Supplementary Material

SIR can be induced in LEDGF/p75-knockdown cells

An additional way of demonstrating the involvement of Rev expressed by unintegrated viral cDNA in promoting SIR is to make use of LEDGF/p75-knockdown cells (Llano *et al.*, 2006). The cellular LEDGF/p75 protein has been shown to be absolutely required for efficient integration of cDNA into host chromosomal DNA (Llano *et al.*, 2006; Maertens *et al.*, 2003; Vandekerckhove *et al.*, 2006). Very little or no integration is observed in viral-infected LEDGF/p75-knockdown cells (Llano *et al.*, 2006; Vandekerckhove *et al.*, 2006), rendering these cells equivalent to normal cells infected with INm virus (see Fig. 1). The results in Supplementary Fig. S2(a) show that, as expected, the integration values obtained following infection of the LEDGF/p75-knockdown cells with wt or VSV-g-coated viruses were very low, even in the presence of infectious viruses at an m.o.i.=10. However, even with these low integration levels, SIR (reduction in integration levels) was observed which followed a pattern similar to that obtained when normal cells were infected with INm virus (see Fig. 1). However, as we have demonstrated previously (Levin *et al.*, 2010), in contrast to the wt HIV-1 cDNA, that of the ΔRev virus can be efficiently integrated into LEDGF/p75-knockdown cells chromosomal DNA (Levin *et al.*, 2010). Thus, an experimental system in which LEDGF/p75-knockdown cells are first infected with a wt virus and secondly with a ΔRev virus should be comparable to the system in which normal cells are first infected with an INm virus and secondly with a ΔRev virus (Fig. 2). Indeed, the SIR pattern (Supplementary Fig. S2b) obtained in the infected LEDGF/p75-knockdown cells was very similar to that shown in Fig. 2, again indicating that Rev-ear expressed from unintegrated DNA confers SIR. The fact that this resistance is transient and the integration level per cell is relatively high clearly indicate that the ΔRev virus cDNA is integrated into the LEDGF/p75-knockdown cells (see also Levin *et al.*, 2010). The timescale of the obtained SIR correlates with the presence viral cDNA and Rev protein (Supplementary Fig. S2c, d).

References

Levin, A., Rosenbluh, J., Hayouka, Z., Friedler, A. & Loyter, A. (2010). Integration of HIV-1 DNA is regulated by interplay between viral Rev and cellular LEDGF/p75 proteins. *Mol Med* **16**, 34–44. Medline

Llano, M., Saenz, D. T., Meehan, A., Wongthida, P., Peretz, M., Walker, W. H., Teo, W. & Poeschla, E. M. (2006). An essential role for LEDGF/p75 in HIV integration. *Science* **314**, 461–464. Medline

Maertens, G., Cherepanov, P., Pluymers, W., Busschots, K., De Clercq, E., Debyser, Z. & Engelborghs, Y. (2003). LEDGF/p75 is essential for nuclear and chromosomal targeting of HIV-1 integrase in human cells. *J Biol Chem* **278**, 33528–33539. Medline

Vandekerckhove, L., Christ, F., Van Maele, B., De Rijck, J., Gijsbers, R., Van den Haute, C., Witvrouw, M. & Debyser, Z. (2006). Transient and stable knockdown of the integrase cofactor LEDGF/p75 reveals its role in the replication cycle of human immunodeficiency virus. *J Virol* **80**, 1886–1896. Medline

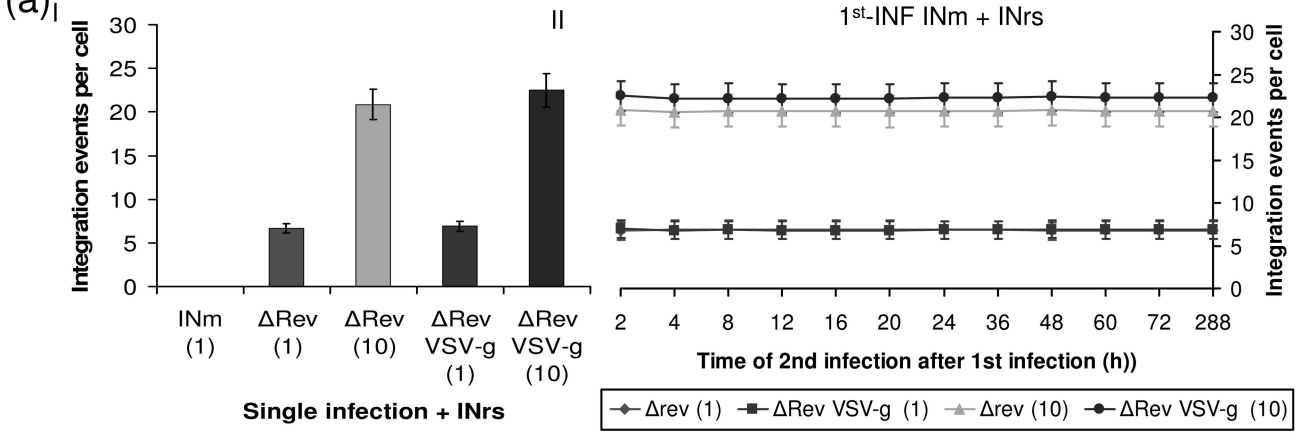
Supplementary Fig. S1. Eradication of SIR – presented in Fig. 2 – by INr peptides. (a) Same as Fig. 2(a) but with the addition of 150 μM INr peptides 2 h before the (i) single infection or (ii) 2nd-INF. (b) Co-immunoprecipitation and Western blot experiments of the experimental systems presented in (a, ii). All experimental details are as described in Methods.

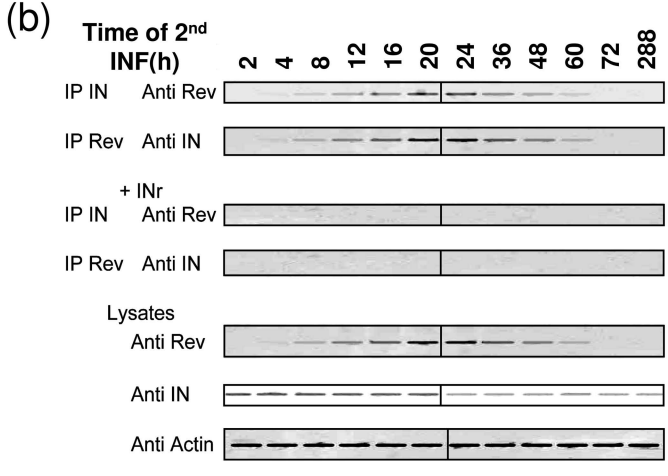
Supplementary Fig. S2. SIR is also observed in LEDGF/p75-knockdown cells. (a) Integration events per cell were estimated in samples taken from LEDGF/p75-knockdown cells that were subjected to (i) single infection or (i) 1st-INF with a wt HIV-1 at an m.o.i.=1 and then 2nd-INF with the indicated wt viruses at an m.o.i.=1 or 10. (b) Same as in (a), but with the indicated ΔRev HIV-1. (b, ii) Inset, magnification of the marked section. (c) Quantitative analysis of viral cDNA in LEDGF/p75-knockdown cells at different times p.i. with wt HIV-1 at an m.o.i.=1. (d, i) Western blot analysis of samples taken from LEDGF/p75-knockdown or wt cells infected with wt HIV-1 at different times p.i. (ii) Bands were quantitatively estimated by Image Gauge V3.0 software from Fujifilm. All other experimental details are as described in Methods.

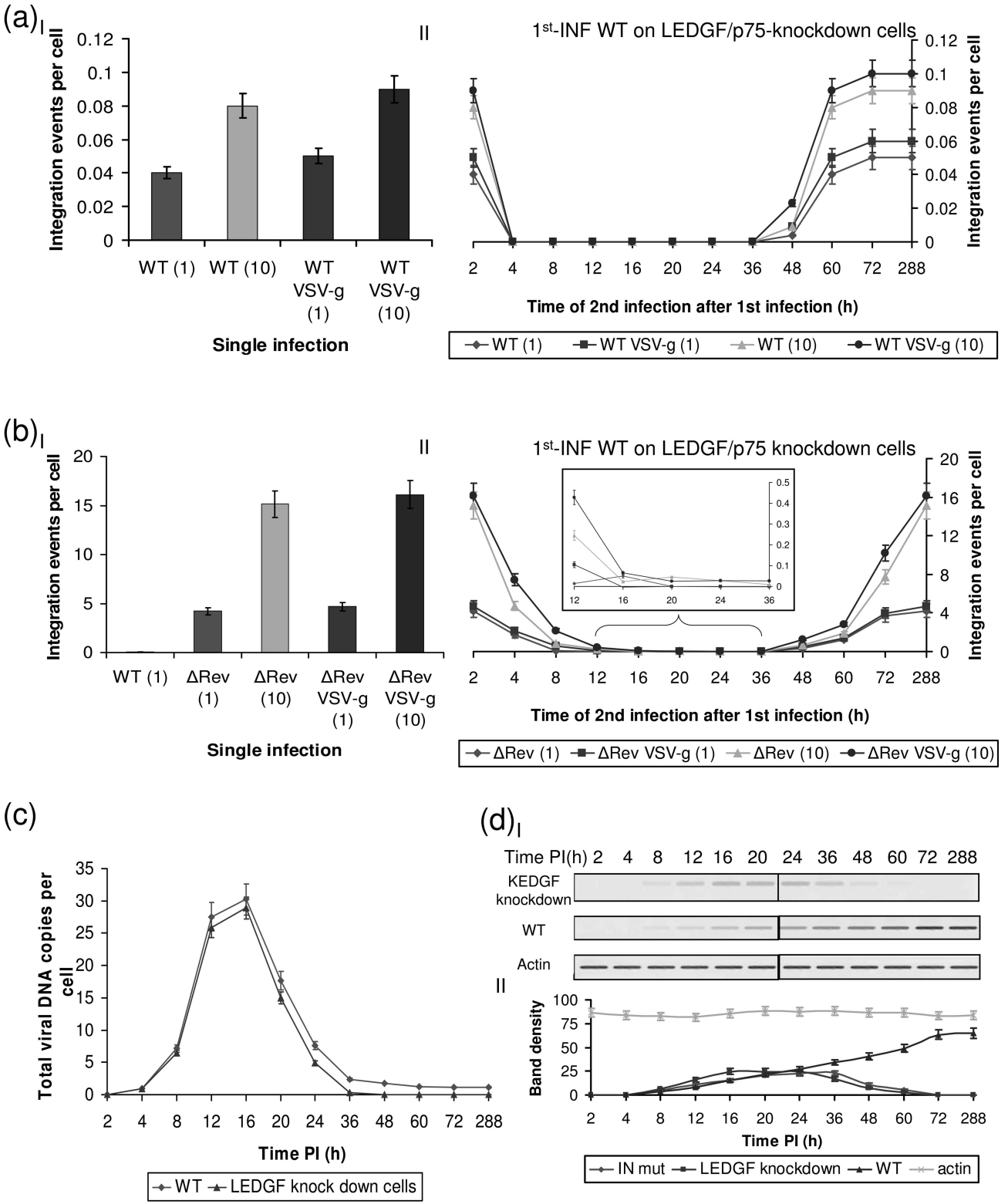
Supplementary Fig. S3. Integration is observed following infection of CD4 cells with VSV-g-coated but not wt HIV. Integration events per cell were estimated following (i) single infection with the indicated viruses or (ii) 1st-INF with the VSV-g-coated HIV-1 at an m.o.i.=1 and then a 2nd-INF with the indicated wt viruses at an m.o.i.=1 or 10, at different time post-1st-INF. All other experimental details are as described in Methods.

Supplementary Fig. S4. Eradication of SIR – presented in Fig. 4(a) – by INr peptides. (a) Same as Fig. 5(a) but with the addition of 150 μM INr peptides 2 h before (i) single infection or (ii) 2nd-INF. (b) Co-immunoprecipitation and Western blot experiments with the systems presented in (a, ii). All experimental details are as described in Methods.

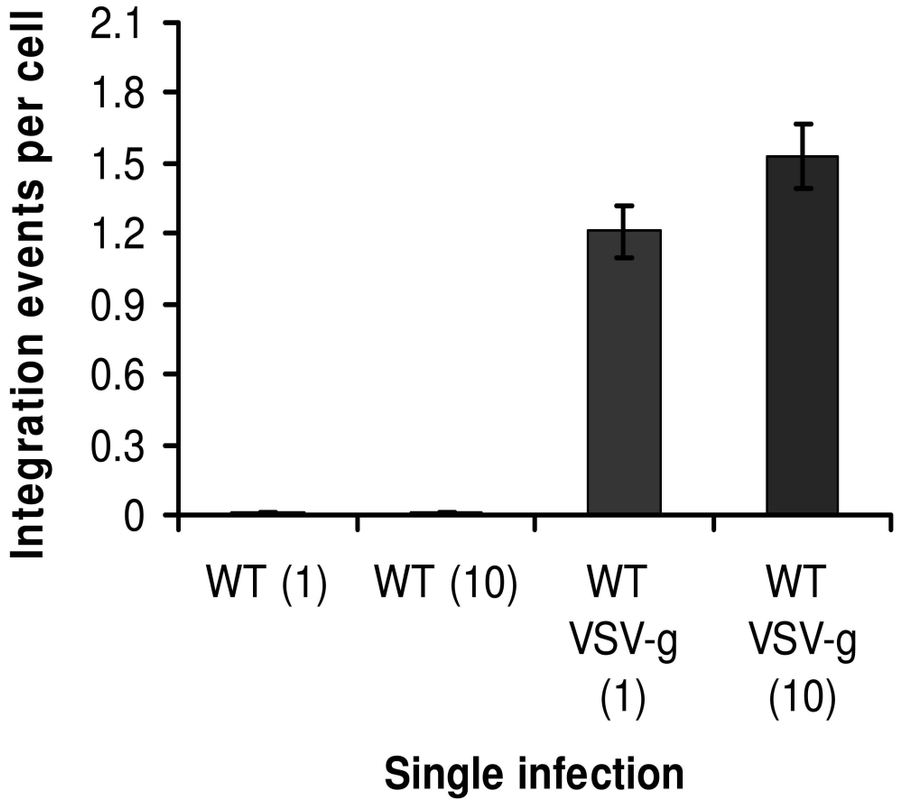
Supplementary Fig. S5. Eradication of SIR – presented in Fig. 4(b) – by INr peptides. (a) Same as Fig. 5b, but with the addition of 150 μM INr peptides 2 h before the (i) single infection or (ii) 2nd-INF. (b) Co-immunoprecipitation and Western blot experiments of the experimental systems presented in (a, ii). All experimental details are as described in Methods.


(a)
I
II
Integration events per cell
30
25
20
15
10
5
0
INm (1)
ΔRev (1)
ΔRev (10)
ΔRev VSV-g (1)
ΔRev VSV-g (10)
Single infection + INrs
1st-INF INm + INrs
2
4
8
12
16
20
24
36
48
60
72
288
Time of 2nd infection after 1st infection (h))
Δrev (1)
ΔRev VSV-g (1)
Δrev (10)
ΔRev VSV-g (10)

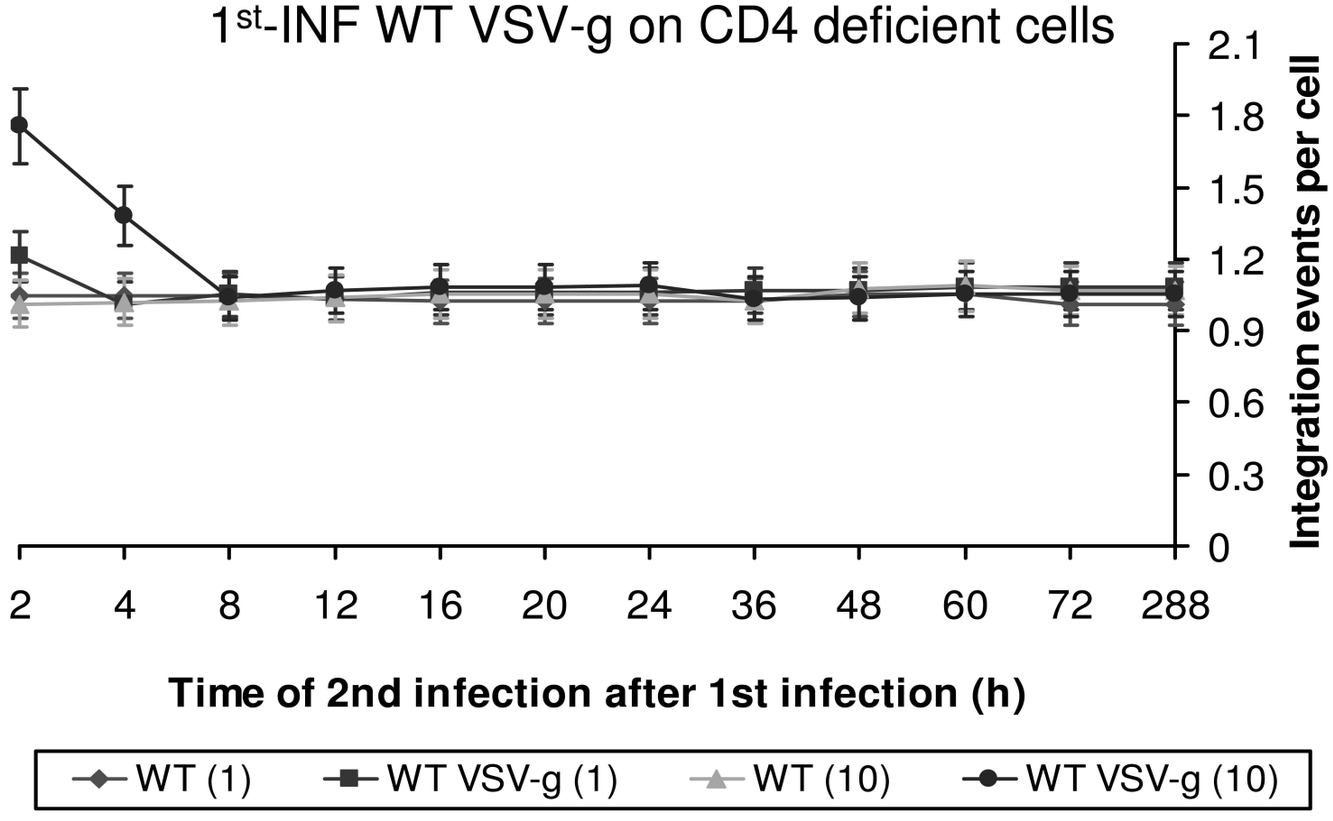

(b)
Time of 2nd INF(h)
2
4
8
12
16
20
24
36
48
60
72
288
IP IN
Anti Rev
IP Rev
Anti IN
+ INr
IP IN
Anti Rev
IP Rev
Anti IN
Lysates
Anti Rev
Anti IN
Anti Actin

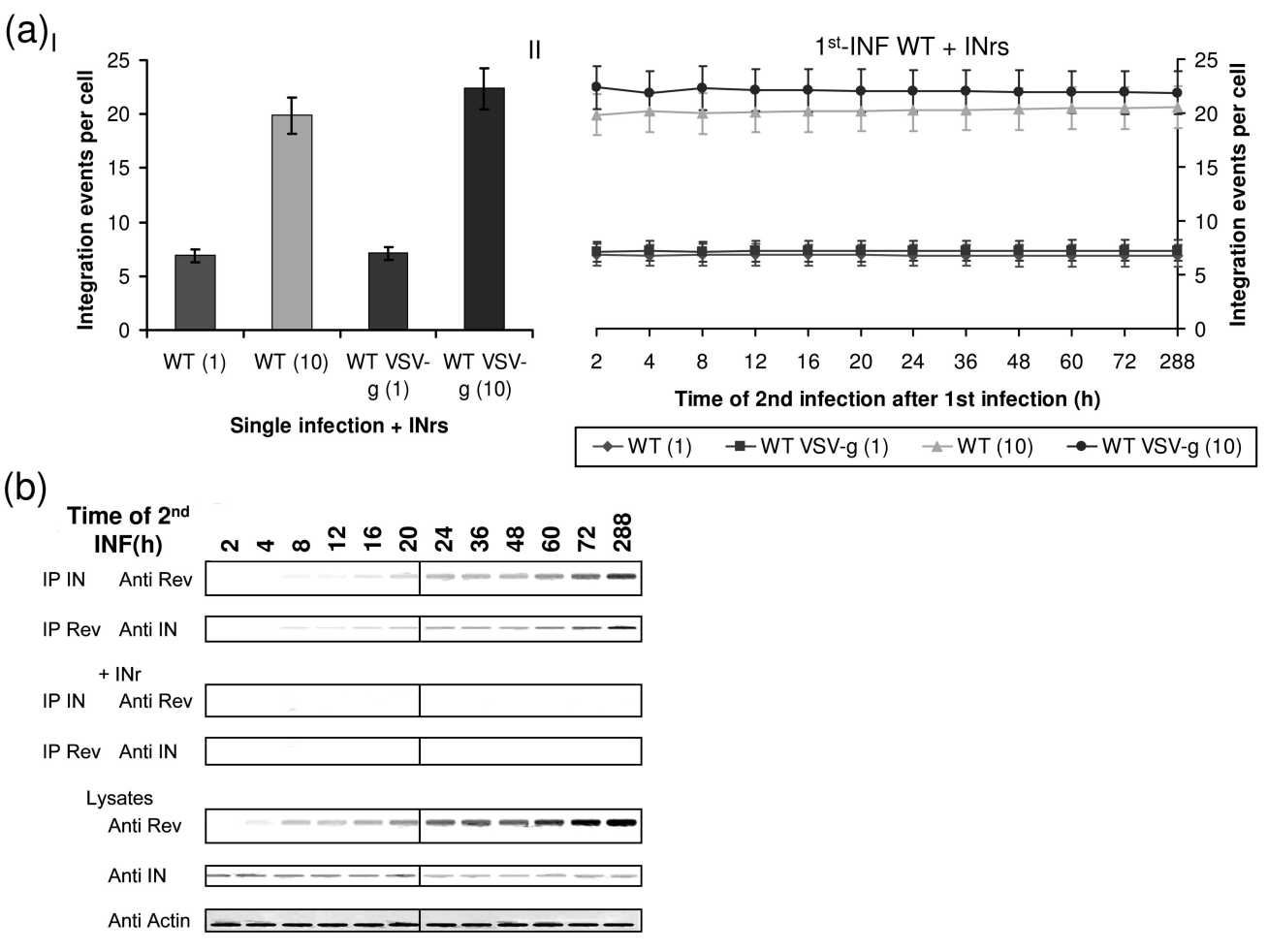

(a)
I
Integration events per cell
0.12
0.1
0.08
0.06
0.04
0.02
0
WT (1)
WT (10)
WT VSV-g (1)
WT VSV-g (10)
Single infection
II
1st-INF WT on LEDGF/p75-knockdown cells
Integration events per cell
2 4 8 12 16 20 24 36 48 60 72 288
Time of 2nd infection after 1st infection (h)
WT (1)
WT VSV-g (1)
WT (10)
WT VSV-g (10)
(b)
I
Integration events per cell
20
15
10
5
0
WT (1)
ΔRev (1)
ΔRev (10)
ΔRev VSV-g (1)
ΔRev VSV-g (10)
Single infection
II
1st-INF WT on LEDGF/p75 knockdown cells
Integration events per cell
0.5
0.4
0.3
0.2
0.1
0
12 16 20 24 36
2 4 8 12 16 20 24 36 48 60 72 288
Time of 2nd infection after 1st infection (h)
ΔRev (1)
ΔRev VSV-g (1)
ΔRev (10)
ΔRev VSV-g (10)
(c)
Total viral DNA copies per cell
35
30
25
20
15
10
5
0
2 4 8 12 16 20 24 36 48 60 72 288
Time PI (h)
WT
LEDGF knock down cells
(d)
I
Time PI(h) 2 4 8 12 16 20 24 36 48 60 72 288
KEDGF knockdown
WT
Actin
II
Band density
100
75
50
25
0
2 4 8 12 16 20 24 36 48 60 72 288
Time PI (h)
IN mut
LEDGF knockdown
WT
actin

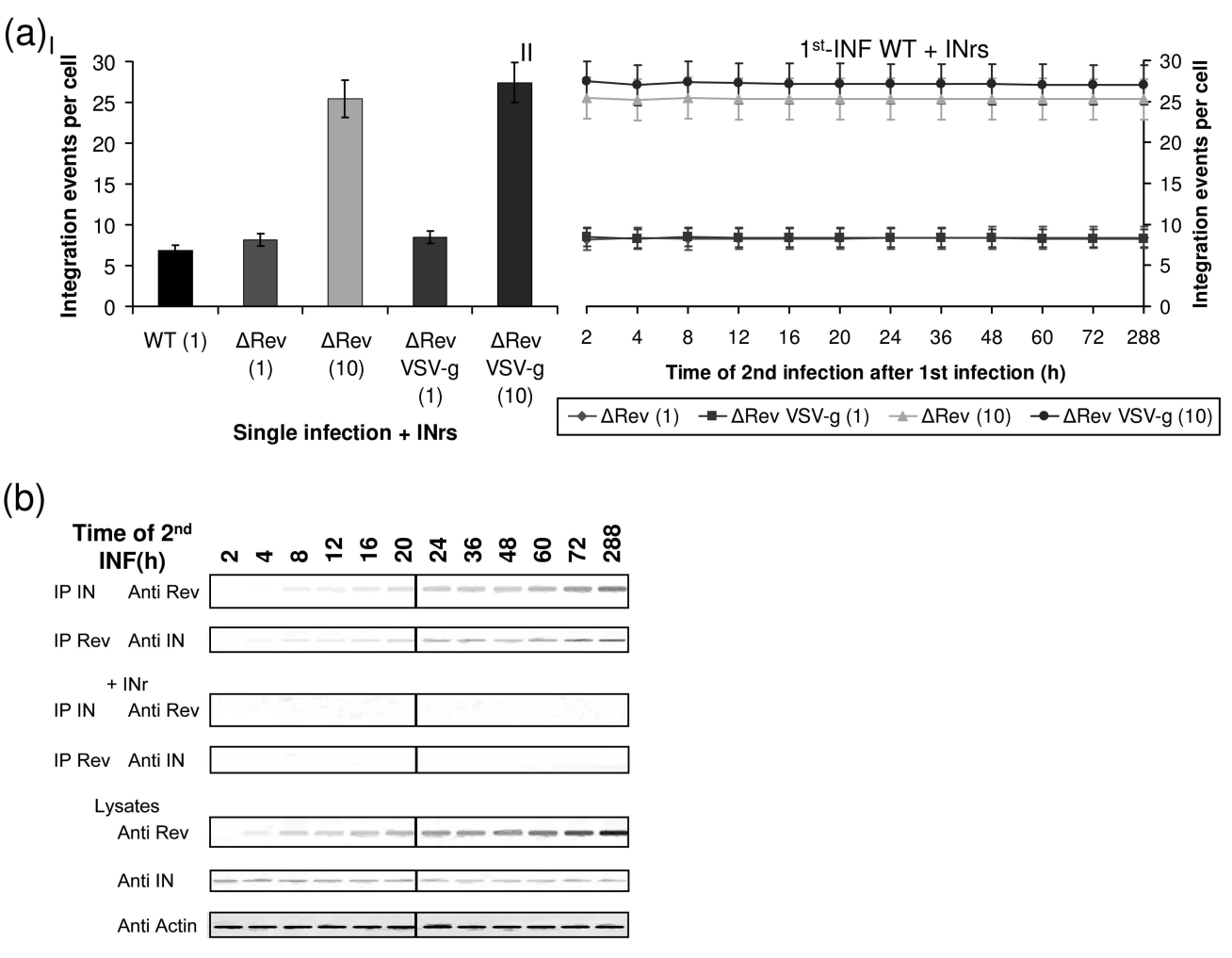
I


Integration events per cell
2.1
1.8
1.5
1.2
0.9
0.6
0.3
0
WT (1)
WT (10)
WT VSV-g (1)
WT VSV-g (10)
Single infection

II


1st-INF WT VSV-g on CD4 deficient cells
Integration events per cell
2.1
1.8
1.5
1.2
0.9
0.6
0.3
0
2
4
8
12
16
20
24
36
48
60
72
288
Time of 2nd infection after 1st infection (h)
WT (1)
WT VSV-g (1)
WT (10)
WT VSV-g (10)


(a)
I
II
Integration events per cell
25
20
15
10
5
0
WT (1)
WT (10)
WT VSV-g (1)
WT VSV-g (10)
Single infection + INrs
1st-INF WT + INrs
2
4
8
12
16
20
24
36
48
60
72
288
Time of 2nd infection after 1st infection (h)
WT (1)
WT VSV-g (1)
WT (10)
WT VSV-g (10)
(b)
Time of 2nd INF(h)
IP IN
Anti Rev
IP Rev
Anti IN
+ INr
Lysates
Anti Actin


(a)
I
II
Integration events per cell
30
25
20
15
10
5
0
WT (1)
ΔRev (1)
ΔRev (10)
ΔRev VSV-g (1)
ΔRev VSV-g (10)
Single infection + INrs
1st-INF WT + INrs
2
4
8
12
16
20
24
36
48
60
72
288
Time of 2nd infection after 1st infection (h)
ΔRev (1)
ΔRev VSV-g (1)
ΔRev (10)
ΔRev VSV-g (10)
(b)
Time of 2nd INF(h)
IP IN Anti Rev
IP Rev Anti IN
+ INr
IP IN Anti Rev
IP Rev Anti IN
Lysates
Anti Rev
Anti IN
Anti Actin