

Figure S1. Gating strategy performed for flow cytometric analysis of T lymphocytes (CD3+ cells) and NKT-like cells (CD3+CD56+ cells) from whole blood samples. **(a)** Lymphocyte gating – lymphocytes were defined as FSC_{low}/SSC_{low} cells (G1). **(b)** T and NKT-like cell gating – T lymphocytes were defined as CD3+ positive population (G2), and NKT-like cells were defined as CD3+CD56+ positive cells (G3). **(c)** Isotype control for TNF-α positive T cells. **(d)** T cells expressing TNF-α were identified in the upper left quadrant. **(e)** TNF-α expression (red line) vs isotype control (blue line) presented in T cells. **(f)** Isotype control for TNF-α -positive NKT-like cells. **(g)** NKT-like cells expressing TNF-α were identified in the upper right quadrant. **(h)** TNF-α expression (red line) vs isotype control (blue line) presented in NKT-like cells. The analysis of IL-6 and IFN-γ expression was performed in the same way. All age groups were analyzed with the same gating strategy.

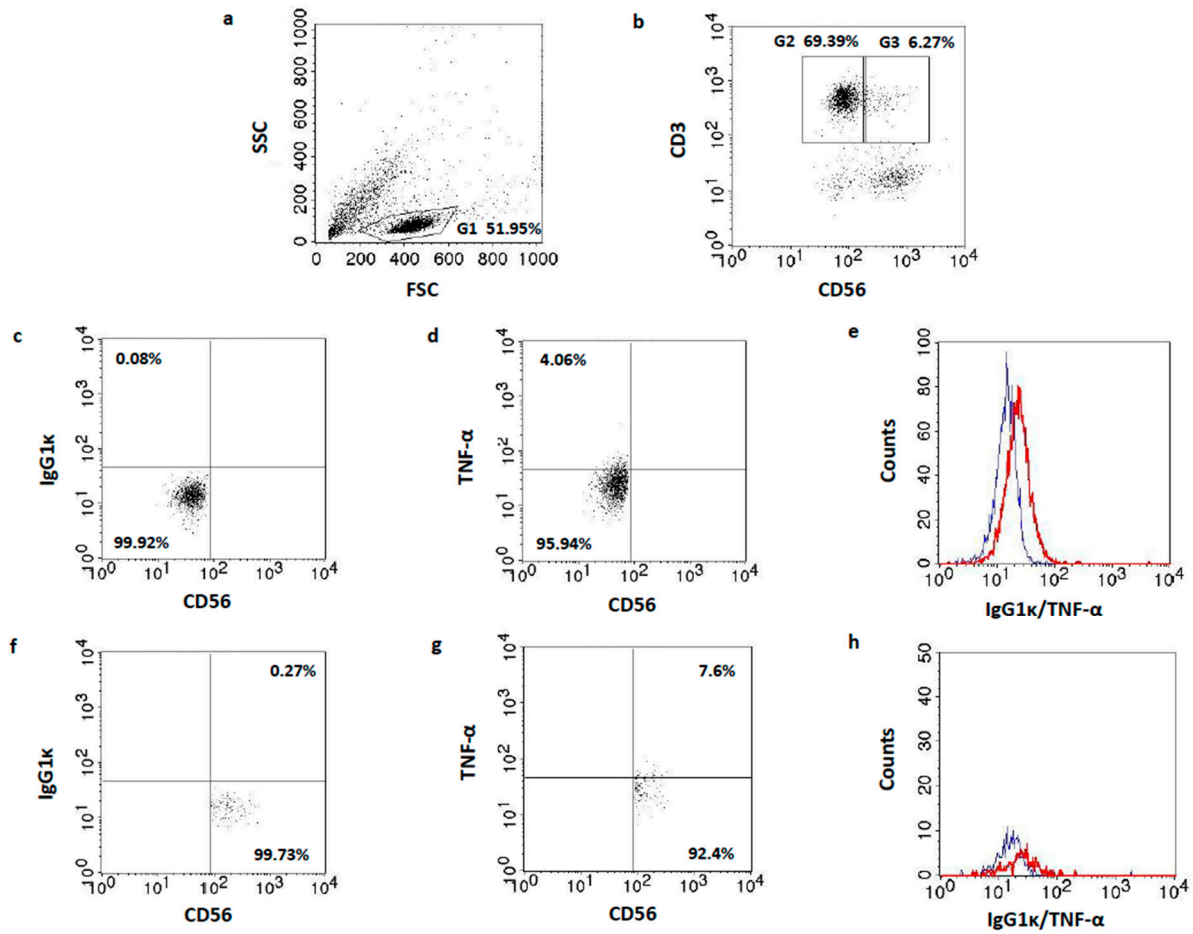


Figure S2. Gating strategy performed for flow cytometric analysis of T lymphocytes (CD3+ cells) and NKT-like cells (CD3+CD56+ cells) from PBMC cultures. **(a)** Lymphocyte gating in the population of PBMCs – lymphocytes were defined as FSC_{low}/SSC_{low} cells (G1). **(b)** T and NKT-like cell gating – T lymphocytes were defined as CD3+ positive population (G2), and NKT-like cells were defined as CD3+CD56+ positive cells (G3). **(c)** Isotype control for TNF- α -positive T cells. **(d)** T cells expressing TNF- α were identified in the upper left quadrant. **(e)** TNF- α expression (red line) vs isotype control (blue line) presented in T cells. **(f)** Isotype control for TNF- α -positive NKT-like cells. **(g)** NKT-like cells expressing TNF- α were identified in the upper right quadrant. **(h)** TNF- α expression (red line) vs isotype control (blue line) presented in NKT-like cells. The analysis of IL-6 and IFN- γ expression was performed in the same way. All age groups were analyzed with the same gating strategy.

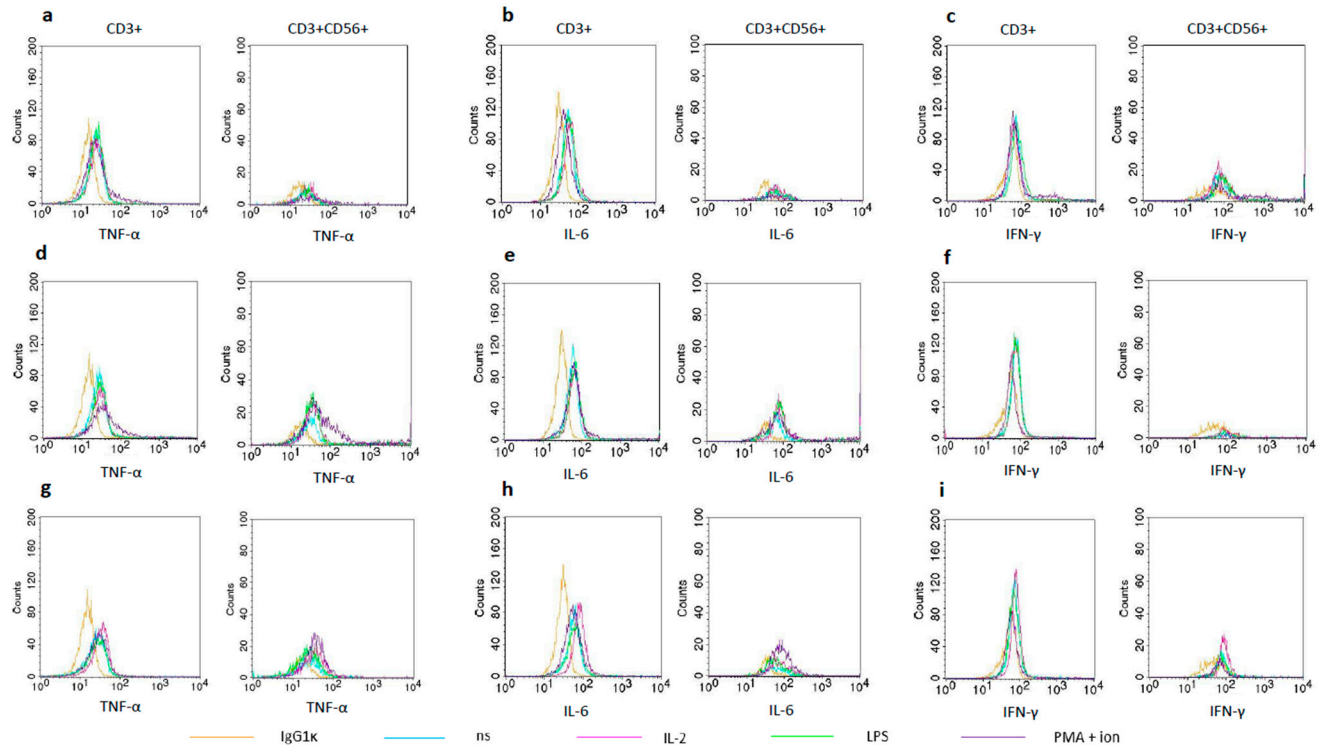


Figure S3. FACS histogram overlay plots for TNF- α , IL-6 and IFN- γ expression in CD3+ and CD3+CD56+ cells presented in the representative samples of the young, old and the oldest group. Each plot shows overlay graphs depicting isotype control (IgG1k – orange line), unstimulated cells (ns – light blue line), cells stimulated with IL-2 (IL-2 – pink line), cells stimulated with LPS (LPS – light green line) and cells treated with PMA and ionomycin (PMA + ion – dark purple line). (a) TNF- α expression in CD3+ cells (left) and CD3+CD56+ cells (right) of the young. (b) IL-6 expression in CD3+ cells (left) and CD3+CD56+ cells (right) of the young. (c) IFN- γ expression in CD3+ cells (left) and CD3+CD56+ cells (right) of the young. (d) TNF- α expression in CD3+ cells (left) and CD3+CD56+ cells (right) of the old. (e) IL-6 expression in CD3+ cells (left) and CD3+CD56+ cells (right) of the old. (f) IFN- γ expression in CD3+ cells (left) and CD3+CD56+ cells (right) of the old. (g) TNF- α expression in CD3+ cells (left) and CD3+CD56+ cells (right) of the oldest. (h) IL-6 expression in CD3+ cells (left) and CD3+CD56+ cells (right) of the oldest. (i) IFN- γ expression in CD3+ cells (left) and CD3+CD56+ cells (right) of the oldest.

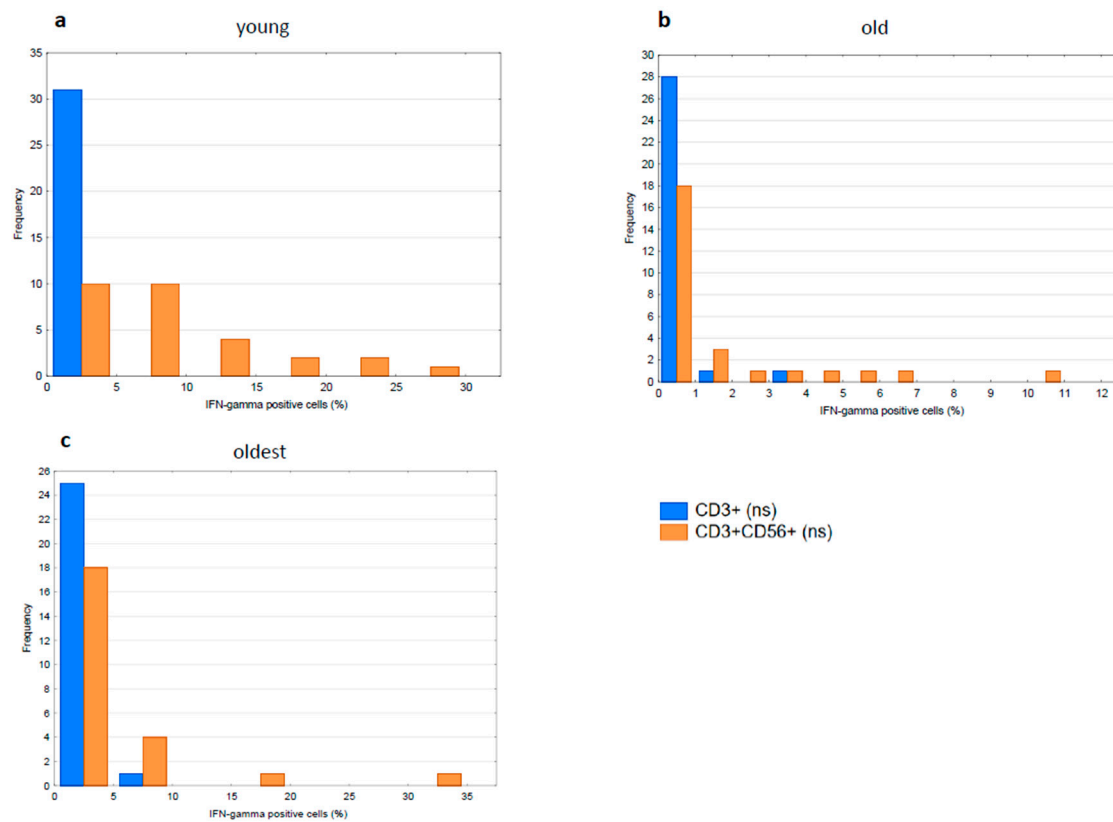


Figure S4. Frequency of samples differing in IFN- γ expression in the population of unstimulated (ns) CD3+ cells (blue color) and CD3+CD56+ cells (orange color) analysed in the group of the young (a), old (b) and the oldest (c).

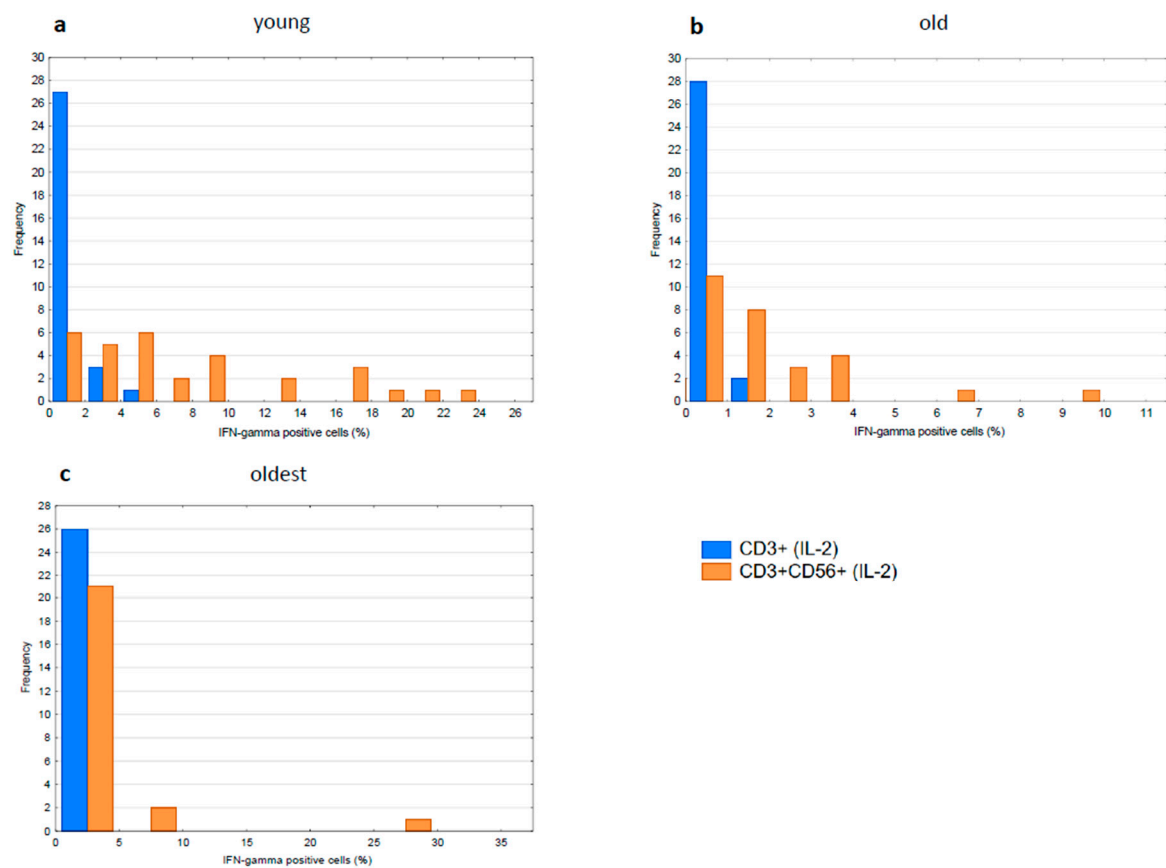


Figure S5. Frequency of samples differing in IFN- γ expression in the population of CD3+ cells (blue color) and CD3+CD56+ cells (orange color) stimulated with IL-2 analysed in the group of the young (a), old (b) and the oldest (c).

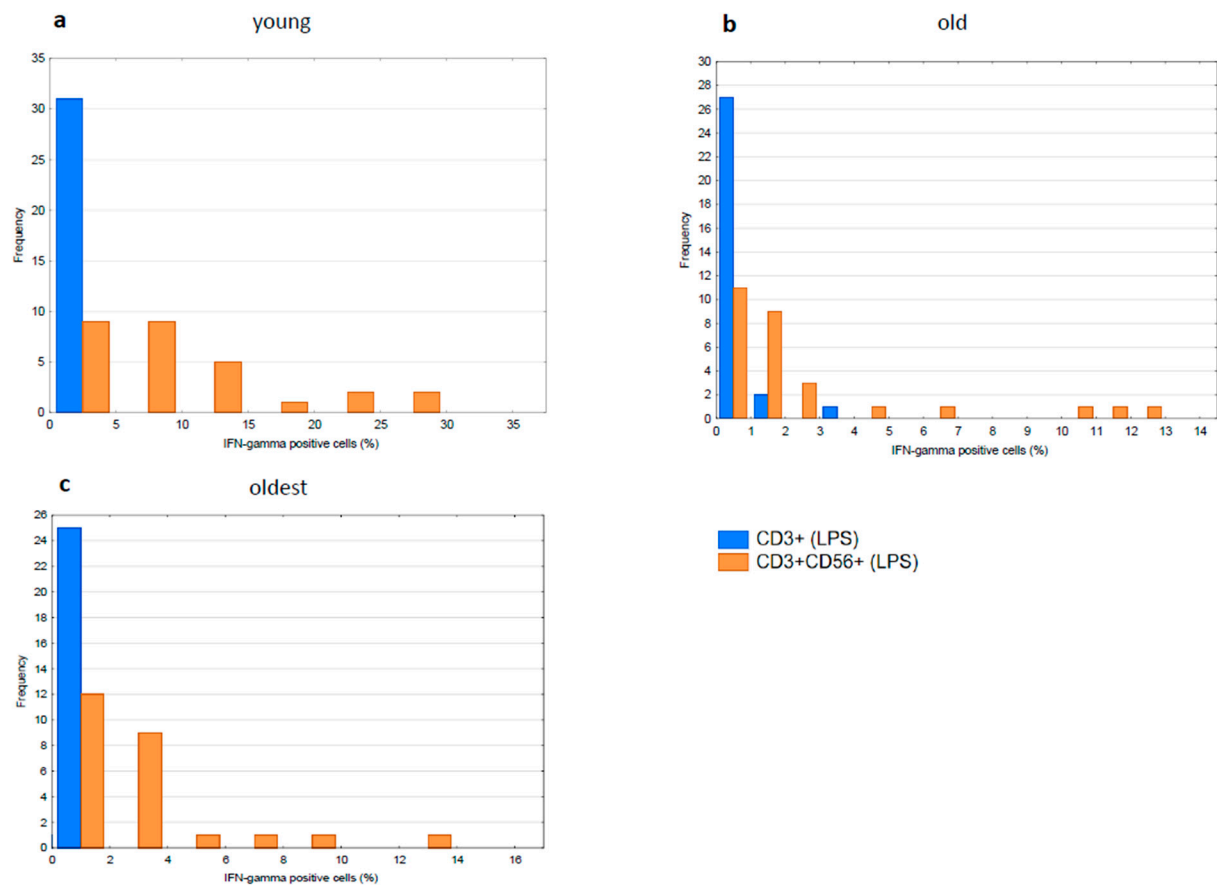


Figure S6. Frequency of samples differing in IFN- γ expression in the population of CD3+ cells (blue color) and CD3+CD56+ cells (orange color) stimulated with LPS analysed in the group of the young (a), old (b) and the oldest (c).

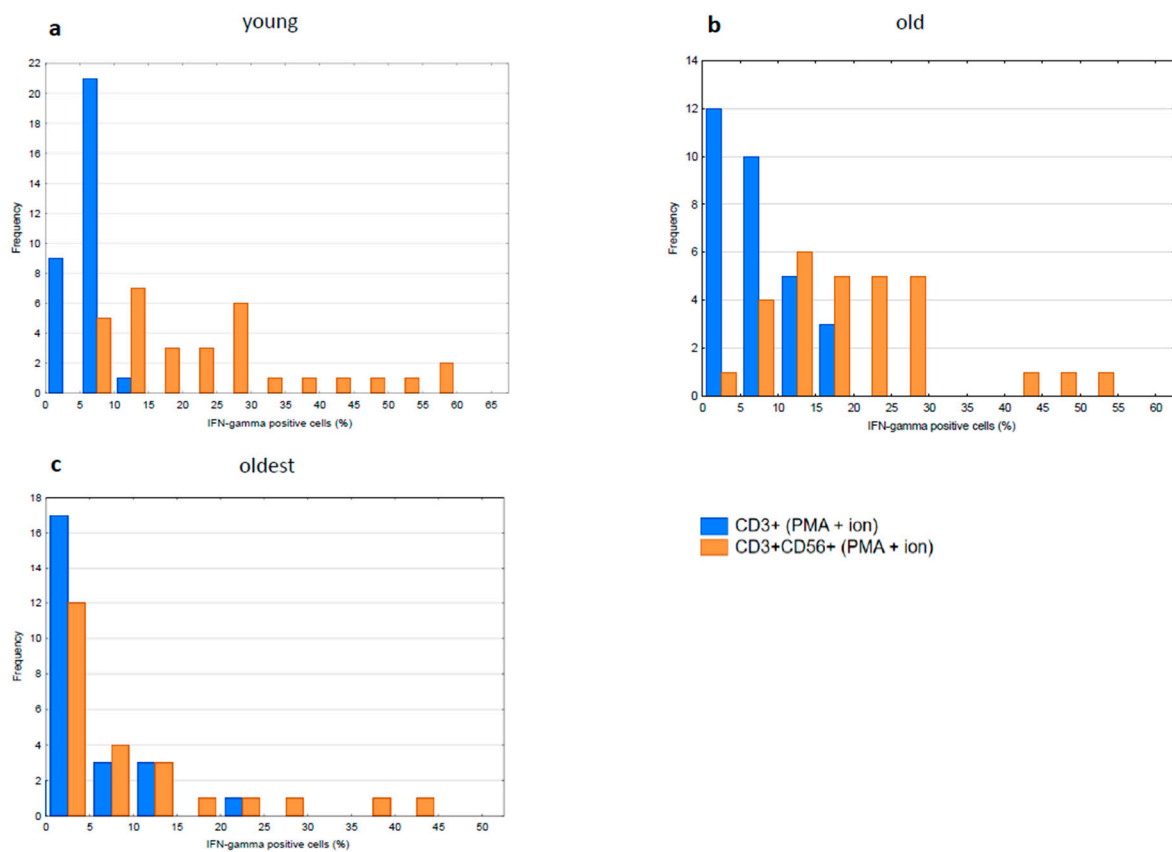


Figure S7. Frequency of samples differing in IFN- γ expression in the population of CD3+ cells (blue color) and CD3+CD56+ cells (orange color) stimulated with PMA and ionomycin analysed in the group of the young (a), old (b) and the oldest (c).