on the Pore Loop 1 side, and Arg241 (blue), forming a positively charged ring on the opposite side (Fig.7a, lower panel).

We have shown previously that point mutations in Pore Loop 1 residues of both human VPS4A and VPS4B inhibit HIV-1 release and infectivity, implying that this element is important for Vps4 protein function[25]. To examine the functional importance of Pore Loop 2 residues, we assayed the efficiency of HIV-1 release and infectivity from cells expressing exogenous wild type VPS4A or VPS4A proteins with mutations in the two most highly conserved Pore Loop 2 arginines (R236A or R246A in VPS4A), the central Pore Loop 2 glutamate (E240A), or the ATP binding site (K173Q, positive control)[16]. As shown in Figure 7c, overexpression of $VPS4A_{R236A}$ and $VPS4A_{R246A}$ inhibited the release of an HIV-1 vector, as indicated by reductions in the levels of the virion-associated MA and CA proteins released into the supernatant (upper panel, compare lanes 4 and 6 to lane 3) and in viral titers (lower panel). In both cases, titer reductions were substantial (48- and 73-fold, respectively, note the log scale in Fig. 7c). Virion release and titers were also impaired by overexpression of the $VPS4A_{E240A}$ mutant, but in this case the reduction was modest (4-fold). Control experiments behaved as expected in that overexpression of the $VPS4A_{K173Q}$ ATP binding mutant strongly inhibited virion release and titer (lanes 7, 470-fold titer reduction), overexpression of the wild type VPS4A protein did not impair virion release (compare lanes 2 and 3), and cellular expression levels of the HIV-1 CA and GFP-VPS4A proteins were equivalent in all cases (panels 2 and 3, respectively). We also demonstrated that the analogous Pore Loop 2 point mutations did not affect yeast $Vps4_{E233Q}$ protein dodecamerization in vitro, implying that these