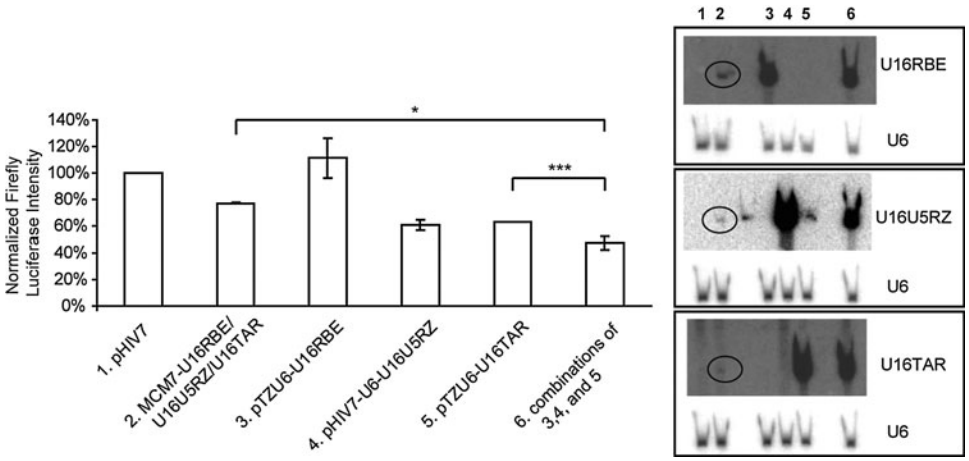
Supplementary Data



SUPPLEMENTARY FIG. S1. Anti-HIV RNA expression correlates with HIV-1 pNL4-3 luciferase knockdown activity. *Left:* HEK 293 cells were transfected at 80% confluency in a 48-well plate with 20 ng of replication-deficient pNL4-3 proviral DNA harboring the firefly luciferase gene (pNL4-3.Luc.R$^-$.E$^-$, NIH AIDS Research and Reference Reagent Program) and 3.2×10^{-2} pmol of plasmids with the anti-HIV RNAs driven either by the U1 or U6 promoter adjusted for constant mass of 300 ng with pBluescript carrier DNA complexed with Lipofectamine 2000 (Invitrogen). The pNL4-3 luciferase construct maintains targets for each of the small RNAs in all the transcripts, both spliced and unspliced, and therefore luciferase readouts can be used as quantitative readouts of viral inhibition. Firefly luciferase output was normalized to the internal control *Renilla* luciferase to account for differences in transfection efficiency. Data presented consist of two independent experiments. *$p<0.05$, ***$p<0.001$. *Right:* HEK 293 cells were transiently transfected at 90% confluency in a 6-well plate with 0.33 pmol of either the MCM7 cassette containing three snoRNAs or single snoRNA expressed with the U6 promoter (pTZ/U6-U16RBE, pHIV7-U6-U16U5RZ, or pTZ/U6-U16TAR), or their combinations adjusted for a constant mass of 4 ng with pBluescript carrier DNA complexed with Lipofectamine 2000 (Invitrogen). Total RNA was extracted 48 hr later with STAT-60 reagent according to the manufacturer's instructions. About 10 μg of total RNA was loaded per lane and RNA was detected with ^{32}P-labeled probes as described in Materials and Methods. Lane 1: pHIV7 (empty), lane 2: MCM7-U16RBE/U16U5RZ/U16TAR, lane 3: pTZ/U6-U16RBE, lane 4: pHIV7-U6-U16U5RZ, lane 5: pTZ/U6-U16TAR, lane 6: combinations of pTZ/U6-U16RBE, pHIV7-U6-U16U5RZ, and pTZ/U6-U16TAR.