Supplemental data:

Supplemental Figure S1

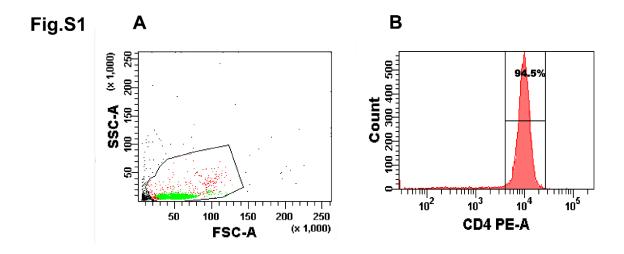


Figure S1: Flow cytometric analysis of human CD4+ T lymphocytes isolated from peripheral blood mononuclear cells (PBMCs): CD4+ T lymphocytes were purified from PBMCs by positive selection using magnetic cell sorting (MACS) CD4+ microbeads (Miltenyi Biotech, Bergisch Gladbach, Germany). Purity of CD4+ T lymphocytes was determined using BD FACS CantoTm II flow cytometer. A) Scatter graph of CD4+ T lymphocytes and B) histogram demonstrating 94.5% purity of isolated CD4+ T lymphocytes.

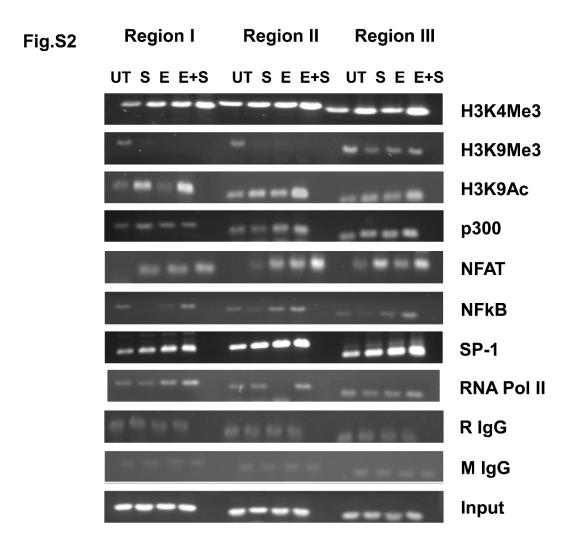


Figure S2: Representative Gel ChIP-PCR analysis: Freshly isolated human CD4+ T cells were untreated (UT) or treated with 25mM ethanol (E) for 24h and then stimulated with anti CD3/CD28 antibodies (1μg/ml) (S and E+S). Semi-quantitative PCR using FasL promoter regions I, II, and III specific primers was performed after chromatin immunoprecipitation (ChIP) with respective antibodies and the product was visualized with ethidium bromide stained agarose gel. Figure S2 shows representative data for H3K4me3, H3K9me3, H3K9Ac modifications, and recruitment of p300, transcription factors NFAT, NFkB, SP-1, and RNA POL II. Isotype specific rabbit IgG (RIgG) or mouse IgG (MIgG) antibodies were used as controls. Diluted fraction of non-immunoprecipitated chromatin was used as input.

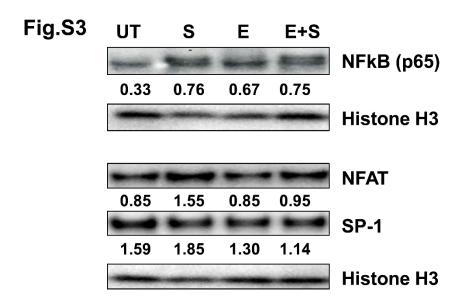


Figure S3: Effect of T cell stimulation and alcohol treatment on transcription factor (TF) activation in CD4+T lymphocytes upon TCR activation: Freshly isolated human CD4+ T cells were untreated (UT) or treated with 25mM ethanol (E) for 24h and then stimulated with anti CD3/CD28 antibodies (1μg/ml) (S and E+S) for 1.5h. Nuclear lysates were analyzed for activation and nuclear translocation of TFs - NFkB (p65), NFAT and Sp-1 levels by Western blot analysis with Histone H3 as loading control. A representative of 3 separate experiments done on CD4+ T cells obtained from different individuals is shown. Densitometry analysis was done and the ratios of TF/histone H3 are shown in the bottom of each panel.