Supplemental figure 1

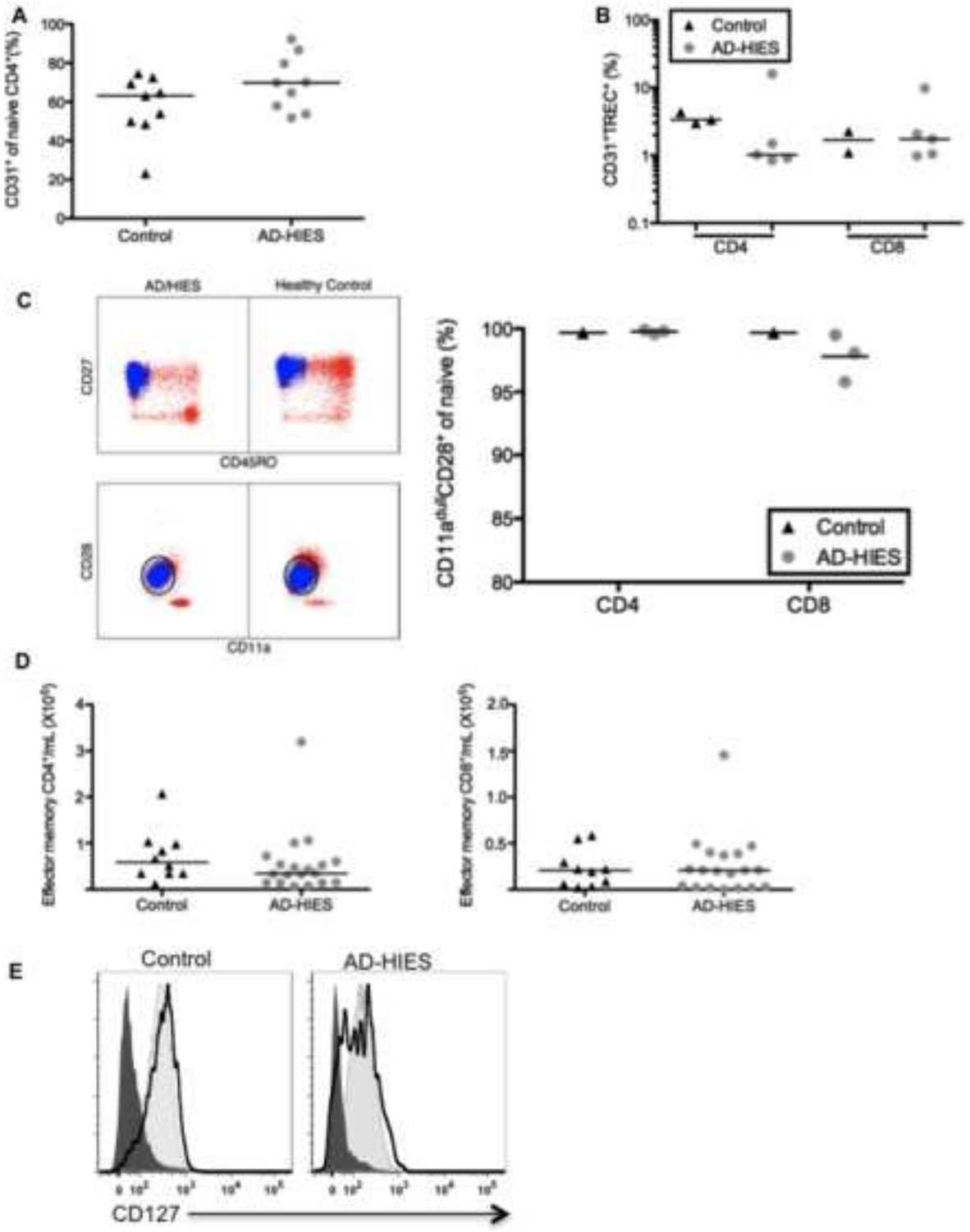

Figure S1. CD27⁺CD45RO⁻ T cells in AD-HIES patients are phenotypically naïve. A. Percentage of recent thymic emigrants (CD31⁺CD27⁺CD45RO⁻) to the CD4 compartment in AD-HIES patients and age-matched controls. Difference is not statistically significant. B. TCR rearrangement excision circles (TRECs) in sorted CD31⁺CD27⁺CD45RO⁻ CD4⁺ and CD8⁺ T cells from AD-HIES patients and controls. Differences are not statistically significant. C. CD28 and CD11a staining of CD27⁺CD45RO⁻(blue) and total CD4⁺ T cells (red) in an AD-HIES patient and control. Percent $CD11a^{dull}CD28^{+}$ cells (encircled) within in the CD27⁺CD45RO⁻(naïve, in blue) are quantified in three AD-HIES patients and controls. D. A. The absolute number of effector (CD27⁻ $CD45RO^{+/-}$) memory T cells did not differ between AD-HIES patients and healthy age-matched controls. Lines represent median values per group and any differences are not statistically significant E. CD127 expression on CD8⁺ subsets. Light grey shaded histogram = naïve, dark grey solid = effector memory, black line = central memory.

Supplemental figure 2

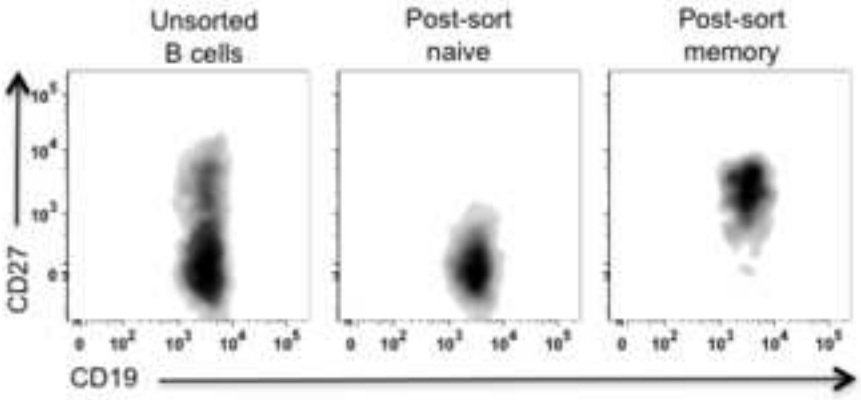

Figure S2. Post-sort analysis of B cell subsets from a mosaic AD-HIES patient. Pre- and post-sort analysis of naïve ($CD19^+CD27^{lo}$) and memory ($CD19^+CD27^{hi}$) B cells from a mosaic AD-HIES patient.

Supplemental figure 3

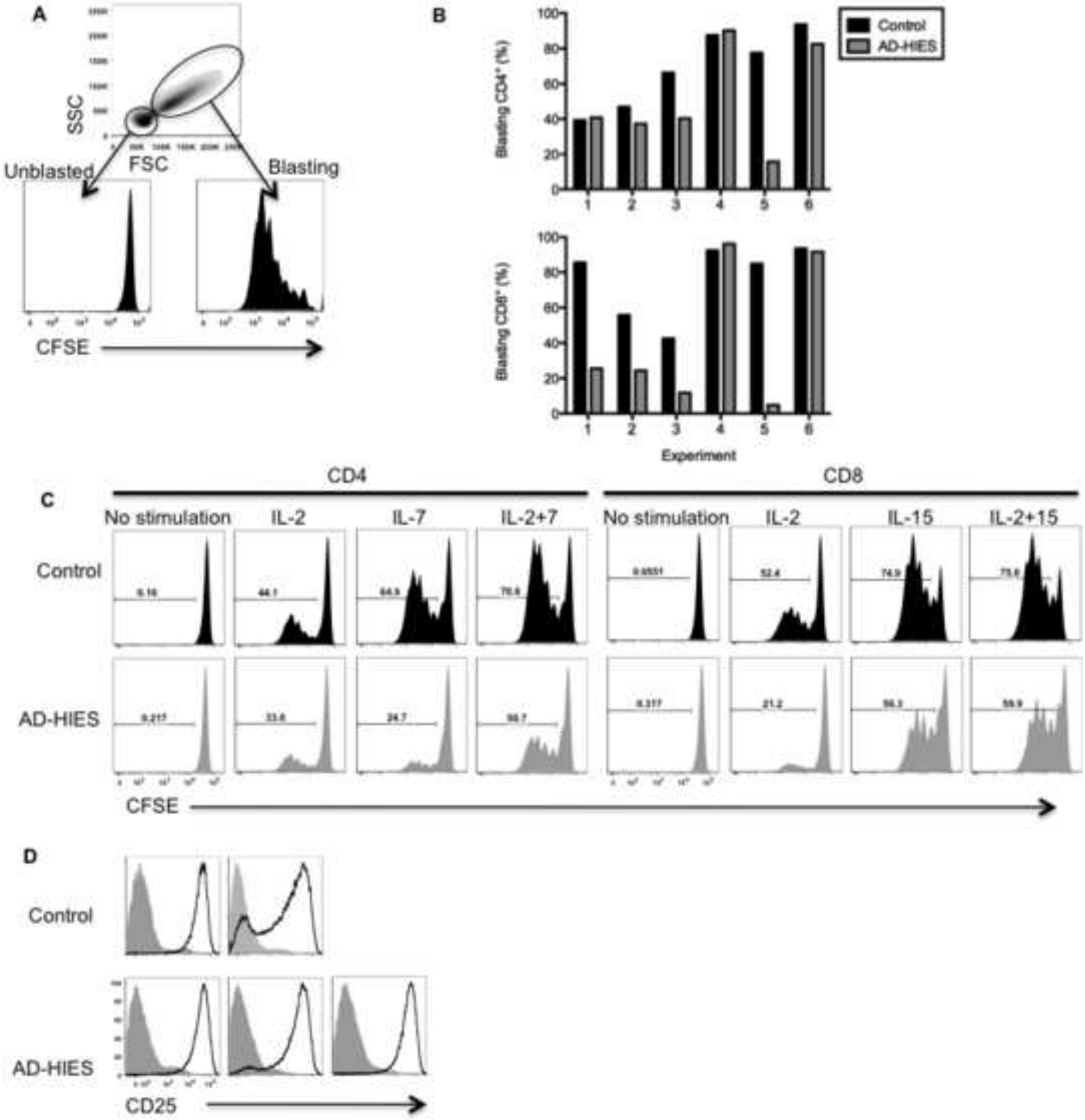

Figure S3. A variable blasting phenotype is observed in AD-HIES patients. A. Sorted naïve CD4⁺ T cells from a control donor were labeled with CFSE and stimulated for 5 days with anti-CD3, anti-CD28, and IL-2. Blasting cells ($FSC^{hi}SSC^{hi}$) cells have proliferated and diluted CFSE wereas unblasted cells ($FSC^{lo}SSC^{lo}$) remain $CFSE^{hi}$. Differences are not statistically significant. B. Quantitation of blasting cells from six controls and six AD-HIES patients following 4-6 days of anti-CD3, anti-CD28, and IL-2 stimulation. C. Representative flow cytometry of CFSE expression in live (aqua viability negative) singlet cells within a lymphocyte gate following five days of stimulation. Data represents one of three independent experiments performed with cells from three AD-HIES patients and controls. D. PBMCs were stimulated with anti-CD3, anti-CD28, and 20 U/mL of IL-2 for 48 hours. Graphs depict CD25 expression on live (aqua viability⁻) CD3⁺ CD4⁺ cells. Solid grey = unstimulated, black line = stimulated. Each set of histograms represents an individual control or AD-HIES patient.

Supplemental figure 4

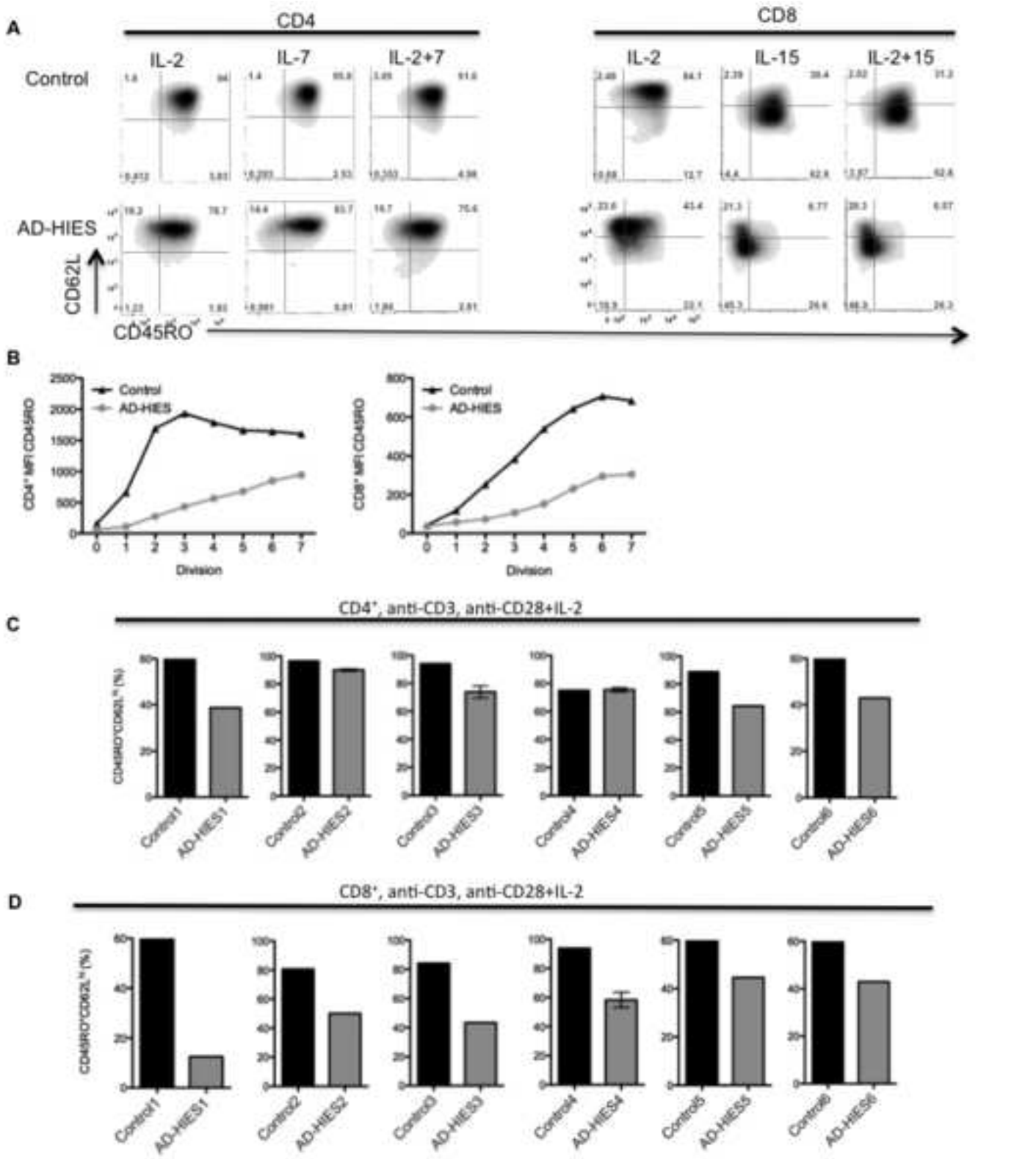

Figure S4. Impaired in vitro central memory differentiation in AD-HIES patients. A. Representative flow cytometry of in vitro differentiated naïve CD4$^+$ and CD8$^+$ T cells. Cells were stimulated with anti-CD3 and anti-CD28 with cytokines as indicated. B. CD45RO expression is lower in AD-HIES dividing CD4$^+$ and CD8$^+$ cells. Cells were stimulated with anti-CD3, anti-CD28, and IL-7 (CD4$^+$) or IL-15 (CD8$^+$). Division subsets were calculated using the proliferation function in FlowJo 9.3.2 and the mean fluorescence intensity of CD45RO was determined in each division. Data is representative of two of three individual experiments with different AD-HIES patients and controls. C and D. Percentage of CD45RO$^+$CD62L^{hi} central memory-like cells following 4-6 days in culture when stimulated in the presence of IL-2. Each graph shows frequencies from an individual experiment. Data is from six individual patients and controls. Error bars result from multiple wells of a condition and represent the standard deviation from the mean.

Supplemental figure 5

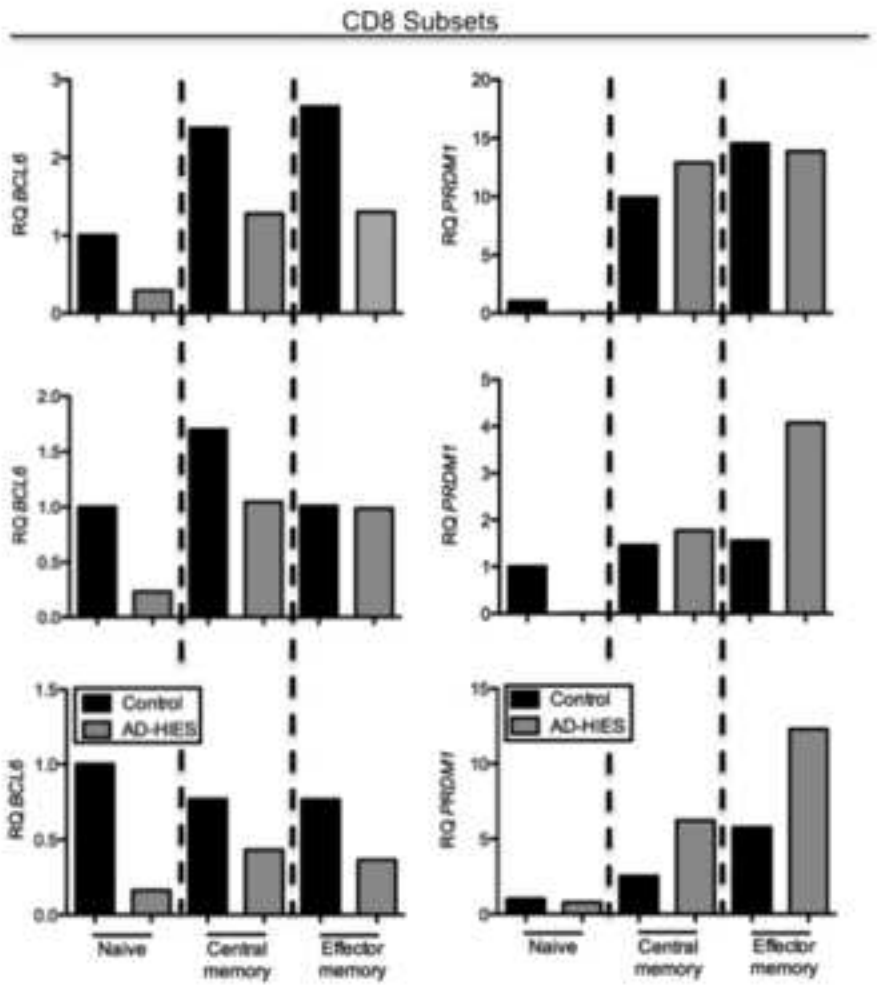

Figure S5. Expression of *BCL6* and *PRDM1* in T cell subsets from additional AD-HIES patients. Expression of *BCL6* and *PRDM1* in CD8$^+$ subsets in three AD-HIES patients and controls. Naïve, central memory, and effector memory subsets were purified by flow cytometry. Transcripts were measured by real-time PCR in triplicate wells relative to *18S*. Each graph represents a unique patient and control pair.

Supplemental figure 6

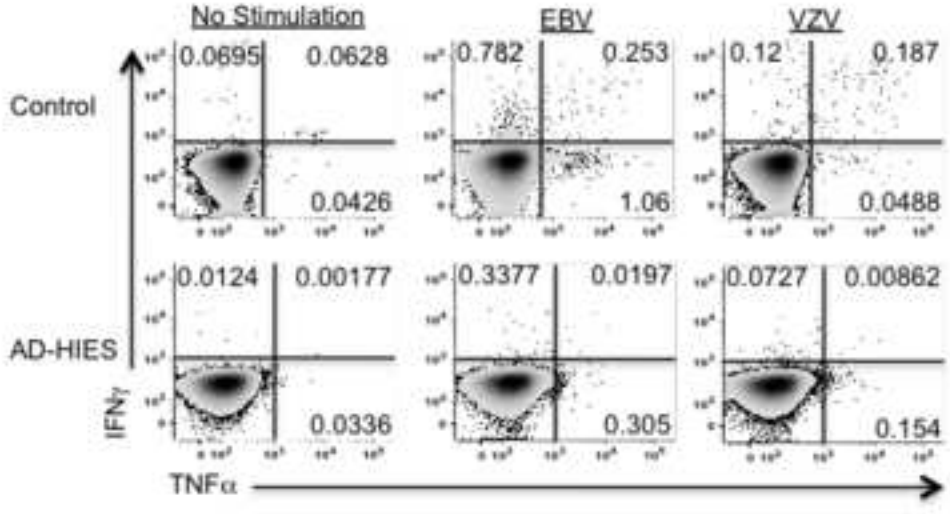

Figure S6. Representative flow cytometry of the CD4 memory response to EBV and VZV. A. PMBCs were thawed and stimulated with EBV and VZV viral lysates. Brefeldin A was added to the cultures after two hours and intracellular cytokines measured following sixteen hours of stimulation. Plots are gated on aqua viability$^-$ singlet lymphocytes, CD3$^+$CD4$^+$CD45RO$^+$CD27$^+$ (central) and CD45RO$^{+/-}$CD27$^-$ (effector) memory populations.