Supplementary Data

Specific transcriptional repression by CBF-1 is a critical factor in the establishment of HIV latency

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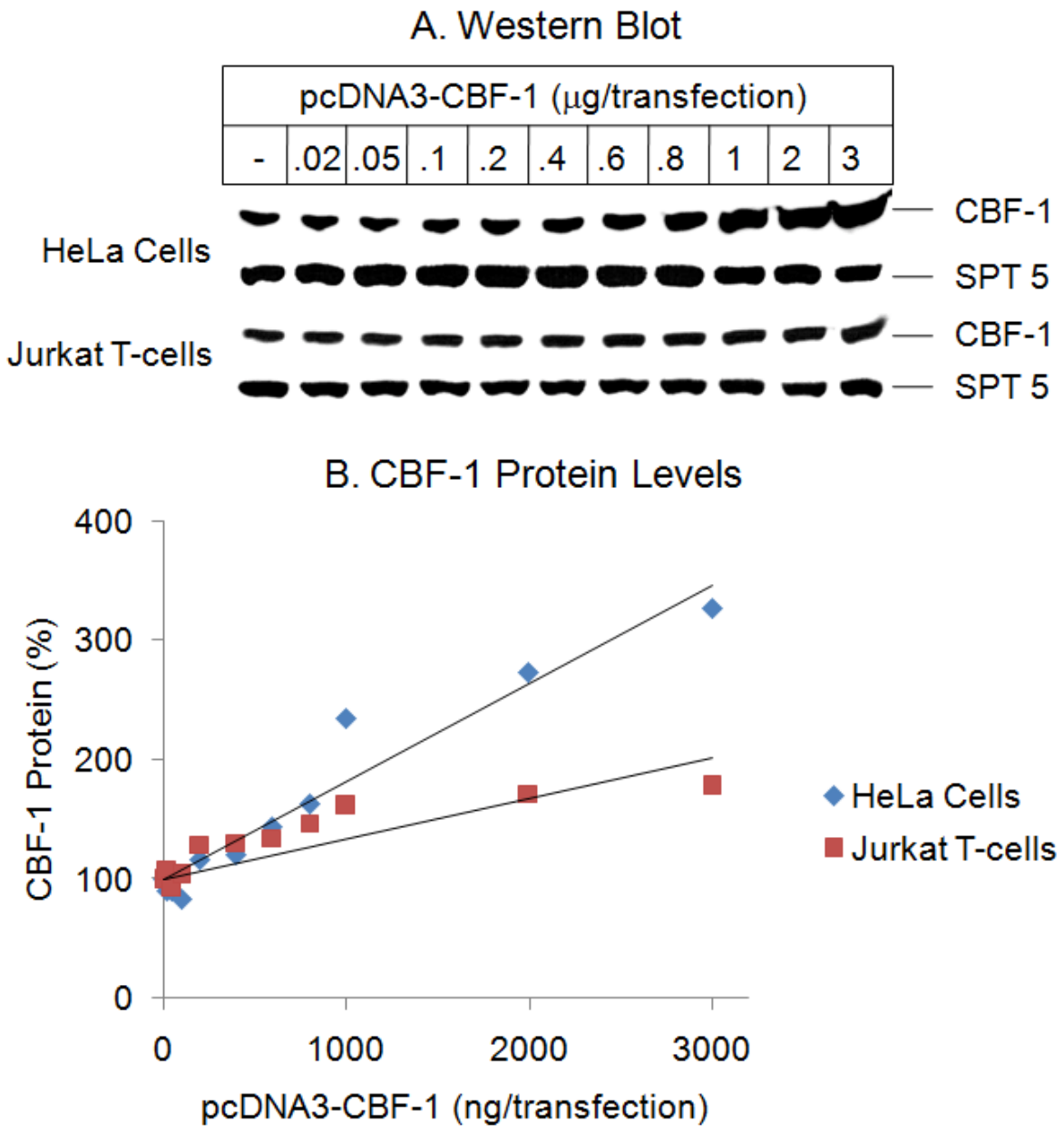

Figure S1. Expression of CBF-1 protein in transfected HeLa and Jurkat T-cells. (A) Western blot of transfected cells. 2 x 10^6 HeLa or Jurkat T-cells were infected with the pcDNA3-CBF-1 vector at plasmid concentrations between 0 and 3000 ng. The levels of CBF-1 protein in nuclear extracts prepared from the transfected cells was then measured by Western blotting using the nuclear protein Spt5 as a marker for the protein recovery. (B) CBF-1 protein levels. The relative amount of CBF-1 protein was determined by densitometry of the Western blot shown in Panel A (Untransfected cells = 100%). There is a linear relationship between the amount of CBF-1 protein expressed and the plasmid concentration using in the transfection assay, but the slopes of curves varies due to the enhanced transfection efficiency of the HeLa cells compared to the Jurkat T-cells.

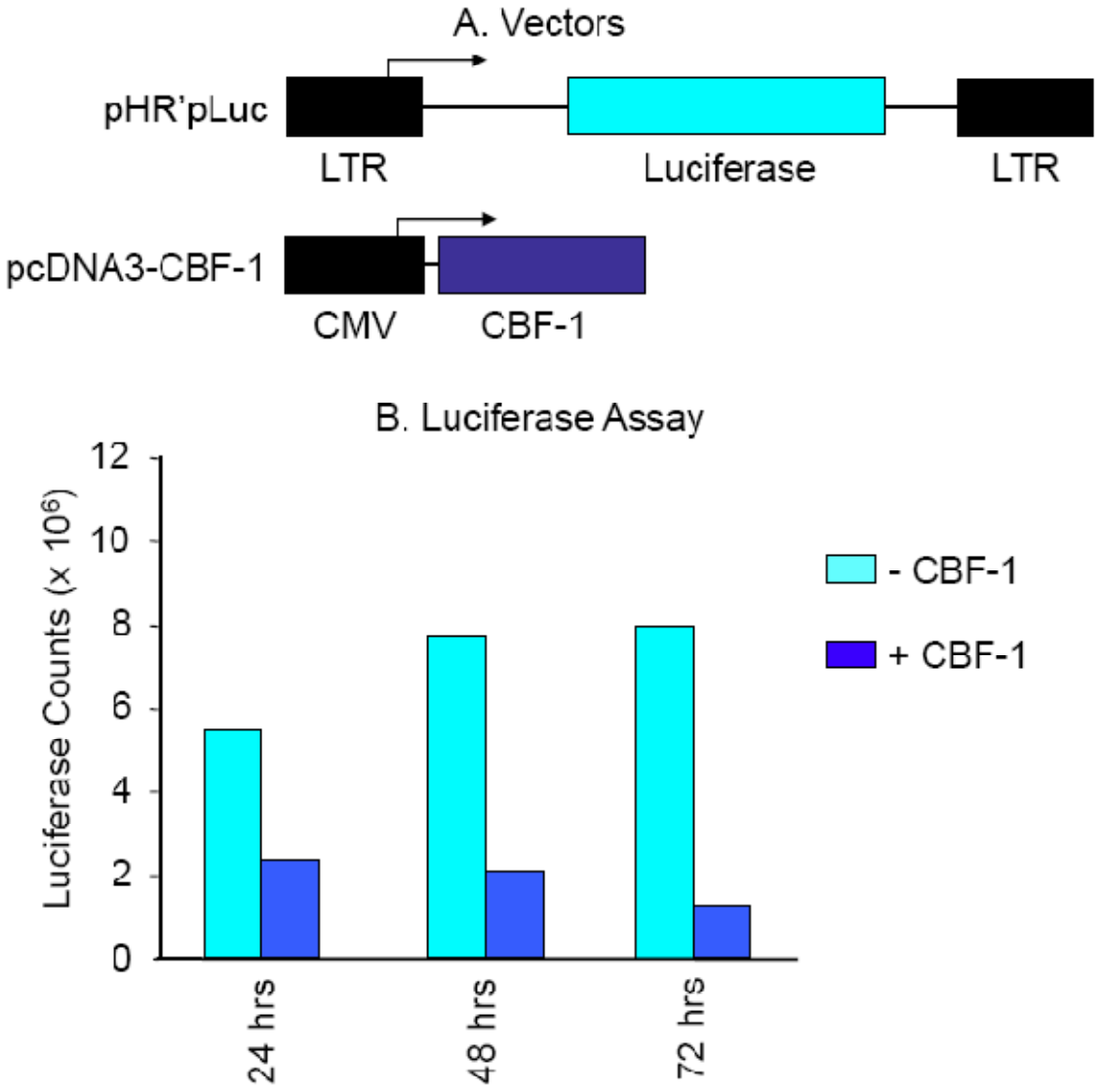

Figure S2. CBF-1 represses HIV transcription independently of Tat. HeLa cells were infected with the pHR'P-Luc lentiviral vector, which contain luciferase reporter gene under HIV-1 LTR promoter, but lacks Tat. The infected cells were then transfected with 3 μg of CBF-1 plasmid. Cell extracts were prepared after the indicated time intervals and analyzed for luciferase activity. Note that CBF-1 expression *in trans* reduced basal HIV expression by up to 4-fold.

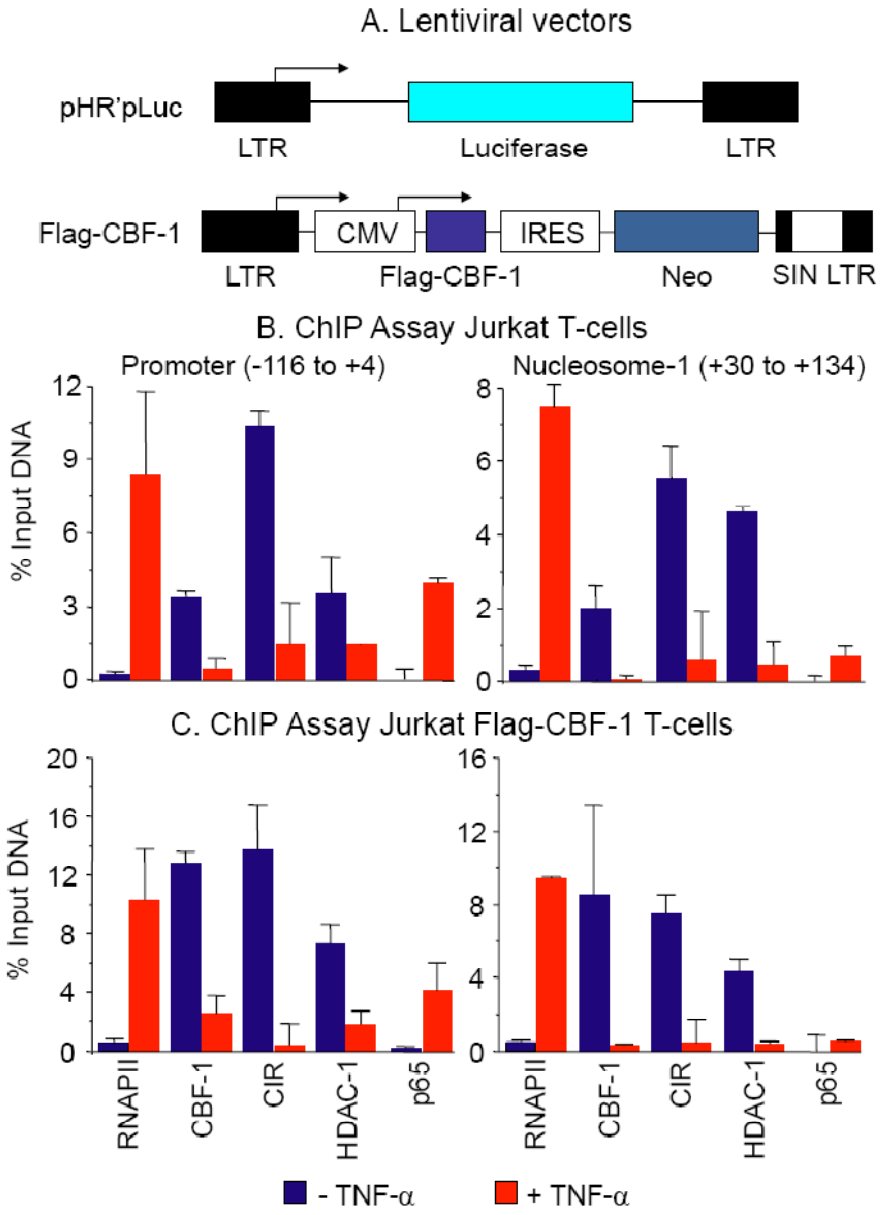

Figure S3. NF-κB is able to displace CBF-1 from HIV LTR *in vivo.* Jurkat cells were infected carrying a latent pHR'pLuc reporter were superinfected with the Flag-CBF-1 vector to increase intracellular CBF-1 levels. The cells were then analysed by ChIP assays before and after stimulation by TNF-α. The overexpression of CBF-1 resulted in higher levels of CBF-1 and co-repressor complexes at the HIV LTR. Activation by TNF-α results in recruitment of NF-κB p65 and RNAP II along with the simultaneous loss of CBF-1, the co-repressor complex, and HDAC-1 in both cell lines. (A) Structure of lentiviral vectors. (B) ChIP assay of Jurkat T-cells and (C) Jurkat-Flag-CBF-1 cells latently infected by pHR'-P-Luc. Cells were analyzed by ChIP assays, either before or after activation by 20 ng/ml TNF-α for 24 hrs. Left panels: Promoter region (-116 to +4). Right panels: Nucleosome-1 region (+30 to +134). Black bars: Minus TNF-α. Red bars: Plus TNF-α.

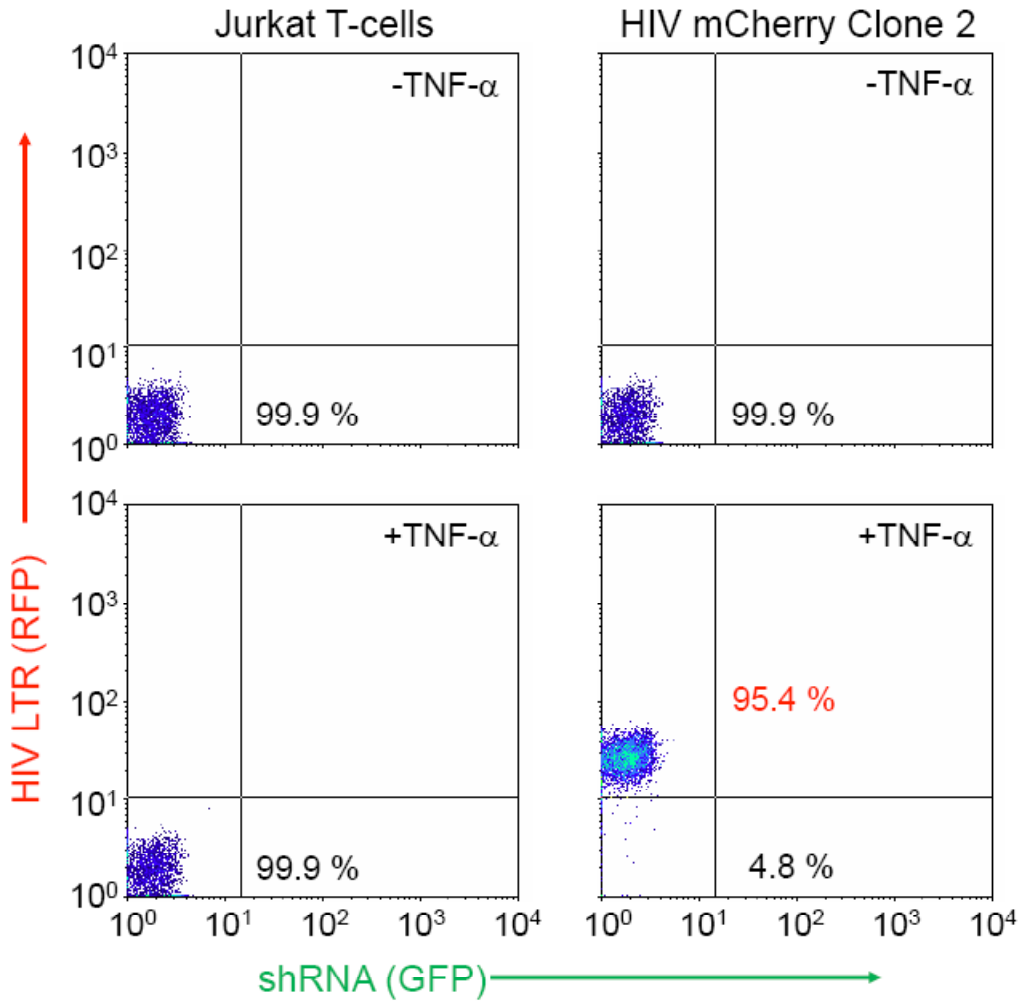

Figure S4. Two color flow analysis of latently infected Jurkat T-cells. HIV mCherry clone 2 is a latently infected Jurkat T-cell line carrying the integrated mCherry (RFP) expressing lentivirus and the H13L Tat mutation. mCherry expression in this cell line is undectable prior to activation by TNF-α. Following TNF-α treatment over 95% of the cells express a high level of mCherry, demonstrating that none of the cells in the population have lost the latent provirus and that the population has homogeneous activation profiles. Time courses demonstrate that the activation of the population is highly synchronous. Top panels: Unstimulated cells. Bottom panels: Cells treated with 20 ng/ml TNF-α for 16 hrs.

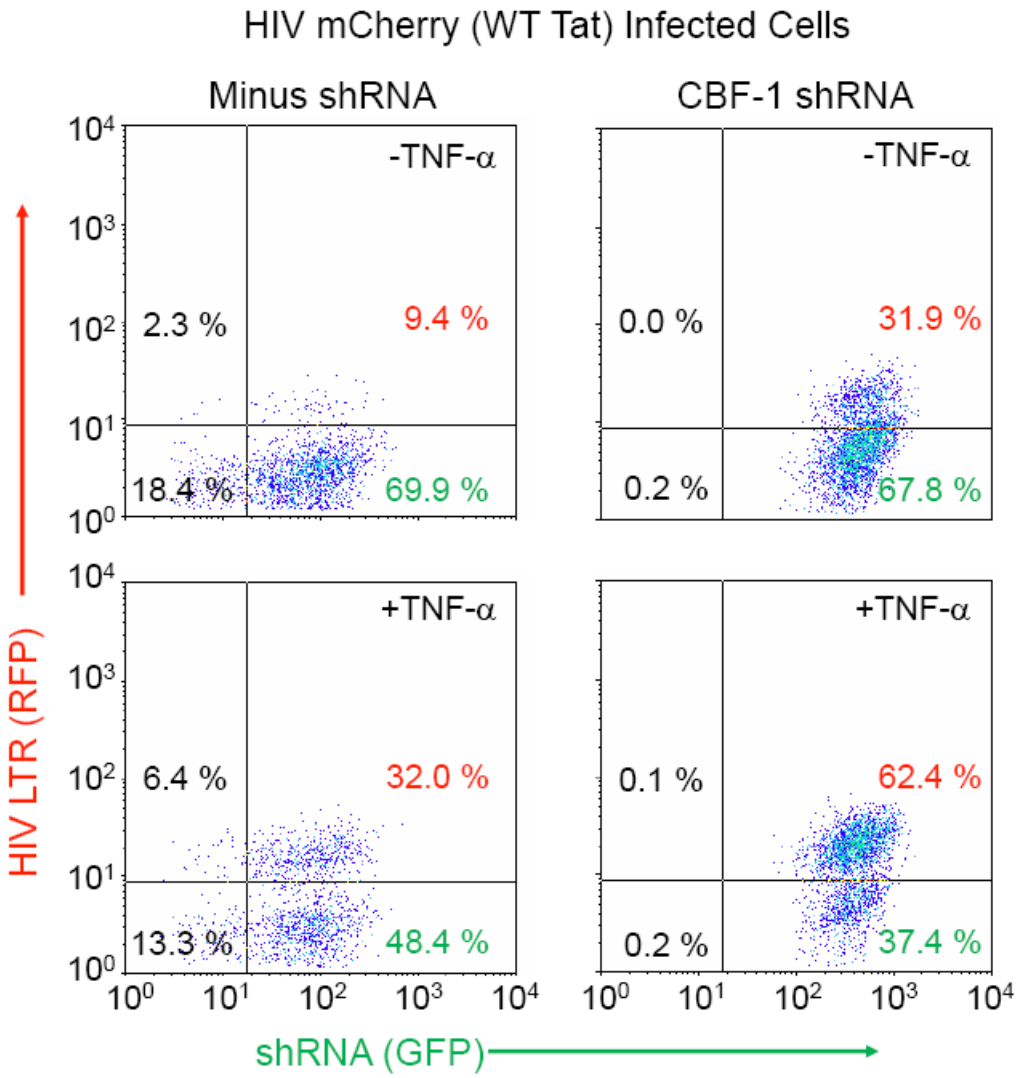

Figure S5. Knockdown of CBF-1 makes T-cells more permissive for HIV gene expression. Cells were infected with lentiviral vectors carrying shRNA to CBF-1 (GFP marker, right panels) or an empty vector control (left panels). The cells were then superinfected with HIV mCherry viruses carrying wildtype Tat after 24 hrs. The cells were infected at an m.o.i. of 1.0 and analyzed by 2 color flow cytometry after an additional 96 hrs. As shown in the upper panels, 32% of CBF-1 knockout cells showed a high level of mCherry expression compared to only 9.4% of control cells. Since restrictions in HIV gene expression can lead to an underestimate of the number of infected cells in the population, the cells were also activated with TNF-α. As shown in the lower panels, approximately 62% of the CBF-1 knockout cells became highly fluorescent after TNF-α treatment, compared to 32% of the control cells. Comparing the ratios of highly fluorescent cells before and after TNF-a induction, we can estimate that 51% of the infected cells in the knockout population showed sustained high levels of HIV transcription, whereas less than 30% of the control cells carried activated proviruses. Top panels: Unstimulated cells. Bottom panels: Cells treated with 20 ng/ml TNF-α for 16 hrs.

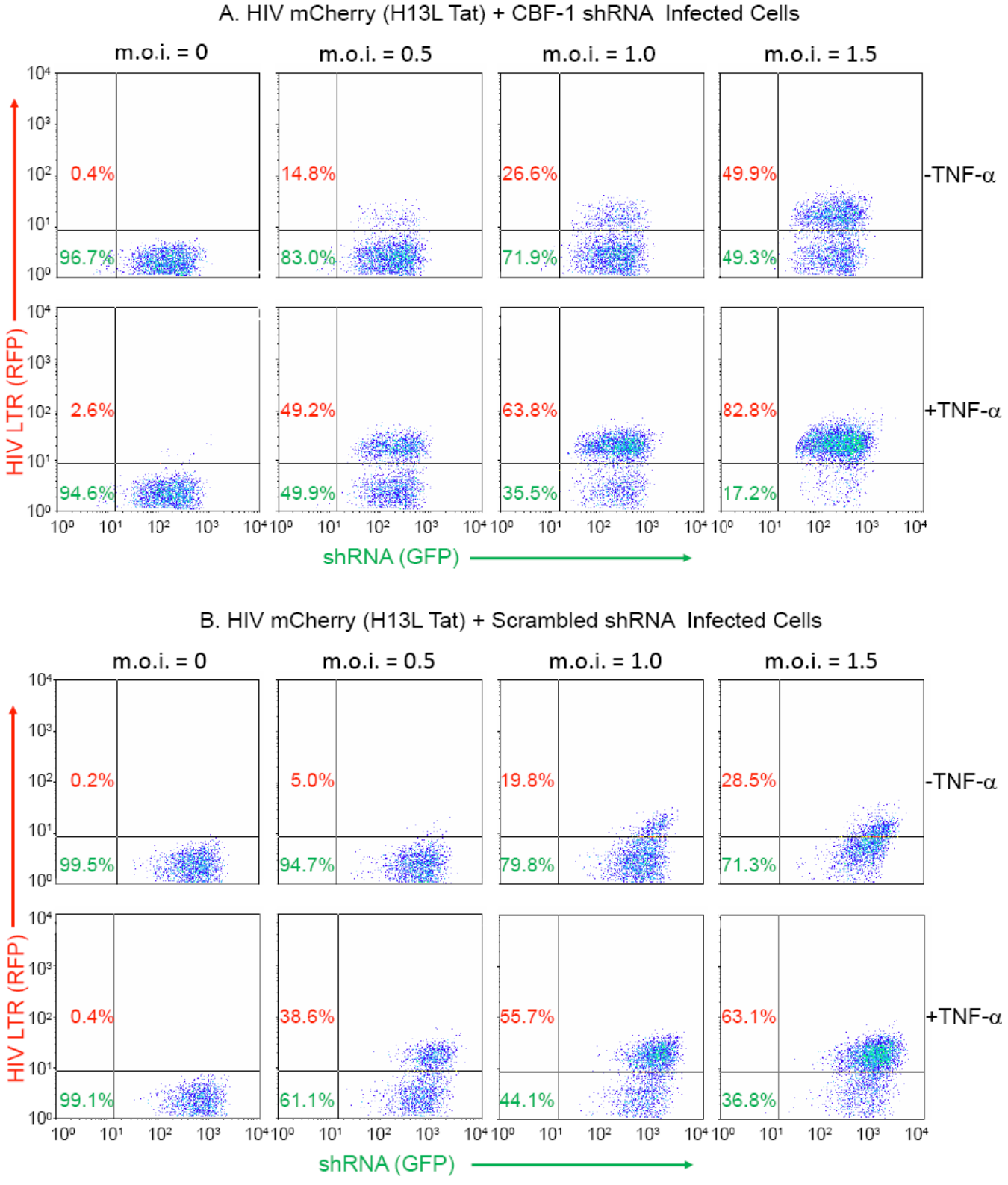
A. HIV mCherry (H13L Tat) + CBF-1 shRNA Infected Cells
m.o.i. = 0
m.o.i. = 0.5
m.o.i. = 1.0
m.o.i. = 1.5
-TNF-α
+TNF-α
0.4%
96.7%
14.8%
83.0%
26.6%
71.9%
49.9%
49.3%
2.6%
94.6%
49.2%
49.9%
63.8%
35.5%
82.8%
17.2%
HIV LTR (RFP)
shRNA (GFP)
B. HIV mCherry (H13L Tat) + Scrambled shRNA Infected Cells
m.o.i. = 0
m.o.i. = 0.5
m.o.i. = 1.0
m.o.i. = 1.5
-TNF-α
+TNF-α
0.2%
99.5%
5.0%
94.7%
19.8%
79.8%
28.5%
71.3%
0.4%
99.1%
38.6%
61.1%
55.7%
44.1%
63.1%
36.8%
HIV LTR (RFP)
shRNA (GFP)

Figure S6. Knockdown of CBF-1 leads to enhanced HIV transcription. This experiment is similar in design to the experiment shown in Figure S4, but the superinfecting virus carried the H13L mutation in Tat. Cells were infected with lentiviral vectors carrying shRNA to CBF-1 (A) or a vector carrying a scrambled shRNA sequence control (B). The cells were then superinfected with HIV mCherry viruses at m.o.i.s between 0.5 and 1.5 and were analyzed by 2 color flow cytometry after 72 hrs. As described previously, the cells were also activated with TNF-α in order to provide an estimate of the total number of infected cells in the population. Note that at every point in the titration the CBF-1 knockout cells show both higher level of mCherry expression (RFP axis) and a higher proportion of cells expressing mCherry than the control cells. For example, at the m.o.i of 1.5, approximately 82% of the cells were infected and approximately 60% of these cells showed high levels of HIV expression in the absence of TNF-α activation. By contrast, in the control populations, approximately 63% of the cells were infected and 45% of these cells were partially activated. Top panels: Unstimulated cells. Bottom panels: Cells treated with 20 ng/ml TNF-α for 16 hrs.

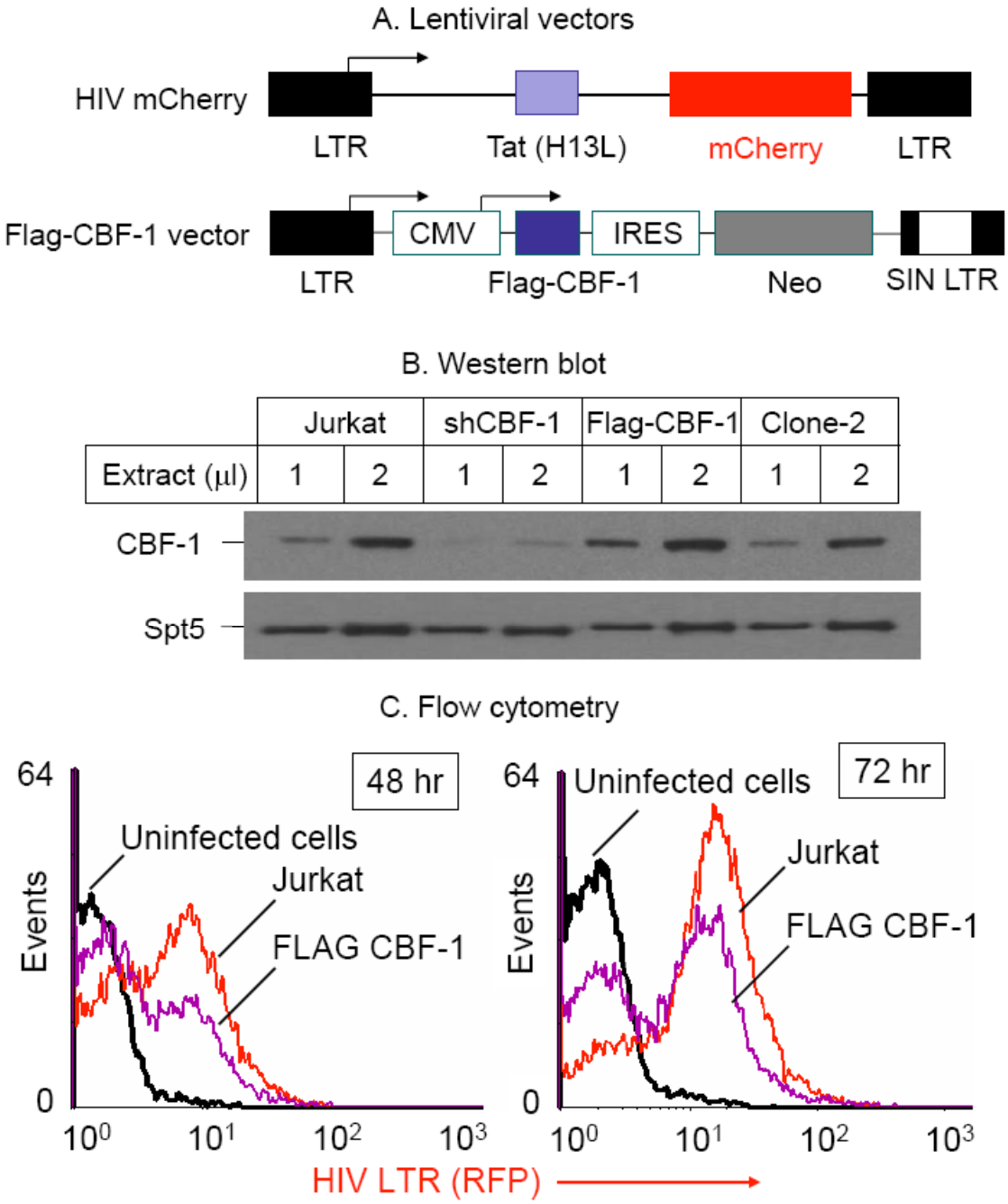

Figure S7. CBF-1 overexpression restricts HIV gene expression in newly infected cells. Jurkat cells, or Jurkat FLAG-CBF-1 cells were infected by the HIV viruses carrying the mCherry reporter at a m.o.i. of approximately 1. (A) Lentiviral vectors. (B) Western blot showing CBF-1 expression levels. (C) Flow cytometry. Left: 48 hrs post infection. Right: 72 hrs post infection. Black line: Uninfected Jurkat T-cells. Purple line: Jurkat FLAG-CBF-1 cells. Red line: Infected Jurkat T-cells.

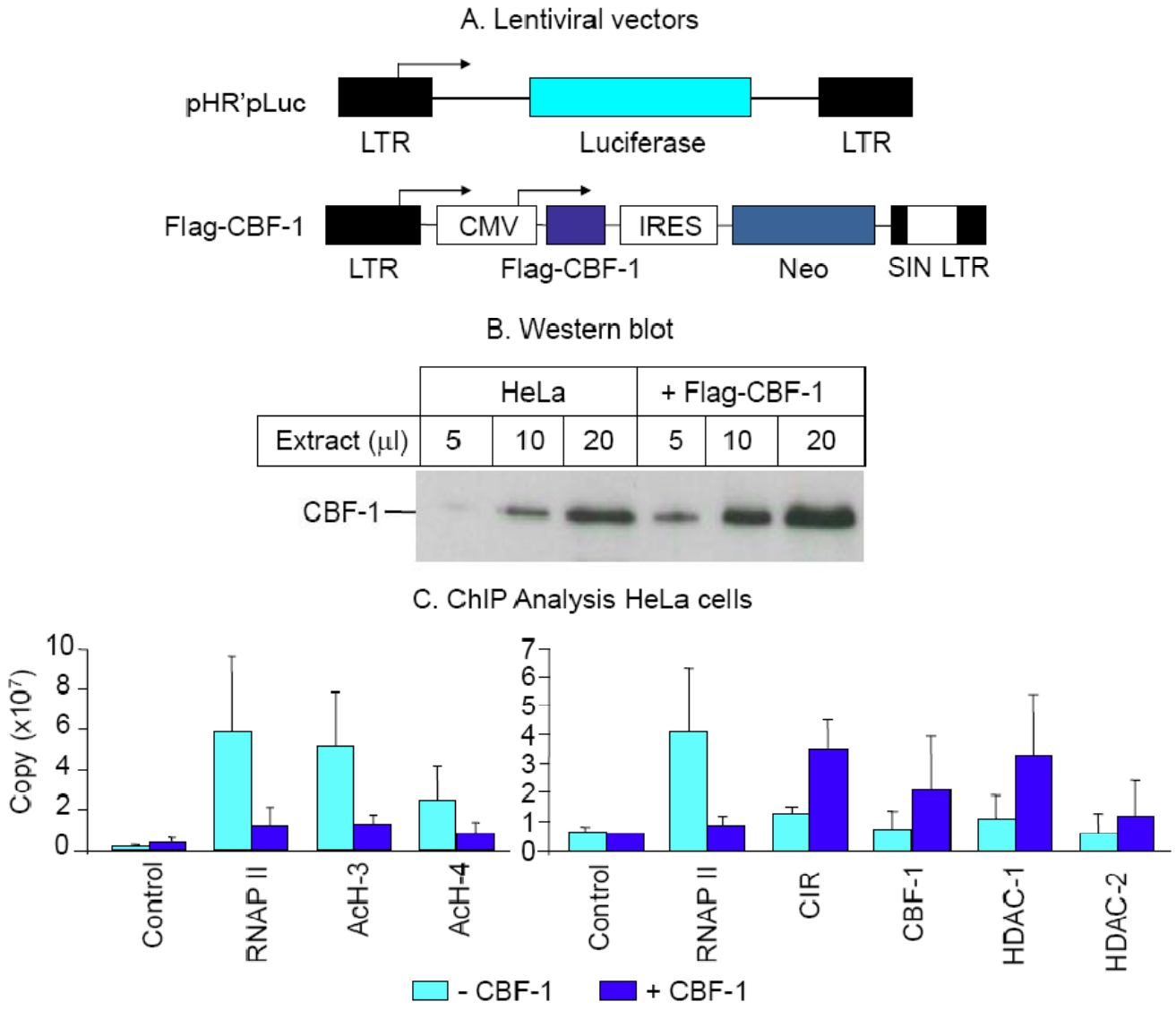

Figure S8. Overexpression of CBF-1 induces repressive chromatin structures at the HIV LTR in HeLa cells. HeLa cells chronically infected with pHR'P-Luc were superinfected with pHR'P-Flag-CBF-1-IRES-Neo and selected using G418 to create HeLa cells carrying the luciferase reporter and over expressing CBF-1. The two cell lines analyzed by chromatin immunoprecipitation (ChIP) assays for the presence of different transcriptional effectors. Five percent of immunoprecipitated material was analyzed by quantitative real-time-PCR and the data was expressed as the relative copy number of recovered DNA per sample. Controls were immunoprecipitated samples using rabbit pre-immune sera. These results clearly demonstrate that in the presence of higher amounts of CBF-1 at LTR, there is a decline of RNAP at the promoter and concomitant recruitment of HDAC-containing corepressor complexes. The CBF-1 copressor complex in turn deacetylates the core histones on nucleosomes and establishes a repressive chromatin structure. These results are very similar to the results obtained using Jurkat T-cells shown in Figure S7, however, since HeLa cells support sustained HIV gene expression for long periods of time there is no need to use freshly infected cells for this experiment. (A) Structure of lentiviral vectors. (B) Western blot showing overexpression of CBF-1. (C) ChIP assay. Left panel: RNAP II and histone antibodies. Right panel: RNAP II and the CBF-1 co-repressor complex.

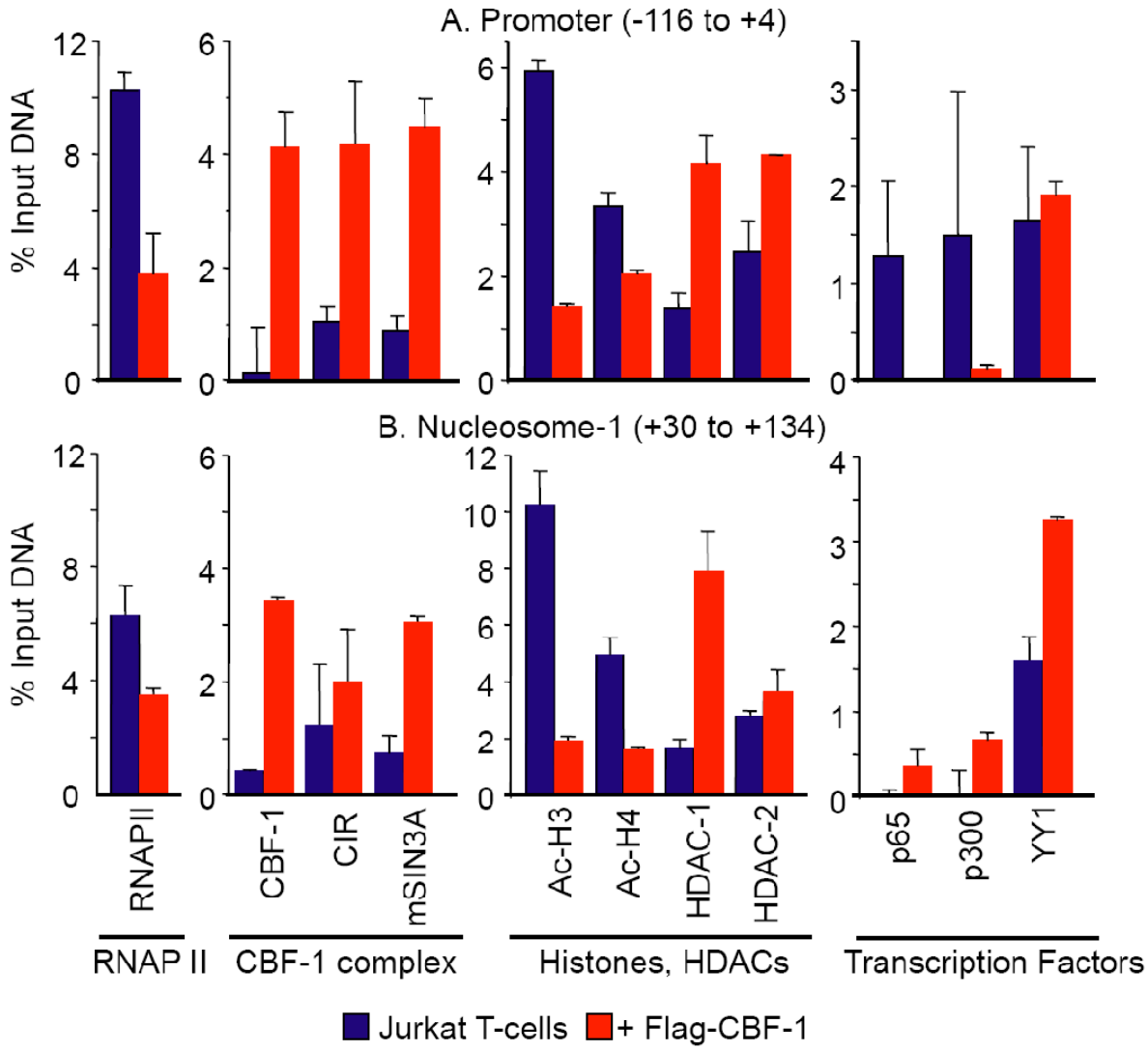

Figure S9. CBF-1 blocks HIV transcription in newly infected cells by recruiting HDACs to the promoter. ChIP assays were performed using Jurkat and Jurkat-Flag-CBF-1 cells freshly infected by the pHR'P-Luc viruses and superinfected with Flag-CBF-1. The Jurkat-Flag-CBF-1 cells showed an 8-fold increase in CBF-1 levels at the promoter and 4-fold higher levels of CIR and mSIN3A corepressor proteins. Recruitment of CBF-1 to the LTR resulted in a two-fold reduction in the levels of RNAP II at the promoter and a 3 to 5-fold reduction in the level of acetylated histone H3 at both Nuc-1 and at the promoter. Similarly there was a 50% reduction in the levels of acetylated histone H4. By contrast, the levels of HDAC-1 increased 2 to 4-fold. There was also a modest increase in the levels of HDAC-2. We also observed reductions in the level of the p65 subunit of NF-κB and of p300 at promoter region down to background levels in the Jurkat-Flag-CBF-1 cells. By contrast, the levels of YY1, which is also able to recruit HDACs to the HIV LTR (Coull et al., 2000), remained relatively constant, suggesting that the increase in HDAC levels that we have observed is due to CBF-1 rather than YY1. Note that because the Flag-CBF-1 vector is a SIN vector, the HIV promoter region has been deleted and it does not interfere with the ChIP analysis. (A) Promoter region (-116 to +4). (B) Nucleosome-1 region (+30 to +134). Black bars: Jurkat T-cells. Red bars: Jurkat T-cells constitutively over-expressing CBF-1 following infection by the Flag-CBF-1 vector.

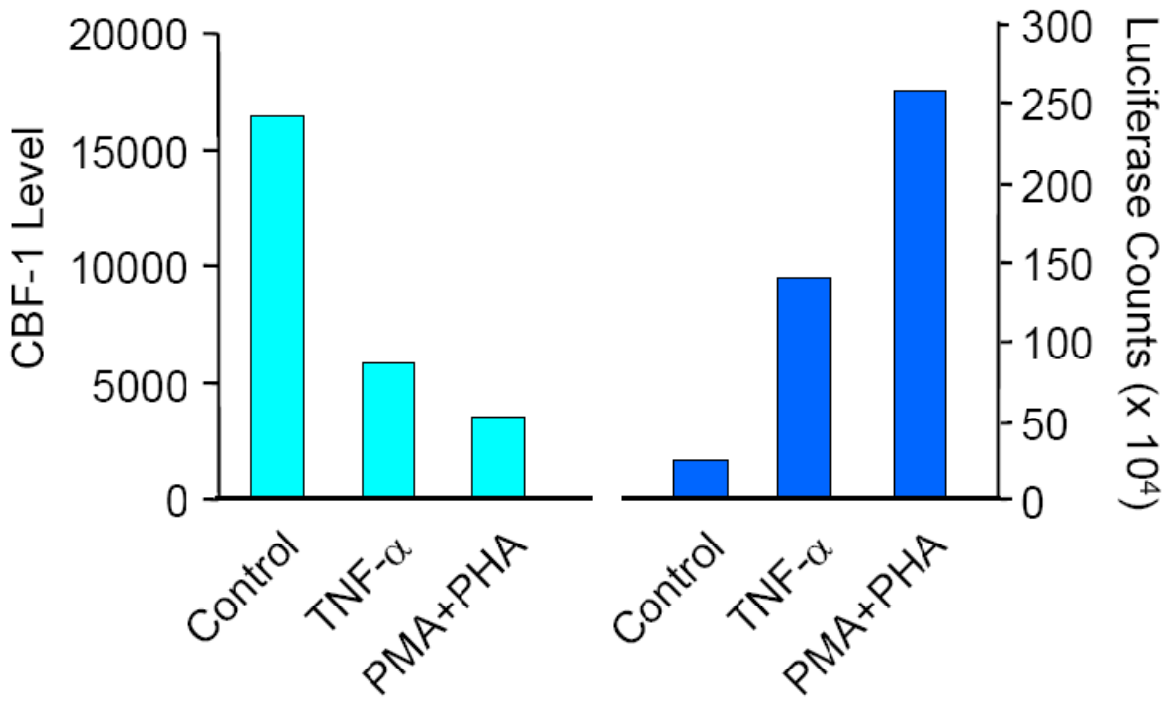

Figure S10. Activation of Jurkat cells by a variety of mitogenic stimuli downregulates CBF-1 mRNA levels. Jurkat-pHR'P-Luc cells were treated with either TNF-α (20 ng / ml) or with PMA (50 ng/ml) plus PHA (10 μg/ml) for 16 hrs, keeping untreated cells as control. Cell extracts were analyzed for luciferase activity (right panel) or used to measure CBF-1 mRNA levels by RT-PCR (left panel). As control for mRNA recovery we also quantified beta actin mRNA levels. In the data shown, CBF-1 gene levels were normalized according to beta actin mRNA levels. Note that induction of T-cells by either method resulted in strong down regulation of CBF-1 mRNA levels.