

Supplementary information

Multiplexed analysis of signalling proteins at the single-immune cell level

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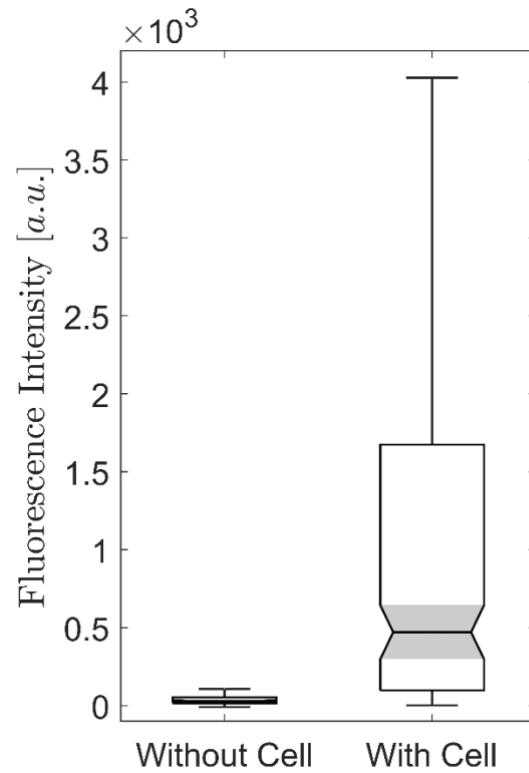


Figure S1. Protein secretion measured in chambers containing at least one cell compared to chambers containing no cell in the same microfluidic chip. The signal in chambers without a cell is very low. We also do not observe diffusion of analytes from a cell-occupied chamber to an adjacent empty chamber, when the pneumatic valves are closed.

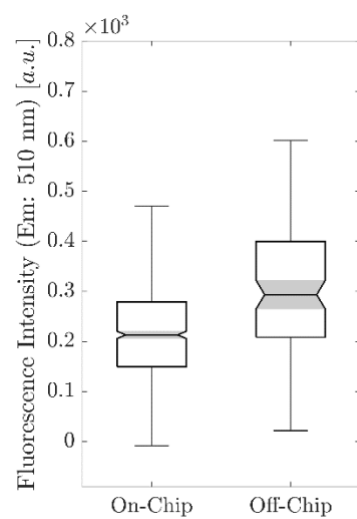


Figure S2. The ROS level in the cells was measured in bulk (off-chip) and in the microchambers (on-chip) with CellROX™ Green.

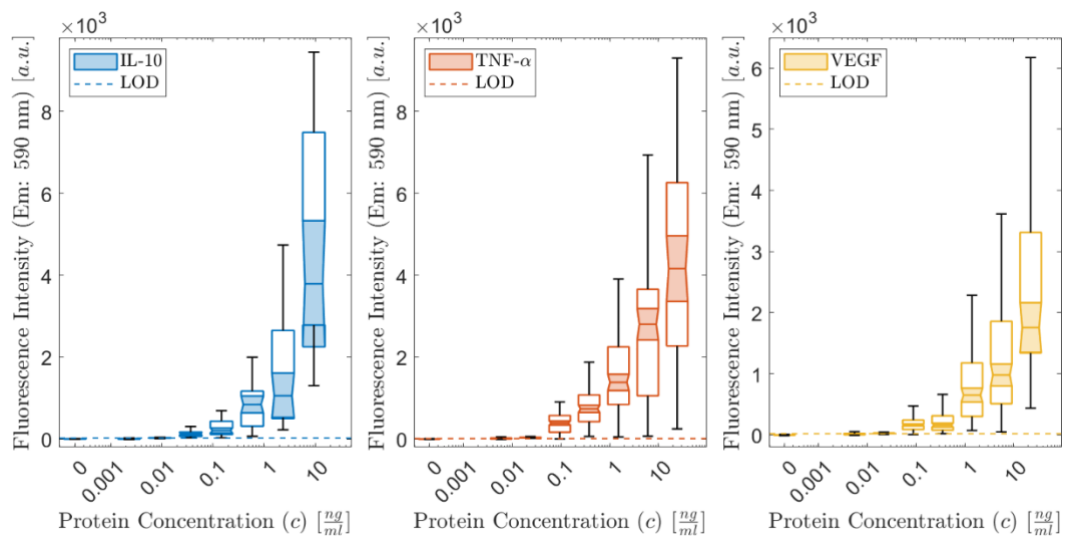


Figure S3. Calibration curves of the three target proteins measured in a volume of 50 μ l on a plate reader. The LODs of the proteins are 0.015 ng/ml, 0.01 ng/ml, and 0.015 ng/ml for IL-10, TNF- α , and VEGF, respectively.

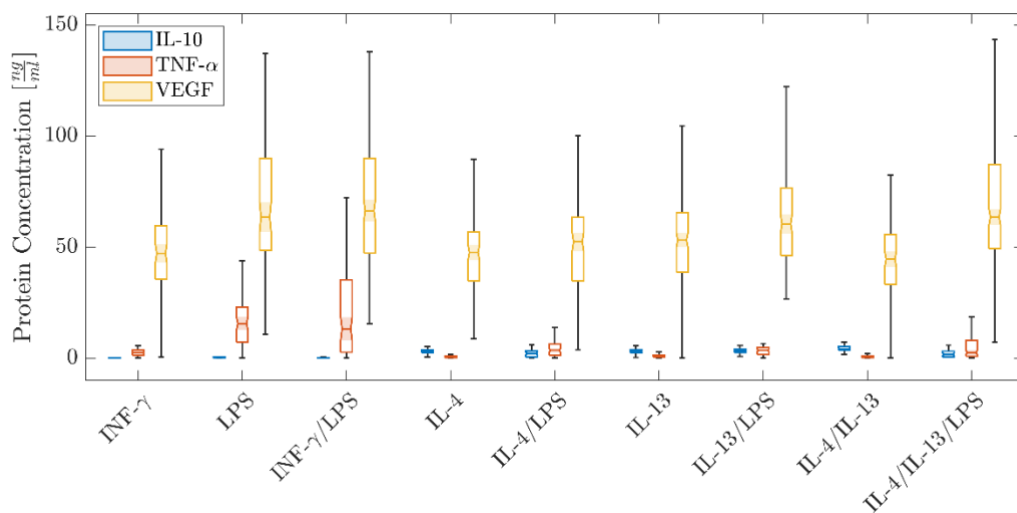


Figure S4. Secretion of IL-10, TNF- α and VEGF from macrophages polarized with different compounds according to the protocol in the main manuscript.

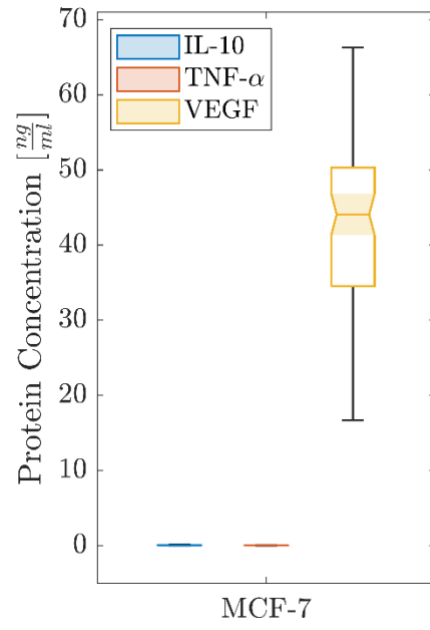


Figure S5. *Off-chip* secretome analysis of the breast cancer cell line MCF-7.

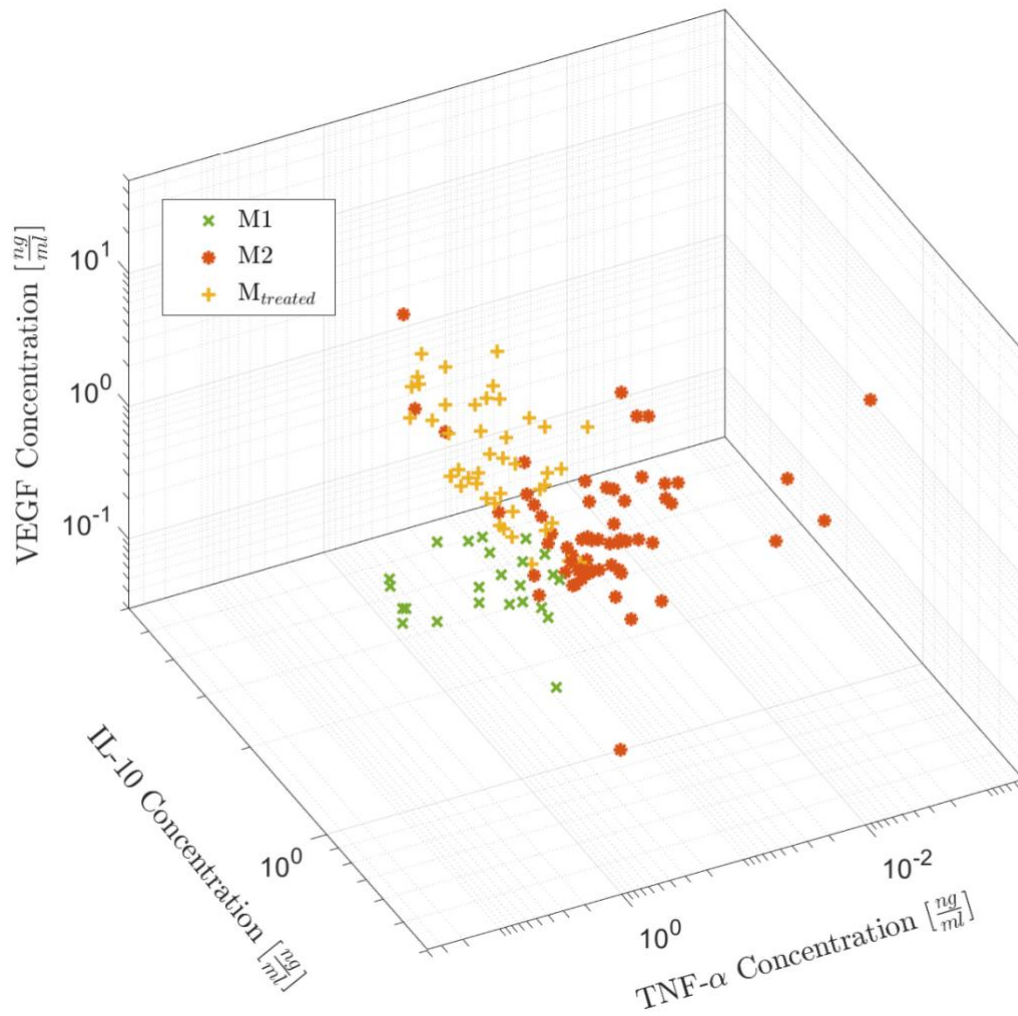


Figure S6. Single-cell secretion profile of polarized macrophages and macrophages treated with supernatant from cancer cells (M_{treated}). The secretion levels of macrophages are depicted for microchambers, where a single cell was co-encapsulated with all three types of beads. Green symbols: M1-polarized macrophages (n=24), red symbols: M2-polarized macrophages (n=68), yellow symbols: M_{treated} (n=45).

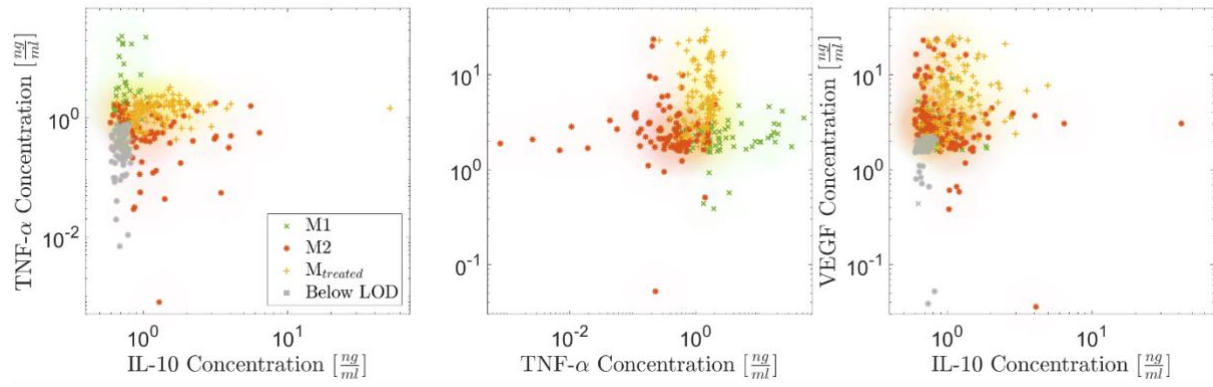


Figure S7. Multiplex expression of the proteins from single macrophages. The plotted data is the same data as in Figure 6c-e including all the outliers.