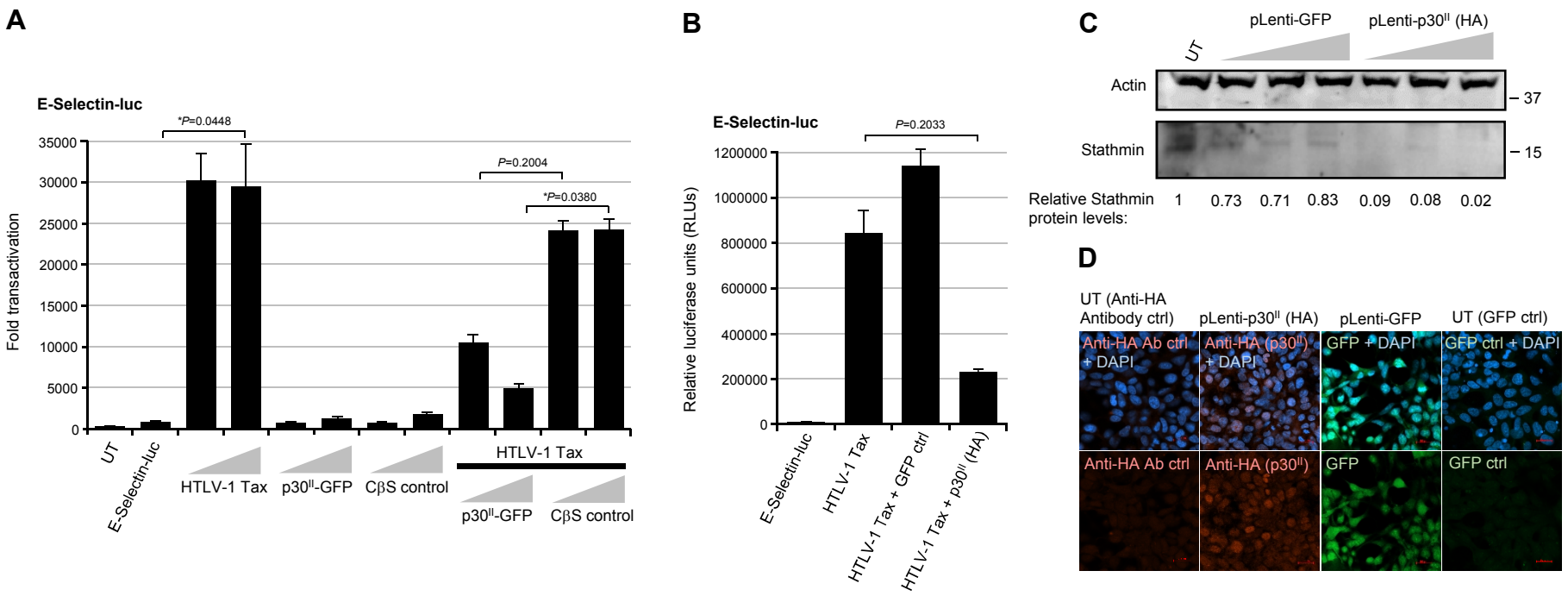
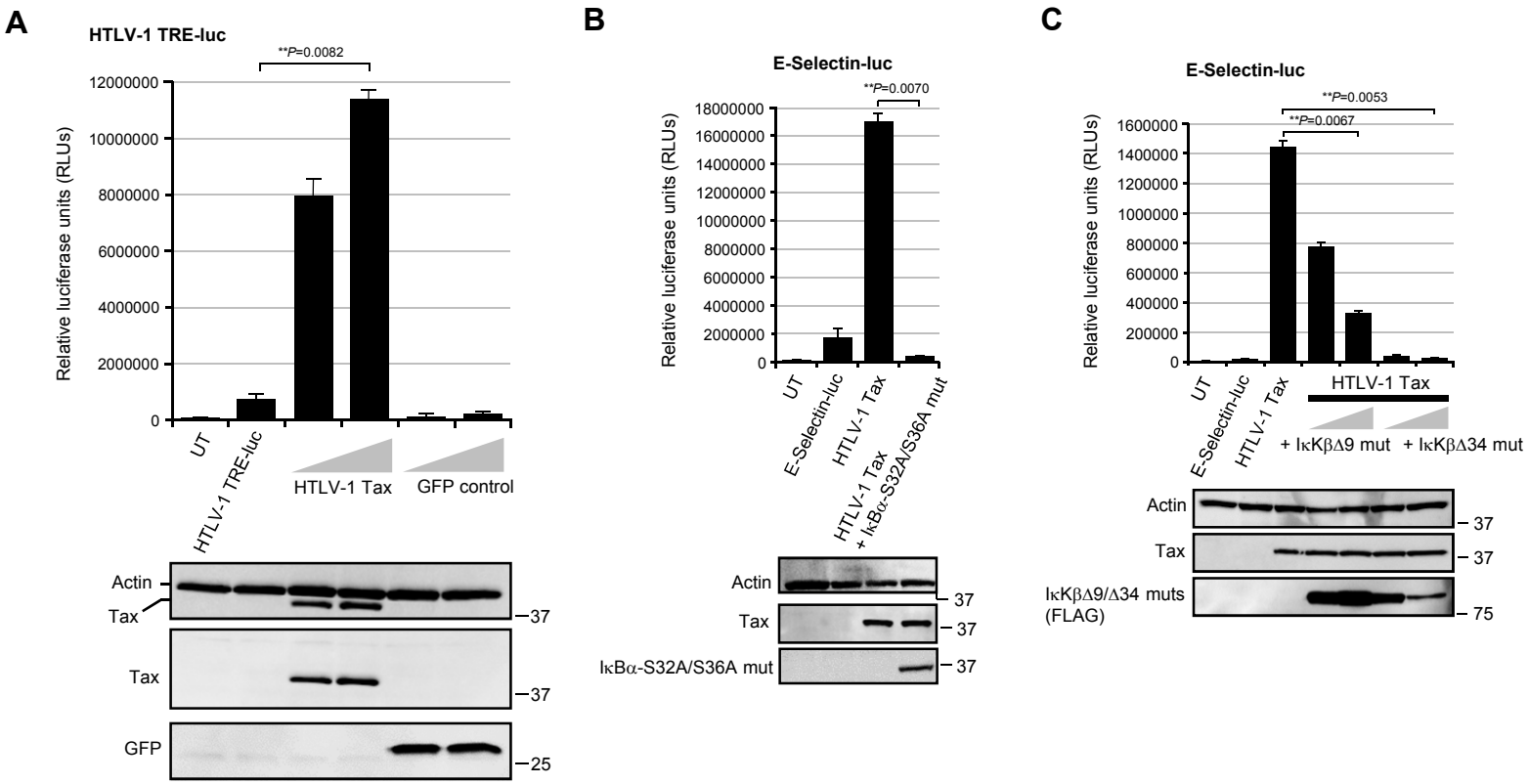
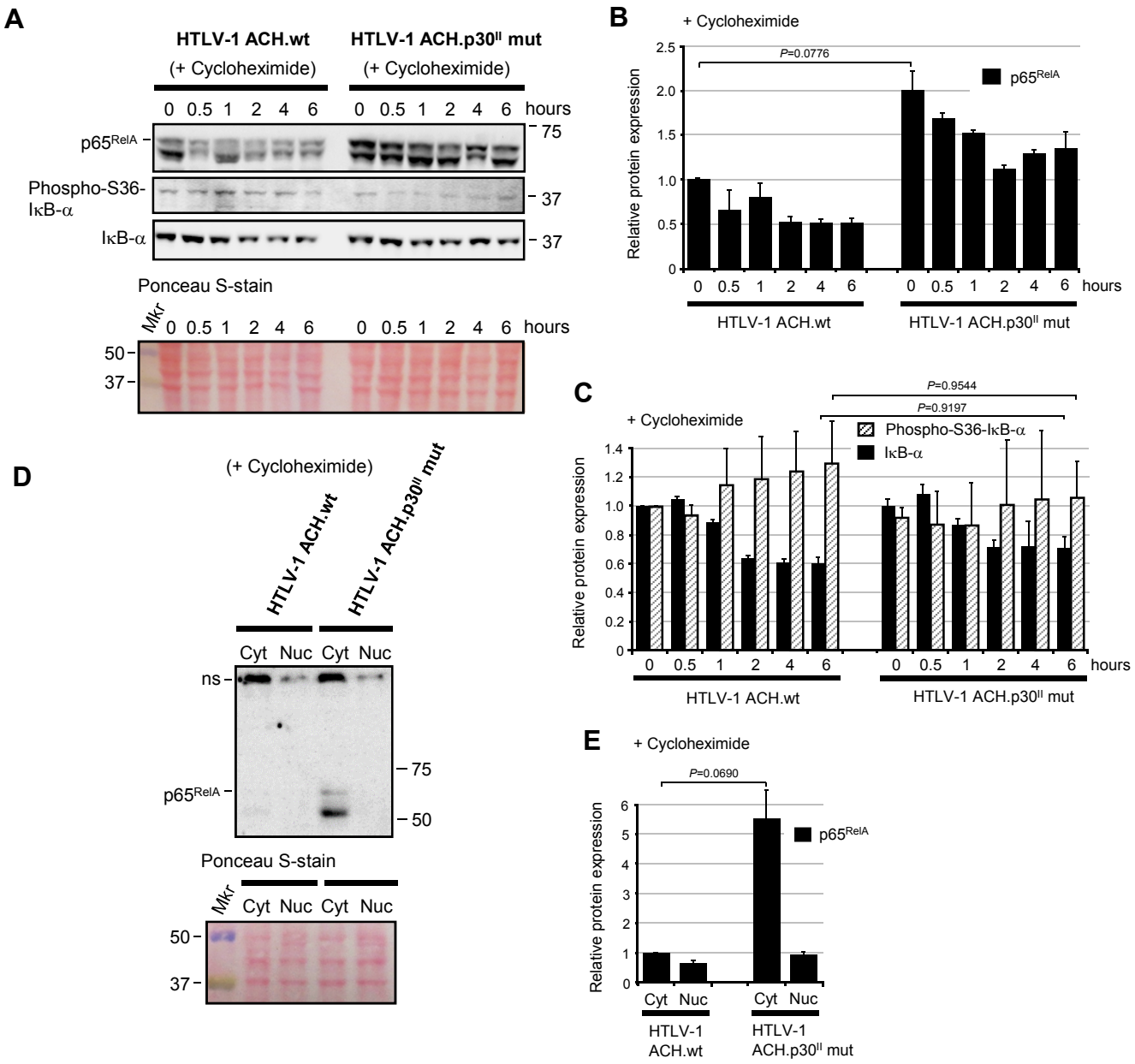


Supplemental Fig. S1. The HA-tagged HTLV-1 p30^{II} protein represses Stathmin expression and inhibits Tax-dependent NF-κB transactivation similar to p30^{II}-GFP. (A) The data from Fig. 1E which shows Tax-dependent transactivation of the *E-selectin* promoter-luciferase reporter plasmid and inhibition by p30^{II}-GFP are represented as 'Fold transactivation' for comparison. (B) 293 HEK cells were cotransfected with the *E-selectin*-luciferase reporter construct and an RcCMV-HTLV-1 Tax expression construct, and then transduced with lentiviral expression vectors for GFP or HTLV-1 p30^{II} (HA-tagged). Relative luciferase activities were measured and normalized for equivalent total cellular proteins. (C) The effects of pLenti-GFP and pLenti-HTLV-1 p30^{II} (HA) upon Stathmin expression were determined by transducing 293 cells with the lentiviral constructs and then performing SDS-PAGE and immunoblot analyses. The relative Stathmin protein levels were quantified by densitometry and normalized to Actin. (D) The expression of HTLV-1 p30^{II} (HA) and GFP in lentiviral transduced cultures was visualized by confocal microscopy either by immunostaining the samples with an Anti-HA tag monoclonal antibody (Abcam) and rhodamine red-conjugated secondary antibody (red signal), or by direct-fluorescence for GFP (green). DAPI nuclear-staining is provided for reference. Untransduced (UT) cells were included as negative controls for the Anti-HA antibody and GFP fluorescence detection (n=3). Scale bar, 20 μm.



Supplemental Fig. S2. Transactivation of the *E-selectin* gene promoter by HTLV-1 Tax is dependent upon NF-κB-signaling. (A) The functionality of the Tax protein used in these studies and its ability to transactivate the CREB-dependent Tax-responsive elements (TREs) within the HTLV-1 promoter was demonstrated by cotransfecting 293 HEK cells with 0.25 μg of an HTLV-1 TRE-luciferase reporter plasmid (Giebler et al., 1997) and increasing amounts (0.25 and 0.5 μg) of RcCMV-HTLV-1 Tax or a pcDNA3.1-GFP control. Relative luciferase activities were measured and normalized for equivalent total cellular protein levels. The expression of the HTLV-1 Tax, GFP, and Actin proteins was detected by SDS-PAGE and immunoblotting. (B and C) Dominant-negative mutants of IκBα or the IκKβ subunit inhibit Tax-induced NF-κB transactivation. 293 cells were cotransfected with an *E-selectin* promoter-luciferase reporter plasmid and either RcCMV-HTLV-1 Tax alone, or together with a phosphorylation/degradation-defective IκBα "super repressor" mutant, IκBα-S32A/S36A (DiDonato et al., 1996) in B. The HTLV-1 Tax, IκBα-S32A/S36A mutant, and Actin proteins were detected by immunoblotting. In C, 293 cells were cotransfected with the *E-Selectin*-luc reporter plasmid and RcCMV-HTLV-1 Tax alone, or with expression constructs (0.25 and 0.5 μg) for the dominant-negative IκKβ subunit deletion mutants, IκKβΔ9 or IκKβΔ34 (Sylla et al., 1998). Relative luciferase activities were then measured and normalized for equivalent total cellular proteins. The HTLV-1 Tax, IκKβΔ9 (FLAG), IκKβΔ34 (FLAG), and Actin proteins were detected by SDS-PAGE and immunoblotting. For A-C, the data represent the mean ± standard deviation (error bars) from three independent experiments.



Supplemental Fig. S3. The HT1080/HTLV-1 ACH.wt proviral clone contains reduced NF-κB p65^{RelA} levels relative to the ACH.p30^{II} mutant. (A) The HT1080/HTLV-1 ACH.wt and ACH.p30^{II} clones were treated with the protein synthesis-inhibitor, cycloheximide (50 μg/ml; Sigma-Aldrich), and the S36-phosphorylation and degradation of IκB-α and expression of the NF-κB p65^{RelA} subunit were detected by SDS-PAGE and immunoblotting. A representative Ponceau S-stained nitrocellulose membrane is shown for comparison. (B and C) The relative levels of p65^{RelA}, S36-phosphorylated IκB-α, and total IκB-α were quantified by densitometry analysis of the immunoblot bands. (D) Nuclear (Nuc) and cytoplasmic (Cyt) extracts were prepared from the HT1080/HTLV-1 ACH.wt and ACH.p30^{II} clones that were treated with cycloheximide for 2 hrs, and the relative levels of p65^{RelA} were detected by immunoblotting. A nonspecific band (ns) is also indicated. A representative Ponceau S-stained membrane is provided for comparison. (E) The expression of p65^{RelA} was quantified by densitometry. All the data is representative of three independent experiments. The data in B, C, and E represent the experimental mean ± standard deviation (error bars).