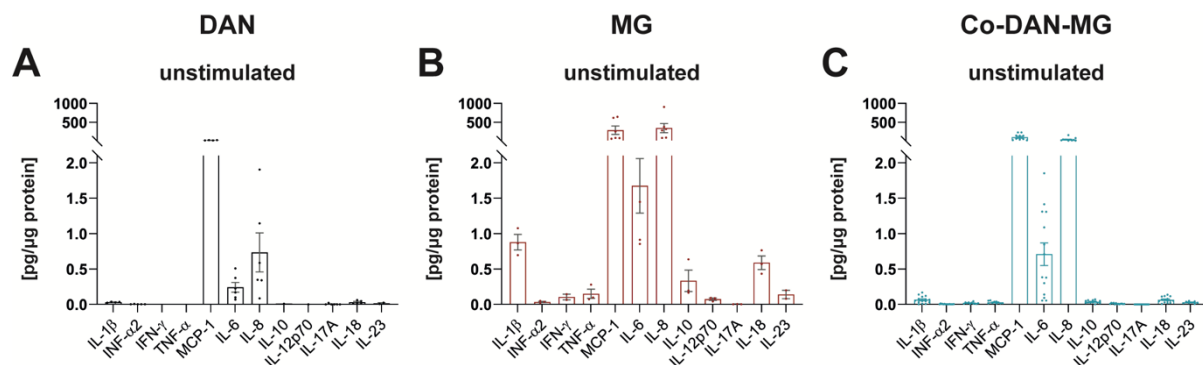
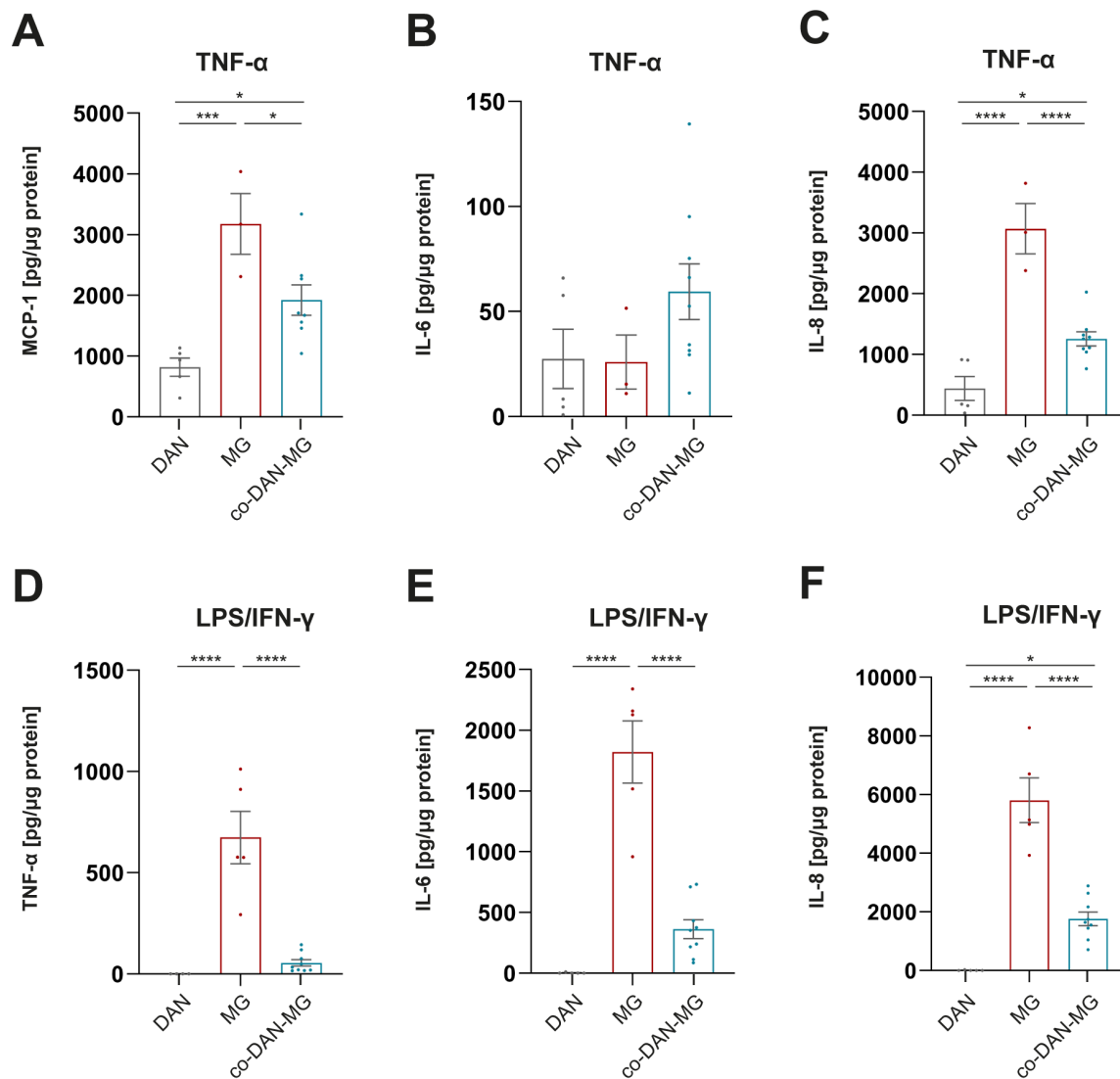


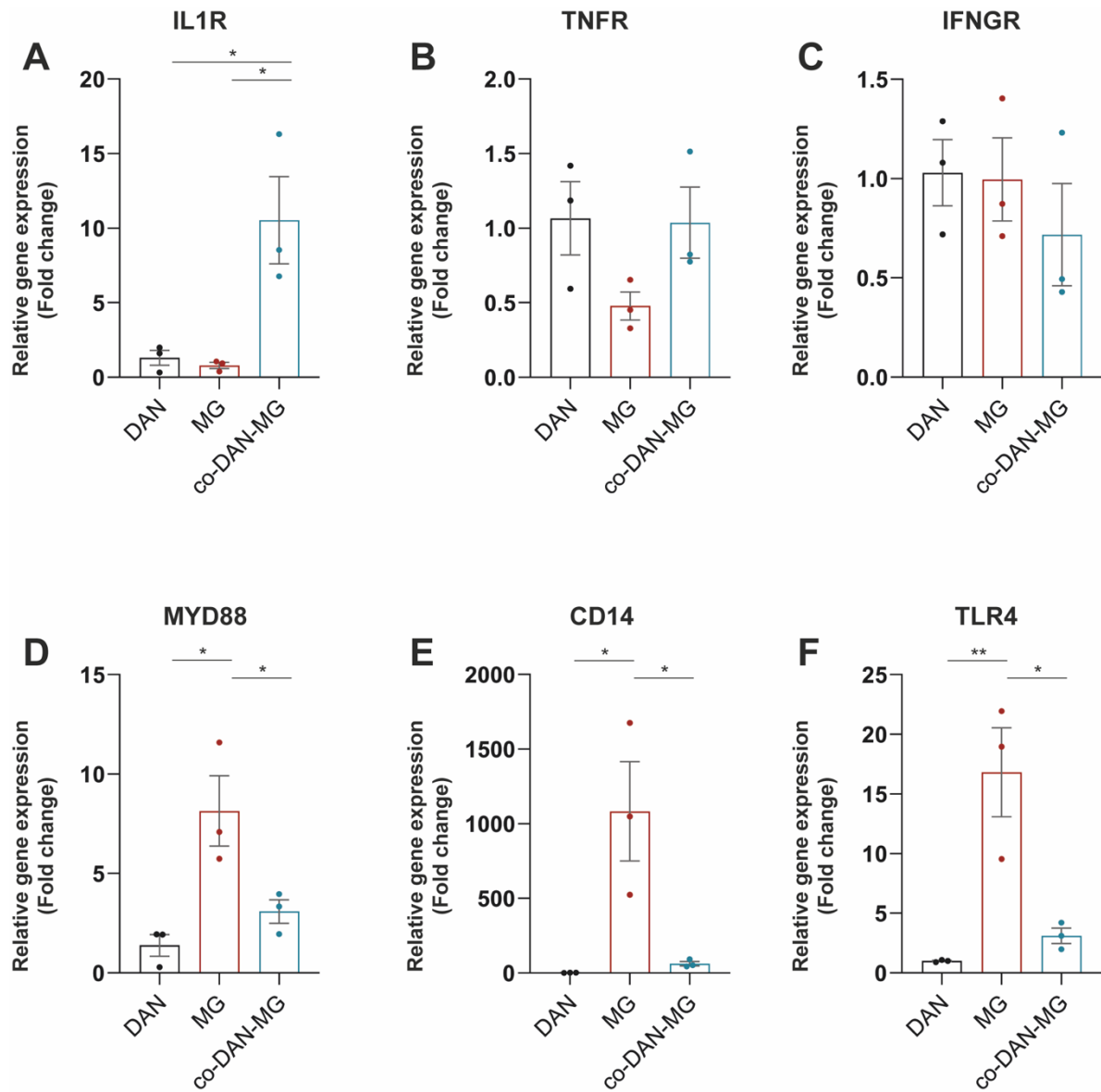
## SUPPLEMENTARY MATERIAL – “Cytokine profiling in human iPSC-derived dopaminergic neuronal and microglial cultures”



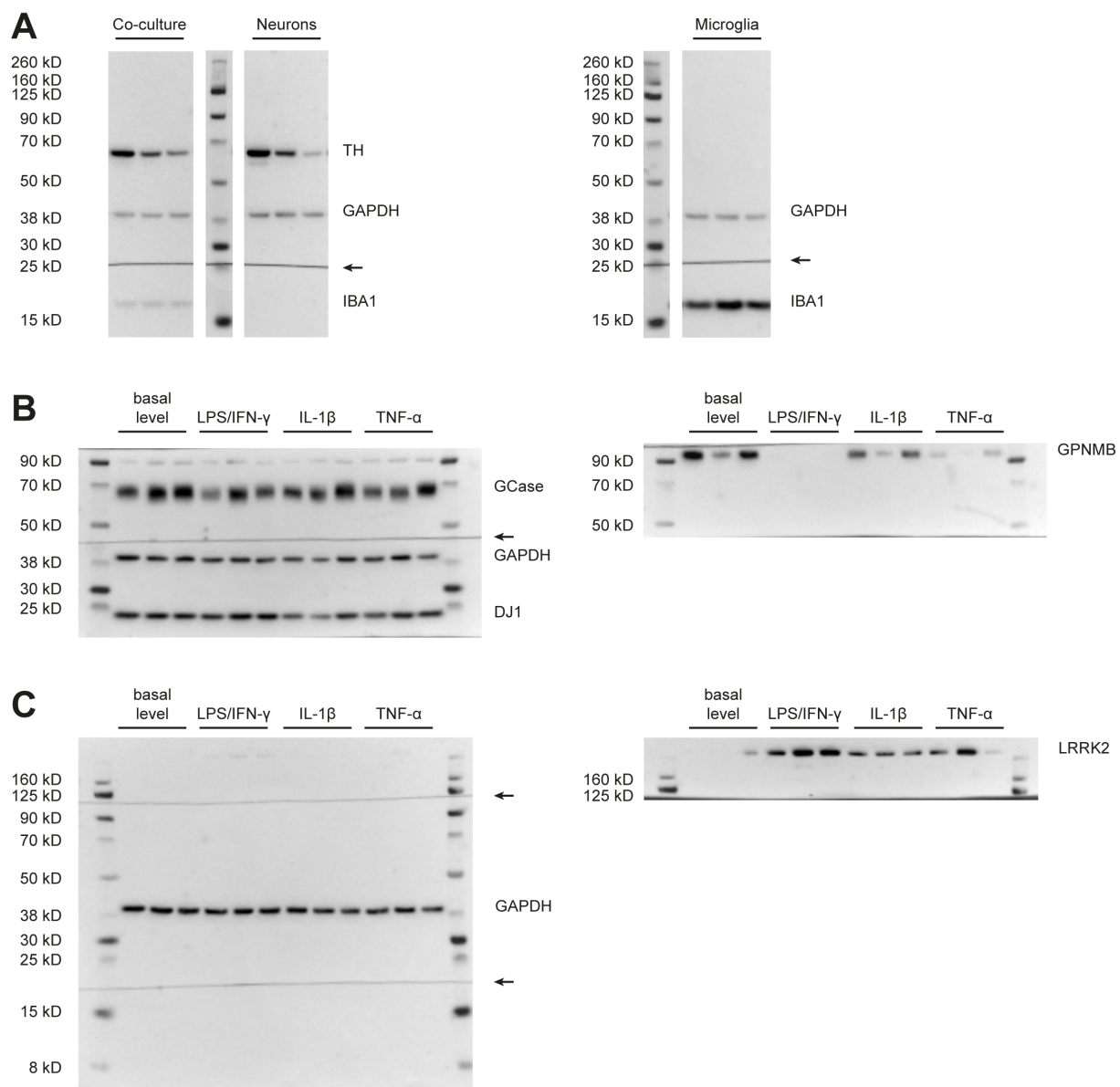
**Figure S1.** Cytokine profiles of iPSC-derived dopaminergic neurons (DAN), microglia (MG), and co-cultures (co-DAN-MG) under basal conditions. Simultaneous measurement of 12 cytokines in cell culture media by FACS using Legendplex Human Inflammation Panel 1 under unstimulated basal conditions in **(A)** DAN, **(B)** MG, and **(C)** co-DAN-MG. Cell culture media from three different lines derived from healthy controls were analyzed: SFC086-03-03, SFC089-03-07 and SCF156-03-01. DAN: DAN: n=5 differentiations of 1-3 lines per each differentiation experiment, MG: n=2 differentiations of 3 lines each, and co-DAN-MG: n=10 differentiations of 1-2 lines per each differentiation experiment. Mean  $\pm$  SEM.



**Figure S2.** Dopaminergic neuron (DAN) and microglia (MG) co-cultures (co-DAN-MG) show a reduced secretion of cytokines compared to MG upon treatment with TNF- $\alpha$  and LPS/IFN- $\gamma$ . **(A)** MCP-1, **(B)** IL-6, and **(C)** IL-8 levels in TNF- $\alpha$  stimulated DAN, MG, and co-DAN-MG. Levels of **(D)** TNF- $\alpha$ , **(E)** IL-6, **(F)** IL-8 after stimulation with LPS/IFN- $\gamma$ . Media samples were assayed with the Legendplex Human Inflammation Panel 1. DAN: n=5 differentiations of 1-3 lines per each differentiation experiment, MG: n=2 differentiations of 3 lines each, and co-DAN-MG: n=10 differentiations of 1-2 lines per each differentiation experiment. Cell culture media were taken from three healthy controls: SFC086-03-03, SFC089-03-07, and SCF156-03-01. Mean  $\pm$  SEM. Asterisks indicate significant difference between different cell models by One-way ANOVA followed by Tukey's multiple comparisons test.



**Figure S3.** Gene expression levels of cytokine receptors. Relative gene expression analysis of **(A)** *Interleukin 1 receptor (IL1R)*, **(B)** *Tumor necrosis factor alpha receptor (TNFR)*, **(C)** *Interferon gamma 1 receptor (IFNGR)*, **(D)** *myeloid differentiation 88 (MYD88)*, **(E)** *Cluster of differentiation 14 (CD14)*, and **(F)** *Toll-like receptor 4 (TLR4)* in unstimulated cultures relative to  $\beta$ -Actin. n=3 different cell lines (SFC086-03-03, SFC089-03-07 and SCF156-03-01). One-way ANOVA followed by Tukey's multiple comparisons test. Mean  $\pm$  SEM.



**Figure S4.** Whole Western blots. Blots show protein levels of **(A)** tyrosine hydroxylase (TH), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), ionized calcium binding protein (IBA1) and **(B)**  $\beta$ -glucocerebrosidase (GCCase), DJ-1, glycoprotein nonmetastatic melanoma protein b (GPNMB), GAPDH and **(C)** Leucine-rich repeat kinase 2 (LRRK2) and GAPDH in stimulated and unstimulated microglia. The blots have been cut and incubated separately with antibodies as indicated by the arrows.

**Table S1.** iPSC lines

| ID of iPSC lines | Sex    | Age at biopsy | Reprogramming method | iPSC clone characterization   |
|------------------|--------|---------------|----------------------|---|
| SFC086-03-03     | female | 57            | Sendai virus         | <a href="https://hpscereg.eu/cell-line/STBCi052-C">https://hpscereg.eu/cell-line/STBCi052-C</a> |
| SFC089-03-07     | female | 64            | Sendai virus         | <a href="https://hpscereg.eu/cell-line/STBCi053-A">https://hpscereg.eu/cell-line/STBCi053-A</a> |
| SFC156-03-01     | male   | 75            | Sendai virus         | <a href="https://hpscereg.eu/cell-line/STBCi101-A">https://hpscereg.eu/cell-line/STBCi101-A</a> |

**Table S2.** Primers (annealing temperature 58° C)

| Gene              | Forward primer sequence (5' to 3') | Reverse primer sequence (5' to 3') |
|-------------------|------------------------------------|------------------------------------|
| <i>Beta-actin</i> | TGAAGTGTGACGTGGACATC               | GGAGGAGCAATGATCTTGAT               |
| <i>CD14</i>       | CTCTGTCCCCACAAGTTCCC               | GGATTCCCGTCCAGTGTCAG               |
| <i>IFNGR1</i>     | GAGACGAGCAGGAAGTCGA                | TGGAATCGCTAACTGGCACT               |
| <i>IL1R</i>       | CGTCCCTGTCCTCTTAACCC               | CACACAAGTCCTCCGTCTCC               |
| <i>MYD88</i>      | CCGCCTGTCTCTGTTCTT                 | TCCTCCTCAATGCTGGGT                 |
| <i>TNFR1</i>      | TCACCGCTTCAGAAAACCAC               | TCACTCCAATAATGCCGGTAC              |
| <i>TLR4</i>       | CTTTATTCCCGGTGTGGCCA               | GCAGGGTCTTCTCCACCTTC               |

**Table S3.** Antibodies

| Primary antibody | Cat. No.   | Dilution | Manufacturer                              |
|------------------|------------|----------|---|
| TH               | AB152      | 1:2000   | Millipore, Burlington, MA, USA            |
| GAPDH            | 2118S      | 1:30000  | Cell Signaling, Danvers, MA, USA          |
| IBA1             | Ab178846   | 1:1000   | Abcam, Cambridge, MA, USA                 |
| TH               | NB 300-110 | 1:500    | Novus Biologicals, Centennial, CO, USA    |
| CD88             | 344302     | 1:200    | Biolegend, San Diego, CA, USA             |
| LRRK2            | Ab133518   | 1:200    | Abcam, Cambridge, MA, USA                 |
| GPNMB            | Sc-271415  | 1:1000   | Santa Cruz Biotechnology, Dallas, TX, USA |
| DJ-1             | 5933S      | 1:4000   | Cell Signaling, Danvers, MA, USA          |
| CD45-APC         | 21810456   | 1:100    | ImmunoTools, Friesoythe, Germany          |
| CD14-PE          | 21620144   | 1:100    | ImmunoTools, Friesoythe, Germany          |
| CD88-PE          | 344304     | 1:100    | Biolegend, San Diego, CA, USA             |