Supplementary Methods

Plasmid Construction

PCR products for TAP, Magoh, RNPS1, UPF3B and Y14 were amplified by RT-PCR from HeLa cell total mRNA using the Titan RT-PCR system (Roche) with the following primers:
TAP: 5'gggcgggatccgccaccatggcggacgaggggaagtcgtacagcgaacacgat and
5'ccgcgaattccttcatgaatgccacttctgggatc,
Magoh: 5'gcggcggaattcgccaccatggagagtgacttttatctgcgt and
5'cgccgctctagagattggtttaatcttgaagtg,
RNPS1: 5'gcggcggaattcgccaccatggatttatcaggagtgaaaaag and
5'cgccgctctagatcgggaggagttggagctgga,
Y14: 5'gcgggggatccgccaccatggcggacgtgctagatcttcac and
5'ccccgcactcgagcgacgtctccggtctggact,
UPF3B: 5'gcgcggggatccgccaccatgaaggaagagaaggagcac and
5'cccgccactcgagcgctcctctcctccttcttt. The cDNA for murine REF was isolated by RT-PCR using mRNA isolated from mouse liver using the following oligonucleotides:
5'ggcggggatccgccaccatggccgacaaaatggacatgtct and
5'gcgggggcctctagactgctggtgtccatccttgcattgta. TAP was subcloned into pcDNA3MycHisA as a *Bam*HI-*Eco*RI fragment, Y14 and UPF3B were subcloned into pcDNA3MycHisB as a *Bam*HI-*Xho*I fragment, RNPS1 and Magoh were subcloned into pCDNA3MycHisA as *Eco*RI-*Xba*I fragments. REF was subcloned into pCDNA3MycHisB on a *Bam*HI-*Xba*I fragment. The human H1 promoter was amplified from HeLa cell genomic DNA and subcloned into pBluescript to generate pSUPER as described previously [1]. The pSUPERTAP vector active against human TAP was generated by annealing the following oligonucleotides:
5'gatccccggcatggctcctgagcatgttcaagagacatgctcaggagccatgccttttttggaaa and
5'agcttttccaaaaaggcatggctcctgagcatgtctcttgaacatgctcaggagccatgccggg which are active against nucleotides 478-496 in human TAP (Acc No: NM_006362) and subcloning into the *Hin*dIII and *Bgl*II sites of pSUPER. pSUPERLUC, active against firefly luciferase

was generated by annealing the following oligonucleotides: 5’gatcccccgtacgcggaatacttcgattcaagagatcgaagtattccgcgtacgtttttggaaa and 5’agcttttccaaaaacgtacgcggaatacttcgatctcttgaatcgaagtattccgcgtacgggg and subcloning into pSUPER. GST-ORF 57 was prepared by amplification with the following oligonucleotides 5’ggcggggatccggaatttcatcagatgatgac and 5’ cgccgcctcgagtcactgagtaggtaagaaaaacag then subcloned into the *Bam*HI and *Xho*I sites of pGEX6P (Amersham). 6xHis tagged ORF 57 (amino acids 8-120) was PCR amplified with the following oligonucleotides: 5’ggcgcggcatatgggaatttcatcagatgatgacttt and 5’cccgcggatcctcagcgtctctgtgcagcagcttctcg. The PCR product was subcloned into the *Nde*I and *Bam*HI sites of pET14b. The GST-mREF2-1 fusion vectors used have been described previously [2]. GFP-TAP and GFP-TAP 1-372 were generated by PCR using the following oligonucleotides 5’gcggcggaattcatggcggacgaggggaagtcgtac, 5’ccgcgcggatcctcacttcatgaatgccacttctgg and 5’cccgcgctctagatcagggcggtaacgtcgtgggggcttc. The PCR products were subcloned into the *Eco*RI and *Bam*HI sites of pEGFPC2. The ORF 57-GFP deletion series was generated by PCR amplication using a series of forward and reverse primers: Δ1F 5’cccaagcttatcatcagatgactttgattcg and Δ1R 5’tgcgtcgacggctttctgaaaggccttg, Δ2R 5’tgcgtcgacctaagaatgctgctacgg, Δ3F 5’cccaagcttctgtttctgcctgtacct and Δ3R 5’tgcgtcgactgagaatacagctgtgtttc, Δ4F 5’cccaagcttcattgcctaatgcaaac and Δ4R 5’tgcgtcgacctgagtaggtaagaaaacag. These oligonucleotides incorporated *Hin*dIII and *Sal*I restriction sites to facilitate cloning of the PCR product into the eukaryotic expression vector, pcDNAGFP (Invitrogen) to yield, p57Δ1-4GFP. To generate pLUCSALRRE a *Kpn*I-*Sal*I fragment corresponding to the envelope region (nucleotides 6347-8897) from HIV-1 (HXB2), containing the splice acceptor for intron 2 of Tat and the Rev response element, was subcloned into the CMV driven expression vector pSA91. A luciferase gene derived from pGEM-luc (Promega) was then subcloned between the *Bgl*II restriction sites within Env (nucleotides 7041-7621). The splice donor for intron 2 of Tat (nucleotides 5977-6224 from HIV-1 -HXB2) was subcloned upstream of the luciferase gene on a *Kpn*I-*Stu*I fragment following PCR amplification with the following primers 5’acttggtaccgaagaagcggagacagcgacg and 5’cgtaggccttgccactgtcttctgctcaaa, to generate the reporter construct pLUCSALRRE. pCMVREV was used for expression of HIV-1

Rev protein [3]. To produce pCINEOREV1-66 a fragment of the REV gene corresponding to amino acids 1-66 was amplified using the following oligonucleotides 5’gccgctctagagccaccatggcaggaagaagcggagacagc and 5’cacccgaattcgtcccagataagtgccaag and subcloned into the *Nhe*I and *Eco*RI sites of pCINEO (Promega). The 18S rRNA probe for Northern analysis was amplified from Hela cell genomic DNA using the following primers 5’tggtcgctcgctcctctcccactt and 5’caggctccctctccggaatcgaac. All plasmids were verified by sequencing of the inserts.

References:

1 Brummelkamp, T.R., Bernards, R. and Agami, R. (2002) A system for stable expression of short interfering RNAs in mammalian cells. Science **296**, 550-553

2 Koffa, M.D., Clements, J.B., Izaurralde, E., Wadd, S., Wilson, S.A., Mattaj, I.W. and Kuersten, S. (2001) Herpes simplex virus ICP27 protein provides viral mRNAs with access to the cellular mRNA export pathway. EMBO J. **20**, 5769-5778

3 Kotsopoulou, E., Kim, V. N., Kingsman, A. J., Kingsman, S. M. and Mitrophanous, K. A. (2000) A Rev-independent human immunodeficiency virus type 1 (HIV-1)-based vector that exploits a codon-optimized HIV-1 *gag-pol* gene. J. Virol. **74**, 4839-4852