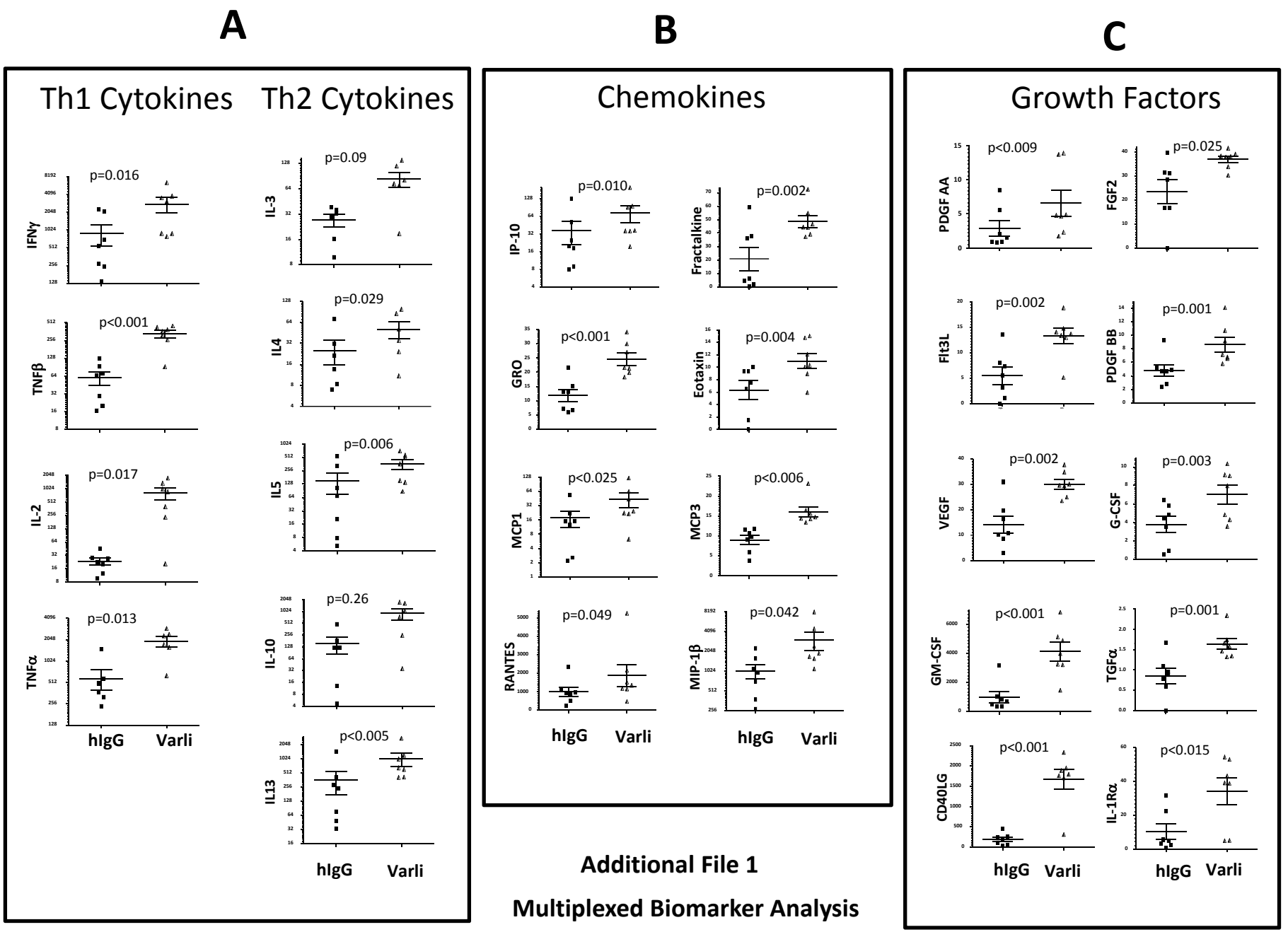


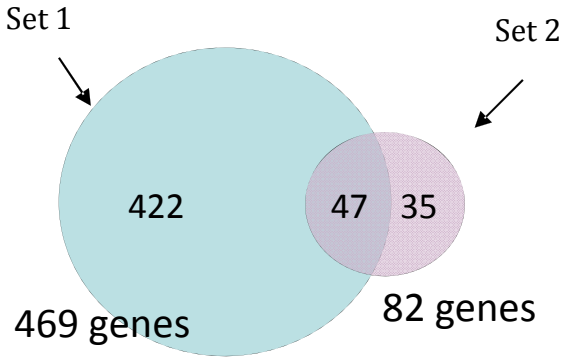
Additional File 1

Multiplexed Biomarker Analysis

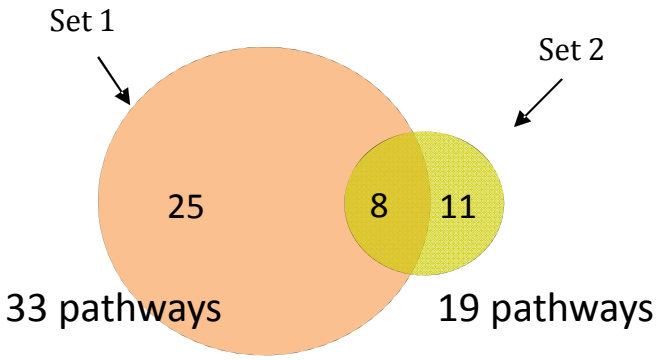
T cells from seven normal donors were isolated and stimulated with varlilumab (filled triangles) or a control human IgG (filled squares) in the presence of OKT3 in independent cultures for 3 days as described under Methods. Cell culture supernatants were harvested and subjected to a 41-plex panel of cytokines (A), chemokines (B) and growth factor (C) analysis. Analyte levels on y-axis are pg/ml.

Additional File 1

A. Unique & Overlapping Genes



B. Unique & Common Pathways (KEGG)



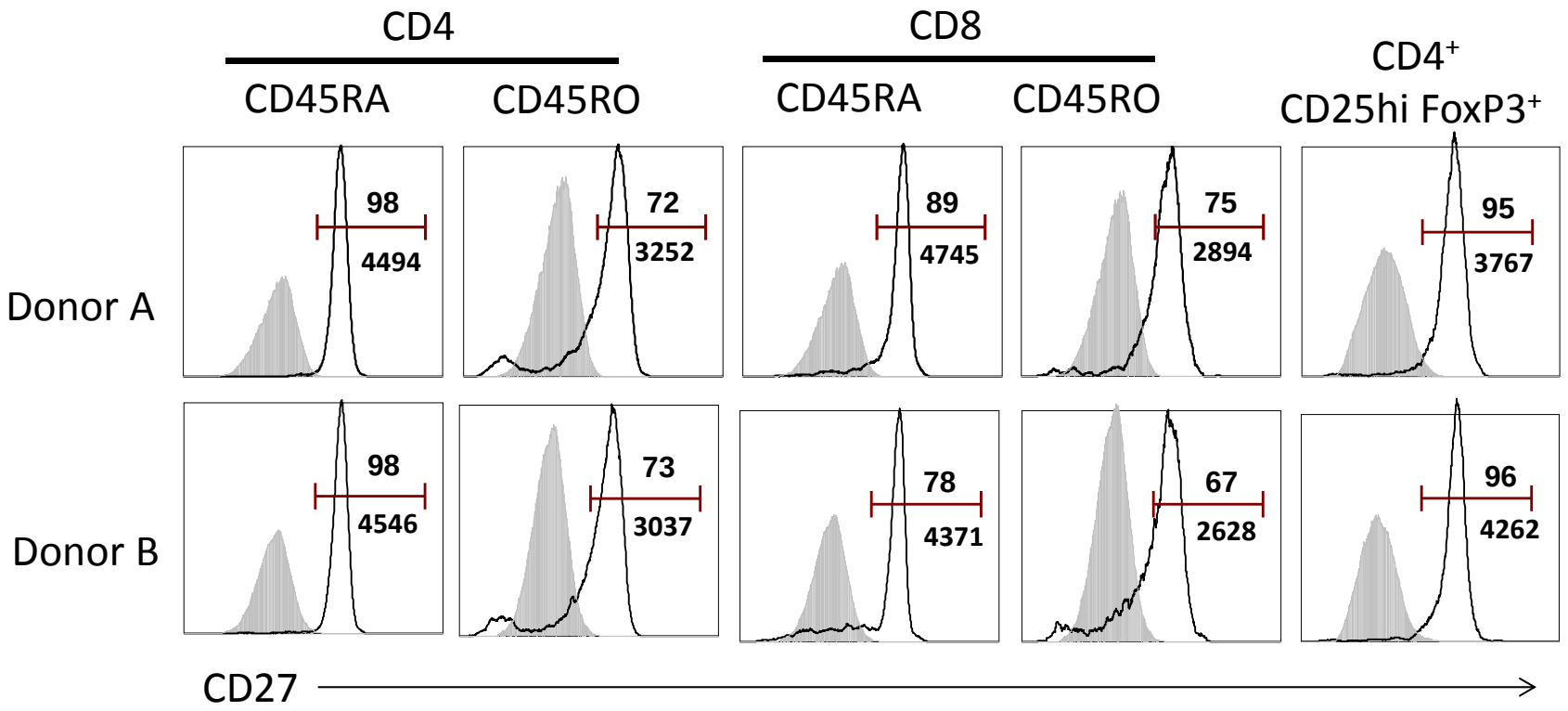
Venn charts summarizing congruence analysis of isolated T cells stimulated with varlilumab using two separate stimulation protocols as described under Methods. Set 1 or standard 72 hour stimulation with OKT3+ varlilumab or OKT3+ Isotype IgG control Ab (n=3); Set 2 or alternate method of 46 hour preactivation step with OKT3 only followed by a brief combined 4 hour stimulation with OKT3+ varlilumab or OKT3+ Isotype IgG control Ab (n=4). The analysis shows 47 genes affected similarly (A) with 8 overlapping KEGG pathways (B) with either stimulation methods. See Tables 1a-c for additional information.

Additional File 2

Genes & Pathways of Varlilumab Stimulation

Additional File 3

Baseline CD27 expression level in T cell subsets



Pan T cells were isolated from normal donor PBMCs using Miltenyi magnetic bead isolation kit and stained for naïve, memory and Treg phenotypic markers. Cells were stained for subset-specific markers and CD27. Shaded histograms are stained with isotype-matched IgG controls. Numbers above the bar denote percentage of cells positive for CD27; numbers below refer to MFI values.