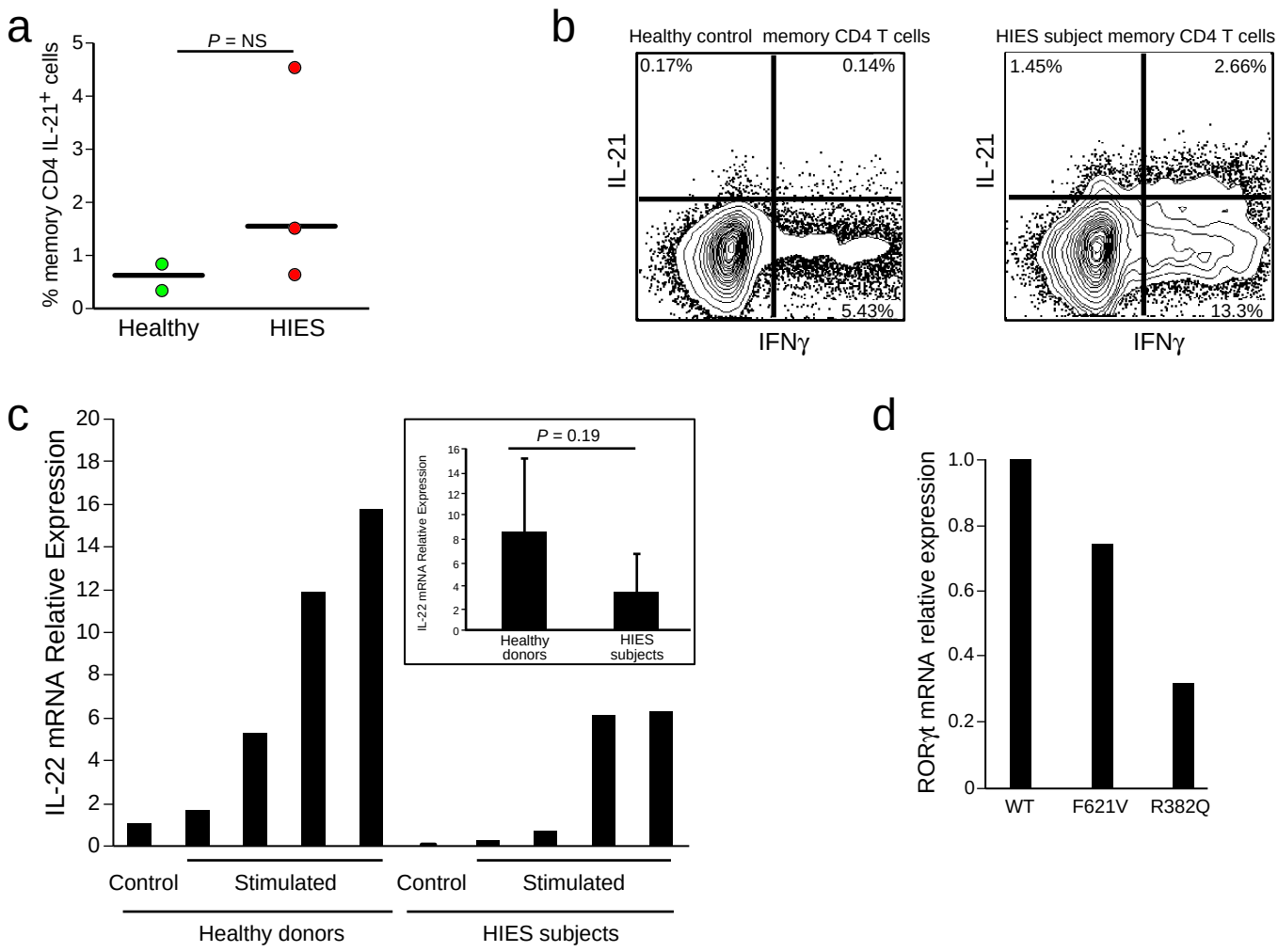
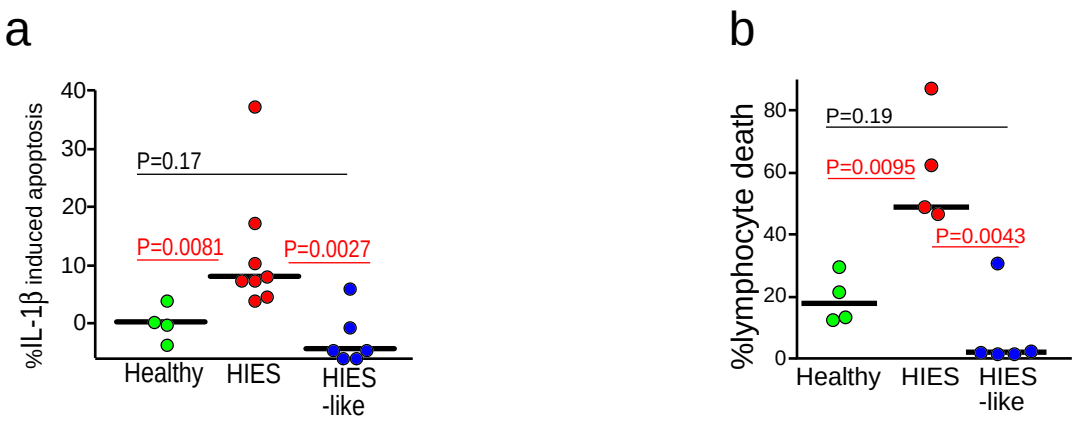
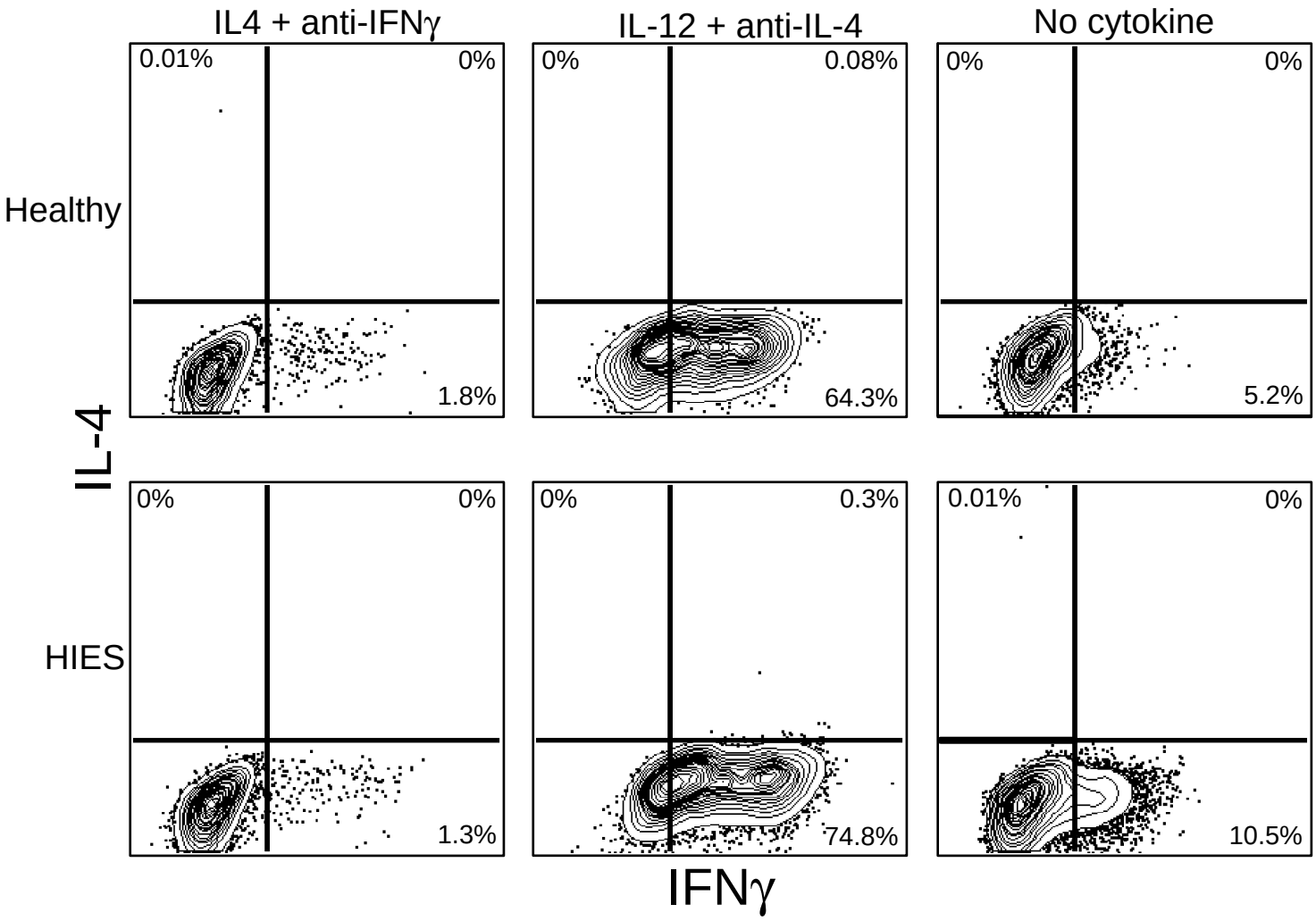


Supplementary Figure 1. IL-21, IL-22 and RORγt production in stimulated PBMC from healthy and HIES subjects. (**a**) PBMC from HIES subjects with *stat*3 mutations (red circles) and healthy individuals (green circles) were stimulated with SEB overnight in the presence of brefeldin A and stained as described in the methods. The frequency of memory CD4 T-cells (gated as described in the methods) that produced IL-21 was then assessed. (**b**) The ability of IL-21 producing cells to co-produce IFNγ was determined in an individual with HIES and a healthy individual. (**c**) Naive CD4 T cells from four healthy donors and four HIES subjects were freshly lysed or stimulated with anti-CD3 and anti-CD28 in the presence of IL-23 for 48 hours as described in the methods. IL-22 and HPRT mRNA expression was detected by quantitative RT-PCR using TaqMan primers and probes purchased from Applied Biosystems. All samples were analyzed in triplicate using the ABI Prism 7500 Fast Real-Time PCR System (Applied Biosystems). Relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method, with expression levels in unstimulated healthy patient cells assigned an arbitrary value of 1. Values shown are mean relative expression levels of triplicate samples. Inset: Average expression levels for the four healthy donors compared to the four HIES subjects. Statistical significance was determined by two-tailed unpaired Student t-test. (**d**) Naïve CD4 T cells were purified by magnetic cell sorting from PBMC from healthy individuals by negative selection using a human naive T cell isolation kit (Miltenyi Biotec). Cells were rested for 12 hours and then five million cells were co-transfected with 2μg of either wild type (WT) or mutant *stat3* and 1μg of pmaxEGFP plasmids using Amaxa Human T cell Nucleofection kit per the manufacturer's instructions (Amaxa GmbH, Cologne Germany). The DNA binding R382Q and SH2 domain F621V *stat3* mutations were tested. Cells were rested in R10 for 8 hours and sorted by flow cytometry for live cells expressing high levels of GFP. Cells were then stimulated with anti-CD3, anti-CD28 and IL-23 for 48 hours, and qRT-PCR for RORγt and HPRT was performed as described in the Methods section. The graph shows RORγt mRNA expression levels of triplicate samples in the mutant gene transfections relative to the wild type gene transfection. Similar results were obtained with PBMC from a second individual.

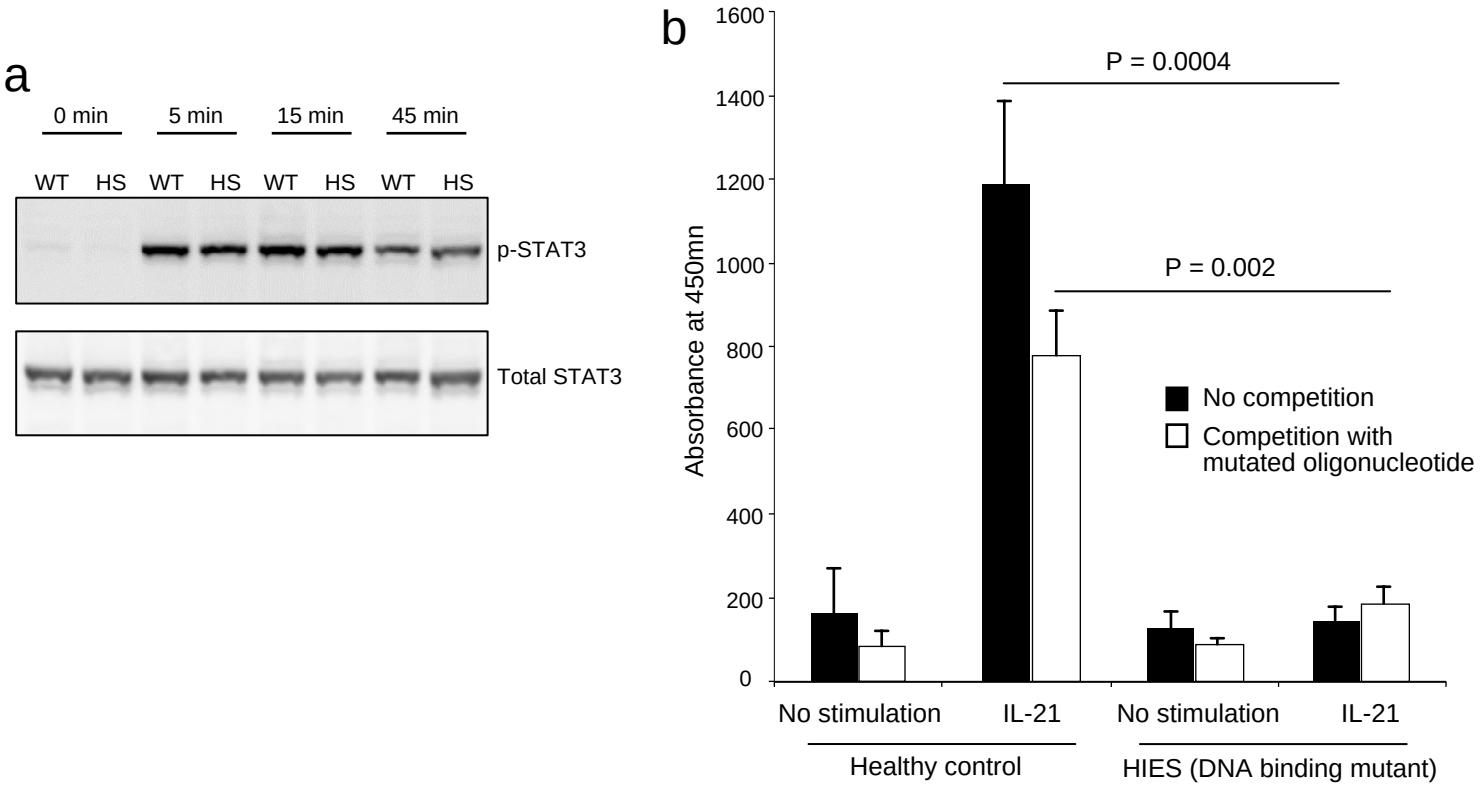


Supplementary Figure 2. Increased T cell apoptosis in HIES subjects compared to healthy donors.

(**a**) PBMC were isolated and placed in R10 overnight in the presence of 20ng/ml IL-1β, or without the addition of any cytokines. Cells were stained for surface markers and with 7-AAD, washed, and then incubated for 15 minutes at 37^{0}C in the presence of 40nM DiOC6 (Invitrogen). The percentage of CD4 T-cell IL-1β-induced apoptosis in PBMC from HIES patients (red circles), healthy controls (green circles) and HIES-like patients (blue circles) was assessed by characteristic staining patterns of DiOC6 and 7-AAD after background subtraction. Statistical significance was determined by the Mann Whitney test. Significant P-values are shown in red and median values with horizontal bars. (**b**) Cell death after overnight stimulation with *S. Aureus* was assessed by positive staining for Vivid as in the methods. Statistical significance was determined by the Mann Whitney test. Significant P-values are shown in red and median values with horizontal bars.



Supplementary Figure 3. Th1/Th2 differentiation of naive T cells from HIES patients is comparable to that in healthy controls. 50,000 $CD27^{+}CD45RO^{-}CD11a^{lo}CD31^{+}$ naive CD4 T cells were flow cytometrically sorted from HIES patients and healthy controls and were stimulated with 25,000 Miltenyi CD2/CD3/CD28 beads in the presence of the indicated cytokines and antibodies for 5 days. Cultures were then restimulated with PMA and ionomycin in the presence of brefeldin A for 4 hours, fixed, permeabilized and stained for intracellular cytokines.



Supplementary Figure 4. Lack of STAT3 DNA binding in HIES subjects.

(**a**) T cells from a DNA-binding mutant HIES subject (HS) and a healthy control (WT) were polyclonally stimulated and expanded in IL-2-containing medium, and then rested in medium without cytokine for 6 hours followed by a 5 – 45 minute stimulation with IL-21 at 100 ng/mL. Cells were subsequently lysed and immunoblotted for the presence of phospho-tyrosine STAT3 and total STAT3. (**b**) T cells from a DNA-binding mutant HIES patient and a healthy control were polyclonally stimulated and expanded in IL-2-containing medium, and rested then in medium without cytokine for 6 hours followed by a 15 minute stimulation with IL-21 at 100 ng/mL. The ability for STAT3 within the whole cell lysate to bind to a candidate oligonucleotide was assayed by EMSA using the TramsAM STAT3 assay kit (Active Motif Carlsbad, CA) according to manufacturers' instructions. Briefly, 2mg of whole cell lysate from both stimulated and unstimulated cells was assayed in the presence or absence of a competitor oligonucleotide or a mutated oligonucleotide. Histograms demonstrate the absorbance at 450nm in excess of the value seen in the presence of the wild type oligonucleotide (used as background). Black bars represent the absence and white bars the presence of the mutated oligonucleotide. P-values were determined using an unpaired t-test.