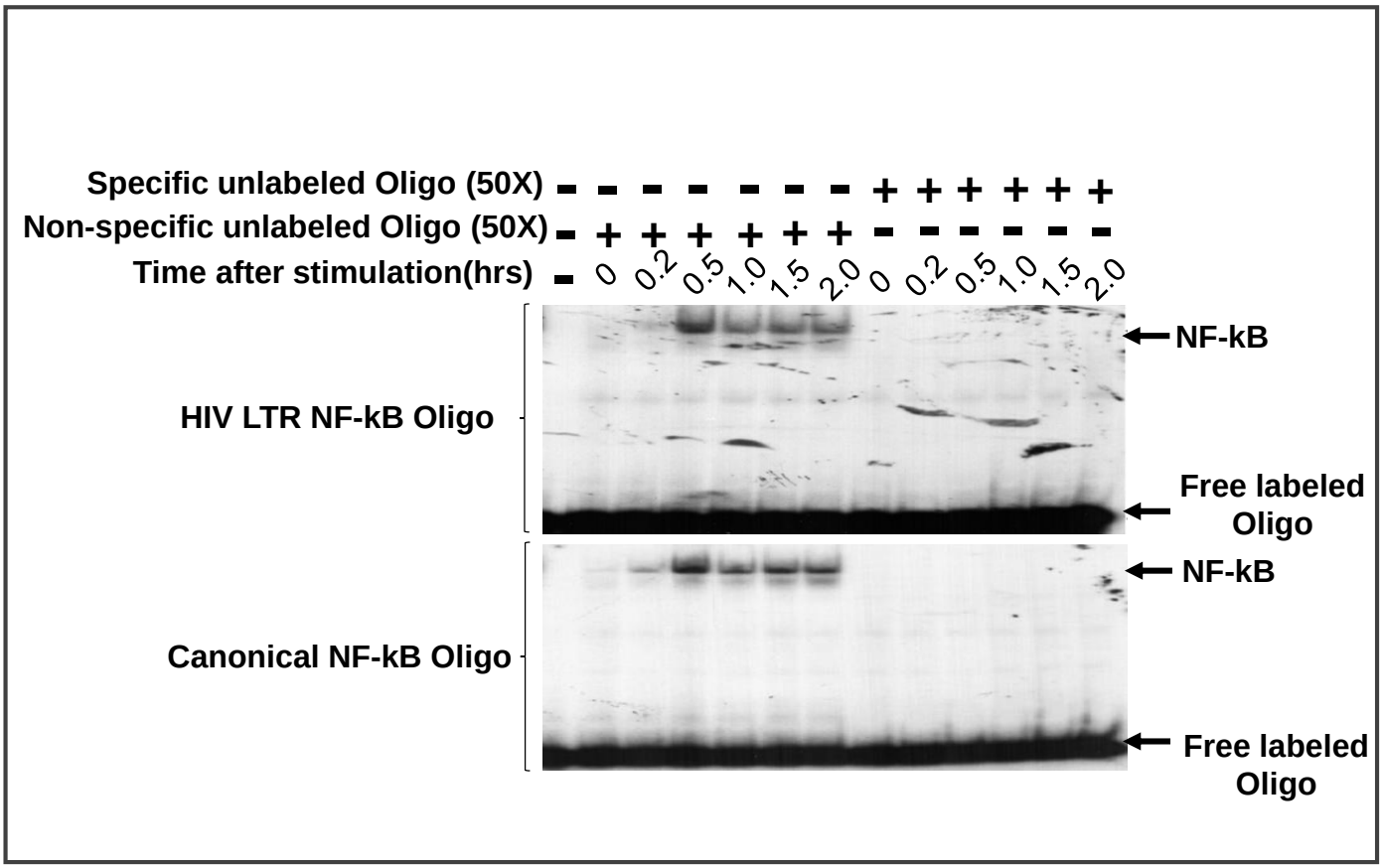
Original Research Article

AP-1 and NF-kB synergize to transcriptionally activate latent HIV upon T-cell receptor activation

Supplementary figure 1



Supplementary figure 1: Electrophoretic mobility gel shift assay using nuclear fraction to determine binding of NF-κB to its cis regulatory elements using oligos derived from the HIV LTR (upper panel) and the Interleukin 2 promoter (lower panel). Note that higher amounts of unlabeled specific oligos competitively prevents NF-κB binding to labeled oligos derived from both the HIV LTR and the IL2 promoter. Specific unlabelled oligos and labelled oligos were the same except that for the labelled oligos, radioactive 32-P was linked to oligos to enable detection of the band shifts by autoradiography.

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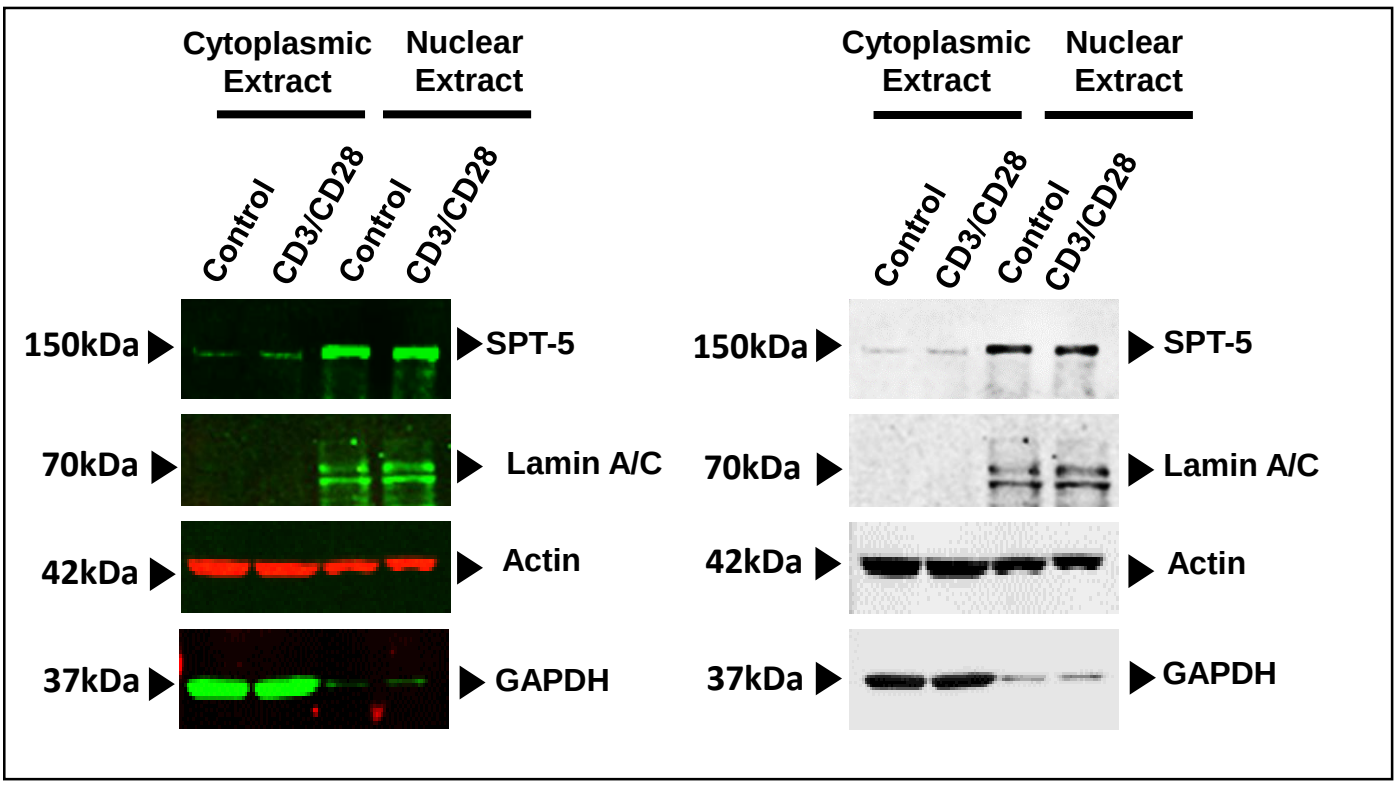
Supplementary figure 2



Supplementary figure 2: Immunoblot showing that SPT-5 is abundantly present in the nuclear extract. HIV latently infected Jurkat cells (2D10 cells) were equally seeded in two Petri dishes (5 million cells / each). One of the plates was treated with anti-CD3/CD28 antibodies for 2hrs to activate cells through the TCR, while the untreated plate was used as unstimulated control. The cells were lysed with CE buffer [0.5% NP-40, supplemented with 10 mM HEPESKOH pH 7.9, 60 mM KCL, 1 mM EDTA, 1 mM DTT,1 mM phenylmethylsulfonylfluoride (PMSF), 10 µg/mL leupeptin, 10 µg/mL aprotinin]. Isolated nuclei were washed thrice with clold PBS and lysed in a NE buffer [250 mM Tris pH7.8, 60 mM KCL, 1 mM EDTA, 1 mM DTT, 1 mM PMSF, 10 µg/mL leupeptin, 10 µg/mL aprotinin, 100 mM NaF, and 200 µM sodium orthovanadate] followed by six freeze-thaw cycles. Equal amounts of Protein (20 µg) was loaded from each sample on SDS-PAGE gel, transferred to a nitrocellulose membrane and detected with antibodies against **SPT-5, Lamin A/C (nuclear marker), GAPDH (cytoplasmic marker) and Actin (both nuclear as well as cellular marker)**.

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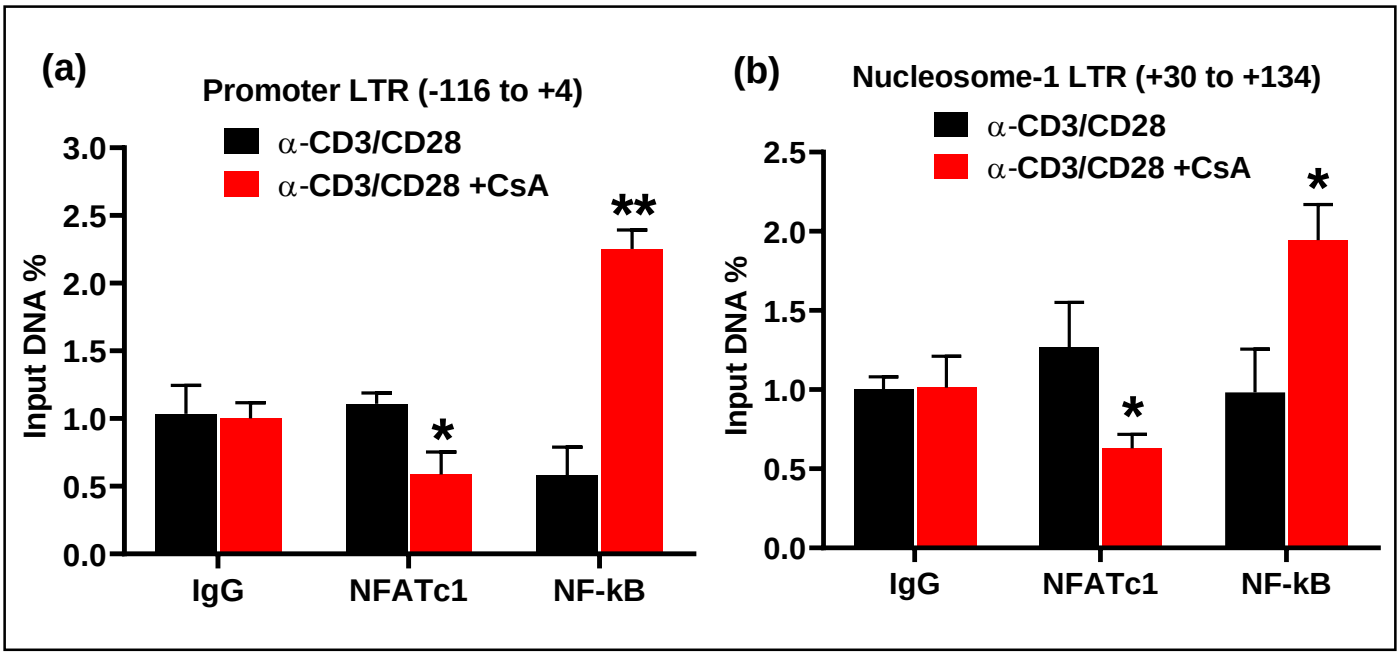
Supplementary figure 3



Supplementary figure 3: Binding of NF-κB and NFAT at HIV LTR in the absence and presence of Cyclosporin A. Chromatin immunoprecipitation (ChIP) analyses were performed using samples treated with and without Cyclosporin A (CsA), an inhibitor of NFAT induction. As indicated in the figure, the ChIP assays were performed using antibodies against NFATC1 and NF-kB (p65), with preimmune IgG, as negative control. The recovered DNA was quantified through quantitative real time PCR (qRT-PCR) using primer sets that specifically amplify the Promoter (-116 to +4) and Nucleosome-1 (+30 to +134) regions of the HIV LTR, in order to demonstrate recruitment of these factors to the HIV LTR. Red bars represent the recruitment of NF-kB and NFATc1 at the HIV LTR in samples treated with Cyclosporin A, while black bars mark the recruitment of these factors at the LTR in untreated samples, as control. Graphs represent the average and standard deviation from three independent and replicate samples. Statistical analysis was calculated with GraphPad Prism 8.0 (GraphPad Software, San Diego, CA, USA). The p value of statistical significance was set at either; $p < 0.05$ (*) or 0.01 (**).