Supplementary Information of Soluble TREM2 Mediates Blood Brain Barrier Permeability through Astrocyte Reactivity

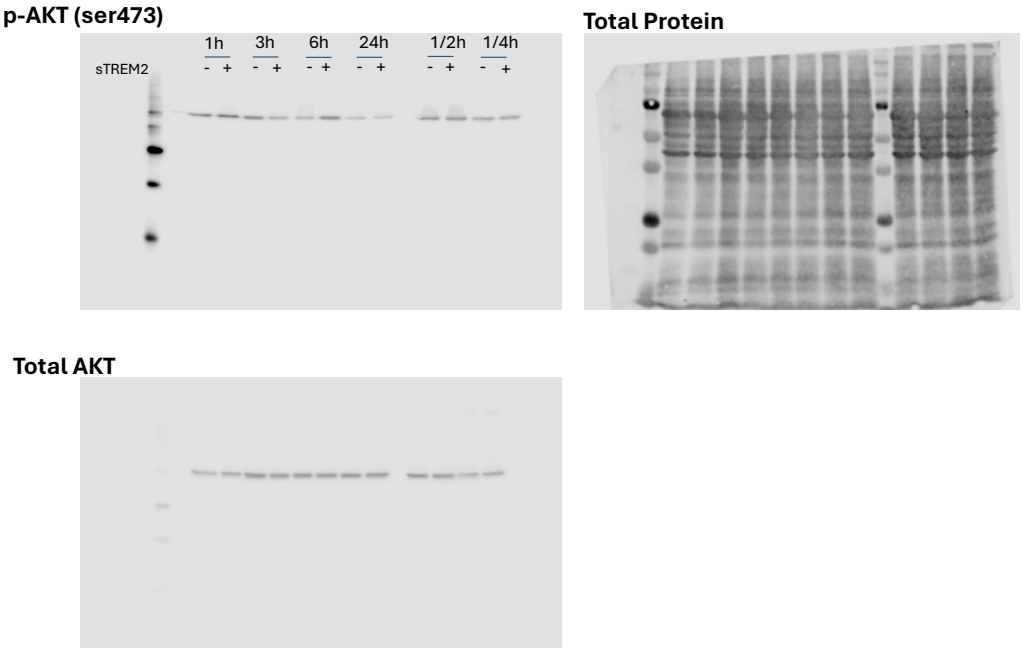

Figure S1. Full western blot image of p-AKT/total AK and Total protein of levels of p-AKT and AKT in human astrocytes treated with soluble TREM2 (sTREM2, 100 nM) for 1, 3, 6, 24, ½ and ¼ h.

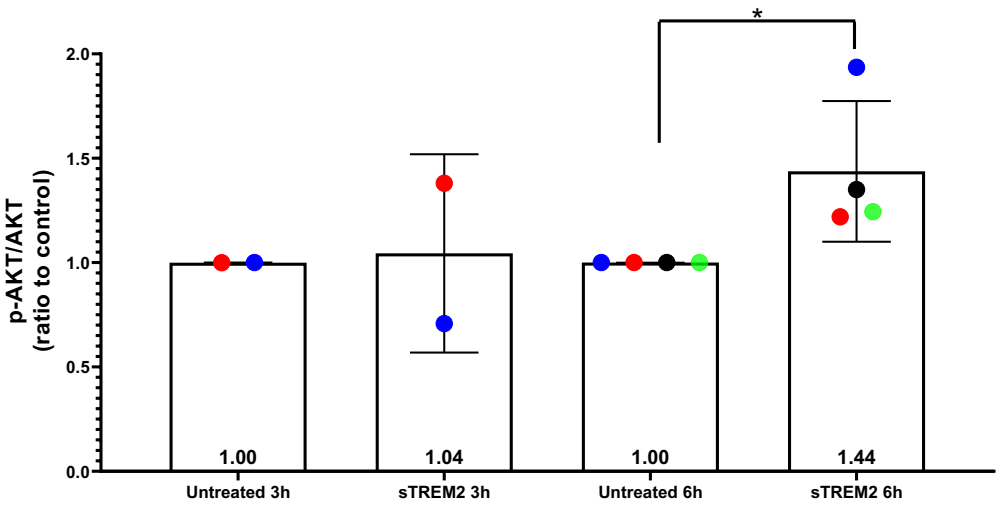

Figure S2. Quantification of phospho-AKT normalized to total AKT (p-AKT/AKT) from astrocytes treated with soluble TREM2 (sTREM2, 100 nM) for 3h (n=2), and 6 h (n=4). Data are presented as mean ± SEM. *$p < 0.05$.

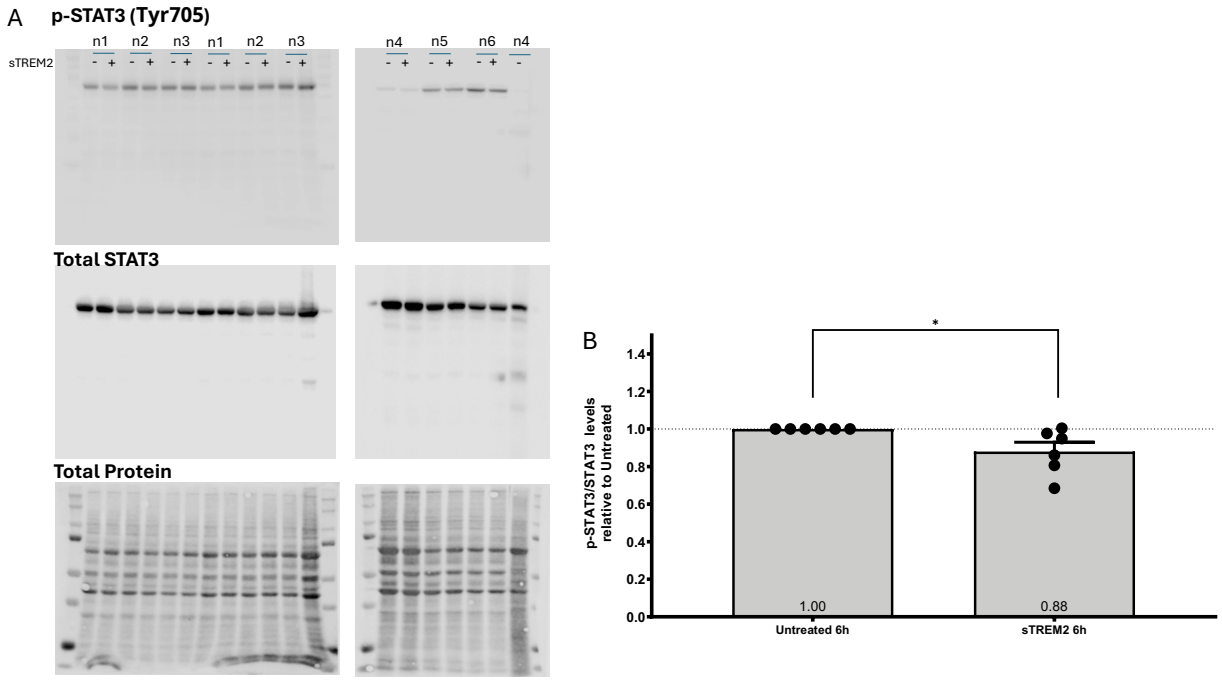

Figure S3. Full western blot image of p-STAT3/total STAT3/ Total protein and analysis of STAT3 activation in astrocytes treated with soluble TREM2 (sTREM2, 100 nM) for 6 h vs vehicle (Untreated). A. Full Blot image blot showing phosphorylated STAT3 (p-STAT3) total STAT3 levels and total protein, including total protein loading, from six independent experiments (n = 6). B. Quantification of the p-STAT3/STAT3 ratio from six independent experiments (n = 6) with statistical analysis. Data are presented as mean ± SEM. *$p < 0.05$ vs. vehicle.

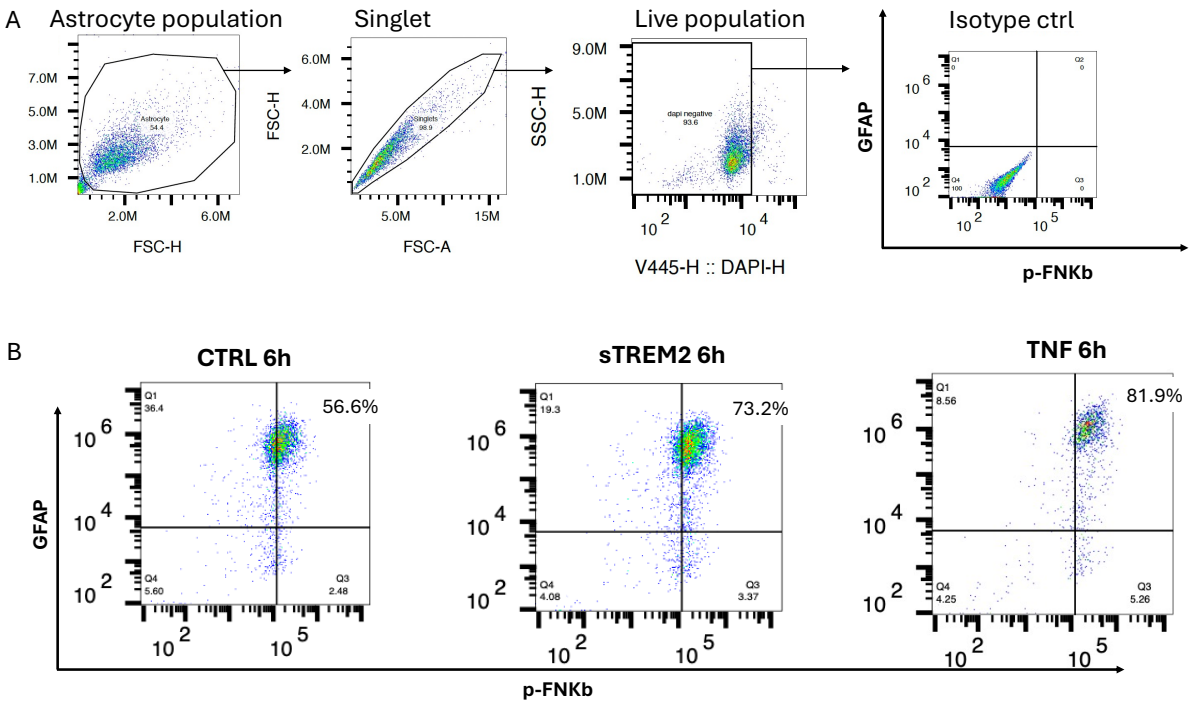

Figure S4 Gating strategy to analyze of GFAP and p-NFκB in astrocytes treated with soluble TREM2 (sTREM2, 100 nM) or TNF-a 4 ng/ml for 6h. A. Sequential gating strategy used to define the negative population based on isotype control. B. Comparison of GFAP and p-NFκB levels across vehicle- and sTREM2-treated astrocytes.

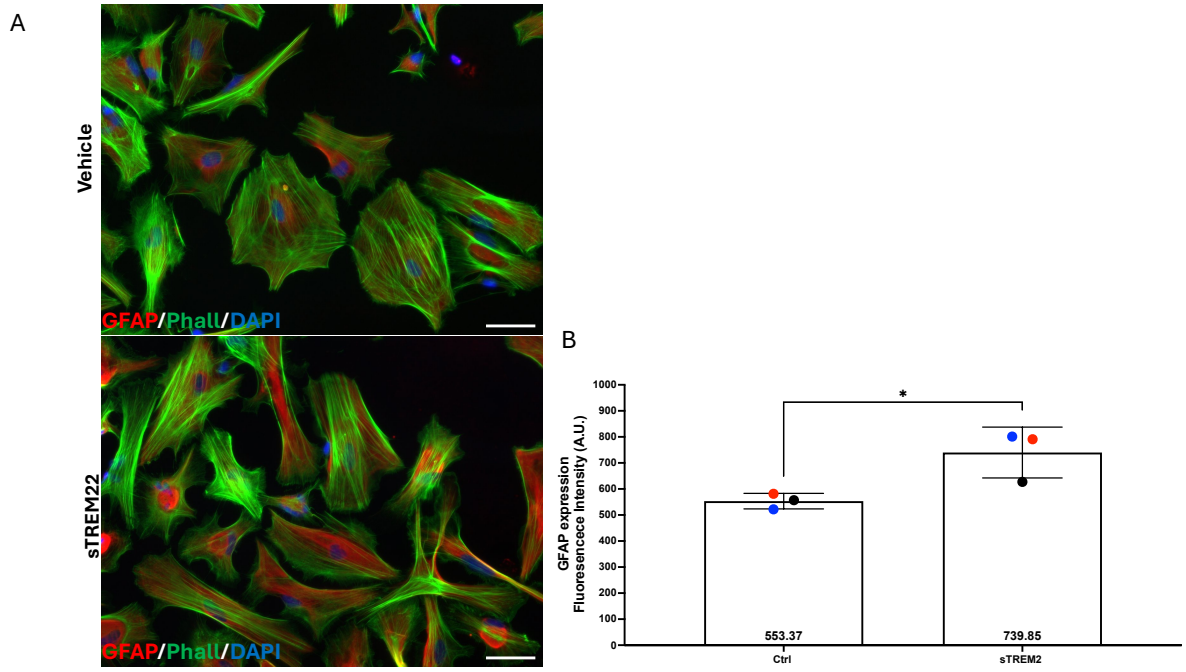

Figure S5. Evaluation of GFAP expression in astrocytes treated with soluble TREM2 (sTREM2, 100 nM) for 6 h. A. Representative immunofluorescence images showing GFAP (red), phalloidin (green), and DAPI (blue). **B.** Quantification of GFAP fluorescence intensity in vehicle- versus sTREM2-treated astrocytes. Data are presented as mean ± SEM from [n = 3] independent experiments. *$p < 0.05$.

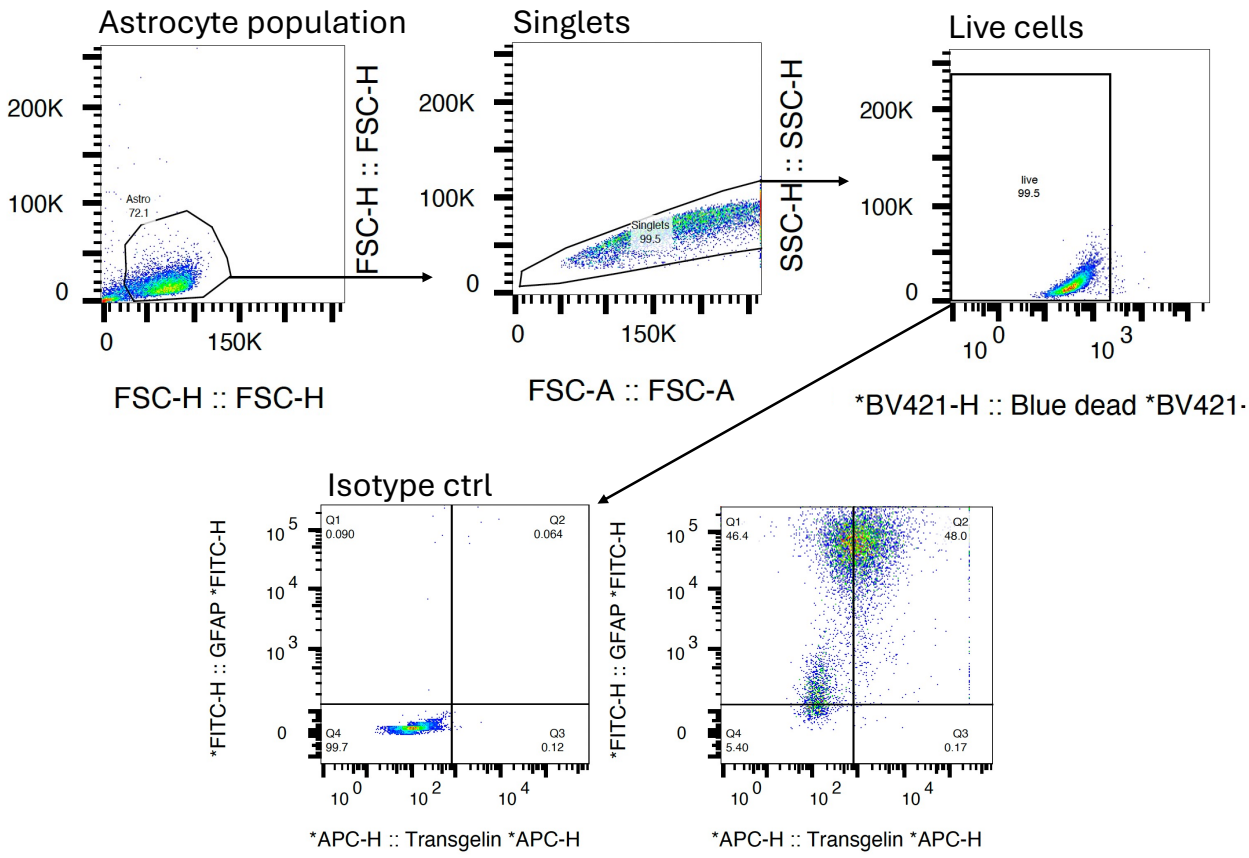

Figure S6. Flow cytometry analysis of Transgelin-2 in a human primary astrocyte. Sequential gating strategy used to define the negative population based on isotype control, with a representative dot plot of Line 1.

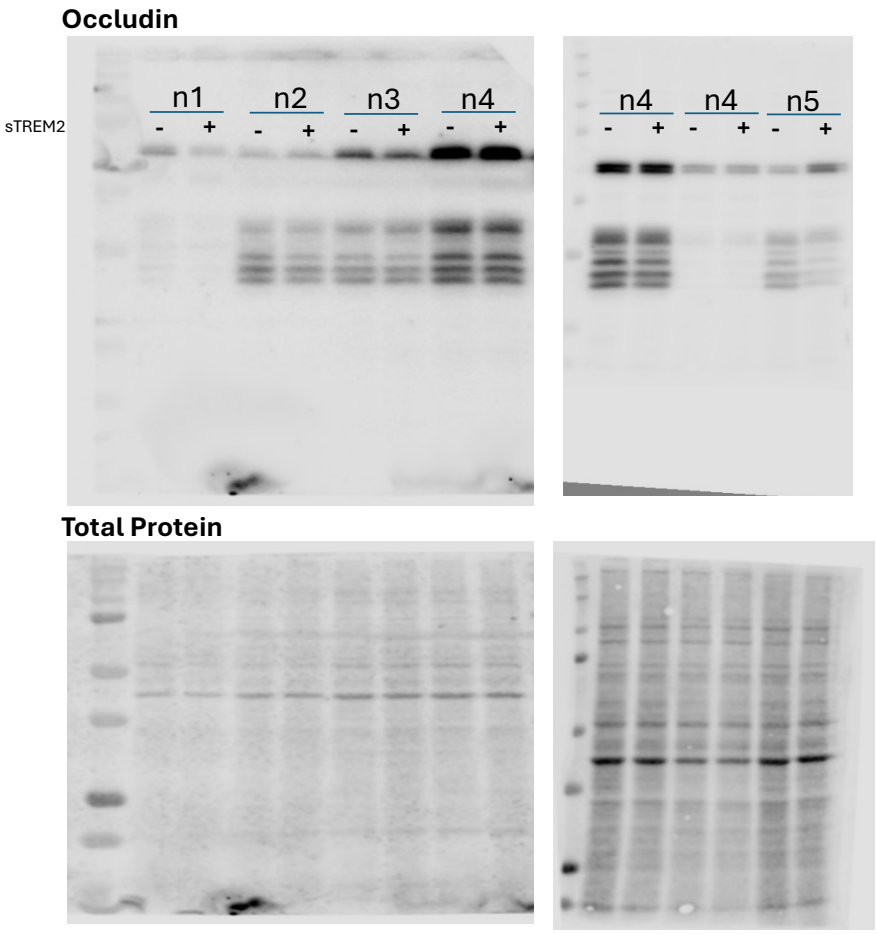

Figure S7. Full Western blot images of Occludin (top membranes) and total protein staining (bottom membranes) from BMVECs in five independent BBB experiments (n = 5) treated with sTREM2 (100 nM, 6 h) or vehicle (untreated). All detected bands, including full-length Occludin (~65–67 kDa) and its cleavage fragments 1 (~50–55 kDa) and 2 (~37–42 kDa), were included in the quantification.

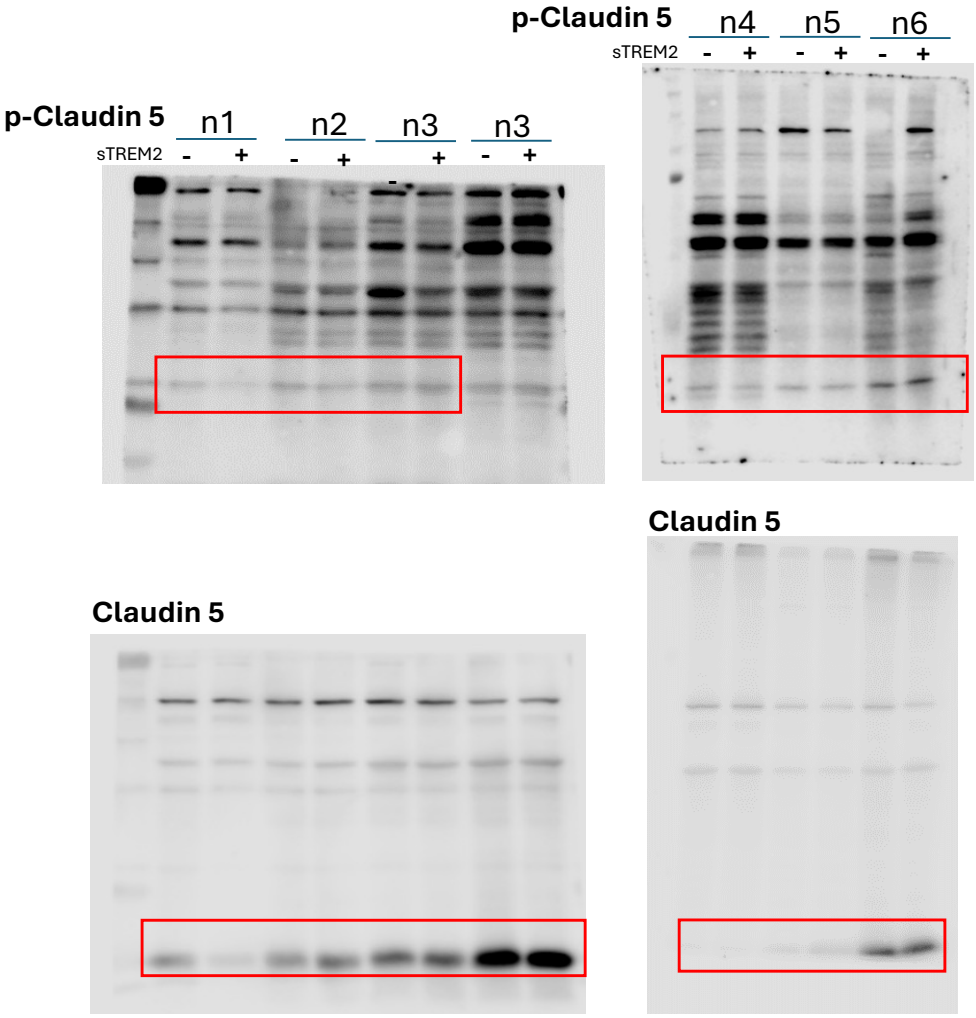

Figure S8. Full Western blot images of phospho-Claudin-5 and total Claudin-5 protein in BMVECs from six independent BBB experiments (n = 6) treated with sTREM2 (100 nM, 6 h) or vehicle control (untreated). The red rectangle indicates the Claudin-5 band at approximately 25 kDa. The upper portion of the membrane corresponding to experiments 1–3 was removed prior to imaging.

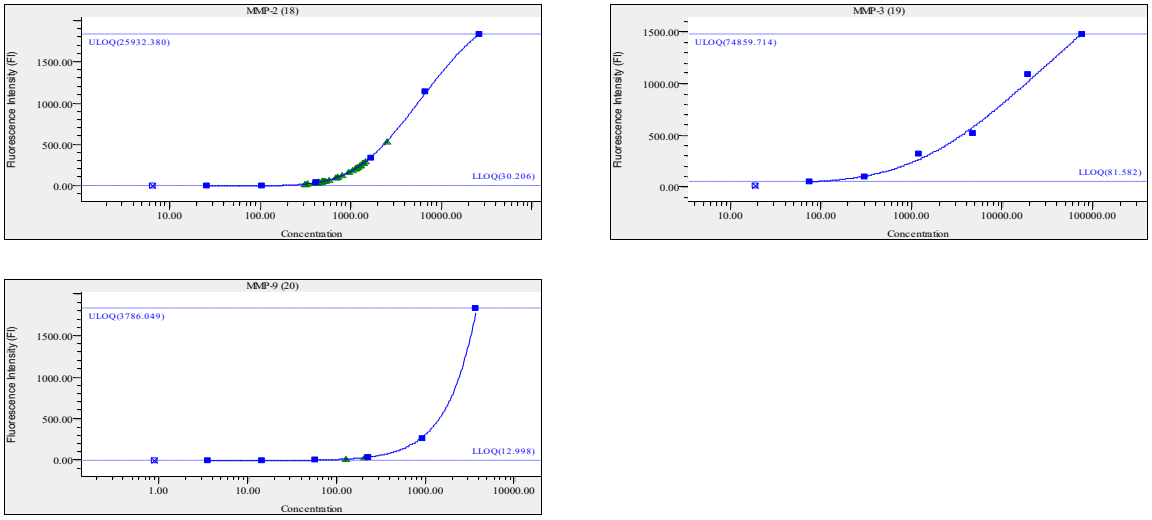

Figure S9. Standard curves for multiplex analysis using the ProcartaPlex™ Human MMP Panel 2, 3-plex for MMP-2, MMP-3, and MMP-9. MMP-2 is shown with detectable concentrations in the standard curve (green triangle), whereas MMP-3 and MMP-9 samples were below the LLOQ (Lower Limit of Quantification) in the analyzed samples and are therefore not represented in the curves.

Target(Bead)	LOD (pg/ml)	LOQ (pg/ml)	Concentration Average of samples (pg/ml)
IL-1β	9.05760282	46.2071279	2.06
IFN-γ	1.09544723	1.94516661	1.51
TNF-α	1.10808089	1.90723439	1.17
MCP-1	36.5465682	154.201453	13225.22
IL-6	7.35426094	54.7869311	14773.35
IL-8	4.58105095	136.58344	4053.98
IL-12p70	1.08968543	3.81253713	0.68
IL-18	1.75969552	4.38411535	4.11
IL-33	8.49090618	19.7965941	5.4

Table S1. Limit of detection (LOD) and LLOQ (Lower Limit of Quantification). The table shows the LOD and LLOQ for each cytokine analyzed using the multiplex ELISA (Bio-Plex Pro Human Cytokine Panel).