vector (Qin et al., 2003) (a gift from David Baltimore, Caltech). Lentiviral vectors packing this shRNA expression construct were produced in 293T cells by calcium phosphate co-transfection (8.1 µg FG12-ALIX shRNA, 8.1 µg pCMV∆R8.91 (Zufferey et al., 1997), and 2.7 µg pMD.G (Ory et al., 1996), 10 cm plate, ~4×10^6). Virus like particles were collected from the cell culture supernatants on the third day posttransfection and concentrated 200-fold by centrifugation through a 20% sucrose cushion.

Purification of Recombinant ALIX Proteins

$ALIX_{Bro1-V}$, $ALIX_{Bro1}$, and $ALIX_V$ were expressed as 6x-His N-terminal fusion proteins in BL21(DE3) Codon+ (RIL) *E. coli* grown in auto-induction media, ZYP-5052 (Studier, 2005), at 37°C for 5-6 hours with vigorous shaking in baffled flasks before moving to 23°C to grow to saturation within 16-18 hours. SeMet $ALIX_V$ was prepared by expression in PASM-5052 media (Studier, 2005). Subsequent purification steps were performed at 4°C unless noted. Cells were lysed with sonication and lysozyme treatment (2.5 mg/ml in 50 mM Tris pH 8.0, 300 mM NaCl, 10 mM imidazole). Clarified supernatant was applied to Ni^{2+}-NTA resin (Qiagen), washed with lysis buffer, and eluted with 25 mM Tris pH 8.0, 100 mM NaCl, 250 mM imidazole. For $ALIX_{Bro1}$, EDTA and DTT were added to 1 mM and the solution was diluted to 50 mM NaCl with ddH_2O. For $ALIX_{Bro1-V}$ and $ALIX_V$, the eluted protein was dialyzed against 25 mM Tris pH 8.0, 100 mM NaCl, 2 mM β-mercaptoethanol while incubating with TEV protease (~18 h, 23°C), and the processed protein was collected as flow-through from a second pass over Ni^{2+}-NTA resin and diluted to 50 mM NaCl. For all three constructs, protein