

$K_d=146\pm 1 \mu\text{M}$), and that the Pro720Leu mutation (GST-ALIX₇₁₄₋₇₂₃, P720L) eliminated binding.

(B) Yeast two hybrid assay showing that human ALIX binds endophilins-1 and -2, and that the interactions are inhibited by the ALIX RP_{757,758}AA mutation. Directed yeast two hybrid assays were performed using the Matchmaker GAL4 Yeast Two Hybrid 3 system (Clontech). AH-109 *Saccharomyces cerevisiae* were co-transformed with pGADT7 or pGBKT7 cloning vectors (Clontech) containing inserts encoding wild type (WT) and mutant human ALIX (Activation Domain fusions, AD) and endophilins-1 and -2 (DNA Binding Domain fusions, DBD). Transformed yeast colonies were grown for three days at 30 °C on YPD plates with -Leu, -Trp selection. 10-100 colonies were pooled, resuspended in a liquid culture of SB (-Leu, -Trp), selected on SB (-Leu, -Trp, -Ade, -His) plates, and allowed to grow for 3 days. Growth on -Leu,-Trp,-His,-Ade media reflects positive binding interactions between ALIX and the endophilins, whereas failure to grow reflects a lack binding activity (ALIX_{RP757,758AA} mutants and negative controls).