Table S2. ALIX Binding to HIV-1 $p6^{Gag}$ and EIAV $p9^{Gag}$

	Estimated Dissociation Constant (μM, 20°C)[a]				
	$ALIX_{Bro1\text{-}V}$ (WT)	$ALIX_V$ (WT)	$ALIX_V$ ($V_{498}D$)	$ALIX_V$ ($F_{676}D$)	$ALIX_V$ ($I_{683}D$)
HIV-1 $p6^{Gag}$	57 ± 21[b]	59 ± 15[b]	NB[c]	NB[c]	>1000[d]
HIV-1 $p6^{Gag}$ ΔYP	>1000[d]	>1000[d]	-	-	-
EIAV $p9^{Gag}$	1.5 ± 0.3[b]	1.2 ± 0.3[b]	18 ± 1	NB[b]	580 ± 20
EIAV $p9^{Gag}$ ΔYP	>1000[d]	>1000[d]	-	-	-

[a]Binding was measured using Biacore 2000 and 3000 optical biosensors (Biacore AB, Uppsala, Sweden) equipped with CM4 sensor chips derivatized with anti-GST antibodies through amine-coupling (Johnsson et al., 1991). GST (control) and GST-ALIX fusion proteins were captured from crude *E. coli* lysates to densities of 1000-1800 RU, and chip surfaces were over-coated with recombinant GST to minimize non-specific interactions. Purified wild-type and mutant HIV-1 $p6^{Gag}$ and EIAV $p9^{Gag}$ were diluted in running buffer (20 mM NaPhosphate, 150 mM NaCl, 0.2 mg/mL BSA, 0.005% P20, pH 7.2), and injected in duplicate from concentrations of 0 μM to 1000 μM. Affinity parameters were obtained by plotting the equilibrium responses against the analyte concentration and fitting to a simple 1:1 binding model (e.g., see Figure 3A)(Myszka, 1999).

[b]Average ± difference between 8 ($ALIX_{Bro1\text{-}V}$) or 6 ($ALIX_V$) independent measurements (errors in other measurements were estimated from statistical fits of the binding data).

[c]NB = No detectable binding.

[d]Weak binding was detectable, but could not be accurately quantified because half-maximal binding was not achieved.