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## Supplementary Materials for

### Mechanism of Vps4 hexamer function revealed by cryo-EM

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> Published 14 April 2017, *Sci. Adv.* **3**, e1700325 (2017) DOI: 10.1126/sciadv.1700325

#### The PDF file includes:

- fig. S1. Vps4 oligomerizes into a hexamer in the presence of ATP.
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- fig. S8. Sequence alignments of Vps4 proteins from *S. cerevisiae*, *Schizosaccharomyces pombe*, *Caenorhabditis elegans*, *Drosophila melanogaster*, and *Homo sapiens*.
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- fig. S11. One wild-type subunit per hexamer is sufficient to maintain full Vps4 hexamer ATPase activity.
- fig. S12. Filament disassembly activity of Vps4.

#### Other Supplementary Material for this manuscript includes the following:

(available at advances.sciencemag.org/cgi/content/full/3/4/e1700325/DC1)

- movie S1 (.mov format). Molecular dynamic flexible fitting into Vps4 open and closed cryo-EM maps.
- movie S2 (.mov format). Morphing motion between Vps4 open and closed models.



**fig. S1. Vps4 oligomerizes into a hexamer in the presence of ATP.** Size exclusion chromatography profile of Vps4<sup>E233Q</sup> in the presence (red curve) and absence of ATP (blue curve). The molecular weights of the species under the elution peak are determined using multi-angle light scattering (MALS) analysis.



**fig. S2. Cryo-EM images of Vps4 oligomer.** (**A** and **B**) Representative cryo-EM images of Vps4 oligomer acquired at  $0^{\circ}$  (A) and  $45^{\circ}$  (B) tilt. (**C** and **D**) Representative reference-free 2D class averages showing particle projections from  $0^{\circ}$  (C) and  $45^{\circ}$  (D) tilt. Scale bar corresponds to 100 Å.

# 183,658 particles extracted from 834/856 (0°/45°-tilted) micrographs



**fig. S3. Flow chart of particle classification and 3D map reconstruction.** Details are provided in the Methods section.



**fig. S4. Map resolution estimation and projection angle distribution.** (**A**) Gold standard Fourier Shell Correlation (FSC) curves for the final 3D maps of Vps4 indicate resolution of 6.1 Å and 6.7 Å for the closed and open conformers, respectively (FSC=0.143 criterion). (**B** and **C**) 3D map re-projection and Euler angle distribution for the closed (B) and open (C) conformers.



**fig. S5. Fitting of the Vps4 hexamer structure into the cryo-EM map.** (A) Closed conformation of Vps4 hexamer after rigid body fitting (left) and flexible fitting (right). The cross correlation coefficient between protein backbone and the cryo-EM map is 0.753 and 0.846, respectively. (B) Open conformation after rigid body fitting (left) and flexible fitting (right). The cross correlation coefficient is 0.800 and 0.886, respectively. The red dashed line circled regions in both (A) and (B) are sample regions whose fitting into the cryo-EM map is significantly improved by flexible fitting over rigid body fitting.



**fig. S6. Molecular dynamics flexible fitting.** (A) Left, cross correlation coefficient between the backbone protein structure and the cryo-EM map for the closed conformation throughout the last three phases of MDFF for each of the three replicate runs. Right, backbone root mean square deviation (RMSD) from the starting structure for the closed conformation during the last three phases of MDFF for each run. (B) The same as in (A) for the open conformation.



**fig. S7. Comparison of the crystal structure and Vps4 hexamer subunit structures.** Comparison between the crystal structure of Vps4 (2QPA, red) and MD-fitted subunit structures in the maps of closed and open hexamer conformers.

		α1' (MIT)α2' (MIT)	
VPS4 YEAST	1	-MSTGDFLTKGIELVQKAIDLDTATQYEEAYTAYYNGLDYLMLALKYEK-NPKSKDLIRA	58
VPS4 SCHPO	1	-MSNPDCLSKAISLVKTAIDNDNAEQYPDAYKYYQSALDYFMMALKYEK-NEKSKEIIRS	58
VPS4 WORM	1	MSVPALOKAIELVTKATEEDTAGRYDOALRLYDOAIEYFLHAIKYESOGDKORNAIRD	58
VPS4 FLY	1	-MAAGTTLOKAIDLVTKATEEDRNKNYAEALRLYEHGVEYFLHTIKYEAOGEKAKDSIRA	59
VPS4 HUMAN	1	MSSTSPNLOKAIDLASKAAOEDKAGNYEEALOLYOHAVOYFLHVVKYEAOGDKAKOSIRA	60
		α3′ (MIT)	
VPS4_YEAST	59	KFTEYLNRAEQLKKHLESEEANAAKKSPSAGSGSNGG-NKKISQEEGEDNGGEDNKKLRG	117
VPS4_SCHPO	59	KVIEYLDRAEKLKVYLQEKNNQISSKSRVSNGNVEGS-NSPTANEALDSDAKKLRS	113
VPS4_WORM	59	KVGQYLNRAEQIKTHLKDGKTQ-KKPVKDGKDDSDEDEDKKKFQD	102
VPS4_FLY	60	KCLQYLDRAEKLKEYLKKGKKKPIKEGGESSAKDDKDKKSDSDDEDGDDPEKKKLQS	116
VPS4_HUMAN	61	KCTEYLDRAEKLKEYLKNKEKKAQKPVKEGQPSPAD-EKGND-SDGEGESDDPEKKKLQN	118
VPS4_YEAST	118	ALSSAILSEKPNVKWEDVAGLEGAKEALKEAVILPVKFPHLFKGNRKPTSGILLYGPPGT	177
VPS4_SCHPO	114	ALTSAILVEKPNVRWDDIAGLENAKEALKETVLLPIKLPQLFSHGRKPWSGILLYGPPGT	173
VPS4_WORM	103	KLSGAIVMEKPNVKWTDIAGLEGAKEALKEAVILPIKFPQLFTGNRKPWQGILLFGPPGT	162
VPS4_FLY	117	KLEDAIVIEKPKVQWSDVAGLDAAKEALKEAVILPIKFPQLFTGKRIPWKGILLFGPPGT	176
VPS4_HUMAN	119	QLQGAIVIERPNVKWSDVAGLEGAKEALKEAVILPIKFPHLFTGKRTPWRGILLFGPPGT	178
		$-\alpha^2 = \beta^2 = \alpha^3 = -\alpha^4 = \alpha^4 = \beta^3 = -\beta^3 = -\beta^3$	
VDGA VEAST	178	CKSVLAKAVATEAN_STEESUSSSDLUSKWMCESEKLUKOLEAMADENKOSTIEIDEVDA	236
VDS4_ILADI	174	CKCYLAKAVATEAC-CTEECICCCI VCKWMCECEDI VCCI FEMADEOKDCI I I DEVDA	230
VID4_BCHIO	163	CKSYLAKAVATEAG-SITT SISSSELVSKAMGEBEKEVKOLT HARLOKT SITT DEIDS	222
VDCA FTV	177	CKOTTAKAVATEAGESTITTSISSSDIMOKADGESEKLVKADI ALAKEMKESTITTDEIDS	222
VPS4_FLI VPS4_HIIMAN	179	GKSYLAKAVATEANNSTFFSISSSDLVSKWIGESEKLVKNLFOLARENKPSIIFIDEIDS	238
····	1.75		200
		α6 _ β5	
VPS4_YEAST	237	LTGTRGEGESEASRRIKTELLVQMNGVGNDSQGVLVLGATNIPWQLDSAIRRFERRIYI	296
VPS4 SCHPO	233	LCGSRSEGESESSRRIKTEFLVQMNGVGKDESGVLVLGATNIPWTLDSAIRRFEKRIYI	292
VPS4_WORM	223	LCSARSDNESESARRIKTEFMVQMQGVGLNNDGILVLGATNIPWILDSAIR <mark>RR</mark> FEKRIYI	282
VPS4_FLY	237	MCSARSDNENDSVRRIKTEFLVQMQGVGNDTDGILVLGATNIPWVLDSAIR <mark>RR</mark> FEKRIYI	296
VPS4_HUMAN	239	LCGSRSENESEAARRIKTEFLVQMQGVGVDNDGILVLGATNIPWVLDSAIR <mark>RR</mark> FEKRIYI	298
		Pn 8n 7n	
	207		250
VPS4_IEAST	297		320
VPS4_SCHPO	293	PLPNAHARARMFELNVGRIPSELTSQDFRELARMTDGISGSDISIVVRDAIMEPVRRIHT	352
VPS4_WORM	283		342
VPS4_FLY	297	PLPEAHARLVMFKIHLGNTTHVLTEQDLKELAGKTEGISGADISIVVRDALMEPVRKVQT	766
			356
VI 04_11011414	299	PLPEPHARAAMFKLHLGTTQNSLTEADFRELGRKTDGYSGADISIIVRDALMQPVRKVQS	356 358
VID4_nomi	299	PLPEPHARAAMFKLHLGTTQNSLTEADFRELGRKTDGYSGADISIIVRDALMQPVRKVQS	356 358
VPS4 YEAST	299 357	PLPEPHARAAMFKLHLGTTQNSLTEADFRELGRKTDGYSGADISIIVRDALMQPVRKVQS	356 358 410
VPS4_YEAST VPS4_SCHPO	299 357 353	PLPEPHARAAMFKLHLGTTQNSLTEADFRELGRKTDGYSGADISIIVRDALMQPVRKVQS 	356 358 410 406
VPS4_YEAST VPS4_SCHPO VPS4_WORM	299 357 353 343	PLPEPHARAAMFKLHLGTTQNSLTEADFRELGRKTDGYSGADISIIVRDALMQPVRKVQS   β6 β7 β8 α10   ATHFKDVSTEDDETRKLTPCSPGDDGAIEMSWTDIEADELKEPDLTIKDFLKAI α   ATHFKEVYDNKSNRTLVTPCSPGDPDAFESSWLEVNPEDIMEPKLTVRDFYSAV   ATHFKHVSGPSPKDPNVIAHDLLTPCSPGDPDAFESSWLEVNPEDIMEPKLANPPLSMODISRSL	356 358 410 406 402
VPS4_YEAST VPS4_SCHPO VPS4_WORM VPS4_FLY	299 357 353 343 357	PLPEPHARAAMFKLHLGTTQNSLTEADFRELGRKTDGYSGADISIIVRDALMQPVRKVQS   β6 β7 β8 α10   ATHFKDVSTEDDETRKLTPCSPGDDGAIEMSWTDIEADELKEPDLTIKDFLKAI ATHFKEVYDNKSNRTLVTPCSPGDPDAFESSWLEVNPEDIMEPKLTVRDFYSAV   ATHFKHVSGPSPKDPNVIAHDLLTPCSPGDPAAIAMNWLDVPGDKLANPPLSMQDISRSL   ATHFKKVSGPSPTNHEEIVNDLVPCSPGDQCAVEMNWMDVPSDKLEEPPVTMRDMLKSL	356 358 410 406 402 416
VPS4_YEAST VPS4_SCHPO VPS4_WORM VPS4_FLY VPS4_HUMAN	299 357 353 343 357 359	PLPEPHARAAMFKLHLGTTQNSLTEADFRELGRKTDGYSGADISIIVRDALMQPVRKVQS - <u>B6</u> ATHFKDVSTEDDETRKLTPCSPGDDGAIEMSWTDIEADELKEPDLTIKDFLKAI ATHFKEVYDNKSNRTLVTPCSPGDPDAFESSWLEVNPEDIMEPLTVRDFYSAV ATHFKHVSGPSPKDPNVIAHDLLTPCSPGDPAIAMNWDVPGDKLANPPLSMQDISRSL ATHFKRVSGPSPTNHEIVNDLLTPCSPGDPGAIEMTWMDVPGDKLEPPVYMRDMLKSL ATHFKKVRGPSRADPNNLVDDLLTPCSPGDPGAIEMTWMDVPGDKLEPPVSMSDMLRSL	356 358 410 406 402 416 418
VPS4_YEAST VPS4_SCHPO VPS4_WORM VPS4_FLY VPS4_HUMAN	299 357 353 343 357 359	PLPEPHARAAMFKLHLGTTQNSLTEADFRELGRKTDGYSGADISIIVRDALMQPVRKVQS - <u>B6</u> ATHFKDVSTEDDETRKLTPCSPGDDGAIEMSWTDIEADELKEPDLTIKDFLKAI ATHFKEVYDNKSNRTLVTPCSPGDPDAFSSWLEVNPEDIMEPKLTVRDFYSAV ATHFKHVSGPSPKDPNVIAHDLLTPCSPGDPHAIAMNWLDVPGDKLLAPPLSMQDISRSL ATHFKKVRGPSRADPNHLVDLLTPCSPGDPGAIEMTWMDVPGDKLLEPVVSMSDMLKSL ATHFKKVRGPSRADPNHLVDLLTPCSPGDPGAIEMTWMDVPGDKLLEPVVSMSDMLRSL	356 358 410 406 402 416 418
VPS4_YEAST VPS4_SCHPO VPS4_WORM VPS4_FLY VPS4_HUMAN	299 357 353 343 357 359	PLPEPHARAAMFKLHLGTTQNSLTEADFRELGRKTDGYSGADISIIVRDALMQPVRKVQS - 86 87 88 all ATHFKDVSTEDDETRKLTPCSPGDDGAIEMSWTDIEADELKEPDLTIKDFLKAI ATHFKEVYDNKSNRTLVTPCSPGDPDAFSSWLEVNPEDIMEPKLTVRDFYSAV ATHFKHVSGPSPKDPNVIAHDLLTPCSPGDPHAIAMNWLDVPGDKLLEPVVSMSDMLKSL ATHFKKVRGPSRADPNHLVDLLTPCSPGDPGAIEMTWMDVPGDKLLEPVVSMSDMLRSL ATHFKKVRGPSRADPNHLVDLLTPCSPGDPGAIEMTWMDVPGDKLLEPVVSMSDMLRSL	356 358 410 406 402 416 418
VPS4_YEAST VPS4_SCHPO VPS4_SCHPO VPS4_WORM VPS4_FLY VPS4_HUMAN VPS4_YEAST	299 357 353 343 357 359 411	PLPEPHARAAMFKLHLGTTQNSLTEADFRELGRKTDGYSGADISIIVRDALMQPVRKVQS - <u>B6</u> <u>B7</u> <u>B8</u> <u>a10</u> ATHFKDVSTEDDETRKLTPCSPGDDGAIEMSWTDIEADELKEPDLTIKDFLKAI ATHFKEVYDNKSNRTLVTPCSPGDPHAIAMNWLDVPGDKLANPPLSMQDISRSL ATHFKKVSGPSPKDPNVIAHDLLTPCSPGDPHAIAMNWLDVPGDKLLEPVVSMSDMLKSL ATHFKKVRGPSRADPNHLVDDLLTPCSPGDPGAIEMTWMDVPGDKLLEPVVSMSDMLRSL <b>a11</b> KSTRPTVNEDDLLKQEQFTRDFGQEGN- 437	356 358 410 406 402 416 418
VPS4_YEAST VPS4_SCHPO VPS4_WORM VPS4_FLY VPS4_HUMAN VPS4_YEAST VPS4_SCHPO	299 357 353 343 357 359 411 407	PLPEPHARAAMFKLHLGTTQNSLTEADFRELGRKTDGYSGADISIIVRDALMQPVRKVQS BG B7 B8 a10 ATHFKDVSTEDDETRKLTPCSPGDDGAIEMSWTDIEADELKEPDLTIKDFLKAI ATHFKEVYDNKSNRTLVTPCSPGDPDAFESSWLEVNPEDIMEPKLTVRDFYSAV ATHFKKVSGPSPTNHEEIVNDLLVPCSPGDPAIAMNWLDVPGDKLANPPLSMQDISRSL ATHFKKVRGPSRADPNHLVDDLLTPCSPGDPGAIEMTWMDVPGDKLLEPVVSMSDMLRSL ATHFKKVRGPSRADPNHLVDDLLTPCSPGDPGAIEMTWMDVPGDKLLEPVVSMSDMLRSL KSTRPTVNEDDLLKQEQFTRDFGQEGN- 437 RKVKPTLNAGDIEKHTQFTKDFGAEG 432	356 358 410 406 402 416 418
VPS4_YEAST VPS4_SCHPO VPS4_WORM VPS4_FLY VPS4_HUMAN VPS4_YEAST VPS4_SCHPO VPS4_WORM	299 357 353 343 357 359 411 407 403	PLPEPHARAAMFKLHLGTTQNSLTEADFRELGRKTDGYSGADISIIVRDALMQPVRKVQS   β6 β7 β8 α10   ATHFKDVSTEDDETRKLTPCSPGDDGAIEMSWTDIEADELKEPDLTIKDFLKAI α   ATHFKEVYDNKSNRTLVTPCSPGDPDAFESSWLEVNPEDIMEPKLTVRDFYSAV α   ATHFKHVSGPSPKDPNVIAHDLLTPCSPGDPAAIAMNWLDVEGDKLANPPLSNQDISKSL α   ATHFKKVSGPSPTNHEEIVNDLLVPCSPGDQGAVEMNWMDVPSDKLFEPPVTMRDMLKSL α   MTHFKKVRGPSRADPNHLVDDLLTPCSPGDPGAIEMTWMDVPGDKLLEPVVSMSDMLRSL α   MINING α α   MINING α α   ATHFKKVRGPSRADPNHLVDDLLTPCSPGDPGAIEMTWMDVPGDKLLEPVVSMSDMLRSL α   MINING α	356 358 410 406 402 416 418
VPS4_YEAST VPS4_SCHPO VPS4_SCHPO VPS4_FLY VPS4_HUMAN VPS4_YEAST VPS4_SCHPO VPS4_WORM VPS4_FLY	299 357 353 343 357 359 411 407 403 417	PLPEPHARAAMFKLHLGTTQNSLTEADFRELGRKTDGYSGADISIIVRDALMQPVRKVQS   β6 β7 β8 α10   ATHFKDVSTEDDETRKLTPCSPGDDGAIEMSWTDIEADELKEPDLTIKDFLKAI α   ATHFKEVYDNKSNRTLVTPCSPGDPDAFESSWLEVNPEDIMEPKLTVRDFYSAV α   ATHFKHVSGPSFKDPNVIAHDLLTPCSPGDPDAFESSWLEVNPEDIMEPKLTVRDFYSAV α   ATHFKKVSGPSPTNHEEIVNDLLVPCSPGDPAAIAMNWLDVPGDKLANPPLSMQDISRSL α   ATHFKKVRGPSRADPNHLVDDLLTPCSPGDPGAIEMTWMDVPGDKLLEPVVSMSDMLRSL α   MINIMA α α	356 358 410 406 402 416 418

fig. S8. Sequence alignments of Vps4 proteins from S. cerevisiae,

Schizosaccharomyces pombe, Caenorhabditis elegans, Drosophila melanogaster, and *Homo sapiens*. Secondary structural elements are shown above the sequences:  $\alpha$ -helix, cylinder;  $\beta$ -strand, arrow; loop, line. The MIT domain is colored orange, large ATPase

domain is colored red, small ATPase domain is colored green and the  $\beta$  domain is colored blue. Residues Trp206, Glu233, Arg288, Arg289, Arg414 and Phe432 are highlighted by yellow boxes.



**fig. S9. Residues at subunit interface III are important for Vps4 oligomerization and ATPase activity.** (**A**) The oligomerization behavior of Vps4<sup>E233Q</sup>, Vps4<sup>E233Q/R414A</sup>, Vps4<sup>E233Q/F432A</sup>, and Vps4<sup>E233Q/R414A/F432A</sup> was analyzed on a Superdex200 size exclusion column at a concentration of 30-50 μM. (**B**) ATPase activity of wild-type Vps4, Vps4<sup>R414A</sup>, Vps4<sup>F432A</sup>, and Vps4<sup>R414A/F432A</sup> were determined. Activity is expressed as μM inorganic phosphate released per μM Vps4 per min. Both single and double mutations result in dramatic decrease in activity.



**fig. S10. Structural comparison of Vps4 subunits in the open and closed conformations.** Left, subunits B-F in both conformers (grey) are aligned with each other and against subunit A in the open conformer (orange). Right, subunit A in the closed conformer (yellow) is aligned against subunit A in the open conformer (orange). All alignments are based on the large ATPase domain.



fig. S11. One wild-type subunit per hexamer is sufficient to maintain full Vps4 hexamer ATPase activity. Vps4<sup>WT</sup> and Vps4<sup>E233Q</sup> were mixed at different ratios with the total protein concentration fixed at 0.5  $\mu$ M (solid line), and the ATPase activity was measured as  $\mu$ M inorganic phosphate released per  $\mu$ M of Vps4<sup>WT</sup> per min. The activity for 0.5  $\mu$ M Vps4<sup>WT</sup> was set as 100%. The dashed lines represent theoretical activity curves if one, two, three, four, five, or six wild-type subunits are required to maintain Vps4 hexamer activity. The experimental curve is in agreement with the theoretical curve for which only 1 of 6 subunits is required for maximal ATPase activity.



**fig. S12. Filament disassembly activity of Vps4.** (**A** and **B**) Electron micrographs of negative stained Vps24-Vps2 filaments after incubation with Vps4 and ADP (A) or Vps4<sup>E233Q</sup> and ADP (B) for 15 min on ice. Scale bars, 20 nm. (**C** and **D**) SDS-PAGE gel analysis of Vps4 mediated disassembly of Vps24-Vps2 filaments.