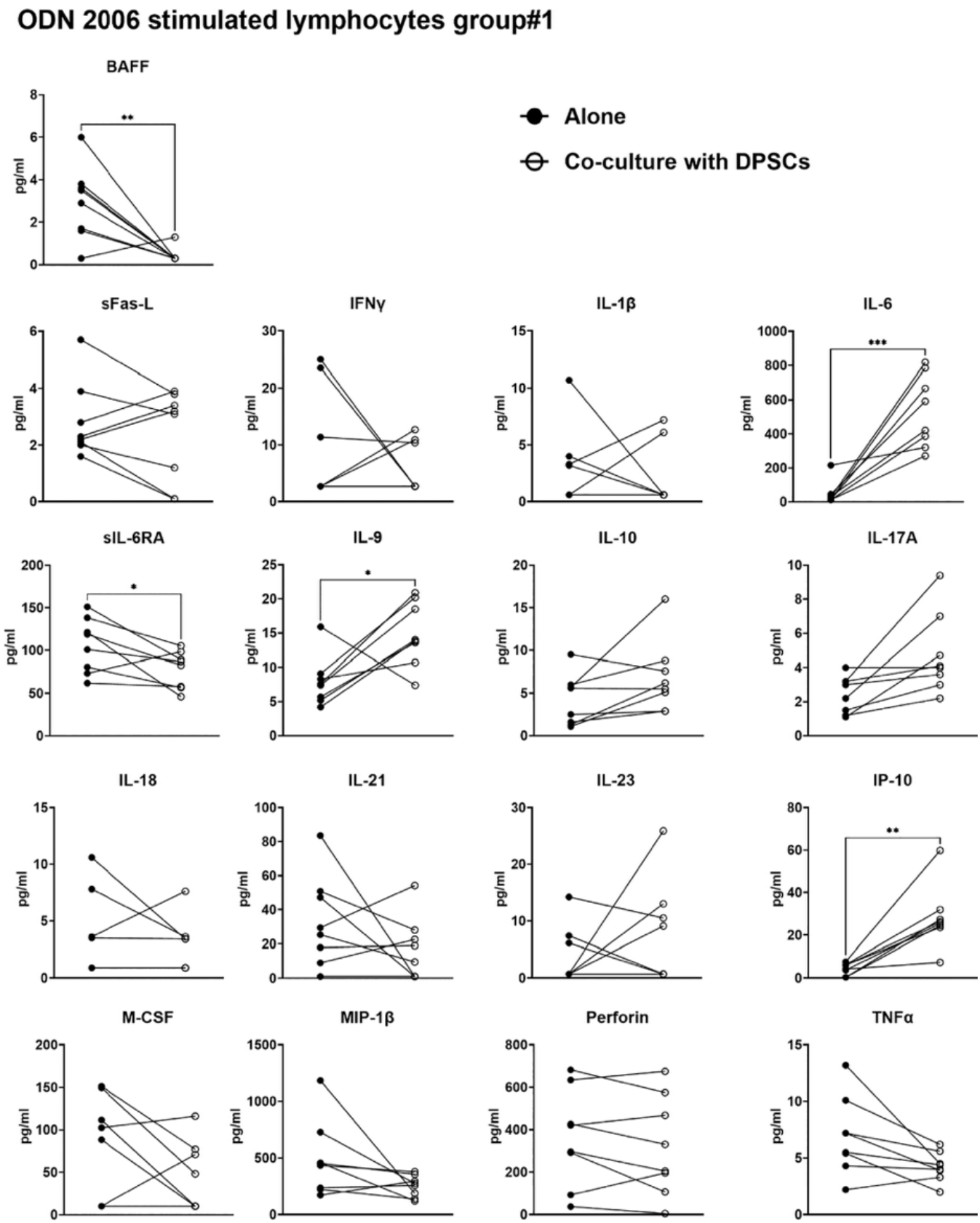
Supplementary Fig. S4. Comparison of the forward- and side-scatter of unstimulated and CD3/CD28 stimulated lymphocytes.



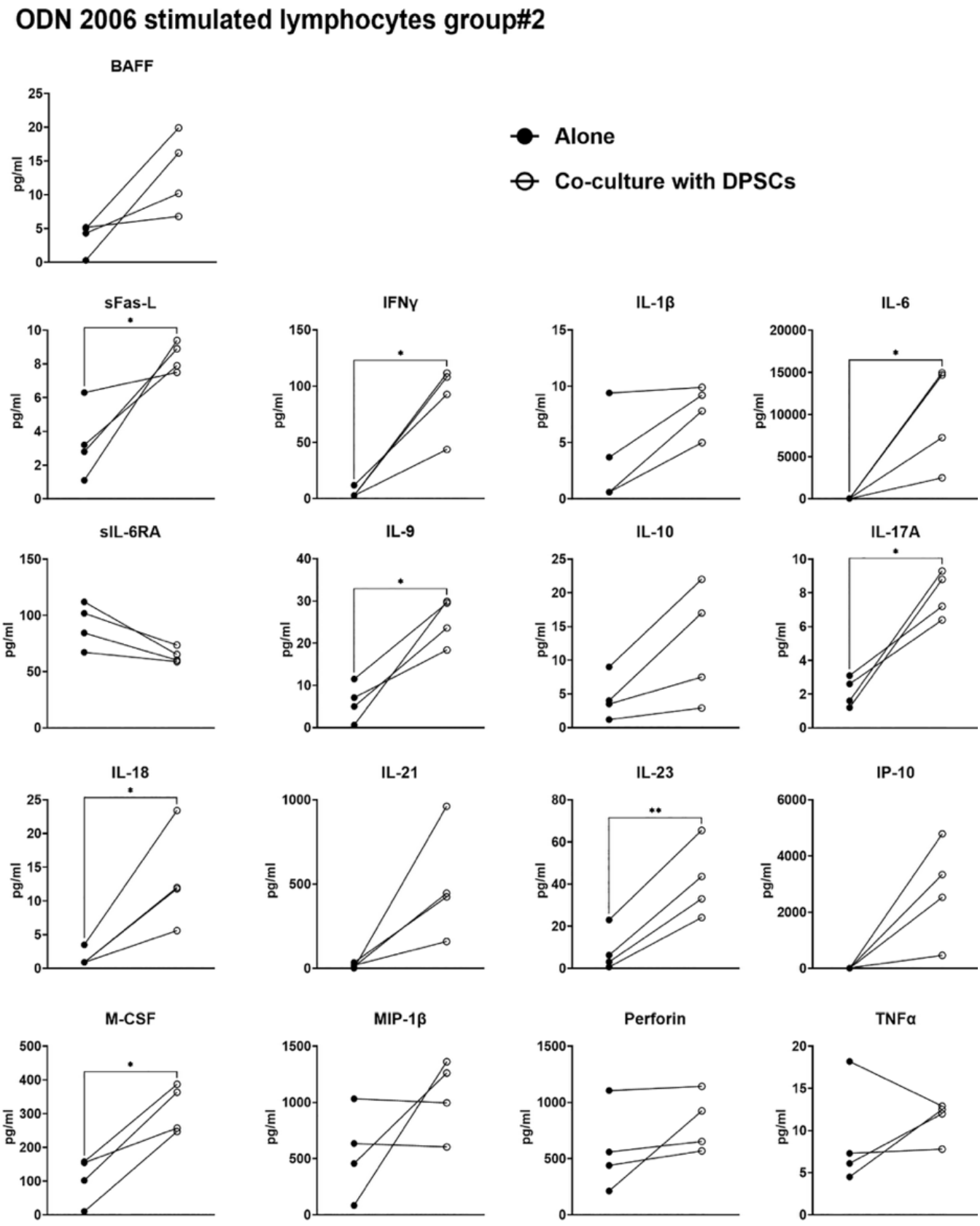
Supplementary Fig. S5. Cytokine concentrations in supernatants of unstimulated lymphocytes from patient group no. 1. Concentrations of 17 cytokines were quantified in supernatants after 48 hours of culture of lymphocytes with and without DPSCs (n=8). Data were analysed with paired Student's t-test. * $p<0.05$, ** $p<0.01$.



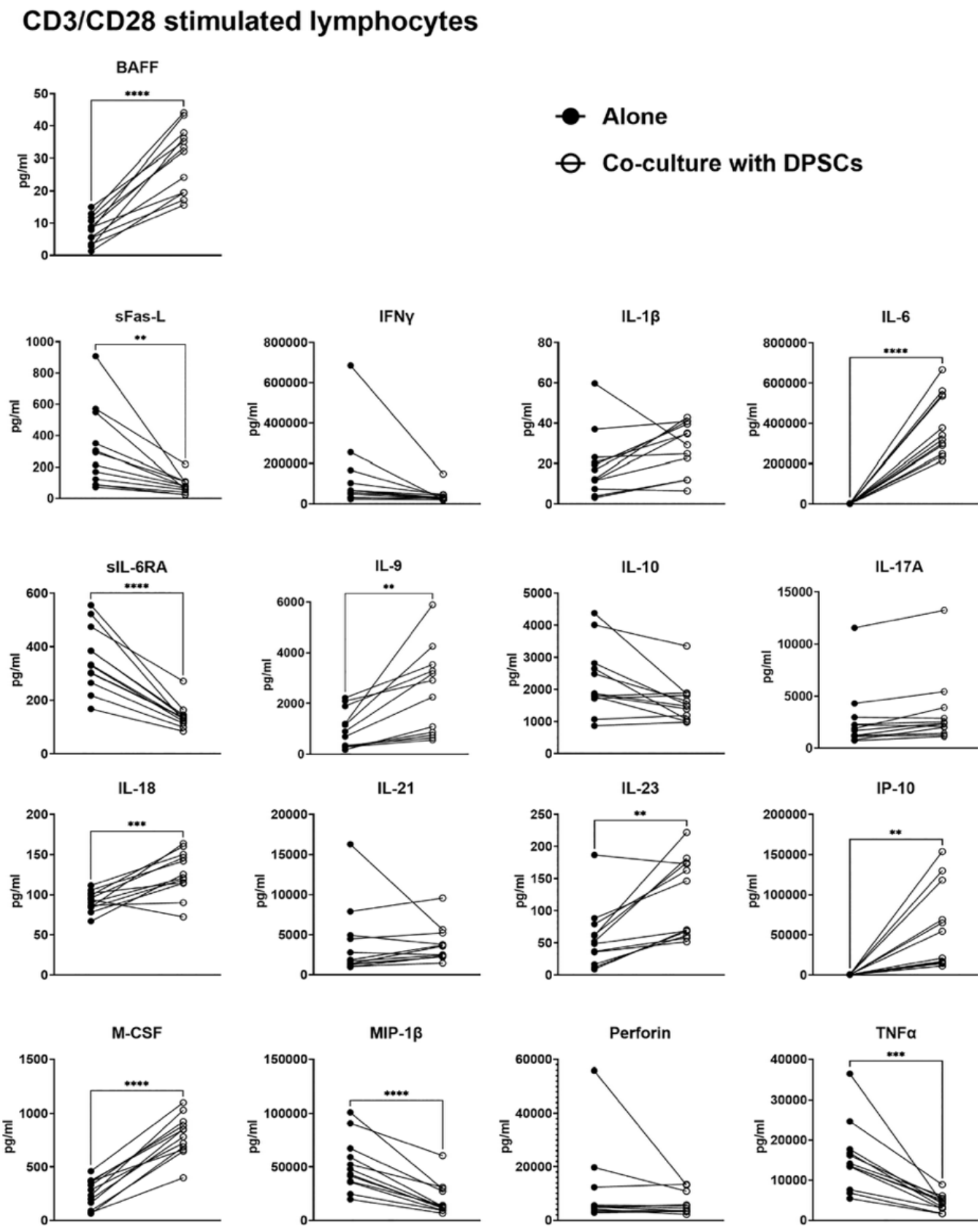
Supplementary Fig. S6. Cytokine concentrations in supernatants of unstimulated lymphocytes from patient group no. 2. Concentration of 17 cytokines were quantified in supernatants after 48 hours of culture of lymphocytes with and without DPSCs (n=4). Data were analysed with paired Student's t-test. * $p<0.05$, ** $p<0.01$.



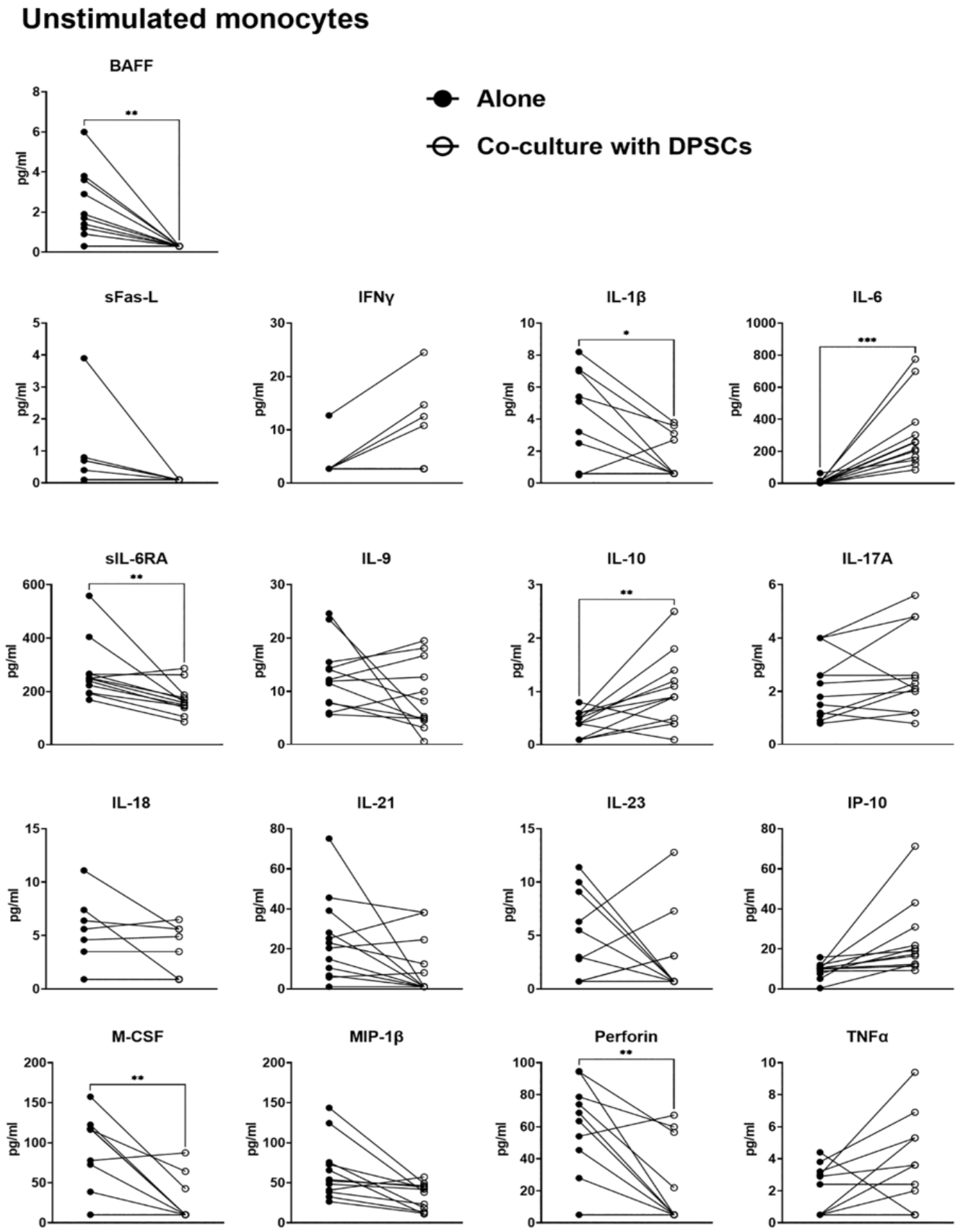
Supplementary Fig. S7. Cytokine concentrations in supernatants of ODN 2006-stimulated lymphocytes from group no. 1. Concentration of 17 cytokines were quantified in supernatants after 48 hours of culture of ODN 2006 stimulated lymphocytes with and without DPSCs (n=8). Data were analysed with paired Student's t-test. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.



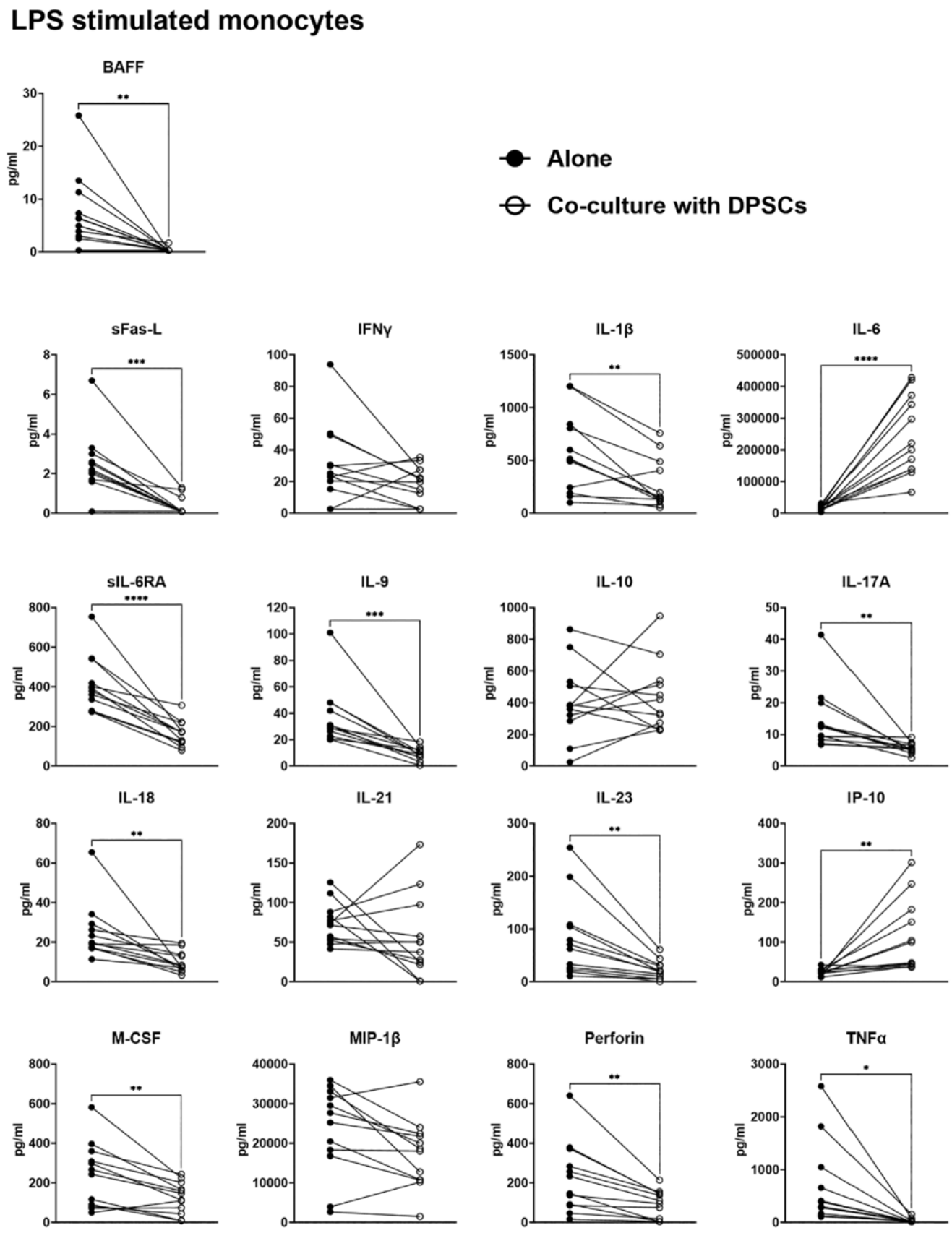
Supplementary Fig. S8. Cytokine concentrations in supernatants of ODN 2006-stimulated lymphocytes from patient group no. 2. Concentration of 17 cytokines were quantified in supernatants after 48 hours of culture of ODN 2006 stimulated lymphocytes with and without DPSCs (n=4). Data were analysed with paired Student's t-test. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.



Supplementary Fig. S9. Cytokine concentrations in supernatants of CD3/CD28-stimulated lymphocytes. Concentration of 17 cytokines were quantified in supernatants after 48 hours of culture of CD3/CD28 stimulated lymphocytes with and without DPSCs (n=12). Data were analysed with paired Student's t-test. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.



Supplementary Fig. S10. Cytokine concentrations in supernatants of unstimulated monocytes. Concentration of 17 cytokines were quantified in supernatants after 48 hours of culture of monocytes with and without DPSCs (n=12). Data were analysed with paired Student's t-test. * $p<0.05$, ** $p<0.01$.



Supplementary Fig. S11. Cytokine concentrations in supernatants of LPS-stimulated monocytes. Concentration of 17 cytokines were quantified in supernatants after 48 hours of culture of LPS stimulatedmonocytes with and without DPSCs (n=12). Data were analysed with paired Student's t-test.
* $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.