

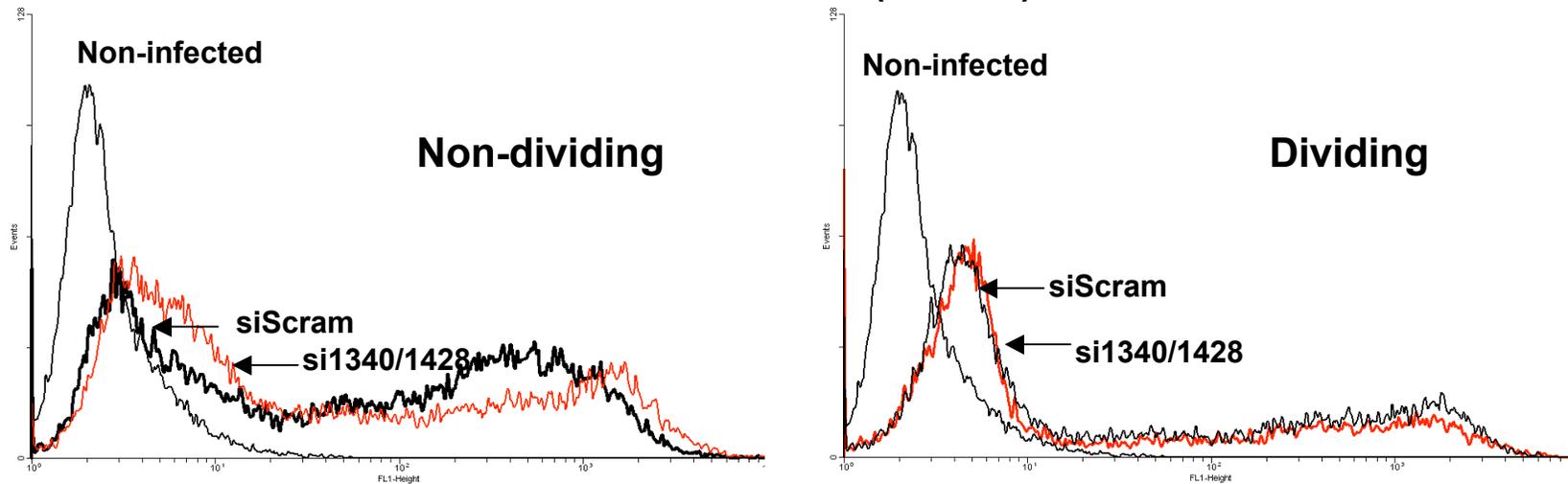
FIG. S1. LEDGF/p75 is a component of functional FIV PICs. This figure complements the data shown for HIV-1 vector PICs in Fig. 7. FIV vector PICs were isolated and used in an *in vitro* DNA integration assay before (left) and after (right) immunoprecipitation with LEDGF/p75 mAb or an irrelevant control mAb (anti-Myc). PICs were reacted with an *in vitro* target DNA (phiX174). Southern blotting was carried out with a probe for the viral genome. Integration products are detected as a higher M.W. band that is a fraction of the non-integrated PICs.

A functional integrase is required, as shown by use of class I FIV IN mutant D66V (the catalytic triad aspartate D66 corresponds to HIV-1 D64) See refs. 64 and 49 for validation of class I properties.

Note first that PICs are readily immunoprecipitated by mAb to LEDGF/p75 but not the control antibody. Second, as in the total fraction at left, integration-competence can be detected.

WT: wild type. mAb: monoclonal antibody.

FIV vector (GiNWF)



HIVeGFP

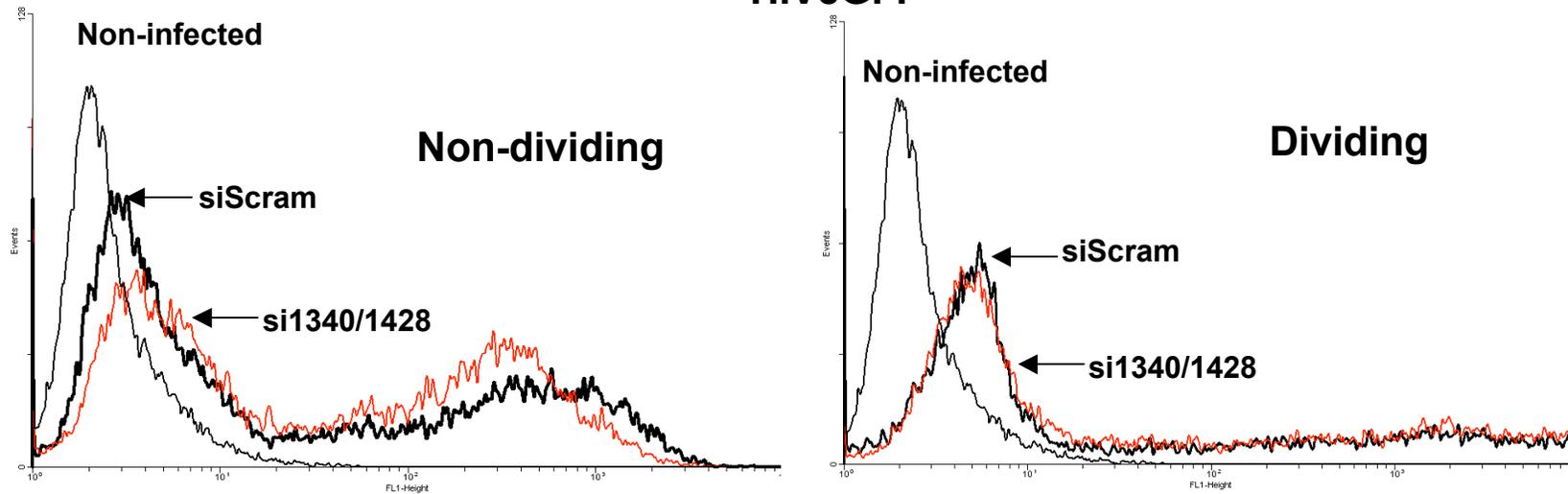
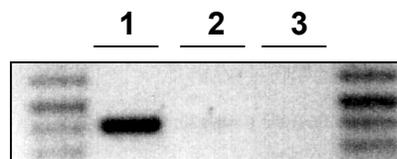


Fig. S2. Lentiviral vector transductions in the presence and absence of LEDGF/p75. Infections were done with LEDGF/p75-deficient si1340/1428 cells (red traces) and control siScram (heavy black traces) with HIVeGFP (bottom) and FIVeGFP (top). Each was done with aphidicolin growth arrest (left) or while proliferating (right) as described in Materials and Methods. Flow cytometry was done at 48 hours after transduction.



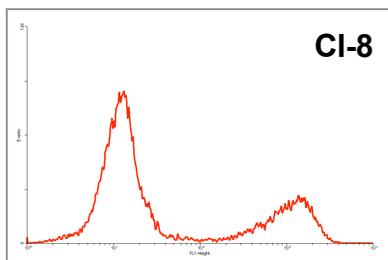
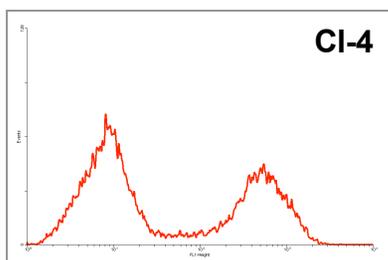
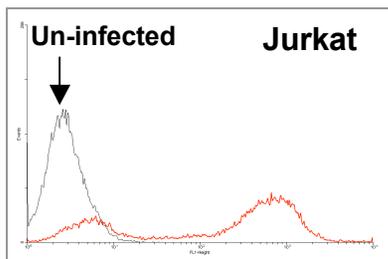
Lane 1: siScramJK

Lane 2: si1340JK cl-2

Lane 3: si1340Jk cl-12

Fig. S3. LEDGF/p75-specific RT-PCR in Jurkat-derived lines (si1340JK clones 2 and 12 and control siScramJK cells). RT reactions were performed with 500 ng of total RNA.

LEDGF/p75-WT cells



LEDGF/p75-deficient cells

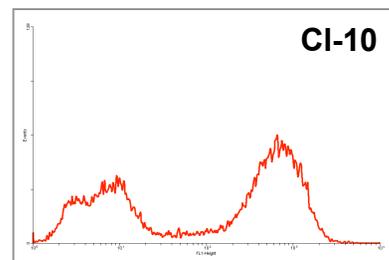
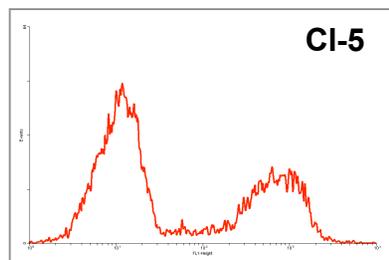
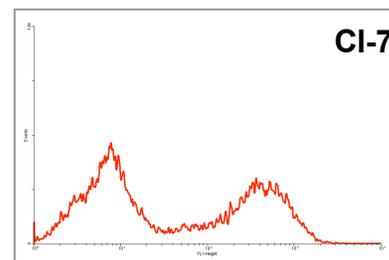
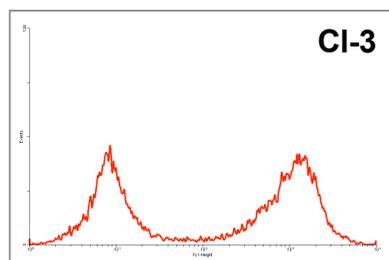
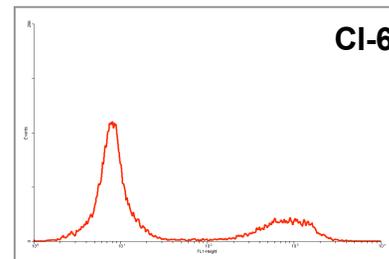
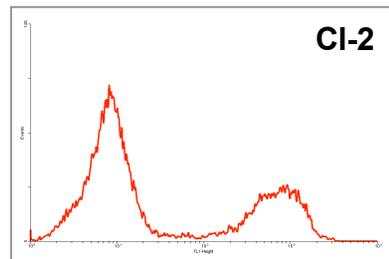
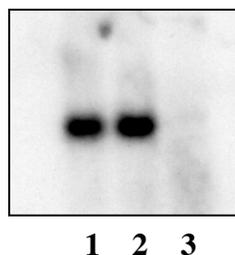


Fig. S4. Single round infection of Jurkat clones with HIVeGFP. GFP fluorescence (x-axis) was analyzed by flow cytometry 48 hours after transduction. No correlation was seen between LEDGF/p75 levels and infectivity (Refer to Fig. 5C for LEDGF/p75 immunoblotting).



Lane 1. si1340JK CL-2

Lane 2. si1340JK CL-2 BC

Lane 3. si1340JK CL-2, uninfected

Fig. S5. Equivalent viral DNA detected in si1340JK CL-2 and si1340JK CL-2 BC cells after infection with a replication-defective HIV-1 reporter virus and 18 days of continuous expansion in culture. Cells were infected at MOI 0.1 with NL43.R-E-.luc(VSV-G) for 16 hours, washed three times and kept in culture under logarithmic growth conditions in which daily cell division occurred. Genomic DNA was analyzed by Southern blotting 18 days after infection. Thirteen micrograms of genomic DNA prepared in parallel from each line were cleaved with Pst I. The probe was a randomly primed ³²P-labeled internal fragment (nt 1415 to 2839) that detects total viral cDNA. Junction blots using EcoR1 digestion with this probe and Alu-PCR studies are in progress. However, at this duration of passage we do not detect unintegrated circular or linear cDNAs in Southern blots or by 2-LTR circle PCR (data not shown).