Supplementary Figure Legends

Supplementary Figure 1. C33A cells were transfected with wt or mutant luciferase construct and pcDNA3 (- Tat) or pcDNA-tat (+ Tat). pRL-CMV was co-transfected as an internal control. The firefly and *Renilla* luciferase activities in cell lysates were determined two day post transfection. Firefly luciferase activity was corrected for *Renilla* luciferase activity. One of >4 experiments is shown, error bars indicate standard deviations.

Supplementary Figure 2. Wild-type and mutant U2-LF, U3, U4 and U6 control constructs predominantly express Gag-Luc. C33A cells were transfected with wt or mutant luciferase construct and pcDNA3 (- tat) or pcDNA-tat (+ tat). pRL-CMV was co-transfected as an internal control. One of three independent experiments is shown. **A.** The firefly and *Renilla* luciferase activities in cell lysates were determined two day post transfection. Firefly luciferase activity was corrected for *Renilla* luciferase activity. Error bars indicate standard deviation. **B.** Equal amounts of *Renilla* luciferase activity were applied to SDS-PAGE. Luciferase protein was detected using Western blot analysis with anti-luciferase antibodies. The Gag-Luc position on the gel is indicated. M indicates mock-transfected cells.

Supplementary Figure 3. Relative representation of the data shown in Figure 6. C33A cells were transfected with wt or mutant luciferase construct in the absence or presence of 100 ng of the pLAI molecular clone. pRL-CMV was co-transfected as an internal control. The firefly and *Renilla* luciferase activities in the lysates were determined two

days post transfection. Firefly luciferase activity was corrected for *Renilla* luciferase activity. CA-p24 was determined in the supernatant and was consistent in all transfections (20.3 ng/ml average, 2.37 ng/ml standard deviation). Wt expression levels were set as 1.

Supplementary Figure 4. C33A cells were transfected with various luciferase constructs in the presence of an active (2A) or inactive (H20N) poliovirus 2A protease-expressing construct. Renilla was expressed from pRL-CMV, HIV-1 indicates the wt LTR-luc construct and ΔHIV-1 a derivative in which nts 80-335 of the HIV-1 5'UTR have been deleted. HCV and ΔHCV indicate a CMV IE-promoter-driven bicistronic firefly-renilla construct (kind gift of Dr. P. Bredenbeek) with an active and inactive hepatitis C virus IRES element as intercistronic region, respectively (82). The firefly and *Renilla* luciferase activities in cell lysates were determined two day post transfection. Expression of each construct in the presence of the H20N construct was set as 1. The only construct that fully withstands 2A co-expression is regulated by the wt HCV IRES. All other constructs show a significantly reduced luciferase expression in the presence of the poliovirus 2A protease. One of three experiments is shown, error bars indicate standard deviation.