Supplemental Figure 1  Arrest of gag maturation by Indinavir Sulfate and selective detection of processed gag p24 by ELISA.

293 T cells were transfected with HIV-1 LAI and maintained in the presence or absence of Indinavir Sulfate (10 µM). At the indicated time intervals, culture supernatants were harvested and analyzed by Western Blotting using a monoclonal antibody (Advanced Biotechnologies) that recognizes both precursor gag p55 and processed gag p24 (upper panel). These supernatants were further examined by an ELISA assay (Beckman Coulter) which selectively detects gag p24 (lower panel).
Supplemental Figure 2  Sensitivity and linearity of HIV-1 2-LTR circle cDNA detection by PCR.

A dilution series comprising between 20 and 200,000 copies of a cloned HIV-1 2-LTR circle junction (Sharkey et al., 2000) was analyzed by real-time PCR as detailed in Materials and Methods. Standard curves for 2 independent experiments, conducted on different days, are indicated.
Supplemental Figure 3  The reverse transcriptase inhibitor nevirapine prevents 2-LTR circle formation during viral dissemination in trans.

Macrophages were infected with VSV-G-pseudotyped HIV-1LAI as detailed in Materials and Methods and at 4 and 6 weeks post infection, PHA-activated peripheral blood lymphocytes were added in the presence and absence of nevirapine (5 µM). After 48 hours, lymphocytes were removed for quantitation of 2-LTR cDNA copy number by PCR.