Cell, Volume *128*

Supplemental Data

Structural and Biochemical Studies of ALIX/AIP1 and Its Role in Retrovirus Budding

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SUPPLEMENTAL EXPERIMENTAL METHODS

Plasmid Construction

ALIX Protein Expression Constructs. ALIX coding sequences were amplified and subcloned from an EST clone (von Schwedler et al., 2003), and ALIX proteins were expressed as either 6x-His or GST N-terminal fusion proteins. For $ALIX_{Bro1\text{-}V}$ (residues 1-698) and $ALIX_{V}$ (residues 360-702), ALIX coding sequences were amplified with 5'*Nde*I and 3'*BamH*I restriction sites and inserted into the pET151/D-TOPO vector (Invitrogen) following the manufacturer's instructions. $ALIX_{Bro1}$ (residues 1-359) was cloned between 5'*Nde*I and 3'*BamH*I restriction sites in a modified pET16b vector (Novagen) designed to contain a TEV protease cleavage site following the 6x-His tag. GST-ALIX expression constructs were generated by inserting ALIX coding sequences between 5'*Nde*I and 3'*BamH*I restriction sites in a pGEX2T vector (GE Healthcare) modified to contain a TEV protease cleavage site and 5'*Nde*I and 3'*BamH*I/*Bgl*II restriction sites following the GST gene (pGEX2T-TEV).

HIV-1 $p6_{Gag}$ and EIAV $p9^{Gag}$ Expression Constructs. Genes encoding $HIV\text{-}1_{NL4\text{-}3}$ $p6^{Gag}$ and EIAV $p9^{Gag}$ were amplified from the proviral R9 and pEV53B plasmids (see below), with 5'*Nde*I and 3'*BamH*I restriction sites, to allow expression as GST fusion proteins