illustrated in Fig. 6a, where the upper model shows the p97-based homology model and the lower model shows the spiraling “hexamer” of crystal form 1 (crystal form 2 is very similar). The p97 D1 homology model predicts that ring formation will be dependent upon residues located at the center of crystallographic Interface 6 (e.g., Leu151), but not upon residues located at the exterior of Interface 6 that are separated when the subunit orientation is adjusted to match the p97 D1 ring (e.g., Trp388, see insets). We therefore probed the validity of the p97 D1 homology model by testing the assembly properties of $VPS4_{\Delta MIT}$ proteins with L151D and W388A point mutations.

As expected, the $Vps4_{\Delta MIT,L151D}$ mutant remained monomeric in both the presence and absence of ATP, indicating that this mutation blocks both dimerization and dodecamerization (compare panels 1 and 2 in Fig. 6b). We note that the inability of this mutant to assemble into any higher order structure implies either that formation of the second ring in the Vps4 dodecamer depends upon forming the first ring (our preferred explanation) or, alternatively, that Leu151 makes important contacts in both rings of the dodecamer. In contrast, the $Vps4_{\Delta MIT,W388A}$ mutant was monomeric in the absence of ATP, but assembled into stable dodecamers upon addition of ATP (panel 3). This result supports the idea that one of the two rings in the $Vps4_{\Delta MIT}$ dodecamer resembles the hexameric ring formed by the homologous p97 D1 domain. This experiment also indicates that the $Vps4_{\Delta MIT}$ dimer is not an obligate on-pathway intermediate for forming the Vps4 dodecamer, because the W388A mutation inhibits dimerization but not dodecamerization. Conversely, we previously showed that the $Vps4_{R352A}$ mutant dimerized normally, but failed to form higher order assemblies in