Interface 1[27]. This conclusion was based upon their report that the Q216A mutation appeared to inhibit $Vps4_{\Delta MIT}$ dimerization, as analyzed by gel filtration chromatography. In our hands, however, $Vps4_{\Delta MIT}$ dimerization was not inhibited by the Q216A mutation or by other mutations within Interface 1. Furthermore, we do not believe that the data presented in Fig. 6 of Hartmann et al. constitute compelling evidence that the Q216A mutation inhibits $Vps4_{\Delta MIT}$ dimerization in solution.

These differences have important implications for models of higher order Vps4 assembly because Hartmann et al. used the Interface 1 dimer as a constraint in creating their model for the fully assembled Vps4 double ring[27]. This constraint compelled a model in which the two Vps4 rings pack in a head-to-head orientation. However, our data indicate that the crystallographic dimer Interface 1 does not actually form in solution, as we find that Interface 1 mutants such as Q216A dimerize in solution (Fig. 5b). Furthermore, we find that the Q216A mutant also dodecamerizes normally, implying that that Gln216 does not make an essential contact in the fully assembled enzyme, as would be required by the head-to-head model (see Supplemental Fig. 3). We also find the head-to-head model unattractive because: 1) it differs from the ring orientations seen in all other well characterized Type II AAA ATPases, 2) it would require that the substrate-binding MIT domains emanate from the center of the double ringed structure (rather than from the end(s), as is seen for substrate binding domains of other AAA ATPases), and 3) it requires the ATPase activities of the two rings to work in opposition, which is difficult to envision if substrates are pumped up and through the rings.