Figure S1

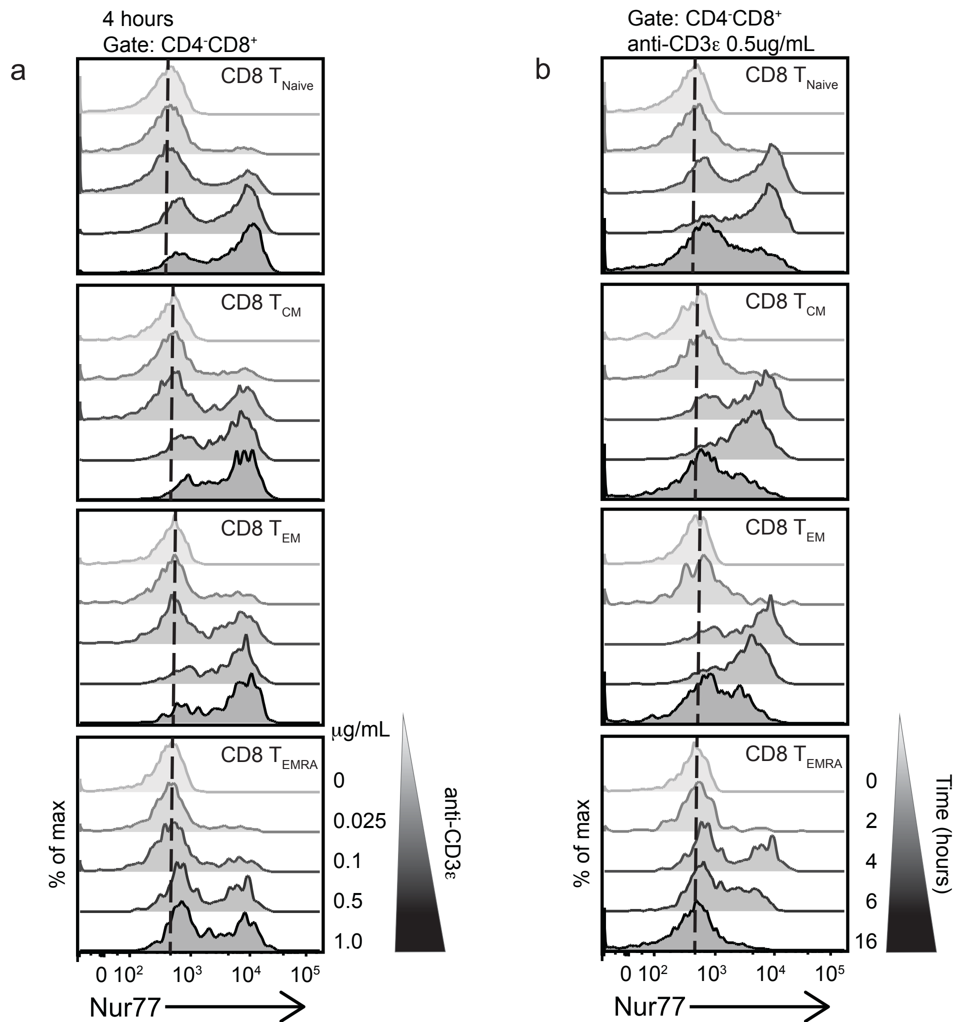

Figure S1. Dose response and temporal induction of Nur77 in CD8 T cell subsets. Histograms represent endogenous intracellular Nur77 in CD8 T cell subsets. **(a)** CD8 RA^+CCR7^+ naïve (T_{Naive}, top panel), RA^-CCR7^+ central memory (T_{CM}, second panel), RA^-CCR7^- effector memory (T_{EM}, third panel), RA^+CCR7^- terminally differentiated memory (T_{EMRA}, bottom panel) human T cell subsets from a mixed population of PBMCs were treated with purified anti-CD3ε mAb for 4 h at the indicated doses (ranging from 0.025 – 1.0 μg ml^{-1}). **(b)** CD8 RA^+CCR7^+ naïve (T_{Naive}, top panel), RA^-CCR7^+ central memory (T_{CM}, second panel), RA^-CCR7^- effector memory (T_{EM}, third panel), RA^+CCR7^- terminally differentiated memory (T_{EMRA}, bottom panel) human T cell subsets from a mixed population of PBMCs were treated with anti-CD3ε 0.5 μg ml^{-1} at the indicated time points (ranging from 2 – 16 hours). Data are representative of two biologically different donors.

Figure S2

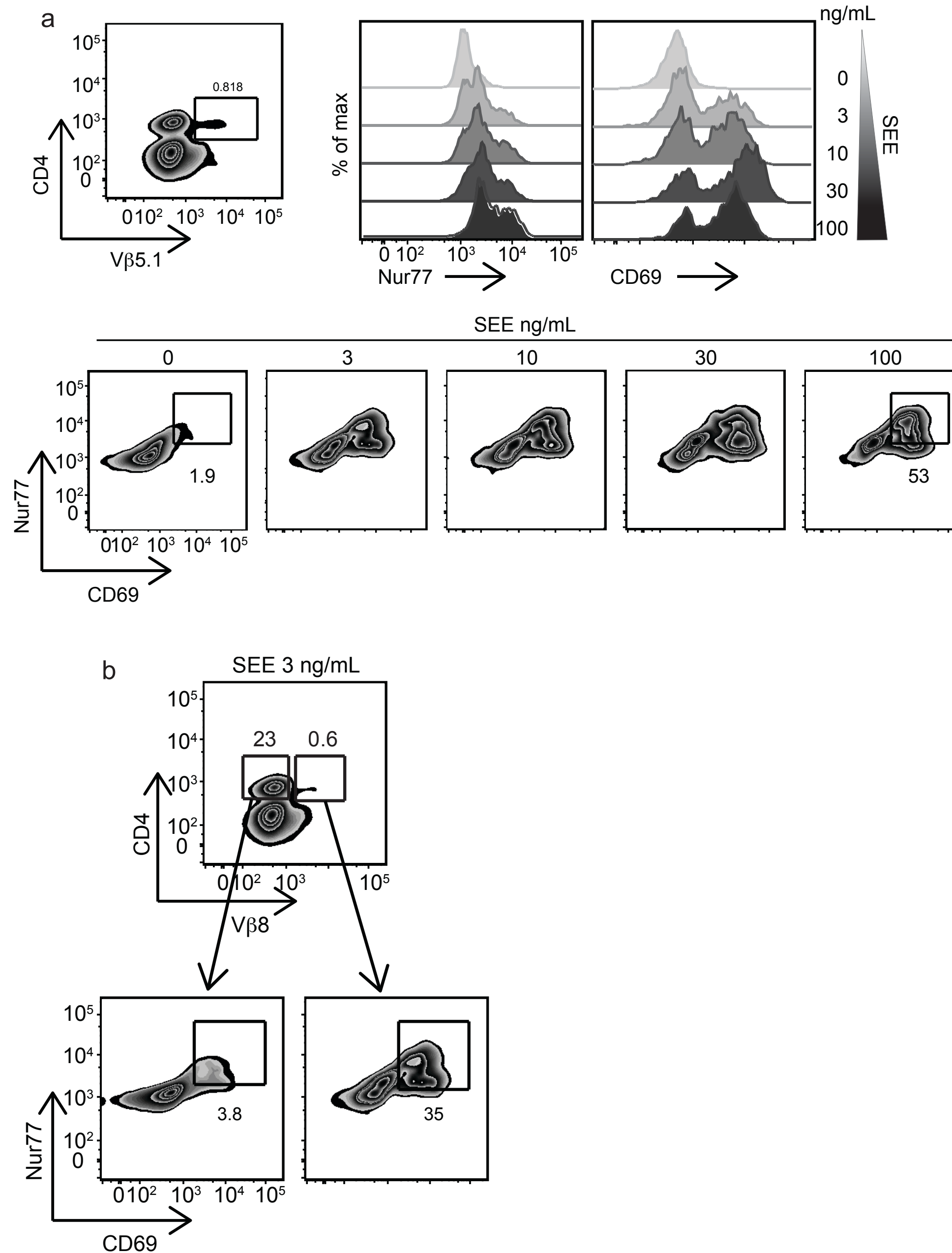

Figure S2. Stimulation with SEE validates Nur77 as a marker for antigen specific signaling in human T cells. Mixed human PBMCs were stimulated with soluble SEE for 16 h and analyzed by FACS. **(a)** Upper left, plot demonstrates gating strategy for $CD4^+V\beta8^+$ subset. Upper right, histograms representative of intracellular Nur77 and surface CD69 dose responses to SEE 3 – 100μg ml^{-1} as indicated in the $CD4^+V\beta8^+$ subset. Bottom row, plots demonstrate Nur77 and CD69 induction in $CD4^+V\beta8^+$ subset to SEE at indicated doses. **(b)** Top plot, $CD3^+$ T cells stained for CD4 and Vβ8 expression to identify double positive and $CD4^+V\beta8^-$ subsets. Bottom plots represent Nur77 and CD69 levels in $CD4^+V\beta8^-$ (left) or $CD4^+V\beta8^+$ (right) subsets stimulated with SEE 3 ng ml^{-1} overnight. Data are representative of at least two independent experiments.

Figure S3

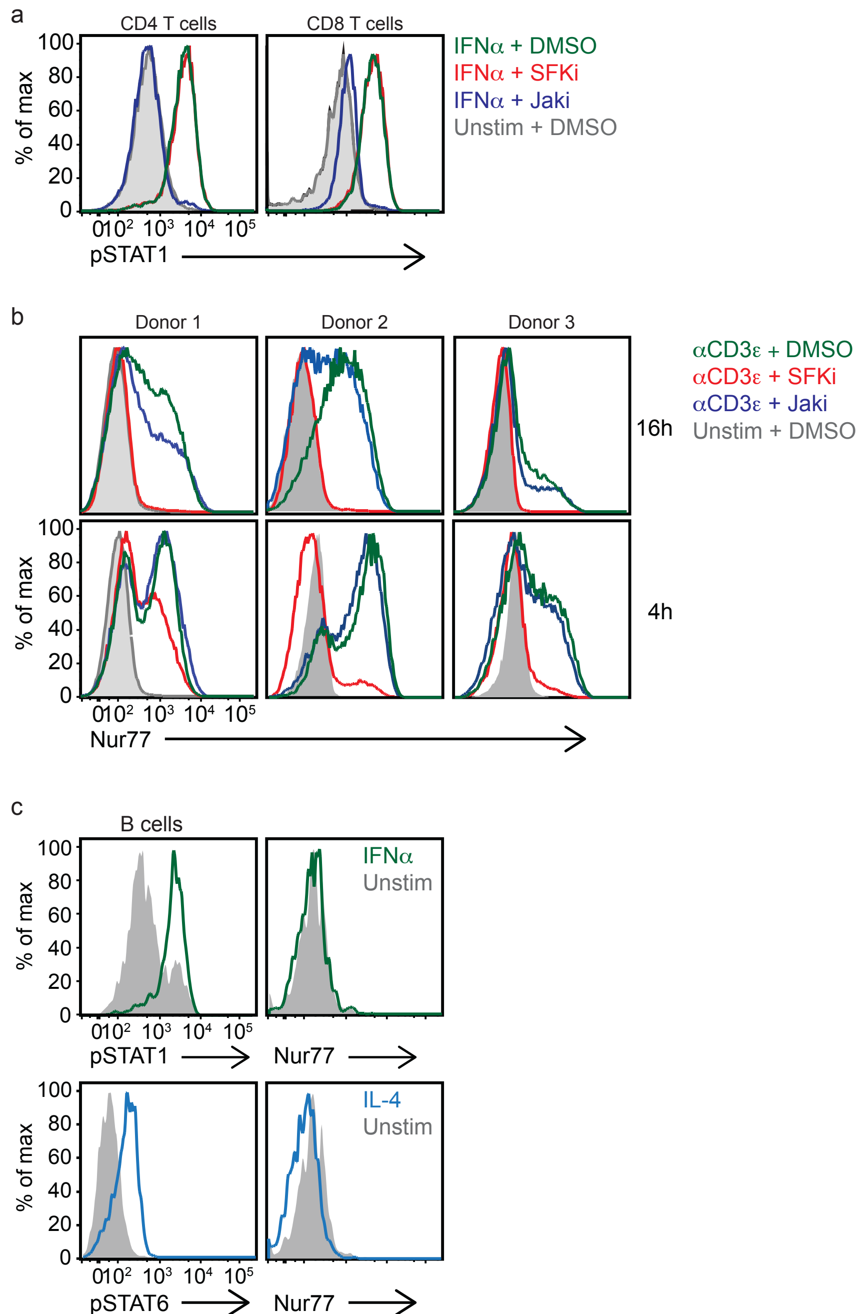

Figure S3. Nur77 induction is insensitive to Jak-STAT signaling pathways in human T and B cells. (a) Histograms represent phosphorylation of STAT1 in CD4 (left panel) or CD8 (right panel) T cells from mixed PBMCs that were pre-treated in the presence or absence of specific inhibitors and then stimulated with IFNα 10kU mL^{-1} for 15 minutes. Filled in grey histograms represent unstimulated samples pretreated with vehicle control (DMSO). **(b)** Histograms represent endogenous Nur77 in CD4 T cells from 3 different donors' mixed PBMCs that were pre-treated in the presence or absence of specific inhibitors and then stimulated for 4 or 16 h with anti-CD3ε 1μg mL^{-1}. Filled in grey histograms represent unstimulated samples pretreated with vehicle control (DMSO). **(c)** Histograms represent STAT phosphorylation and Nur77 in B cells after treatment with IFNα 10kU mL^{-1} or IL-4 10ng mL^{-1} for 15 minutes (pSTAT) or 6 hours (Nur77). Filled in grey histograms represent unstimulated samples treated with media alone. Data are representative of three biologically different donors. (SFKi – src family kinase inhibitor, PP2; Jaki – janus kinase inhibitor, tofacitinib).

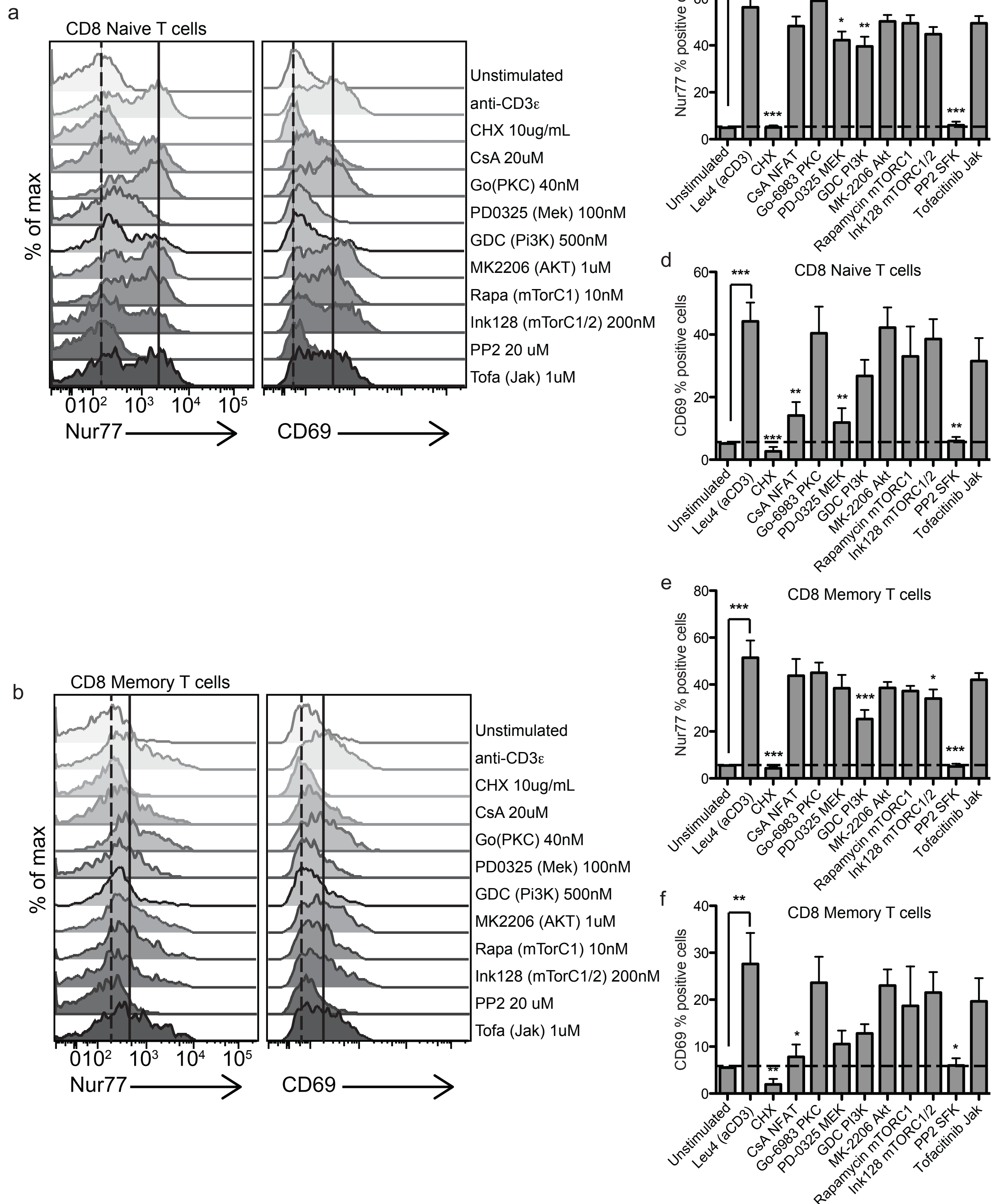

Figure S4. Nur77 integrates TCR signaling pathways in CD8 naïve and memory T cells. Histograms represent Nur77 and CD69 induction in $CD4^{-}CD8^{+}$ naïve **(a)** and memory T cells **(b)** treated with anti-CD3ε in the presence or absence of specific inhibitors for 4 hours. Data in figures S5a-b are representative of at least 5 biologically different donors. **(c-f)** Bar graphs represent Nur77 or CD69 percent positive cells in CD8 naïve **(c,d)** or memory T cells **(e,f)** treated with anti-CD3ε in the presence or absence of specific inhibitors for 4 hours. Horizontal dashed lines in S5c-f mark Nur77 and CD69 percent positive of unstimulated cells. Positive gates were set at the highest 5% of unstimulated cells. Values in S5c-f are the mean of 5-6 biologically different donors +/- SEM. One-way ANOVA was used to compare unstimulated samples (treated with DMSO vehicle control) and inhibitor treatment groups to Leu4 + inhibitor vehicle control (DMSO). *$p<0.05$, **$p<0.01$, ***$p<0.001$