Supplemental Figure 1.
Secondary structure of hVPS4B (above) and sequence alignments of VPS4 proteins from different species as well as four other representative members of the meiotic clade of AAA ATPases. The color coding of secondary structures matches that of Figs. 1A,B, 2A,B, and 6. The color coding of active site residues matches that of Figure 4C. Dashed lines denote disordered loops in the hVPS4B crystal structure. Boxes highlight the apparent conservation of the following structural motifs: $\beta'$ strand (Box 1), kink in helix $\alpha 9$ (Pro 352 in hVPS4B, only conserved in the VPS4 proteins, Box 2), $\beta$ domain (only conserved in the VPS4 proteins, Box 3), helix $\alpha 10$ (Box 4). Sequence alignments were initially generated by ClustalW (Higgins, D., Thompson, J., Gibson, T., Thompson, J.D., Higgins, D.G., Gibson, T.J. *Nucleic Acids Res.* (1994) 22, 4673-4680), and then manually adjusted based upon the known hVPS4B structure.

Supplemental Figure 2.
Space filling highlighting interface residues of p97 D1 and hVPS4B (modeled). Interface residues were defined by the algorithm of Sobolev V., Sorokine A., Prilusky J., Abola E.E. and Edelman M. (1999) “Automated analysis of interatomic contacts in proteins. *Bioinformatics*, 15, 327-332”. In each panel, the left figure shows two adjacent protein subunits and the right figures show the same subunits rotated by 90° in opposite directions about vertical axes so that the view is directly into the surface of the interface. Color coding is as follows: dark blue/dark gold, interface residues that are conserved or identical between p97 D1 and hVPS4B, light blue/light gold, interface residues that are not conserved or identical. Interface residues that are circled and labeled cause hereditary spastic paraplesia when mutated in the meiotic AAA ATPase, spastin (Lindsey, J. C., Lusher, M. E., McDermott, C. J., White, K. D., Reid, E., Rubinsztein, D. C.,
Supplemental Figure 3.

ATP induces assembly of human VPS4 hVPS4B_{126-444}. Gel filtration chromatography of hVPS4B_{126-444} E235Q (7-26 μM) in the absence of nucleotide (above) or preincubated 2 h with 1 mM ATP (below). The N-terminally truncated hVPS4B_{126-444}E235Q protein was used in these studies because it assembled more reversibly than did the full length protein.

Protein concentrations are shown, and elution positions of molecular weight standards are shown above. In the absence of nucleotide, hVPS4B_{126-444} E235Q (MW 37 kDa) eluted as two distinct species that apparently corresponded to monomers and dimers (MW_{est} ~43 and ~115 kDa upper panel). In the presence of 1 mM ATP, however, the protein formed an additional higher order complex (MW_{est} ~380 kDa). 1 mM ADP induced an intermediate assembly phenotype (data not shown).

Note that the largest protein assembly, which we believe corresponds to a double ring structure, corresponds in size to ~380 kDa and forms in the presence of ATP and high hVPS4B_{126-444} concentrations, but not at lower protein concentrations or in the absence of ATP.
This behavior is very similar to that observed for the yeast protein, except that yVps4p assembles at somewhat lower concentrations (∼1 µM) than hVPS4B (∼25 µM).

Chromatography was performed on a Superose HR6 column (Amersham Biosciences) in HR6 buffer. Peaks corresponding to TEV are denoted by asterisks (confirmed by SDS-PAGE and mass spectrometry).

Supplemental Figure 4. LIP5 binds GST-hVPS4B_{126-444} in a GST pulldown experiment.

The indicated proteins were mixed, GST components purified by affinity chromatography on a glutathione sepharose matrix, and bound proteins were visualized by SDS-PAGE with Coomassie blue staining. Lane 1: Molecular weight (MW) markers, Lane 2: GST alone (reference), Lane 3: GST + LIP5 (negative control), Lane 4: GST-hVPS4B_{126-444} alone (reference). Lane 5: GST-hVPS4B_{126-444} + LIP5 (demonstration of binding). The different proteins are identified at right.

30 ml cultures of *E. coli* expressing either GST alone, GST-hVPS4B_{126-444}, or His_{10}-LIP5 were pelleted, resuspended in 5 ml lysis buffer (50 mM Tris HCl pH 7.4, 50 mM NaCl, 5 mM BME), and lysed by addition of 75µl of 10 mg/ml lysozyme (20 min incubation, 4°C), followed by addition of 100µl of 5% deoxycholate (20 more min). Cells were sonicated for 1 min and the soluble proteins collected following centrifugation for 45 min at 13.2 x g. Binding reactions (663 µl) were performed in binding buffer (20 mM Tris HCl pH 8.0, 100 mM NaCl, 2 mM MgCl₂, 2 mM CaCl₂, 5 mM BME, 0.02% NP40, and 5% glycerol) containing: 16 µM GST or GST-hVPS4B_{126-444}, 70 µM LIP5, and 100 µl glutathione agarose slurry (Amersham Biosciences). After incubation for 1 h at 4°C, unbound proteins were removed by washing with buffer (3 x 1.2
ml). Bound proteins were eluted from the matrix by boiling in 100 µl of 2x SDS-PAGE buffer and detected by SDS-PAGE.
Fig. S2
Fig. S4