A

Th1 Cytokines

- IFNγ
  - p=0.016
- TNFβ
  - p<0.001
- IL-2
  - p=0.017
- TNFα
  - p=0.013
- IL-10
  - p=0.006
- IL-13
  - p=0.005

Th2 Cytokines

- IL-3
  - p=0.09
- IL-4
  - p=0.029
- IL-5
  - p=0.26
- IL-10
  - p=0.005

B

Chemokines

- IP-10
  - p=0.010
- Fractalkine
  - p=0.002
- GRO
  - p<0.001
- Eotaxin
  - p=0.004
- MCP1
  - p<0.006
- MIP-1β
  - p=0.042
- RANTES
  - p<0.001
- MCP3
  - p=0.049
- VEGF
  - p=0.002
- G-CSF
  - p=0.003
- GM-CSF
  - p=0.001
- IL-10
  - p<0.015
- CD4+IL-4
  - p<0.001
- IL-1β
  - p<0.009

C

Growth Factors

- PDGF AA
  - p=0.001
- PDGF BB
  - p=0.025
- FGFR2
  - p=0.002
- Flt3L
  - hIgG
- Varli
- hIgG

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.
Venn charts summarizing congruence analysis of isolated T cells stimulated with varilumab using two separate stimulation protocols as described under Methods. Set 1 or standard 72 hour stimulation with OKT3+ varilumab 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+ varilumab 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 Varilumab Stimulation
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.