

1 **Co-evolution of Eukaryotic-like Vps4 and ESCRT-III Subunits in the**  
2 **Asgard Archaea**

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30 Running Head: Vps4 and ESCRT-III in Asgard archaea

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36 **ABSTRACT** (246 words)

37 The emergence of the endomembrane system is a key step in the evolution  
38 of cellular complexity during eukaryogenesis. The Endosomal Sorting  
39 Complex Required for Transport (ESCRT) machinery is essential and  
40 required for the endomembrane system functions in eukaryotic cells.  
41 Recently, genes encoding eukaryote-like ESCRT protein components have  
42 been identified in the genomes of Asgard archaea, a newly proposed  
43 archaeal superphylum that is thought to include the closest extant  
44 prokaryotic relatives of eukaryotes. However, structural and functional  
45 features of Asgard ESCRT remain uncharacterized. Here we show that Vps4,  
46 Vps2/24/46, and Vps20/32/60, the core functional components of the Asgard  
47 ESCRT, co-evolved eukaryote-like structural and functional features.  
48 Phylogenetic analysis shows that Asgard Vps4, Vps2/24/46, and  
49 Vps20/32/60 are closely related to their eukaryotic counterparts. Molecular  
50 dynamic simulation and biochemical assays indicate that Asgard Vps4  
51 contains a eukaryote-like Microtubule Interacting and Transport (MIT)  
52 domain that binds the distinct type-1 MIT Interacting Motif and type-2 MIT  
53 Interacting Motif in Vps2/24/46, and Vps20/32/60, respectively. The Asgard  
54 Vps4 partly, but much more efficiently than homologs from other archaea,  
55 complements the *vps4* null mutant of *Saccharomyces cerevisiae*, further

56 supporting the functional similarity between the membrane remodeling  
57 machineries of Asgard archaea and eukaryotes. Thus, this work provides  
58 evidence that the ESCRT complexes from Asgard archaea and eukaryotes  
59 are evolutionarily related and functionally similar. Thus, despite the apparent  
60 absence of endomembranes in Asgard archaea, the eukaryotic ESCRT seems  
61 to have been directly inherited from an Asgard ancestor, to become a key  
62 component of the emerging endomembrane system.

63

64 **IMPORTANCE** (105 words)

65 The discovery of Asgard archaea has changed the exiting ideas on the  
66 origins of Eukaryotes. Researchers propose that eukaryotic cells evolve from  
67 Asgard archaea. This hypothesis partly stems from the presence of multiple  
68 eukaryotic signature proteins in Asgard archaea, including homologues of  
69 ESCRT proteins that are essential components of the endomembrane system  
70 in eukaryotes. However, structural and functional features of Asgard ESCRT  
71 remain unknown. Our study provides evidence that Asgard ESCRT is  
72 functionally comparable to the eukaryotic counterparts suggesting that,  
73 despite the apparent absence of endomembranes in archaea, eukaryotic  
74 ESCRT was inherited from an Asgard archaeal ancestor, alongside the  
75 emergence of endomembrane system during eukaryogenesis.

76 **Keywords:** Endomembrane system, Asgard archaea, ESCRT,

77 Eukaryogenesis, Evolution.

78

## 79 **INTRODUCTION** (4595 words)

80 Eukaryogenesis is a major, long-standing puzzle in evolutionary  
81 biology because the specifics of the evolutionary process leading to the  
82 eukaryotic cellular complexity are far from being clear. One of the key  
83 distinctions of eukaryotic cells from the cells of prokaryotes is the presence  
84 in the former of the sophisticated endomembrane system. Undoubtedly, the  
85 emergence of the endomembrane system was a milestone event in  
86 eukaryogenesis because it is a pre-requisite of the intracellular  
87 compartmentalization which is a hallmark of eukaryotic cells (1). The  
88 Endosomal Sorting Complex Required for Transport (ESCRT) machinery is  
89 an essential component of the eukaryotic endomembrane system that, as  
90 such, has been thought to be restricted to eukaryotic cells (2). For instance,  
91 *Saccharomyces cerevisiae* ESCRT consists of five main subcomplexes:  
92 ESCRT-0, -I, -II, -III, and Vps4 (3-5). Of these, the Vps4 and ESCRT-III  
93 subunits are central players in ESCRT function that mediates remodelling  
94 and scission of endomembranes (6, 7). The ESCRT-III subunits can be  
95 further divided into two classes, termed Vps2/24/46 and Vps20/32/60, and  
96 both participate in either directly or indirectly forming membrane-bound  
97 polymeric assemblies that sever membrane necks (8). On the other hand,  
98 Vps4, an ATPase, promotes ATP-dependent disassembly of the ESCRT-III

99 polymers, thus ensuring the ESCRT-III subunits turnover. Several studies  
100 have shown that the N-terminal Microtubule Interacting and Transport  
101 (MIT) domain of Vps4 recognizes and interacts with the type-1 MIT  
102 Interacting Motif (MIM1) that is present in the Vps2/24/46 class ESCRT-III  
103 subunits and type-2 MIT Interacting Motif (MIM2) present in the  
104 Vps20/32/60 subunits. These recognition models are essential for the  
105 biological function of ESCRT-III and Vps4 (9-11).

106 The cell division (Cdv) systems discovered in some archaeal orders,  
107 such as Sulfolobales and Desulfurococcales within the TACK  
108 (Thaumarchaeota, Aigarchaeota, Crenarchaeota, and Korarchaeota)  
109 superphylum include a homolog of eukaryotic Vps4 (CdvC) and several  
110 homologs of eukaryotic ESCRT-III subunits (CdvBs) (12, 13). Given these  
111 homologies and because in eukaryotes, the MIT-MIM2 interactions occurred  
112 between CdvC and CdvB (12, 14, 15), the crenarchaeal Cdv system has  
113 been proposed to be the evolutionary ancestor of eukaryotic ESCRT (16).  
114 However, this evolutionary relationship remains uncertain. One reason for  
115 the uncertainty is that CdvBs lack the well-characterized MIM1, and the  
116 absence of the MIT-MIM1 interaction is likely to reflect major functional  
117 differences between crenarchaeal Cdv and eukaryotic ESCRT (12, 17). Such  
118 differences might indicate that, although the two systems consist of

119 homologous subunits, the Cdv system is not the direct ancestor of eukaryotic  
120 ESCRT.

121 The recently discovered Asgard archaea (including Lokiarchaeota,  
122 Thorarchaeota, Heimdallarchaeota, Odinararchaeota, and Helarchaeota) have  
123 been proposed to include the closest archaeal relatives of eukaryotes. This  
124 proposition stems, partly, from the findings that the Asgard genomes encode  
125 a broad repertoire of Eukaryotic Signature Proteins (ESPs) that are far more  
126 prevalent in Asgard than they are in other archaea (18-21). Among these  
127 ESPs are highly conserved homologs of eukaryotic ESCRT-I, -II, -III, and  
128 Vps4. Notably, the presence of these proteins in Asgard archaea that was  
129 originally demonstrated on metagenomics assemblies has been confirmed by  
130 analysis of the first closed Asgard genome, ruling out the possibility of a  
131 eukaryotic contamination (12, 18, 19, 22).

132 Here, we explore the phylogenetic relationships among the ESCRT-III  
133 components, reconstitute and biochemically characterize the Asgard Vps4,  
134 and test its potential biological function in the heterologous *S. cerevisiae*  
135 endomembrane system. The combined phylogenetic, genetic and  
136 biochemical analyses reveal close relationships between the ESCRT-III  
137 subunits and Vps4 of Asgard archaea and eukaryotes, to the exclusion of  
138 other archaea.



139

## 140 **RESULTS**

### 141 **Eukaryotic-like ESCRT-III subunits and Vps4 in Asgard archaea**

142       Given that the ESCRT-III subunits are tightly linked to the functional  
143 complexity of ESCRT (12), we first performed a detailed sequence  
144 comparison and phylogenetic analysis of the Vps2/24/46 and Vps20/32/60  
145 as well as the Vps4 ATPase from Asgard archaea based on the available  
146 genomic data(18, 19). In the unrooted maximum-likelihood phylogenetic  
147 tree Vps2/24/46 and Vps20/32/60, the Asgard proteins form a cluster with  
148 eukaryotic homologs that is separated from the archaeal (TACK) CdvB  
149 cluster by a long branch (Fig. 1A, Fig. S1 and Table S1), supporting the  
150 notion that Asgard archaea possess "eukaryote-like" ESCRT-III subunits.  
151 All the Asgard Vps20/32/60 proteins form a strongly supported clade with  
152 the eukaryotic Vps20/32/60 which is compatible with a direct ancestral  
153 relationship. The Asgard Vps2/24/46 proteins formed three clades one of  
154 which (Odinarchaeota, Lokiarchaeota, and Thorarchaeota) clustered with the  
155 eukaryotic homologs whereas the remaining two (Heimdallarchaeota) placed  
156 near the root of the Asgard-eukaryote branch (Fig. 1A). This tree topology is  
157 likely to result from acceleration of evolution in Heimdallarchaeota.

158       In addition to the phylogenetic results, we found that the Asgard

159 Vps2/24/46 contained leucine-rich motifs located in the C-terminal helix  
160 and resembling the C-terminal MIM1 that are conserved in eukaryotes  
161 although some leucine residues were substituted by isoleucine in the Asgard  
162 homologs (Fig. 1B) (9). The C-terminal regions of the Asgard Vps20/32/60  
163 contain proline-rich motifs that resemble MIM2 although do not fully  
164 conform to the MIM2 consensus in eukaryotes and TACK archaea (10, 11).  
165 Taken together, the results of phylogenetic analysis and motif search for  
166 ESCRT-III subunits not only demonstrate the Asgard-eukaryote affinity but  
167 also show that the ancestors of the Vps2/24/46 and Vps20/32/60 groups have  
168 already diverged in Asgard archaea, antedating eukaryogenesis.

169 It appears likely that Vps4 structurally and functionally co-evolved with  
170 ESCRT-III subunits in Asgard archaea. To explore the evolution of Vps4, an  
171 unrooted maximum-likelihood phylogenetic tree was constructed for the  
172 group of ATPases including CdvC from the TACK superphylum, Asgard  
173 Vps4, and the so called eukaryotic "meiotic clade" comprised of Vps4,  
174 Katanin 60, and Spastin (23). As in the ESCRT-III subunit tree, the Asgard  
175 Vps4 formed a branch with the eukaryotic homolog that was separated by a  
176 long, strongly supported branch from the archaeal CdvC branch (Fig. 2A and  
177 Fig. S2). The Asgard Vps4 did not form a single clade, but rather four  
178 clades, all of which were located close to the root of the Asgard-eukaryote

179 branch.

180 Despite their high divergence demonstrated by the lack of monophyly  
181 in the phylogenetic tree (Fig. 2A and Fig. S2), all Asgard Vps4 contain the  
182 eukaryotic-like "arginine collar" that consists of three conserved arginine  
183 residues (Fig. S3A). In eukaryotes, this motif is located in the pore loop 2  
184 of Vps4 and is involved in the ESCRT-III filaments translocation to the  
185 central pore of the Vps4 hexamer for disassembly (Fig. S3B) (24, 25).

186 Because Vps4 recognizes ESCRT-III subunits via the MIT domain, we  
187 specifically analyzed the phylogeny of the MIT domains of the Vps4  
188 proteins from Asgard archaea, eukaryotes, and TACK archaea. The tree  
189 demonstrates a clear affiliation of Asgard with eukaryotes that, in this case,  
190 form a clade with one of the MIT domain from Heimdallarchaeota (Fig. 2B  
191 and Fig. S4). Affiliation with Heimdallarchaeota has been previously  
192 observed for many Asgard genes(19, 26, 27).

193 To further structurally characterize MIT domain in Asgard Vps4, we  
194 constructed stable models of full length Vps4 from  
195 Heimdallarchaeota\_LC\_3, Odinararchaeota\_LCB\_4, Thorarchaeota\_AB\_25,  
196 and Lokiarchaeum\_GC14\_75 using homology modeling and molecular  
197 dynamics simulation, and compared these with the *S. cerevisiae* Vps4  
198 structure. As a control for the Asgard Vps4, we include CdvC from

199 *Sulfolobus solfataricus*\_P2 (cluster I in Fig. 2A) and Bathyarchaeota (cluster  
200 II in Fig. 2A). All MIT domains of Asgard Vps4 and TACK CdvC adopted  
201 a three-helix bundle structure that is closely similar to the *S. cerevisiae*  
202 MIT domain structure although the helices in both the Asgard and TACK  
203 structures are somewhat shorter than in the *S. cerevisiae* structure (Fig.  
204 2Bb and Fig. S5).

205 Taken together, the above data suggest that the evolution of Asgard  
206 Vps4, especially their MIT domain, was accompanied by the functional  
207 divergence of the ESCRT-III subunits. Thus, although the Asgard Vps4  
208 proteins are highly diverged, the results of sequence comparison,  
209 phylogenetic analysis and structural modeling are compatible with  
210 coevolution of Vps4 with ESCRT-III subunits and an ancestral relationship  
211 between the membrane remodeling machineries of Asgard and eukaryotes.  
212 Furthermore, it can be predicted that Asgard Vps2/24/46 and Vps20/32/60  
213 form ESCRT-III-like filaments similar to those in eukaryotes.

214

### 215 **Interactions between Asgard Vps4 and ESCRT-III subunits**

216 As previously described, unlike the CdvBs, Asgard Vps2/24/46 and  
217 Vps20/32/60 share the same eukaryotic ESCRT-III secondary structure, and  
218 these organizations are probably responsible for their ability bind to Vps4

219 like their eukaryotic counterparts(12). The isothermal titration calorimetry  
220 (ITC) was adopted to verify that the MIT domain of Asgard Vps4 bind to  
221 Vps2/24/46 and Vps20/32/60, respectively (Fig. S6). To characterize the  
222 interactions between Asgard Vps4 and ESCRT-III subunits, the respective  
223 binding free energies were estimated by MM-GBSA calculations (Table  
224 S2A) (28, 29). The binding free energies of Vps4-Vps2/24/46 in  
225 Heimdall\_LC\_3, Odin\_LCB\_4, Thor\_AB\_25, and Loki\_GC14\_75 were  
226 calculated as -39.02, -61.85, -71.81, -72.24 kcal/mol, respectively. All these  
227 values, although compatible with stable binding, are lower than the binding  
228 free energy of Vps4-Vps2 (-82.98 kcal/mol) in *S. cerevisiae*, suggesting that  
229 the affinity of Asgard Vps4 for Vps2/24/46 is weaker than that of *S.*  
230 *cerevisiae* Vps4 for Vps2. The binding free energies for Asgard Vps4-  
231 Vps20/32/60 differed to a greater degree indicating variation in the affinities  
232 (Table S2B). The Thor\_AB\_25 value of -119.97 kcal/mol was substantially  
233 greater than the binding free energy of the Vps4-Vps20 interaction in *S.*  
234 *cerevisiae* (-88.30 kcal/mol), the values for Heimdall\_LC\_3 (-81.43  
235 kcal/mol) and Loki\_GC14\_75 (-88.89 kcal/mol) comparable to those for *S.*  
236 *cerevisiae*, and that for Odin\_LCB\_4 (-49.53 kcal/mol) much lower than in  
237 *S. cerevisiae*.

238 We further analyzed the structural basis for the MIT domain of Asgard

239 Vps4 binding to the putative MIM1 and MIM2 of Vps2/24/46 and  
240 Vps20/32/60, respectively, by using MM-GBSA calculations(28). The key  
241 amino acids that contribute to the Vps4 MIT domain binding to the  
242 Vps2/24/46 MIM1 in Heimdall\_LC\_3, Odin\_LCB\_4, and Thor\_AB\_25 are  
243 mainly located in Helix 2 and Helix 3 of the MIT domain similar to the  
244 location of MIM1-interacting residues in *S. cerevisiae* Vps4 (Fig. 3A and  
245 Table S3). These findings are consistent the MIM1 peptide binding at the  
246 interface between Helix 2 and Helix 3 of the MIT domain as observed in  
247 eukaryotes (10, 30). In Loki\_GC14\_75, the key amino acid residues are  
248 located in Helix 1 and Helix 2, suggesting a distinct interaction mode.

249 The key residues involved in the MIT-Vps20/32/60 interactions are  
250 spread among Helix 1, Helix 2, and Helix 3, in positions closely similar to  
251 those involved in the MIT-Vps20 interactions in *S. cerevisiae* (Fig. 3B and  
252 Table S4). Thus, the MIM2 peptide is predicted to bind the grooves formed  
253 by the three-helix bundle rather than Helix 1 and Helix 3 only as also  
254 observed for the eukaryotic ESCRT-III (10, 11). Taken together, these  
255 findings indicate that the interactions of the Asgard Vps4 MIT domain with  
256 the MIM1 (in Vps2/24/46) and MIM2 (in Vps20/32/60) motifs closely  
257 resemble the corresponding interactions in eukaryotes.

258

259 **Asgard Vps4 phenotypically complements *vps4* null mutation in *S.***  
260 ***cerevisiae***

261 We further sought to determine whether the Asgard and eukaryotic  
262 Vps4 ATPases were functionally interchangeable. To this end,  
263 Heimdall\_LC\_3, Odin\_LCB\_4, Thor\_AB\_25 and Loki\_GC14\_75 were  
264 tested for the ability to complement the *S. cerevisiae vps4* null mutation. As  
265 a control for the Asgard Vps4, we performed the complementation assays  
266 with CdvC from *S. solfataricus\_P2* and Bathyarchaeota. Briefly, we re-  
267 codon-optimized the coding sequences of Asgard Vps4 and TACK CdvC for  
268 expression in *S. cerevisiae*, and, respectively, assembled the coding  
269 sequences into transcription units of pPOT-RFP vector that contains a native  
270 promoter region of *S. cerevisiae* BY4741 *vps4* (a 500 bp DNA sequence  
271 region upstream from the ATG start codon of this gene) and a *S. cerevisiae*  
272 Cytochrome c isoform 1 (CYC1) terminator using the YeastFab Assembly  
273 method.(31) The assembly products were transformed into *S. cerevisiae*  
274 *vps4Δ* by the LiAc/PEG method(32). As previously described, in *S.*  
275 *cerevisiae*, *vps4* null mutation resulted in temperature-sensitive growth  
276 defect, causing growth arrest at 39 °C (33, 34). We found that the Asgard  
277 Vps4 could slightly suppress the growth defect of *vps4Δ* at 39 °C (Fig. 4A).  
278 Remarkably, however, after incubation at 39 °C for 96 h, the growth of

279 *vps4Δ* bearing Asgard Vps4 showed substantial, although variable,  
280 restoration at 30 °C, in a sharp contrast with *vps4Δ* for which no restoration  
281 was observed (Fig. 4A). Nevertheless, both CdvCs showed only minimal  
282 growth restoration of *vps4Δ* at 39 °C and a limited enhancement of viability  
283 at 30° C; complementation with these proteins was substantially less efficient  
284 than that observed with their Asgard counterparts. Furthermore, the *S.*  
285 *cerevisiae* Vps4, Asgard Vps4, and CdvCs were re-codon-optimized,  
286 synthesized, and respectively, cloned into a pCold-TF vector (Takara Bio Co  
287 Ltd., Japan). After expression in *Escherichia coli* BL21, proteins were  
288 purified by Mag-Beads His-Tag Protein Purification Kit (BBI Co., Ltd,  
289 China). The biochemical experiments *in vitro* show that these purified  
290 proteins are active ATPases both at 30 °C and 39 °C (Fig. 4B). This  
291 observation eliminates the possibility that the poor complement result of  
292 CdvCs was due to the lack of ATPases activity at 39 °C, and is compatible  
293 with the involvement of the ATPase activity of Asgard Vps4 in sustaining  
294 the viability of the *S. cerevisiae vps4Δ* mutant under non-permissive  
295 conditions.

296 As previously described, *vps4* null mutation could induce an formation  
297 of an aberrant prevacuolar compartment adjacent to the vacuoles, known as  
298 class E compartment, due to the block of intracellular protein trafficking (3,



299 30, 33). To further demonstrate that Asgard Vps4 is functionally analogous  
300 to its eukaryotic counterpart, we observed the vacuoles in the *S. cerevisiae*  
301 cells bearing Asgard Vps4. As expected, the characterized class E  
302 compartment vacuolar morphology was clearly observed in the *S. cerevisiae*  
303 *vps4Δ* cells, and this defect was almost completely rescued by *S. cerevisiae*  
304 Vps4 (Fig. 4C). We found that the Vps4 of Heimdall\_LC\_3, Odin\_LCB\_4,  
305 Thor\_AB\_25, and Loki\_GC14\_75 also partially complemented the aberrant  
306 vacuoles in the *vps4* null mutant, with the reduction of class E compartment  
307 of about 80% of that observed with the native *S. cerevisiae* Vps4 (Fig. 4C).  
308 However, the enlarged vacuoles induced in *vps4Δ* strain were not markedly  
309 eliminated by the Asgard Vps4. Taken together, these findings show that the  
310 Asgard Vps4 are functionally more similar to the eukaryotic homologs than  
311 homologs from other archaea.

312

## 313 **DISCUSSION**

314 In this work, we combined computational approaches, including  
315 sequence comparison, phylogenetic analysis and structural modeling, with  
316 genetic and biochemical experiments to investigate the evolutionary and  
317 functional relationships between the ESCRT-III machineries of Asgard  
318 archaea and eukaryotes. Phylogenetic analyses of both the ESCRT-III

319 subunits and Vps4 ATPase show a clear affinity between Asgard archaea  
320 and eukaryotes, to the exclusion of the other archaea. Moreover, the  
321 divergence of the two groups of ESCRT-III subunits already occurred in  
322 Asgard archaea.

323 The results of amino acid sequence analysis and structural modeling are  
324 best compatible with the coevolution of Vps4 with the ESCRT-III subunits.  
325 In particular, the interaction between the MIT domain of Vps4 and the  
326 MIM1- and MIM2-like of the ESCRT-III subunits appears to have evolved  
327 already in Asgard archaea.

328 The findings of the computational analysis are complemented by our  
329 experimental results. In particular, we show that Asgard Vps4 is capable of  
330 complementing the *S. cerevisiae* vps4 null mutant much more efficiently  
331 than homologs from Crenarchaeota and Bathyarchaeota. This enhanced  
332 functionality might be underpinned by the evolution of distinct, "eukaryote-  
333 like" structural features, such as the arginine collar that is involved in the  
334 disassembly of ESCRT-III polymers.

335 Taken together, all these findings are compatible with the direct origin of  
336 the eukaryotic ESCRT machinery from the Asgard ancestor. In a broader  
337 evolutionary context, the ESCRT complex likely evolved in the common  
338 ancestor of the TACK and Asgard superphyla whereas its further elaboration

339 occurred in the Asgard lineage. The key event apparently was the  
340 duplication of CdvB that seems to combine features of Vps2/24/46 and  
341 Vps20/32/60 (12), with subsequent functional diversification of the subunits  
342 and coevolution with Vps4.

343 An intriguing outstanding question is the function of the ESCRT  
344 machinery in the Asgard archaea. There is no indication that these (or any  
345 other) archaea possess intracellular membranes (22), so the ECRT-III  
346 proteins and Vps4 are likely to be involved in cell division as demonstrated  
347 for the Cdv proteins of Crenarchaeota. However, the specialization of the  
348 ESCRT-III subunits might provide for the formation of eukaryotic-like  
349 filaments that could be involved not only in the inside-out fission to produce  
350 membrane vesicles that have been observed in the MK-D1 strain, but also  
351 the outside-in fission that allows the Asgard archaea to engulf their bacterial  
352 metabolic partners. The latter capability is critical for the ‘Entangle-Engulf-  
353 Enslave model’ of eukaryogenesis (22). Further molecular and cell  
354 biological study of the Asgard membrane remodeling apparatus, even if  
355 challenging due to the recalcitrance of these organisms to growth in culture,  
356 should shed light on the origin of the eukaryotic endomembrane system, one  
357 of the key aspects of eukaryogenesis.

358

## 359 **MATERIALS and METHODS**

### 360 **Bioinformatics analysis**

361 All the protein sequences were obtained either by NCBI accession number  
362 or by BLAST search(35) the non-redundant protein sequences against local  
363 Nr database. The protein sequences were aligned using MUSCLE  
364 (V3.8.1551)(36), trimmed with TrimAl (V1.4)(37) before construction of  
365 phylogenetic trees using IQ-Tree (V1.6.5)(38). The indicated functional  
366 domains of proteins were analyzed by Interpro  
367 (<https://www.ebi.ac.uk/interpro/>) and NCBI's conserved domain database.

### 368 **Homology modeling and docking study**

369 We searched the Vps4, Vps2/24/46, and Vps20/32/60 sequences belonging  
370 to *S. cerevisiae*, Lokiarchaeum\_GC14\_75, Thorarchaeota\_AB\_25,  
371 Heimdallarchaeota\_LC\_3, and Odinararchaeota\_LCB\_4 from NCBI database  
372 and CdvC sequences belonging to to *Sulfolobus solfataricus*\_P2, and  
373 Bathyarchaeota (<http://www.ncbi.nlm.nih.gov>, accession number:  
374 KZV07689.1, P36108.2, NP\_013794.1; KKK42121.1, KKK42122.1,  
375 KKK44605.1; OLS30569.1, OLS30568.1, OLS30800.1; OLS27542.1,  
376 OLS27541.1, OLS27540.1; OLS18192.1, OLS18193.1, OLS18194.1,  
377 AAK41192.1, WP\_119819537.1, respectively) to build homology model.  
378 The Cryo-EM structure of Vps4 (E233Q) hexamer belonging to *S. cerevisiae*

379 was obtained from the Protein Data Bank (PDB code: 5XMI)(39), and the  
380 subunit B was chosen for modeling template and the missing residues (1-  
381 118) were built at the I-TASSER server  
382 (<http://zhanglab.ccmb.med.umich.edu/I-TASSER>). Sequence alignments and  
383 homology modelings of Vps4 for Lokiarchaeota, Thorarchaeota,  
384 Heimdallarchaeota, and Odinararchaeota with unknown structures were  
385 carried out on MODELLER program(40), downloaded and installed from  
386 salilab server ([https://salilab.org/modeller/download\\_installation.html](https://salilab.org/modeller/download_installation.html)). The  
387 three-dimensional structures of Vps2/24/46 and Vps20/32/60 for *S.*  
388 *cerevisiae* and four Asgard archaea were also built at the I-TASSER server.  
389 Among several three-dimensional models generated using homology  
390 modeling and ab initio method, the best model was selected after a series of  
391 refining and minimization and molecular dynamic simulation employing  
392 ff14SB force fields parameters by AMBER 16.0 package(41). Then the  
393 complexes of Vps2/24/46 and Vps20/32/60 against Vps4 were simulated  
394 using the ZDOCK server(42). Ten top docking poses were generated.

### 395 **Molecular dynamic (MD) simulation**

396 The parallel version of AMBER 16.0 package was used to prepare the  
397 complex files and conduct MD simulations employing ff14SB force fields  
398 parameters. The ionizable residues default protonation states in AMBER

399 16.0 were assigned. All MD simulations were carried out by applying cubic  
400 periodic boundary conditions (PBC) and in an explicit water box of TIP3P  
401 water molecules(43) with a minimum distance of 10.0 Å between complex  
402 surface and water box boundary. The Na<sup>+</sup> or Cl<sup>-</sup> counterions were added in  
403 sufficient number to neutralize any net charges of the structures above. All  
404 of chemical bond lengths of hydrogen-heavy atoms were restrained by the  
405 SHAKE algorithm(44). A cutoff radius of 10.0 Å was set for both non-  
406 bonded electrostatic and van der Waals interactions. Long-range electrostatic  
407 forces were taken into account using Particle Mesh Ewald (PME)  
408 method(45). Langevin dynamics and Langevin piston methods were applied  
409 to keep the temperature (300 K) and the pressure (1 bar) of the system  
410 constant, respectively. The time step was set to 2.0 fs.

411 The solvated systems were minimized using PMEMD.CUDA module  
412 enabled NVIDIA graphics processing units (GPUs)(46, 47) in three stage,  
413 keeping the solute fixed and just minimized the positions of the water and  
414 counterions firstly with 100 kcal/(mol·Å<sup>2</sup>) restraints and then reduced to 10  
415 kcal/(mol·Å<sup>2</sup>), and lastly for the entire system without any restraining force.  
416 Each stage was conducted with 10000 steps of steepest descent algorithm  
417 followed by 1000 steps conjugate gradient minimization to get rid of any  
418 unfavorable steric contacts for both solvent and protein molecules. Then, a

419 NVT simulation was conducted to slowly heat the systems temperature from  
420 0 K to 300 K over a period of 500 ps, and density equilibrated for 2000 ps  
421 with a weak restraint applied to the whole protein at 1 atm and 300 K.  
422 Finally, all restraints were removed, and production MD simulations were  
423 carried out at constant pressure (1 atm) and temperature (300 K) in NPT  
424 ensemble. For each system, MD simulation was performed for 500 ns and  
425 repeated thrice with different random number, and a total of 1.5  $\mu$ s trajectory  
426 was analyzed by CPPTRAJ module (48).

#### 427 **Calculations of binding free energy**

428 The binding free energies of Vps2/24/46 and Vps20/32/60 against Vps4  
429 were calculated by molecular mechanics-generalized Born surface area  
430 (MM-GBSA) method(28, 29). All energy components were calculated using  
431 500 snapshots that were extracted every 200 ps during the last 100 ns of  
432 each MD simulation trajectory. The configurational entropy was not  
433 considered in the approach as it is extremely time-consuming. So the  
434 binding free energy in the solvent environment can be expressed as:

$$435 \Delta G_{\text{bind}} = \Delta E_{\text{ele}} + \Delta E_{\text{vdw}} + \Delta G_{\text{np}} + \Delta G_{\text{ele}}$$

436 The  $\Delta E_{\text{ele}}$ ,  $\Delta E_{\text{vdw}}$ ,  $\Delta G_{\text{np}}$ ,  $\Delta G_{\text{ele}}$  represented electrostatic energy in the gas  
437 phase, Van der Waals energy in the gas phase, non-polar solvation energy,  
438 and polar solvation energy, respectively. All energy terms were calculated

439 using MM-GBSA calculations, and the  $\Delta G_{\text{ele}}$  is estimated by GB model(29),  
440 and the  $\Delta G_{\text{np}}$  is calculated from the solvent accessible surface area (SASA)  
441 of the molecules by molsurf, with the values 0.00542 and 0.92 for  
442 SURFTEN and SURFOFF, respectively(49). The decomposition of binding  
443 free energies were calculated at residue-pair level for a further investigation  
444 of the complexes interactions using the MM-GBSA decomposition  
445 program(50, 51) implemented in AMBER 16.0.

446 **Protein expression in *Escherichia coli* BL21 and purification for**  
447 **biochemical assays *in vitro***

448 The Vps4, Vps2/24/46, and Vps20/32/60 coding sequences belonging to *S.*  
449 *cerevisiae*, Lokiarchaeum\_GC14\_75, Thorarchaeota\_AB\_25,  
450 Heimdallarchaeota\_LC\_3, and Odinararchaeota\_LCB\_4, and CdvC coding  
451 sequences belonging to *Sulfolobus solfataricus*\_P2, and Bathyarchaeota  
452 from NCBI database (<http://www.ncbi.nlm.nih.gov>, accession number:  
453 KZV07689.1, P36108.2, NP\_013794.1; KKK42121.1, KKK42122.1,  
454 KKK44605.1; OLS30569.1, OLS30568.1, OLS30800.1; OLS27542.1,  
455 OLS27541.1, OLS27540.1; OLS18192.1, OLS18193.1, OLS18194.1,  
456 AAK41192.1, WP\_119819537.1, respectively) were codon optimized by  
457 *GeneDesgin* (<http://54.235.254.95/gd/>) for expression in *E. coli* BL21,  
458 synthesized (BGI Genomics Co., Ltd), and, respectively, cloned into a



459 pCold-TF vector (Takara Bio Co Ltd., Japan) that includes an N-terminal  
460 His tag and a soluble trigger factor chaperone tag. The *E. coli* BL21 (Takara  
461 Bio Co Ltd, Japan) beared the recombinant vectors were inoculated in LB  
462 medium containing 100 µg/ml carbenicillin, and incubated at 37°C until the  
463 OD<sub>600</sub> reached at 0.6-0.8, and then the isopropyl-d-1-thiogalactopyranoside  
464 was added at the final concentration of 0.5 mM, followed by incubation at  
465 15 °C for 18-24 h. The cells pellets were collected and resuspended in 20 ml  
466 binding buffer (20 mM phosphate buffer (pH 7.4), 500 mM NaCl, 50 mM  
467 imidazole, 1 mM dithiothreitol, 1 mM lysozyme, and 1 mM  
468 phenylmethylsulfonyl fluoride), followed by ultrasonic decomposition. Next,  
469 the target proteins were purified by Mag-Beads His-Tag Protein Purification  
470 Kit (BBI Co., Ltd, China) with wash buffer (20 mM phosphate buffer (pH  
471 7.4), 500 mM NaCl, 100 mM imidazole, and 0.1% NP-40) and elution buffer  
472 (20 mM phosphate buffer (pH 7.4), 500 mM NaCl, and 500 mM imidazole).  
473 Finally, the purified proteins were concentrated to 1-2 ml in phosphate  
474 buffered saline (PBS, pH 7.4) by 30K Amicon Ultra-15 (Millipore Co Ltd.,  
475 USA). Concentrations of these proteins were determined by Bradford  
476 Protein Assay Kit (Beyotime Bio Co Ltd., China). The purified Vps4,  
477 Vps2/24/46, and Vps20/32/60 belonging to *S. cerevisiae*,  
478 Lokiarchaeum\_GC14\_75, Thorarchaeota\_AB\_25,

479 Heimdallarchaeota\_LC\_3, and Odinarchaeota\_LCB\_4 were used for  
480 Isothermal titration calorimetry assay. The purified Vps4 belonging to *S.*  
481 *cerevisiae*, Lokiarchaeum\_GC14\_75, Thorarchaeota\_AB\_25,  
482 Heimdallarchaeota\_LC\_3, and Odinarchaeota\_LCB\_4, and Cdv belonging  
483 to *Sulfolobus solfataricus*\_P2, and Bathyarchaeota were used for ATPase  
484 activity assay.

#### 485 **Isothermal titration calorimetry assay**

486 Isothermal titration calorimetry assay (ITC) was carried out at 25°C using an  
487 ITC200 system (MicroCal, USA). The Vps4 MIT domain (3 µM in PBS  
488 buffer) was placed in cell and titrated with 19 injections of 10 µl of  
489 Vps2/24/46 or Vps20/32/60 (33 µM in PBS buffer) at 2 min intervals. The  
490 heat of ligand dilution into buffer was subtracted from the reaction heat,  
491 after removing the data of 1<sup>st</sup> injection. Data analysis was carried out using  
492 Origin 7.0 (MicroCal, USA).

#### 493 **ATPase activity assay**

494 The ATPase activity was determined by a slightly modified malachite green  
495 assay(52). In short, the purified proteins (4 µM) were incubated with  
496 reaction buffer (1 mM ATP, 20 mM HEPES pH 7.4, 100 mM NaCl, 10 mM  
497 MgCl<sub>2</sub>, 1 mM DTT) in a total volume of 50 µl at the indicated temperature  
498 for 90 min, and was immediately stopped by liquid nitrogen. Then, the

499 reaction mixture was added with 100 µl of malachite green color buffer (14  
500 mM ammonium molybdate, 1.3 M HCl, and 1.5 mM malachite green) and  
501 50 µl of 21% (w/v) citric acid, followed by incubation at room temperature  
502 for 30 min. Finally, the reaction mixture that turned green was attributed to  
503 the free phosphate released by Vps4 ATP hydrolysis. Additionally, the  
504 control experiments were identical to the treatment group, except that the  
505 mixture of Vps4 and reaction buffer was immediately treated with liquid  
506 nitroge before addition of malachite green color buffer and citric acid; and  
507 these experiments were to eliminate the interference of irrelevant free  
508 phosphate. Also, the empty vector was served to prove that the ATP  
509 hydrolysis is due to Vps4.

#### 510 ***S. cerevisiae* strains and cultivation**

511 The *S. cerevisiae* strain BY4741 (*MATa leu2Δ0 met15Δ0 ura3Δ0 his3Δ1*)  
512 and its derivative *vps4* null mutant strain YPR173Ca (designated the *S.*  
513 *cerevisiae*Δ*vps4* in this study) were from *S. cerevisiae* deletion mutant  
514 library (53). *S. cerevisiae* cells were routinely cultured in YPD medium (10  
515 g/L yeast extract, 20 g/L peptone, 20 g/L glucose) or SC-Ura medium (6.7  
516 g/L YNB, 0.01µmol/L Fe(NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub>, 20 g/L glucose, and complete amino  
517 acids without uracil) at 30 °C unless otherwise noted. The solid media were  
518 identical to those of YPD or SC-Ura except that agar was present.

## 519 **Complementation Assay**

520 The Vps4 coding sequences belonging to Lokiarchaeum\_GC14\_75,  
521 Thorarchaeota\_AB\_25, Heimdallarchaeota\_LC\_3, and  
522 Odinarchaeota\_LCB\_4, and CdvC coding sequences belonging to *Sulfolobus*  
523 *solfataricus*\_P2, and Bathyarchaeota (NCBI accession number:  
524 KKK42121.1, OLS30569.1, OLS27542.1, OLS18192.1, AAK41192.1,  
525 WP\_119819537.1, respectively) were codon optimized by *GeneDesgin*  
526 (<http://54.235.254.95/gd/>) for expression in *S. cerevisiae*, before synthesis by  
527 BGI Genomics Co., Ltd (54). To eliminate the interference of transcriptional  
528 level factor, a native promoter region of *S. cerevisiae* BY4741 *vps4* (a 500  
529 bp DNA sequence region upstream from the ATG start codon of this gene)  
530 was used to drive the coding sequences. Then, we assembled the coding  
531 sequences, the *S. cerevisiae vps4* native promoter, and a *S. cerevisiae* CYC1  
532 (Cytochrome c isoform 1) terminator into a pPOT-RFP vector according to a  
533 developed YeastFab Assembly protocol(31). Besides, the pPOT-RFP vector  
534 containing the entire *S. cerevisiae* BY4741 *vps4* with its native promoter  
535 and the CYC1 terminator were transformed into the *vps4* null mutant *S.*  
536 *cerevisiae*(32), and this reconstituted strain was designated the "+*S.*  
537 *cerevisiae*". In this study, both the *S. cerevisiae* and *S. cerevisiae vps4* $\Delta$  were  
538 transformed with the pPOT-RFP vector as the control.

539 **FM-64M staining**

540 *S. cerevisiae* cells of each strain were cultured in SC-Ura medium at 30 °C  
541 and normalized to an OD<sub>600</sub> of 0.5-0.8. Then, the *S. cerevisiae* cells were  
542 stained with 80 μM FM-64M (AAT Bioquest Co Ltd., China) at 30 °C for 20  
543 min, and next cultured for 120 min after washes with medium. Finally, the *S.*  
544 *cerevisiae* cells were examined under a N-STORM fluorescence microscope  
545 (Nikon Co Ltd., Japan).

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559

## 560 **AUTHOR' CONTRIBUTIONS**

561 Zhongyi Lu and Meng Li conceived and designed the experiments. Zhongyi  
562 Lu, Tianyi Li, Siyu Zhang, and Jinqian Li performed the experiments.  
563 Huiying Chu and Guohui Li designed the molecular dynamics strategy. Ting  
564 Fu performed the simulations. Zhongyi Lu, Ting Fu, and Huiying Chu  
565 analyzed the data. Yang Liu and Junbiao Dai contributed  
566 reagents/materials/analysis tools. Zhongyi Lu, Ting Fu, Eugene Koonin and  
567 Meng Li wrote and all authors edited and approved the paper.

568

## 569 **CONFLICT OF INTEREST**

570 The authors declare no conflict of interest.

571

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### Figures Legends

737 **FIG 1. Phylogenetic and amino acid sequence analysis of the ESCRT-**

738 **III-related subunits in archaea and eukarya.** (A) Unrooted maximum-

739 likelihood phylogenetic tree of the ESCRT-III-related subunits in archaea

740 and eukarya. The information of the Asgard Vps2/24/46 and Vps20/32/60

741 can be found in Table S1. Part of the bootstrap values are shown on nodes.

742 (B) Predicted MIM1 and MIM2 in Asgard Vps2/24/46 and Vps20/32/60,

743 respectively. The information of proteins used here can be found in Table S1.

744 The ESCRT-III core domain, C-terminal helix, and MIM1 and MIM2 are

745 presented.

746

747 **FIG 2. Phylogenetic and structural analysis of the Asgard Vps4.**

748 (A) Unrooted maximum likelihood phylogenetic analysis of the Vps4-

749 related in archaea and eukarya. The information of the Asgard Vps4 can be

750 found in Table S1. Part of the bootstrap values are shown on nodes. (B)

751 Phylogenetic (a) and structural (b) analysis of the Asgard Vps4 MIT domain.  
752 The sequences of CdvC MIT domain are used as the outgroup to further  
753 confirm the phylogenetic relationship of the MIT domain in eukaryotic and  
754 Asgard Vps4. The antiparallel three-helix bundle of MIT domains is shown  
755 explicitly.

756

757 **FIG 3. Comparison of the Vps4 (surface representation, grey) in**  
758 **complex with ESCRT-III subunits (ribbon representation, blue) in**  
759 **Asgard archaea.**

760 The MIM1 and MIM2 are shown in orange (stick representation, orange)  
761 and highlighted in red in close-up views (space filling representation). The  
762 black letters indicated main residues in MIT domains that contribute to the  
763 interaction. The Vps4 MIT domain in complex with (A) Vps2/24/46 and (B)  
764 Vps20/32/60 subunits in *S. cerevisiae*, Heimdall\_LC\_3, Odin\_LCB\_4,  
765 Thor\_AB\_25, and Loki\_GC14\_75 are indicated.

766

767 **FIG 4. Functional complementation of *Saccharomyces cerevisiae* vps4**  
768 **null mutants by Asgard Vps4.**

769 (A) Complementation of the high-temperature-sensitive growth defect of  
770 vps4 mutant cells. Five microliters of a series of 10-fold dilutions derived

771 from a starting suspension of an OD<sub>600</sub> of 10<sup>-1</sup> was inoculated into SC-Ura  
772 medium. (B) The ATPase activity of *S. cerevisiae* Vps4, Asgard Vps4 and  
773 Cdvs at 30 °C and 39 °C were, respectively, confirmed by a malachite green  
774 assay. The substrates would turn from golden to green owing to the  
775 inorganic phosphate released from ATP hydrolysis by Vps4 under the  
776 indicated condition. (C) The class E compartments in *S. cerevisiae* vps4 null  
777 mutants were largely abrogated by Asgard Vps4. The vacuolar morphologies  
778 in the indicated strains were visualized by fluorescent microscopy.  
779 Arrowhead highlights the class E compartment in vps4 null mutant. Scale  
780 bar=10 μm. Quantification of class E compartment in the indicated strains.  
781 The results represented the means from three independent replicates (20  
782 cells per experiment), and standard deviations are indicated with error bars.  
783 Statistical significance was assessed by one-way analysis of variance with  
784 Bonferroni's multiple-comparison test. \*\*,  $P < 0.01$ .

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## Supplemental information

792 **FIG S1. Phylogenetic analysis of ESCRT-III-related proteins in the**  
793 **eukarya and archaea.** The tree was reconstructed by maximum likelihood  
794 analysis using 156 representative amino acid sequences based on LG+G4  
795 model (recommended by the "TESTONLY"), with option "-bb 1000", and  
796 the bootstrap values are shown on nodes.

797

798 **FIG S2. Phylogenetic analysis of Vps4-related proteins in eukarya and**  
799 **archaea.** The tree was reconstructed by maximum likelihood analysis using  
800 76 representative amino acid sequences based on LG+I+G4 model  
801 (recommended by the "TESTONLY"), with option "-bb 1000", and the  
802 bootstrap values are shown on nodes.

803

804 **FIG S3. Predicted "arginine collar" in Vps4 of Asgard archaea and**  
805 **eukarya.** (A) The Walker A, Walker B, Sensor I, ARG finger, and Sensor II  
806 are conserved across all the indicated sequences, and are shown to confirm  
807 the location of "arginine collar". Conserved arginine residues of "arginine  
808 collar" are highlighted (red shading and red letters, respectively). The  
809 information of proteins used here can be found in Table S1. (B) The top and  
810 bottom views of the hexameric ring (grey) were constructed by

811 Heimdall\_LC\_3 Vps4 (white) as the example to demonstrate the location of  
812 "arginine collar", including R222, R231, and R232.

813

814 **FIG S4. Phylogenetic analysis of the Microtubule Interacting and**  
815 **Transport domain in Vps4-related proteins.** The tree was reconstructed  
816 by maximum likelihood analysis using 48 amino acid sequences based on  
817 LG+I+G4 model (recommended by the "TESTONLY"), with option "-bb  
818 1000", and the bootstrap values are shown on nodes.

819

820 **FIG S5. The number of Vps4 and CdvC Microtubule Interacting and**  
821 **Transport domain amino acid residues in alpha conformation of**  
822 **Asgard archaea, *Saccharomyces cerevisiae* and TACK archaea during**  
823 **molecular dynamic simulations.** The curves of the numbers of Vps4 MIT  
824 domain amino acid residues in alpha conformation of Asgard and *S.*  
825 *cerevisiae*, which were calculated from the last 200 ns MD simulation  
826 trajectories, were plotted against simulation time.

827

828 **FIG S6. ITC binding profiles of Asgard Vps4 Microtubule Interacting**  
829 **and Transport domain titrated with Asgard Vps2/24/46 and**  
830 **Vps20/32/60.** (A) The curve of Heimdall\_LC\_3 TF-Vps4-MIT titrated with

831 Heimdall\_LC\_3 TF-Vps2/24/46 was fit to Sequential Binding Sites;  $\Delta H_1=-$   
832  $4.73 \times 10^5$  cal mol<sup>-1</sup>;  $\Delta H_2=9.31 \times 10^5$  cal mol<sup>-1</sup>. The curve of Heimdall\_LC\_3-  
833 Vps4-MIT titrated with Heimdall\_LC\_3 TF-Vps20/32/60 was fit to  
834 Sequential Binding Sites;  $\Delta H_1=2.78 \times 10^6$  cal mol<sup>-1</sup>;  $\Delta H_2=-2.31 \times 10^6$  cal mol<sup>-1</sup>.  
835 (B) The curve of Odin\_LCB\_4 TF-Vps4-MIT titrated with Odin\_LCB\_4  
836 Vps2/24/46 was fit to One Set of Sites;  $\Delta H=3.92 \times 10^5$  cal mol<sup>-1</sup>. The curve of  
837 Odin\_LCB\_4 TF-Vps4-MIT titrated with Odin\_LCB\_4 TF-Vps20/32/60  
838 was fit to One Set of Sites;  $\Delta H=3.31 \times 10^5$  cal mol<sup>-1</sup>. (C) The curve of  
839 Thor\_AB\_25 TF-Vps4-MIT titrated with Thor\_AB\_25 TF-Vps2/24/46 was  
840 fit Sequential Binding Sites;  $\Delta H_1=3.25 \times 10^8$  cal mol<sup>-1</sup>;  $\Delta H_2=1.29 \times 10^6$  cal  
841 mol<sup>-1</sup>. The curve of Thor\_AB\_25 TF-Vps4-MIT titrated with Thor\_AB\_25  
842 TF-Vps20/32/60 was fit to One Set of Sites;  $\Delta H=1.62 \times 10^6$  cal mol<sup>-1</sup>. (D)  
843 The curve of Loki\_GC14\_75 TF-Vps4-MIT titrated with Loki\_GC14\_75  
844 TF-Vps2/24/46 was fit to Sequential Binding Sites;  $\Delta H_1=-1.21 \times 10^5$  cal mol<sup>-1</sup>;  
845  $\Delta H_2=4.22 \times 10^5$  cal mol<sup>-1</sup>. The curve of Loki\_GC14\_75 TF-Vps4-MIT  
846 titrated with Loki\_GC14\_75 TF-Vps20/32/60 was fit to One Set of Sites;  
847  $\Delta H=1.42 \times 10^6$  cal mol<sup>-1</sup>. Binding to a TF control surface was negligible (not  
848 shown).

849

850 **TABLE S1. Summary of proteins used in this study.**

851

852 **TABLE S2. The predicted binding free energies between Vps4 and**  
853 **ESCRT-III subunits (Vps2/24/46 (A), and Vps20/32/60 (B)).**

854

855 **TABLE S3. The dominant amino acid residues of Vps4 Microtubule**  
856 **Interacting and Transport domain involved in binding with Vps2/24/46**  
857 **are listed.**

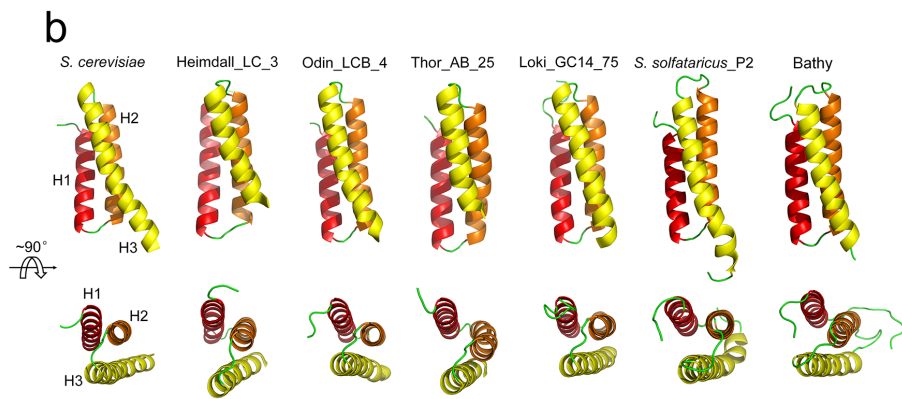
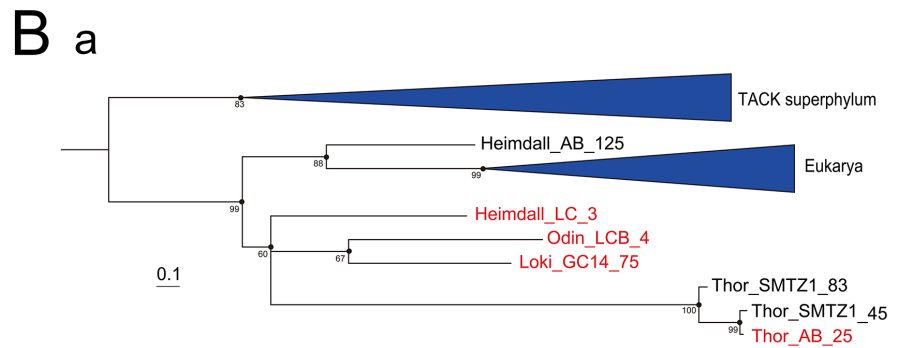
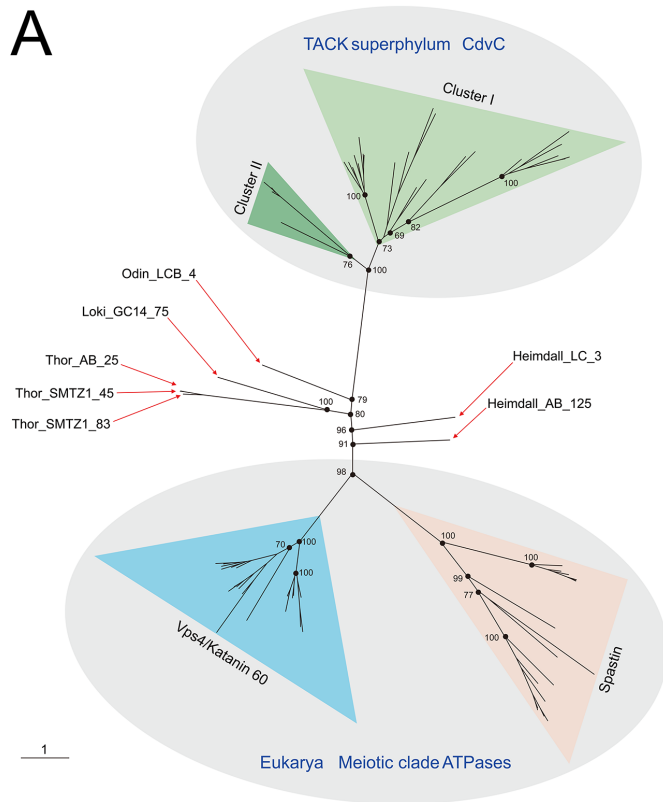
858

859 **TABLE S4. The dominant amino acid residues of Vps4 Microtubule**  
860 **Interacting and Transport domain involved in binding with Vps20/32/60**  
861 **are listed.**

862



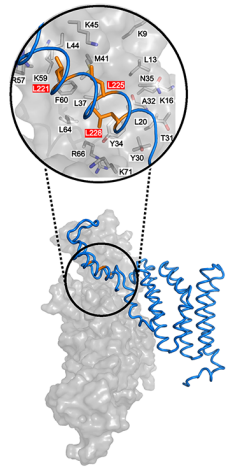




**A**

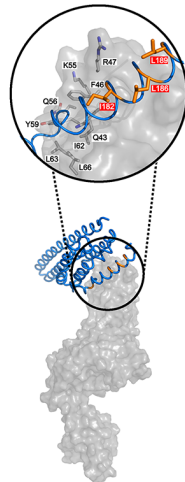
*S. cerevisiae*

Vps4 MIT vs Vps2



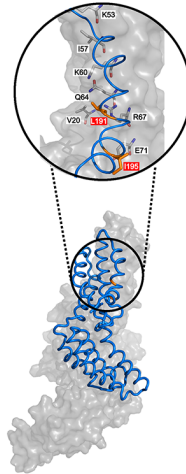
Heimdall\_LC\_3

Vps4 MIT vs Vps2/24/46



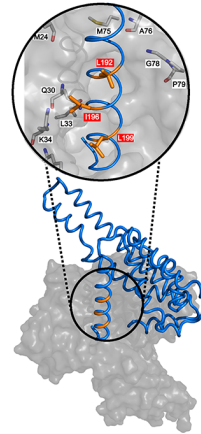
Odin\_LCB\_4

Vps4 MIT vs Vps2/24/46



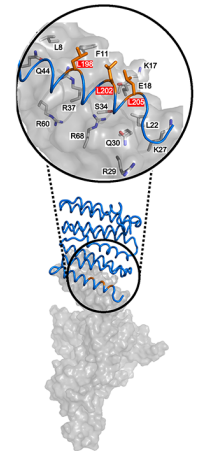
Thor\_AB\_25

Vps4 MIT vs Vps2/24/46



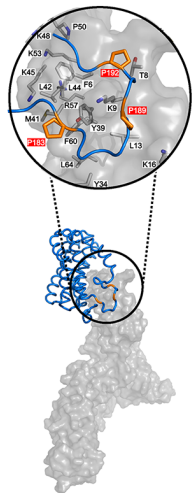
Loki\_GC14\_75

Vps4 MIT vs Vps2/24/46

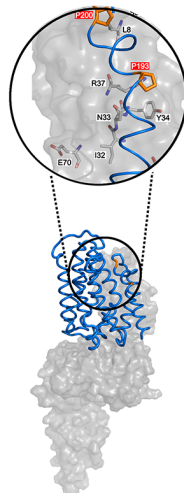


**B**

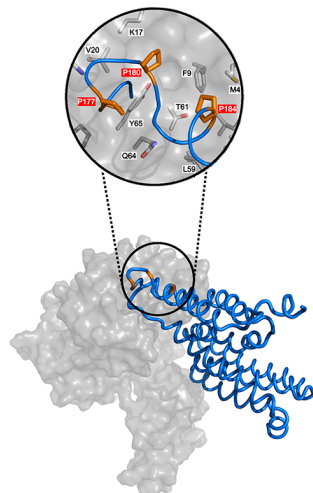
Vps4 MIT vs Vps20



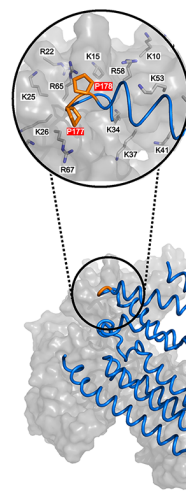
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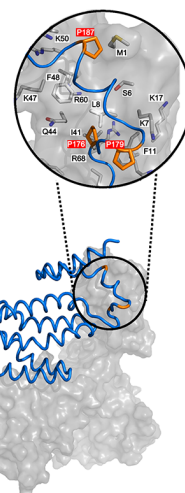
Vps4 MIT vs Vps20/32/60



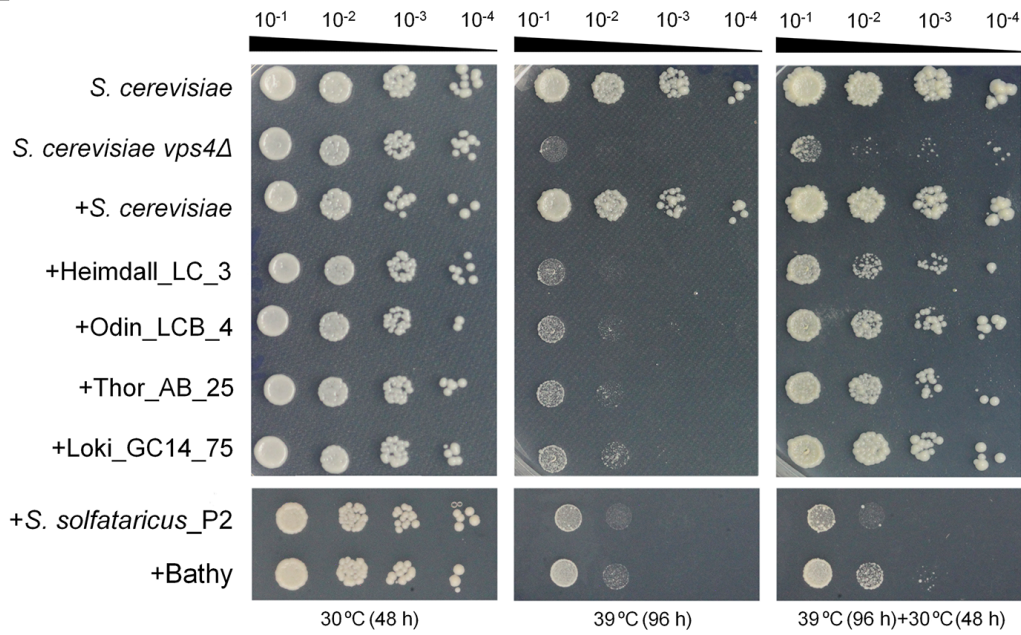
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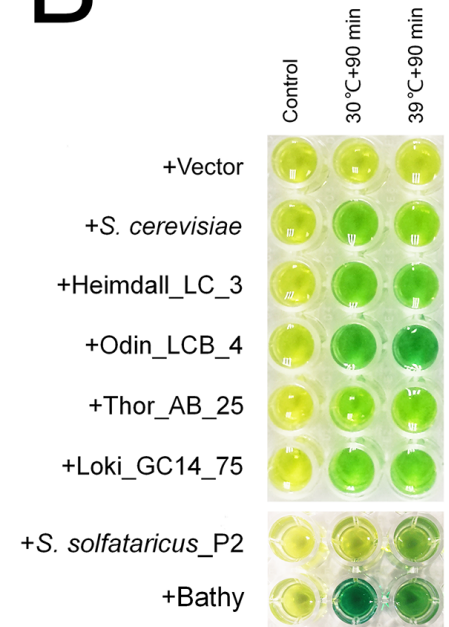
Vps4 MIT vs Vps20/32/60



**A**



**B**



**C**

