

that upon addition of ATP, the Vps4 $_{\Delta MIT, Q216A}$  protein converts from a dimer to a dodecamer.

**Supplemental Figure 4.** Relationship between the p97 D1 hexamer interface and the modeled Vps4 hexamer interface.

(a) Structures of the crystallographically defined p97 D1 hexamer (left) and the analogous interfaces in the p97 homology model of the Vps4 hexamer (right). The inset shows a detailed view of the major Vps4 interface. Residues that are located within the interface and are identical between Vps4 and p97 D1 are highlighted in red, and conservative changes are highlighted in blue.

(b) Sequence alignments of residues within Vps4 Interface 6 and equivalent regions of the p97 D1 and spastin AAA ATPase cassettes. The aligned regions span both sides of Interface 6 (helix  $\alpha 1$ -strand  $\beta 1$ , upper sequence and helix  $\alpha 8$ - $\alpha 9$ , lower sequences). Colored residues contribute to the interdomain interface between subunits in the p97 D1 hexamer, and the color coding is the same as in part (a). Residues that mediate interdomain contacts in the p97 D1 but are not identical in Vps4 are underlined.