Supplementary Information

Supplementary Figure 1:

Secondary structure and domain architecture of *S. cerevisiae* Vps4p and multiple sequence alignment with human VPS4A, human VPS4B, and archaeal Vps4 homologs from *S. solfataricus*, *A. hospitalis*, *T. barophilus* and *C. symbiosum*. Sequences were aligned using ClustalW [1]. Secondary structure elements for yeast Vps4p are indicated above the sequence based on mapping of PDB ID: 3EIE [2] and PDB ID: 2V6X [3] on UniProt ID: P52917 using the PDB Protein Feature View [4]. The bar to the right of the sequence alignment denotes domain boundaries for yeast Vps4p. The coloring scheme corresponds to colors used in the sequence alignment and secondary structure assigment.

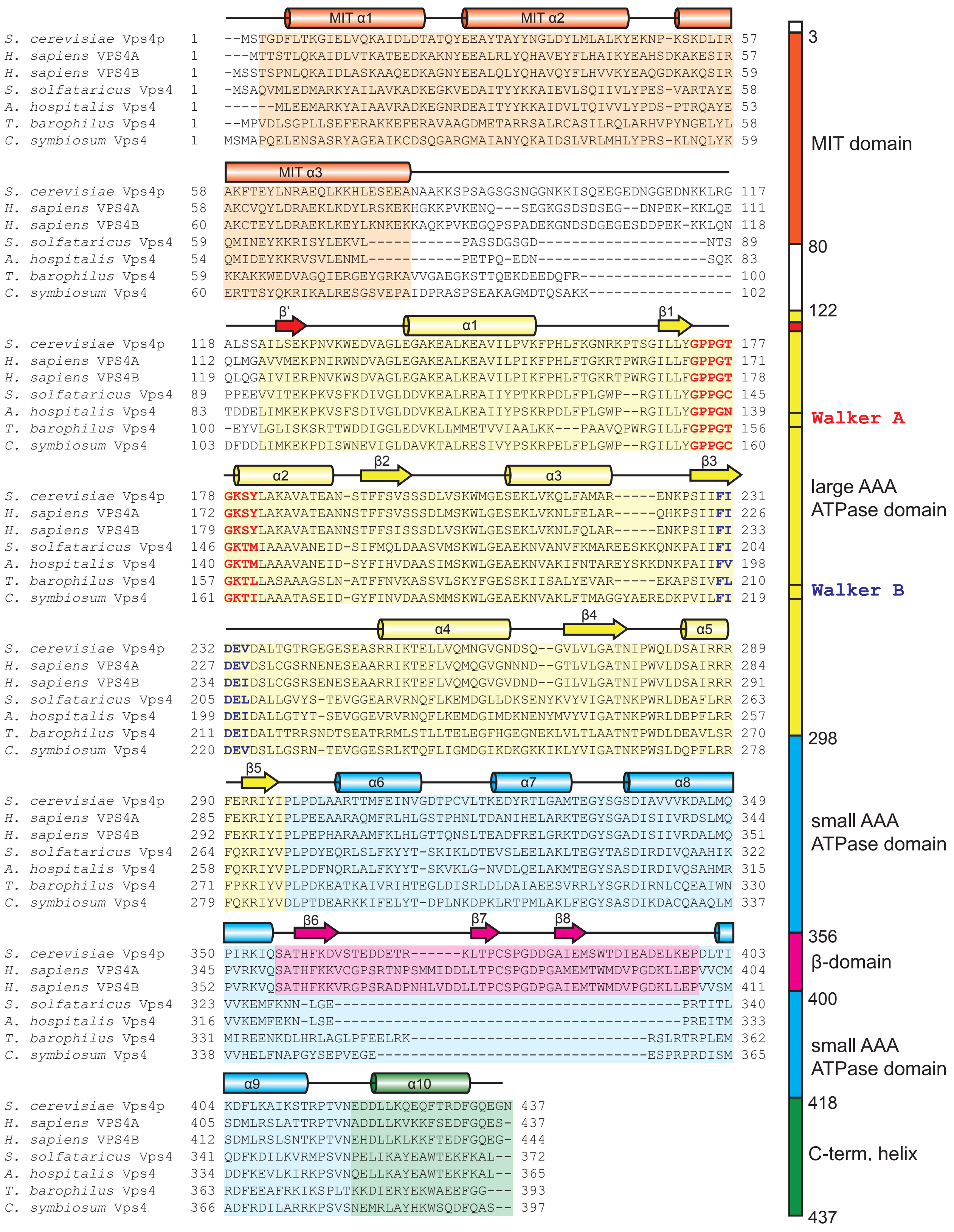
Supplementary Figure 2:

Characterization of AhosVps4 oligomerization by size exclusion chromatography. (A) Chromatograms of AhosVps4 (red) and AhosVps4(E206Q) (blue) injected at concentrations of 100 μM. In the absence of nucleotide (top panel), both proteins migrate close to the retention volume expected for dodecamers. When the buffer was supplemented with 2 mM magnesium chloride and 1 mM ATP (bottom panel), both proteins migrated as hexamers, with AhosVps4(E200Q) eluting slightly earlier than AhosVps4.

Supplementary Figure 3:

AhosVps4 crystals contain the full-length protein. SDS-PAGE analysis of AhosVps4 crystals (lane 3) produces a band at the same apparent molecular mass as the 42 kDa full-length AhosVps4 control (compare lane 3 with different concentrations of full-length AhosVps4 in lanes 1 and 2).

Supplementary Figure 1:



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S. cerevisiae Vps4p     1   --MSTGDFLTKGIELVQKAIDLDTATQYEEAYTAYYNGLDYLMLALKYEKNP-KSKDLIR 57
H. sapiens VPS4A        1   ---MTTSTLQKAIDLVTKATEEDKAKNYEEALRLYQHAVEYFLHAIKYEAHSDKAKESIR 57
H. sapiens VPS4B        1   -MSSTSPNLQKAIDLASKAAQEDKAGNYEEALQLYQHAVQYFLHVVKYEAQGDKAKQSIR 59
S. solfataricus Vps4    1   -MSAQVMLEDMARKYAILAVKADKEGKVEDAITYYKKAIEVLSQIIVLYPES-VARTAYE 58
A. hospitalis Vps4      1   ------MLEEMARKYAIAAVRADKEGNRDEAITYYKKAIDVLTQIVVLYPDS-PTRQAYE 53
T. barophilus Vps4      1   --MPVDLSGPLLSEFERAKKEFERAVAAGDMETARRSALRCASILRQLARHVPYNGELYL 58
C. symbiosum Vps4       1   MSMAPQELENSASRYAGEAIKCDSQGARGMAIANYQKAIDSLVRLMHLYPRS-KLNQLYK 59

S. cerevisiae Vps4p     58  AKFTEYLNRAEQLKKHLESEEANAAKKSPSAGSGSNGGNKKISQEEGEDNGGEDNKKLRG 117
H. sapiens VPS4A        58  AKCVQYLDRAEKLKDYLRSKEKHGKKPVKENQ---SEGKGSDSDSEG--DNPEK-KKLQE 111
H. sapiens VPS4B        60  AKCTEYLDRAEKLKEYLKNKEKKAQKPVKEGQPSPADEKGNDSDGEGESDDPEK-KKLQN 118
S. solfataricus Vps4    59  QMINEYKKRISYLEKVL------------PASSDGSGD-------------------NTS 89
A. hospitalis Vps4      54  QMIDEYKKRVSVLENML------------PETPQ-EDN-------------------SQK 83
T. barophilus Vps4      59  KKAKKWEDVAGQIERGEYGRKAVVGAEGKSTTQEKDEEDQFR------------------ 100
C. symbiosum Vps4       60  ERTTSYQKRIKALRESGSVEPAIDPRASPSEAKAGMDTQSAKK----------------- 102

S. cerevisiae Vps4p     118 ALSSAILSEKPNVKWEDVAGLEGAKEALKEAVILPVKFPHLFKGNRKPTSGILLYGPPGT 177
H. sapiens VPS4A        112 QLMGAVVMEKPNIRWNDVAGLEGAKEALKEAVILPIKFPHLFTGKRTPWRGILLFGPPGT 171
H. sapiens VPS4B        119 QLQGAIVIERPNVKWSDVAGLEGAKEALKEAVILPIKFPHLFTGKRTPWRGILLFGPPGT 178
S. solfataricus Vps4    89  PPEEVVITEKPKVSFKDIVGLDDVKEALREAIIYPTKRPDLFPLGWP--RGILLYGPPGC 145
A. hospitalis Vps4      83  TDDELIMKEKPKVSFSDIVGLDDVKEALKEAIIYPSKRPDLFPLGWP--RGILLYGPPGN 139
T. barophilus Vps4      100 -EYVLGLISKSRTTWDDIGGLEDVKLLMMETVVIAALKK---PAAVQPWRGILLFGPPGT 156
C. symbiosum Vps4       103 DFDDLIMKEKPDISWNEVIGLDAVKTALRESIVYPSKRPELFPLGWP--RGILLYGPPGC 160

S. cerevisiae Vps4p     178 GKSYLAKAVATEAN-STFFSVSSSDLVSKWMGESEKLVKQLFAMAR-----ENKPSIIFI 231
H. sapiens VPS4A        172 GKSYLAKAVATEANNSTFFSVSSSDLMSKWLGESEKLVKNLFELAR-----QHKPSIIFI 226
H. sapiens VPS4B        179 GKSYLAKAVATEANNSTFFSISSSDLVSKWLGESEKLVKNLFQLAR-----ENKPSIIFI 233
S. solfataricus Vps4    146 GKTMIAAAVANEID-SIFMQLDAASVMSKWLGEAEKNVANVFKMAREESKKQNKPAIIFI 204
A. hospitalis Vps4      140 GKTMLAAAVANEID-SYFIHVDAASIMSKWLGEAEKNVAKIFNTAREYSKKDNKPAIIFV 198
T. barophilus Vps4      157 GKTLLASAAAGSLN-ATFFNVKASSVLSKYFGESSKIISALYEVAR-----EKAPSIVFL 210
C. symbiosum Vps4       161 GKTILAAATASEID-GYFINVDAASMMSKWLGEAEKNVAKLFTMAGGYAEREDKPVILFI 219

S. cerevisiae Vps4p     232 DEVDALTGTRGEGESEASRRIKTELLVQMNGVGNDSQ--GVLVLGATNIPWQLDSAIRRR 289
H. sapiens VPS4A        227 DEVDSLCGSRNENESEAARRIKTEFLVQMQGVGNNND--GTLVLGATNIPWVLDSAIRRR 284
H. sapiens VPS4B        234 DEIDSLCGSRSENESEAARRIKTEFLVQMQGVGVDND--GILVLGATNIPWVLDSAIRRR 291
S. solfataricus Vps4    205 DELDALLGVYS-TEVGGEARVRNQFLKEMDGLLDKSENYKVYVIGATNKPWRLDEAFLRR 263
A. hospitalis Vps4      199 DEIDALLGTYT-SEVGGEVRVRNQFLKEMDGIMDKNENYMVYVIGATNKPWRLDEPFLRR 257
T. barophilus Vps4      211 DEIDALTTRRSNDTSEATRRMLSTLLTELEGFHGEGNEKLVLTLAATNTPWDLDEAVLSR 270
C. symbiosum Vps4       220 DEVDSLLGSRN-TEVGGESRLKTQFLIGMDGIKDKGKKIKLYVIGATNKPWSLDQPFLRR 278

S. cerevisiae Vps4p     290 FERRIYIPLPDLAARTTMFEINVGDTPCVLTKEDYRTLGAMTEGYSGSDIAVVVKDALMQ 349
H. sapiens VPS4A        285 FEKRIYIPLPEEAARAQMFRLHLGSTPHNLTDANIHELARKTEGYSGADISIIVRDSLMQ 344
H. sapiens VPS4B        292 FEKRIYIPLPEPHARAAMFKLHLGTTQNSLTEADFRELGRKTDGYSGADISIIVRDALMQ 351
S. solfataricus Vps4    264 FQKRIYVPLPDYEQRLSLFKYYT-SKIKLDTEVSLEELAKLTEGYTASDIRDIVQAAHIK 322
A. hospitalis Vps4      258 FQKRIYVPLPDFNQRLALFKYYT-SKVKLG-NVDLQELAKMTEGYSASDIRDIVQSAHMR 315
T. barophilus Vps4      271 FPKRIYVPLPDKEATKAIVRIHTEGLDISRLDLDAIAEESVRRLYSGRDIRNLCQEAIWN 330
C. symbiosum Vps4       279 FQKRIYVDLPTDEARKKIFELYT-DPLNKDPKLRTPMLAKLFEGYSASDIKDACQAAQLM 337

S. cerevisiae Vps4p     350 PIRKIQSATHFKDVSTEDDETR------KLTPCSPGDDGAIEMSWTDIEADELKEPDLTI 403
H. sapiens VPS4A        345 PVRKVQSATHFKKVCGPSRTNPSMMIDDLLTPCSPGDPGAMEMTWMDVPGDKLLEPVVCM 404
H. sapiens VPS4B        352 PVRKVQSATHFKKVRGPSRADPNHLVDDLLTPCSPGDPGAIEMTWMDVPGDKLLEPVVSM 411
S. solfataricus Vps4    323 VVKEMFKNN-LGE---------------------------------------PRTITL 340
A. hospitalis Vps4      316 VVKEMFEKN-LSE---------------------------------------PREITM 333
T. barophilus Vps4      331 MIREENKDLHRLAGLPFEELRK---------------------------RSLRTRPLEM 362
C. symbiosum Vps4       338 VVHELFNAPGYSEPVEGE-------------------------------ESPRPRDISM 365

S. cerevisiae Vps4p     404 KDFLKAIKSTRPTVNEDDLLKQEQFTRDFGQEGN 437
H. sapiens VPS4A        405 SDMLRSLATTRPTVNADDLLKVKKFSEDFGQES- 437
H. sapiens VPS4B        412 SDMLRSLSNTKPTVNEHDLLKLKKFTEDFGQEG- 444
S. solfataricus Vps4    341 QDFKDILKVRMPSVNPELIKAYEAWTEKFKAL-- 372
A. hospitalis Vps4      334 DDFKEVLKIRKPSVNQELLKAYEAWTEKFKAL-- 365
T. barophilus Vps4      363 RDFEEAFRKIKSPLTKKDIERYEKWAEEFGG--- 393
C. symbiosum Vps4       366 ADFRDILARRKPSVSNEMRLAYHKWSQDFQAS-- 397
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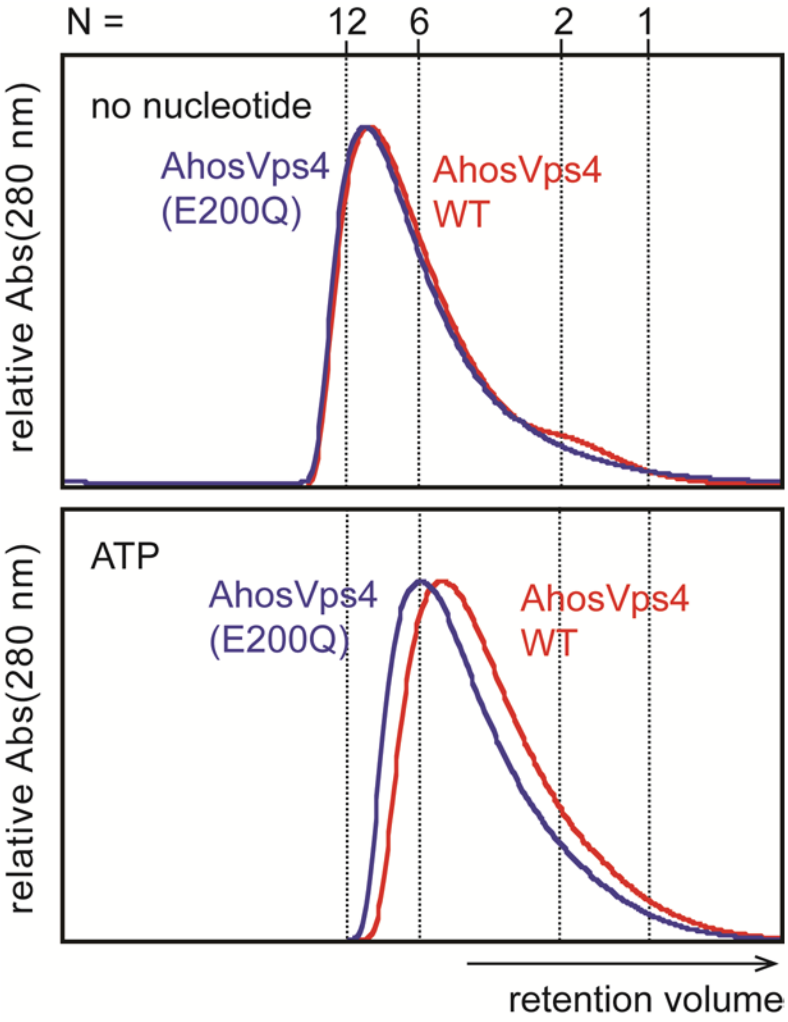
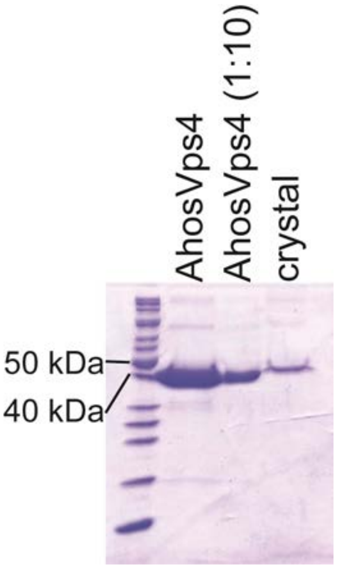
Supplementary Figure 2:



Supplementary Figure 3:



Supplementary Table 1: Bacterial and yeast expression vectors

Plasmid name	DNASU ID	internal ID (Sundquist, Hill)	
pET151-D-Topo-Vps4p	ScCD00586084	WISP13-150	CPH1421
pET151-D-Topo-Vps4p(E233Q)	ScCD00586086	WISP13-151	CPH1443
pET151-D-Topo-Vps4p(L151D)	ScCD00586088	WISP12-144	CPH2511
pET151-D-Topo-Vps4p(R352A)	ScCD00586089	WISP12-145	CPH2512
pET151-D-Topo-Vta1	ScCD00586091	WISP12-146	CPH1501
pET151-PP-SsoVps4	SsCD00586092	WISP11-593	CPH2233
pET151-PP-SsoVps4(E206Q)	SsCD00586093	WISP11-594	CPH2236
pET151-PP-SsoVps4(E206Q, 85-372)	SsCD00586094	WISP13-152	CPH1469
pET151-D-Topo-SsoVps4(Y121D)	SsCD00586095	WISP13-153	CPH2414
pET151-PP-SsoVps4(F328A)	SsCD00586096	WISP13-154	CPH2388
pET151-PP-SsoVps4(R262A)	SsCD00586097	WISP13-155	CPH2555
pET151-PP-SsoVps4(R263A)	not submitted	WISP13-156	CPH2658
pJExpress414-AhosVps4	AhCD00586085	WISP11-484	CPH2306
pJExpress414-AhosVps4(E200Q)	AhCD00586087	WISP12-67	CPH2392
pRS415MET+GFP-CPS	ScCD00597455	WISP07-94	CPH2844
pRS416+Vps4p	to be assigned	WISP07-95	CPH2845
pRS416+Vps4p(L151D)	ScCD00597456	WISP12-168	CPH2846
pRS416+Vps4p(R352A)	to be assigned	WISP13-79	CPH2847

Supplementary References:

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2. Gonciarz, M. D., Whitby, F. G., Eckert, D. M., Kieffer, C., Heroux, A., Sundquist, W. I. & Hill, C. P. (2008). Biochemical and structural studies of yeast Vps4 oligomerization. *J Mol Biol* **384**, 878-95.
3. Obita, T., Saksena, S., Ghazi-Tabatabai, S., Gill, D. J., Perisic, O., Emr, S. D. & Williams, R. L. (2007). Structural basis for selective recognition of ESCRT-III by the AAA ATPase Vps4. *Nature* **449**, 735-9.
4. Velankar, S., McNeil, P., Mittard-Runte, V., Suarez, A., Barrell, D., Apweiler, R. & Henrick, K. (2005). E-MSD: an integrated data resource for bioinformatics. *Nucleic acids research* **33**, D262-5.