Supplementary information

Materials and Methods

Generation of a moesin Cre-Lox system

pMC-Cre (Bothe et al., 2000), expressing the Cre recombinase, was stably introduced into Rat2 cells by cotransfection with HIV-1-neo, CSNW (Hatziioannou et al., 2003), using FuGENE 6 transfection reagent (Roche). Cells were then subjected to selection in G418 (500 μg/ml) 48 h after transfection until colony formation was observed. Four neomycin-resistant clones were then expanded and tested for resistance to MoMLV-puro or HIV-1-puro virus. Deletion of N-Msn-zeo was monitored by PCR using primers flanking the cloning site (primers 10 and 11 in Table S2 in the Supplemental data).

Generation of cells stably expressing unfused moesin variants

Unfused moesin variants were amplified and subcloned into pcDNA3.1$^+$ vectors (Invitrogen) using the following primers (see Table S2 in the Supplemental data); full-length moesin (primers 1 and 2) or C-terminally truncated moesin (primers 1 and 4). 3x10^6 Rat2 cells were then transfected with 4μg of the moesin constructs or the control empty pcDNA3.1$^+$ vector as described (Naghavi et al., 2005). 48 h post-transfection cells were selected in G418 (500 μg/ml) and several resistant clones of each stable line were further tested in transduction assays.

Production of HIV-1 pseudotyped particles containing Vpr-Blam

HIV-1 containing Vpr-Blam virus pseudotyped with VSV-G envelope was produced by transfection of 293T cells using 1 μg p8.91, 1 μg pMDG, 1.5 μg pCSPW and 3 μg pCMV4-3 Blam-Vpr vector (Cavrois et al., 2002) as described (Naghavi et al., 2005). The virus was concentrated by ultracentrfiguation at 17,000g for 2 h a 4°C and tittered by

infection of Rat2 cells and colony counting after selection in puromycin (1.5 μg /ml) as described (Naghavi et al., 2005).

Vpr-Blam fussion assay

Target cells were plated at 2×10^5 cells/well in a 48-well dish one day before infection. Infection was carried out for 6 h at 37°C using 100 μl HIV-1-Vpr-Blam virus in the presence of 8 μg /ml polybrene. Infected cells were washed using CO2-independent media and targeted with 100 μl of CCF2-AM (Invitrogen) for 1 h at room temperature (RT) in the dark as described (Cavrois et al., 2002). Cells were then washed with 200 μl development media and incubated at RT for 16 h in the dark. The number of blue (cleaved CCF2) cells were determined by FACS after excitation at 405 nm.

References

Bothe, G.W., Haspel, J.A., Smith, C.L., Wiener, H.H. and Burden, S.J. (2000) Selective expression of Cre recombinase in skeletal muscle fibers. *Genesis*, **26**, 165-166.

Hatziioannou, T., Cowan, S., Goff, S.P., Bieniasz, P.D. and Towers, G.J. (2003) Restriction of multiple divergent retroviruses by Lv1 and Ref1. *Embo J*, **22**, 385-394.

Figure Legends

Figure S1. Outline of the cDNA library screening strategy.

Figure S2. N-Msn-zeo cDNA is responsible for the resistance to retroviral infection. Cre recombinase was stably introduced into one of the virus-resistant Rat2 N-Msn-zeo stable lines isolated, RL4, by cotransfection with HIV-1-neo. Four neomycin resistant clones (RL4-Cre:1, 7, 10 and 11) were expanded and tested for resistance to MoMLV-puro (A) or HIV-1-puro (B). Wt Rat2 fibroblasts, Rat2; Rat2 cells containing empty pBabe-HAZ

vector, Rat2-HAZ; control virus resistant cells; R4-7. (C-D) Deletion of N-Msn-zeo and presence of the control GAPDH DNAs were monitored by PCR. Position of the PCR-DNAs diagnostic of N-Msn-zeo (C) and GAPDH (D) are indicated; M, marker DNAs. Data are typical of those obtained in at least three independent experiments.

Figure S3. Both full-length or C-terminally truncated untagged moesin induce retroviral resistance in Rat2 cells. Cells stably expressing full length, Msn:6, or C-terminally truncated moesin, N-Msn:5, or stably transduced with the empty vector, pcDNA:9, were incubated with various amount of HIV-1-puro (A) or MoMLV-puro (B) viruses. Wt Rat2 cells, Rat2; the control virus resistant line, R4-7. Cells were selected and the number of puromyin resistant colonies was counted 10 days after infection. Data are typical of those obtained in at least three independent experiments. (C) Quantitative RT-PCR showing the level of transgene moesin expression in Rat2 cells. Cytoplasmic RNA prepared from the same cell lines used in the transduction assay was converted to ds cDNA and used as template for RT-PCR using primers unique to transgene moesin cDNA sequences. The fold-change ratio between the Msn or N-Msn lines and the control Rat2-HAZ line are median copy numbers normalized to GAPDH copy numbers obtained in duplicate from two independent RNA preparations.

Figure S4. Vpr-Blam fussion assay suggests a post entry block to infection. (A) Wt Rat2 fibroblasts, Rat2; the control virus-resistant R4-7 cells; or the Rat2 lines containing pBabe-HAZ vector expressing N-Msn-zeo, RL4 either uninfected (upper panels) or infected (lower panels) with HIV-1-Vpr-Blam virus pseudotyped with VSV-G envelope protein were targeted with CCF2-AM. The next day the number of green (uncleaved CCF2) versus blue (cleaved CCF2) cells were determined by FACS after excitation at

430 nm and 405 nm, respectively. Similar results were obtained in at least three independent experiments. (B) Titration of the viral block using HIV-1-Vpr-Blam virus on rat cells. Wt Rat2 cells, R4-7, RL4 or cells stably expressing full length, Msn:6, or C-terminally truncated moesin, N-Msn:5 were infected with 10-fold serially diluted HIV-1-Vpr-Blam virus. Cells were selected and the number of puromyin resistant colonies was counted 10 days after infection.

Figure S5. Both full-length or C-terminally truncated moesin block replication of HIV-1 pseudotyped with amphotropic envelope glycoprotein. Cells stably expressing unfused full length moesin, Msn:6, or zeocin fused C-terminally truncated moesin, RL34, or stably transduced with the empty vector, pcDNA:9, were incubated with various amount of a HIV-1 virus carrying a amphotropic envelope protein (HIV-1-ampho-puro). Wt Rat2 cells, Rat2; the control virus resistant line, R4-7. Cells were selected and the number of puromyin resistant colonies was counted 10 days after infection. Similar results were obtained in at least three independent experiments.

Figure S6. Moesin cDNA is responsible for the loss of stable microtubules in the moesin overexpressing Rat2 lines. Two neomycin resistant clones, generated from the N-Msn-zeo overexpressing line RL4, in which the cDNA was excised, RL4-cre:1, or retained, RL4-cre:10, or Rat2 cells containing empty pBabe-HAZ vector, Rat2-HAZ, were fixed and stained with a rabbit polyclonal antibody for stable Glu-MTs and a rat mAb for dynamic Tyr-MTs followed with incubation with fluorescently labeled secondary antibodies. Images were taken as described in Materials and Methods.

Table S1. Genes recovered from the cDNA library screen.

Gene	Accession number	# hits
Moesin	NM_030863	11
E130018b19Rik protein	XM_219363	4
Putative nuclear protein	XM_217470	2
Prolyl 4-hydroxylase α	XM_340798	1
Sorting nexin 6	XM_341993	1
Splicing factor 3b, subunit 2	XM_215182	1
Chromosome 11 clone RP23-271013	AC084407	1
Clone IMAGE: 6919104	BC058445	1
B230396K1Rik	XM_205737	1
BAC clone PR24-84013	AC135633	1
Chromosome 1 Clone PR11-198A7	AL596342	1

Table S2. Primers used for amplification and quantification of moesin.

Primer #	Primer ID	Sequences
1	Msn-S1-EcoRI	5'GCTGATGAATTCGCCACCATGCCGAAGACGATCAGTGTTC3'
2	Msn-A1-NotI	5'GCAACTGCGGCCGCCTACATGGACTCAAATTCATCAATGCG3'
3	Msn-A2-NotI	5'GCAACTGCGGCCGCCATGGACTCAAATTCATCAATGCG3'
4	Msn-A3-NotI	5'GCAACTTGGTCAACTTGGCGCGGCCGCCCT3'
5	Zeo-A-XbaI	5'GCAACGTCTAGATCAGTCCTGCTCCTCGGCCAC3'
6	pCMV-forward	5'CGCAAATGGGCGGTAGGCGTG3'
7	Moesin-S1	5'GGAGATGTATGGTGTGAAC3'
8	pHAZ-AP (2041)	5'GACCACTCGGCGTACAGCTCGTCCAGGC3'
9	Msn-A6	5'GCAGAAAGAACAGGCGCTGG3'
10	pHAZ-SP	5'GCTTATCCATATGATGTTCCAGATT3'
11	pHAZ-AP (1906)	5'AAGTCCCGGGAGAACCCGAGCCGGTCG3'

Underlined sequences are the restriction enzyme sites.