ATPγS nucleotides (Figures 2a, b). In these two molecules, the nucleotide binds in a site between the large and small domains, and makes all of the expected contacts with the Walker A/P Loop (^{173}GPPGTGKSY^{181}), Walker B (^{230}FIDEVD^{235}), and sensor 1 (^{275}ATN^{277}) elements[18] (Figure 2a). In particular, the adenine ring adopts the anti conformation, and stacks between Tyr181 (Walker A) and Met307 (helix 6) [40; 41; 42; 43; 44]. The ATPγS triphosphate hydrogen bonds with backbone amide NH groups in the P Loop (^{173}GPPGTGKSY^{181}, underlined residues make H bonds), and the phosphates also contact the conserved Lys179 (Walker A/P Loop, β-phosphate salt bridge), Asn277 (sensor 1, γ-sulfate) and Pro175 (P Loop, γ-sulfate). A bound Mg^{2+} ion is coordinated by oxygen atoms from the β and γ phosphates, and by the conserved side chains of Ser180 (P Loop) and Asp232 (Walker B). The structure is consistent with the observation that the K179A mutation inhibits ATP binding (and higher order assembly)[16]. The Walker B element also contains another highly conserved acidic residue (Glu233), which is thought to function as the catalytic base that activates a nucleophilic water[16; 25]. This residue was mutated to Gln in the $Vps4_{\Delta MIT}$ constructs to minimize ATP hydrolysis, and the Gln233 side chain projects out of the active site where it contacts Ser198 and Ser200. We have not attempted to compare the positions of the active site nucleotides and side chains with those of the ADP-bound Vps4 structure because both complexes were solved at modest resolution and because the bound ADP ligand has a very high B factor (179 $Å^2$)[28].

Our remaining two Vps4 structures do not exhibit clear density for bound ATPγS nucleotides. Specifically, Molecule C in crystal form 2 has density in the phosphate binding sites that likely corresponds to an ethylene glycol molecule (which