

shown), indicating that appreciable concentrations of monomers could accumulate under these low protein and non-equilibrium conditions.

**Figure 6.** Mutagenesis test of the Vps4<sub>ΔMIT</sub> hexamer homology model.

(a) The “p97 D1 Homology” model for a Vps4<sub>ΔMIT</sub> hexameric ring within the dodecameric enzyme (upper) is shown together with the arrangement of Vps4 along the crystallographic six-fold screw axis (lower). The p97 D1 Homology Model was created by superimposing Vps4<sub>ΔMIT</sub> subunits onto the crystal structure of the hexameric ring of the homologous p97 D1 protein<sup>44</sup>. The Trp388 (green) and Leu151 (orange) residues are shown explicitly in homology model and crystal structure to illustrate how the Leu151 residue contributes to the interface in both models whereas the Trp388 residue contributes to the interface in the crystal structure but not in the p97 D1 Homology Model (see insets).

(b) Gel filtration chromatograms of wild type and mutant Vps4<sub>ΔMIT</sub> proteins in the absence (black) or presence (green) of ATP. For reference, elution positions for monomeric (1), dimeric (2), and dodecameric (12) proteins are shown as dotted vertical lines, and the elution positions of molecular weight standards are shown below the chromatograms. Note that upon addition of ATP, the wild type Vps4<sub>ΔMIT</sub> protein converts from a dimer to a dodecamer, Vps4<sub>ΔMIT,L151</sub> remains a monomer, and Vps4<sub>ΔMIT,W388A</sub> converts from a monomer to a dodecamer.

**Figure 7.** VPS4A Pore Loop 2 residues are required for HIV budding.