from a pGEX2T vector (GE Healthcare) modified to contain 5'*Nde*I and 3'*BamH*I cloning sites (Garrus et al., 2001; von Schwedler et al., 2003). ALIX point mutants were generated by the Quickchange (Stratagene) method, following the manufacturer's protocol.

GST-CHMP4A Expression Construct. The GST-CHMP4A expression construct used in GST pulldown experiments was described previously (von Schwedler et al., 2003). Note, however, that the CHMP4A and CHMP4B designations have been reversed from our previous publication to follow the convention of Katoh et al. (Katoh et al., 2003).

ALIX Mammalian Expression Vector. FLAG-tagged human ALIX DNA was amplified and an *Nde*I-*BamH*I fragment was subcloned into a pCI-neo vector (Promega) engineered to express the protein with an N-terminal FLAG epitope tag. ALIX mutants were constructed using the quick change method following manufacturer's instruction (Stratagene).

Virus Expression Constructs. HIV-1 proviral expression constructs were based on HIV-1_{NL4-3} R9ΔApa (Swingler et al., 1997) (a gift from Didier Trono, University of Lausanne). The HIV-1 $p6^{Gag}$ ΔPTAP construct has been described previously (Garrus et al., 2001). The HIV-1 $p6^{Gag}$ ΔYP was constructed using megaprimer mutagenesis to introduce the $_{36}YP_{37}$ to $_{36}SR_{37}$ mutation in $p6^{Gag}$ without altering the overlapping pol reading frame. The vector pEV53B (Olsen, 1998) (a gift from John Olsen, UNC Chapel Hill) was used to produce EIAV virus like particles, and the $_{23}YP_{24}$ to $_{23}SR_{24}$ mutation was introduced using the megaprimer method.

shRNA Lentiviral Vector for ALIX Depletion. An shRNA targeting human ALIX nucleotides 1765-1783 (Chen et al., 2005), was built into the FG12 lentiviral delivery