



Supplementary Figure S2. Analysis of the CRM1-dependent NES of BDV X protein. (A) Output results of the analysis of the sequence of X protein using NetNES. The NetNES algorithm (T. la Cour et al., Protein Eng. Des. Sel., 17(6):527-36, 2004) identified a NES sequence in X N-terminal sequence between residues 5 and 16. The graph (top) illustrate NES score along the entire X sequence, whereas the bottom table gives individual NES scores for the first 18 residues of the protein. (B) Alignment and comparison of the putative X Protein NES with the consensus sequence for NES and with known NES (tumor suppressors p53, BRCA and CDC25B, HIV1 Rev protein and the protein kinase inhibitor (PKI) protein). Bold letters represent residues in the core region (LXL). (C) Co-immunoprecipitation experiments for X and CRM1. HEK 293T cells were transfected with a BDV X expressing plasmid (pCAG-X). 48 h after transfection, cell lysates were processed for immunoprecipitation with an anti-CRM1 antibody, followed by protein G-coupled magnetic beads, as described in the methods. Non-transfected cells (-) were used as a control. The bead eluates, together with fractions of total cell lysates (input) were analyzed by western blotting against CRM1 and BDV X proteins.