# Simultaneous stimulation of Peripheral Blood Mononuclear Cells with CpG ODN2006 and α-IgM antibody leads to strong immune responses in monocytes without affecting B cell activation
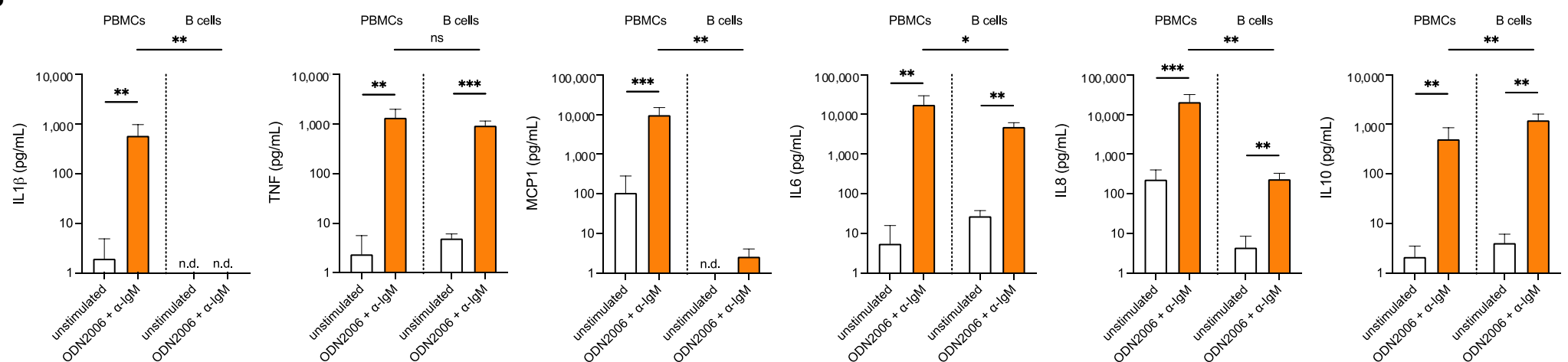
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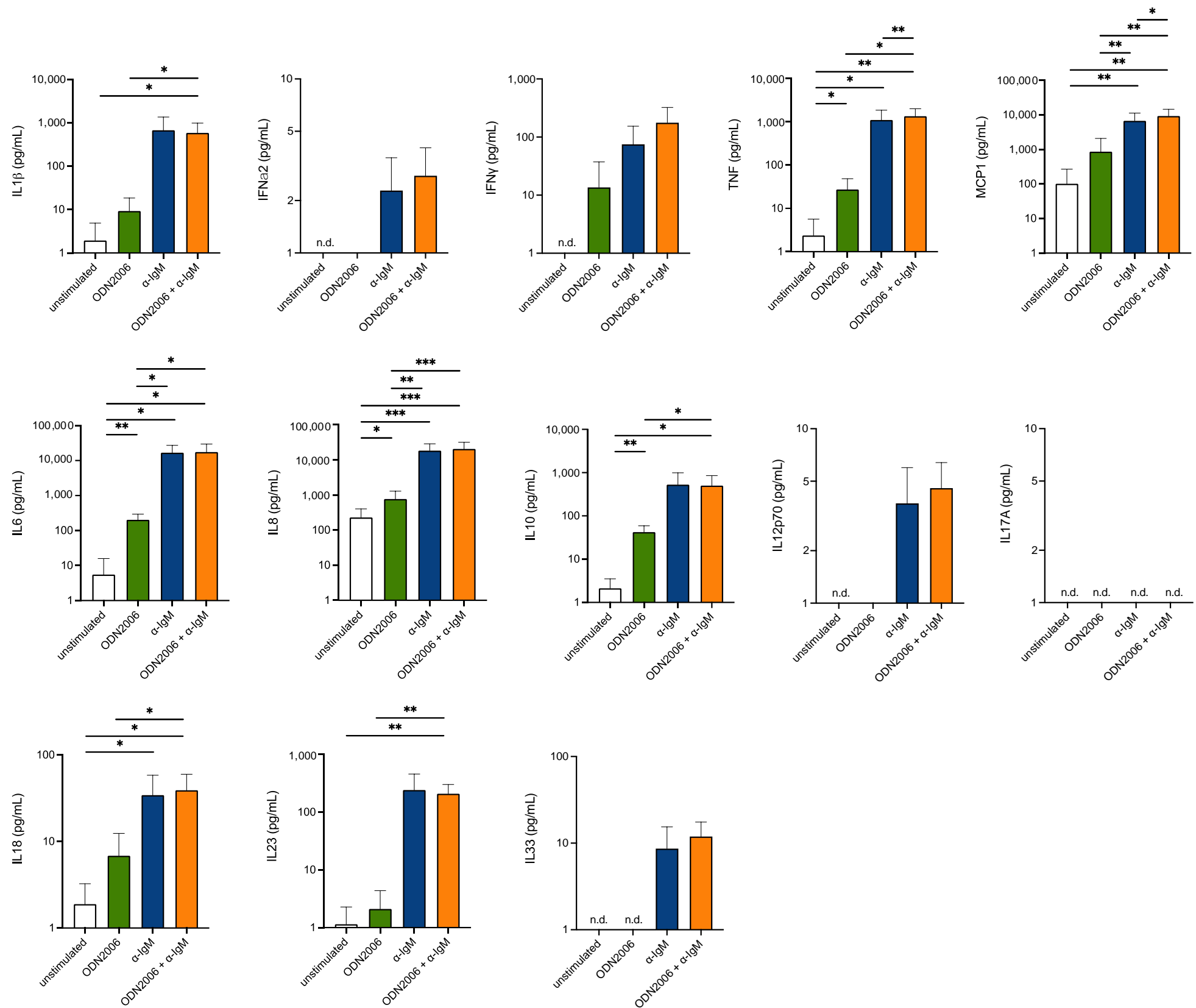
Supplementary Figure S1: **Stronger secretion of many cytokines after stimulation of mixed PBMCs with α-IgM compared to ODN2006. A**) Secreted IL1β, IFNα2, IFNγ, TNF, IL10, IL12p70, IL17A, IL18, IL23, IL33 (measured via Legendplex, n=6) and MCP1, IL6, IL8 (measured via ELISA, n=9-11) in supernatant of mixed PBMCs after ODN2006, α-IgM or ODN2006 + α-IgM stimulation for 24 h. Data already displayed in Figure 1 and B. **B**) Bar diagram of secreted IL1β, TNF and IL10 (measured via Legendplex, PBMCs n=7, B cells n=5) and MCP1, IL6, IL8 (measured via ELISA, PBMCs n=7, B cells n=5) in supernatant of mixed PBMCs vs isolated B cells after 24 h of stimulation with ODN2006 and α-IgM. Data already displayed in Figure 1B and D. N.d.: not detectable. No statistical test was performed for cytokines whose level was not detectable in unstimulated sample. One-Way ANOVA with Geisser-Greenhouse correction and Tukey's multiple comparisons test was used for analysing data including more than two stimulation conditions (A). Paired t test was used for the comparison of unstimulated vs. stimulated conditions within the same cell type (B). For comparing data from PBMCs with data from B cells, unpaired t test was applied, since different donors were used (B); *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001.
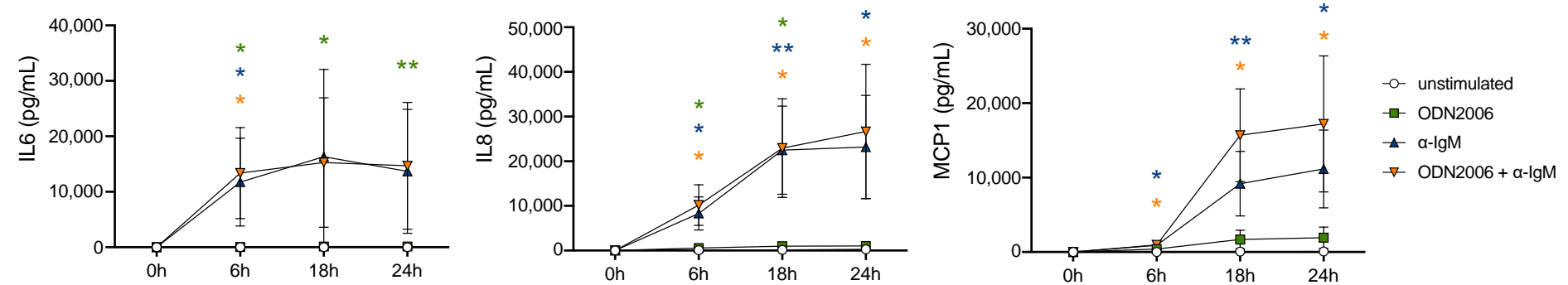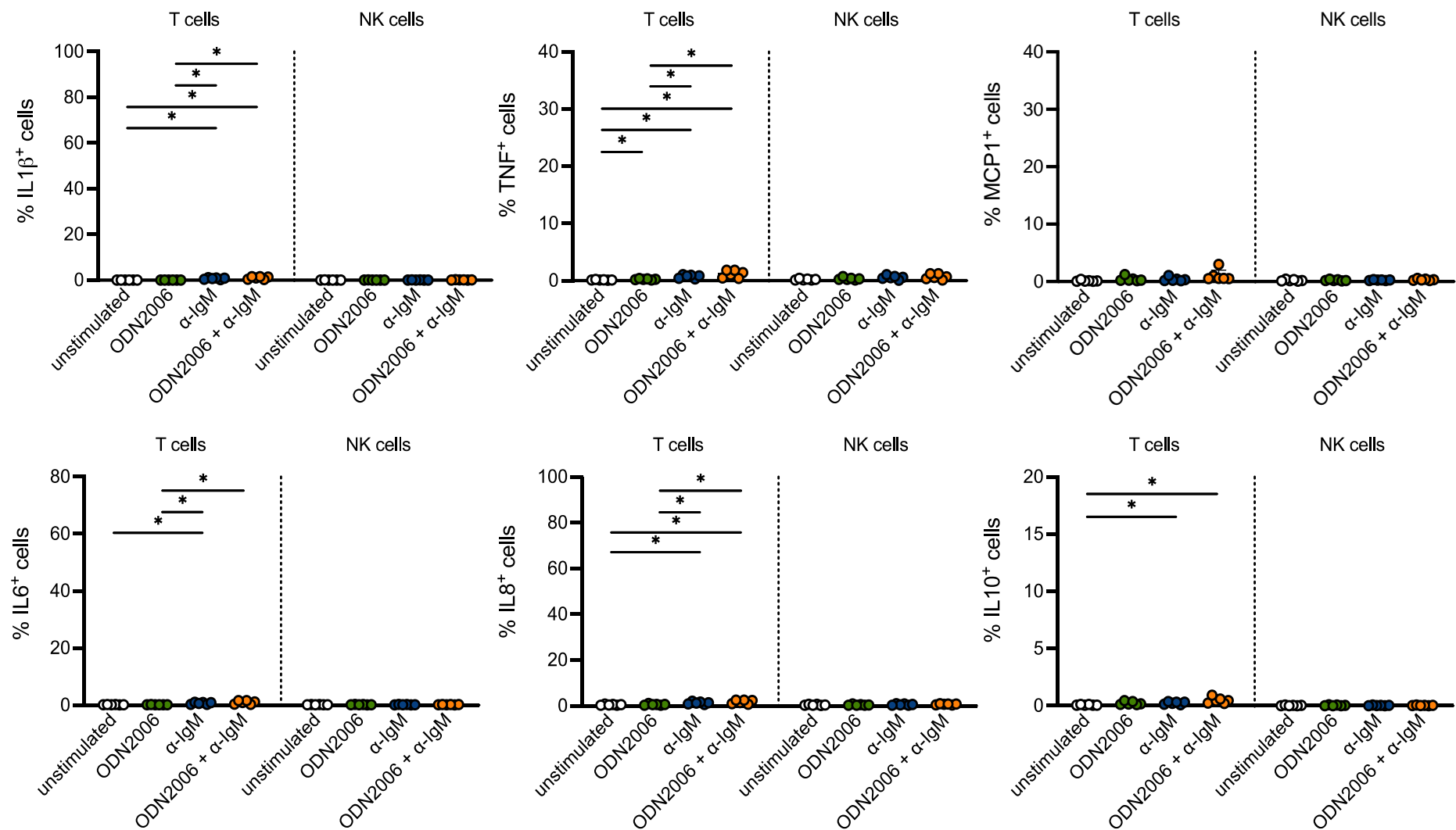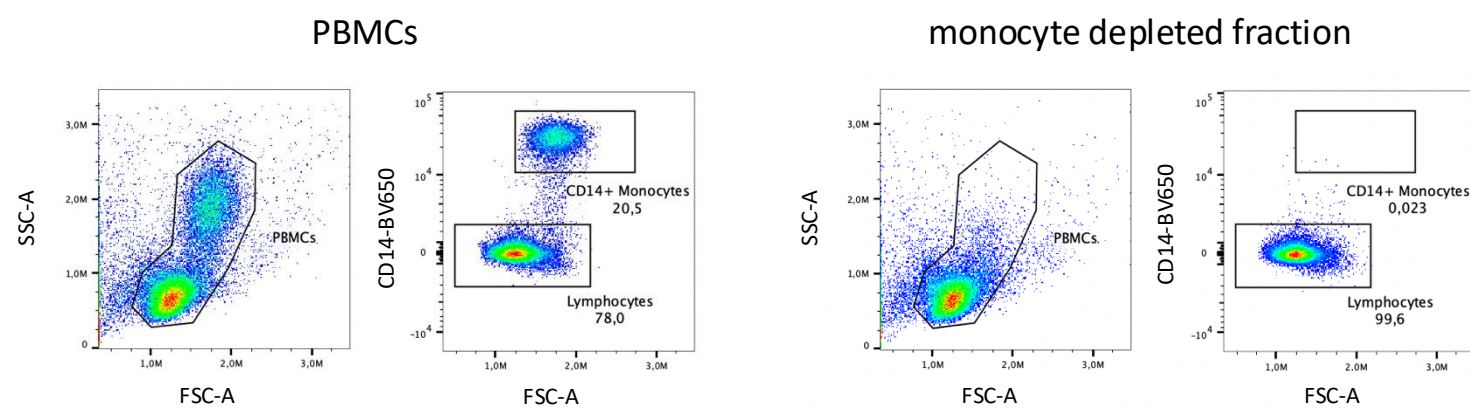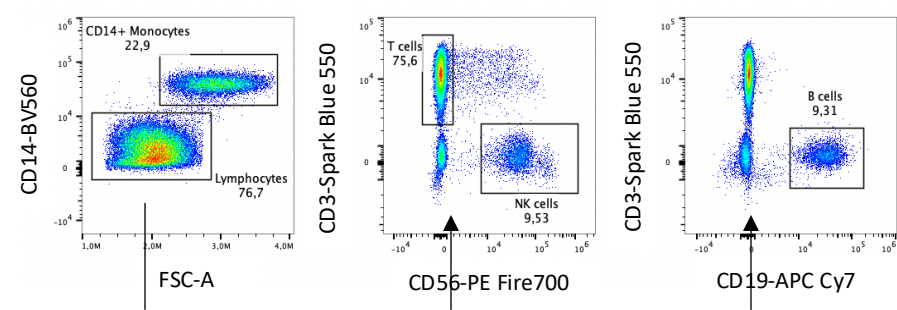
Supplementary Figure S2: Effects of ODN2006 and α-IgM on responses of monocytes mainly B cell independent. A) Time course measurement of secreted IL6, IL8, MCP1 in supernatant of mixed PBMC culture over 0, 6, 18 and 24 h via ELISA (n=6; statistics always refer to the unstimulated sample of the same timepoint). **B)** Intracellular cytokine staining for IL1β, TNF, MCP1, IL6, IL8 and IL10 in T cells and NK cells after ODN2006, α-IgM or ODN2006 + α-IgM stimulation for 6 h (IL1β, TNF, IL6, IL8) or 24 h (MCP1, IL10) via flow cytometry staining (n=6). **C)** Representative flow cytometry plots of mixed PBMCs and monocyte depleted fraction. Two-Way ANOVA with Geisser-Greenhouse correction and Tukey's multiple comparisons test was used for analysing data including more than two stimulation conditions and different time points (A). One-Way ANOVA with Geisser-Greenhouse correction and Tukey's multiple comparisons test was used for analysing data including more than two stimulation conditions but only one time point (B); *p ≤ 0.05, **p ≤ 0.01.
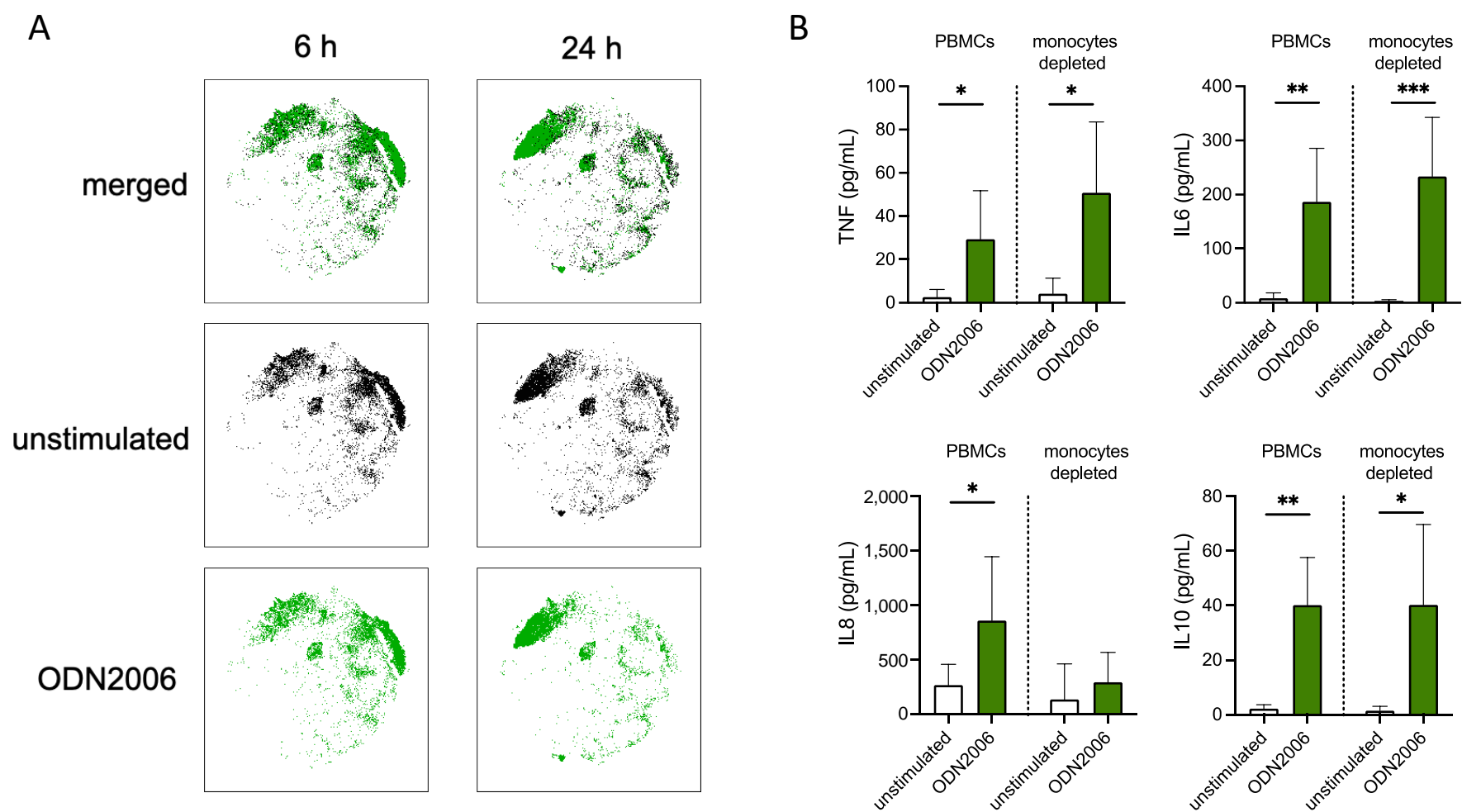
Supplementary Figure S3: ODN2006 did not influence the cytokine secretion of monocytes. A) t-SNE plots from samples stained for intracellular cytokines which were unstimulated (black) or stimulated with ODN2006 (green) for 6 h (left) and 24 h (right) after gating on CD14+ monocytes (n=6). **B**) Secreted TNF, IL6, IL8 and IL10 in supernatant of mixed PBMCs and monocyte depleted fraction after ODN2006 stimulation for 24 h measured with Legendplex for TNF and IL10 (n=6; statistical asterisks above bars refer to the unstimulated sample of the same cells) and ELISA for IL6 and IL8 (n=7-8; statistical asterisks above bars refer to the unstimulated sample of the same cells). Paired t test was used for the comparison of unstimulated vs. stimulated conditions within the same cell type as well as for comparing data from PBMCs with data from B cells (B); *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001.
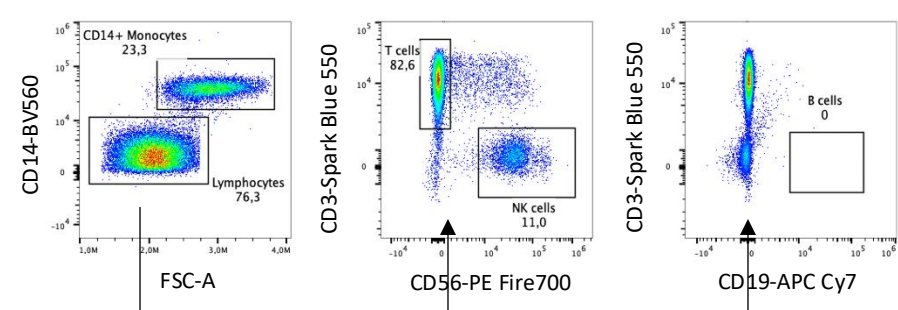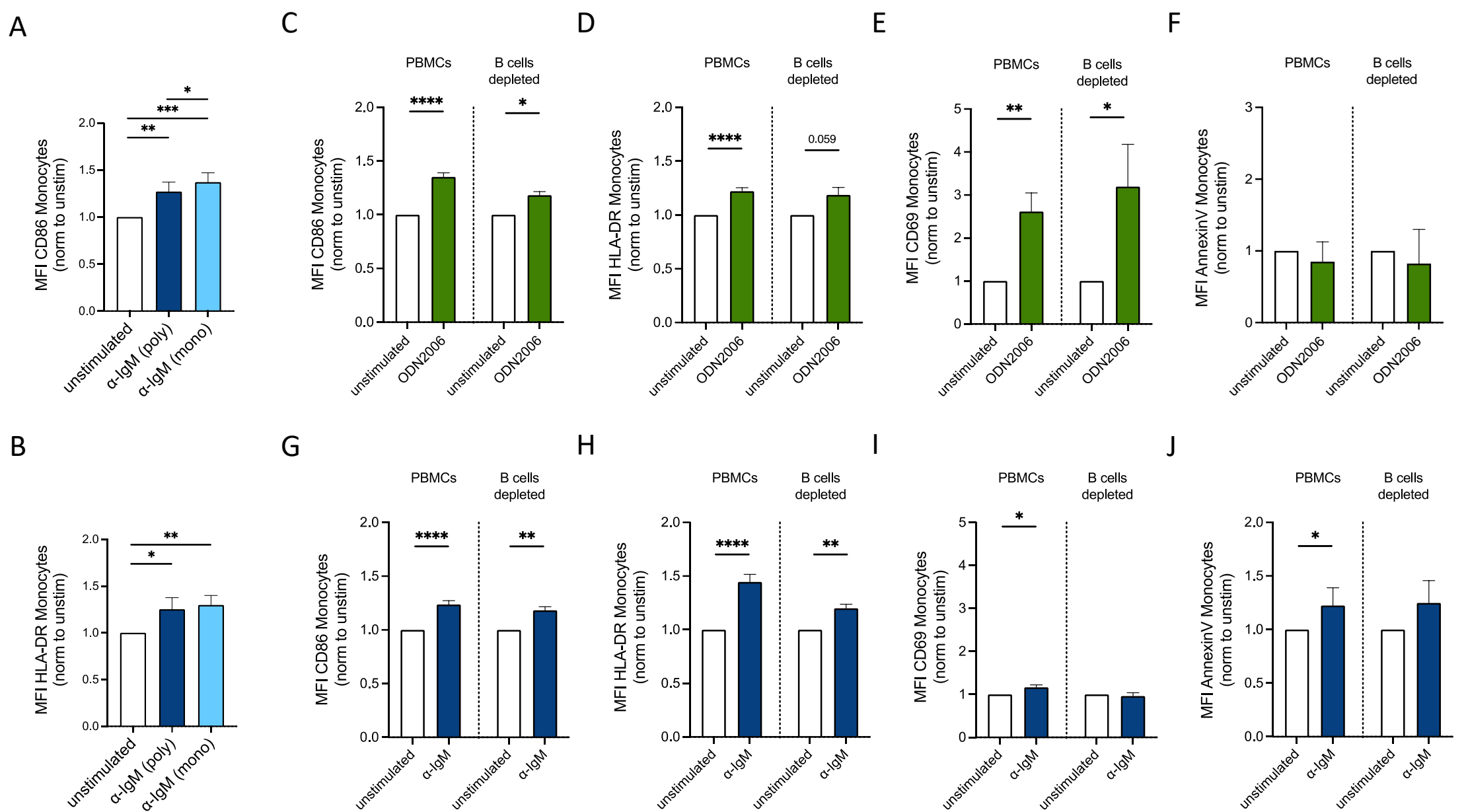
Supplementary Figure S4: Representative flow cytometry plots of mixed PBMCs (left) and B cell depleted fraction (right).

Supplementary Figure S5: ODN2006 and α-IgM affected activation marker expression on monocytes independently of B cells. **A-B)** Expression of CD86 (A) and HLA-DR (B) on monocytes after 6 h of stimulation with monoclonal and polyclonal α-IgM in culture of mixed PBMCs (n=6). **C-F)** Expression of CD86 (C), HLA-DR (D) and CD69 (E) on monocytes after 6 h and AnnexinV on monocytes after 24 h (F) of ODN2006 stimulation in culture of mixed PBMCs vs B cell depleted fraction (n=6-12). **G-J)** Expression of CD86 (G), HLA-DR (H) and CD69 (I) on monocytes after 6 h and AnnexinV on monocytes after 24 h (J) of α-IgM stimulation in culture of mixed PBMCs vs B cell depleted fraction (n=6-12). One-Way ANOVA with Geisser-Greenhouse correction and Tukey's multiple comparisons test was used for analysing data including more than two stimulation conditions (A-B). Paired t test was used to compare two conditions (C-J); *p ≤ 0.05, **p ≤ 0.01, ***p ≤ 0.001, ****p ≤ 0.0001.