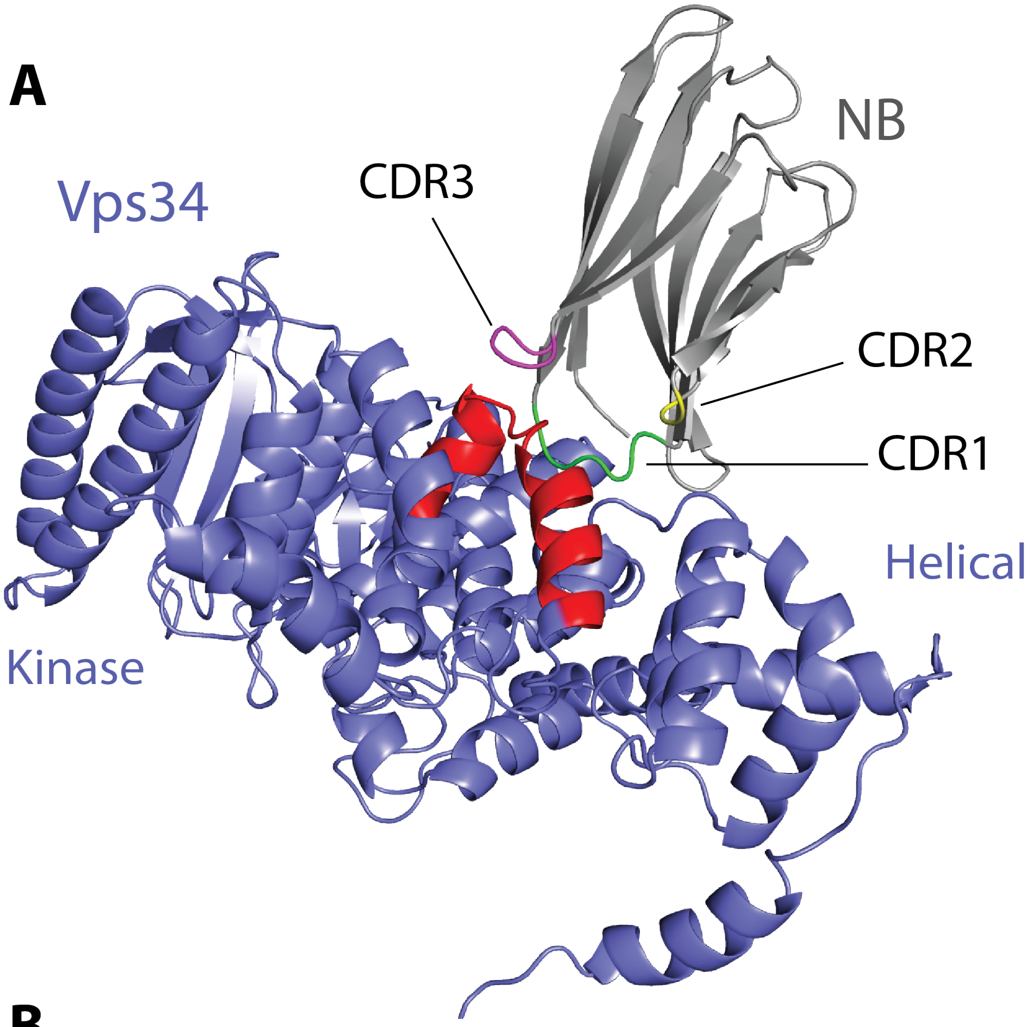
Supplementary Materials

Figs. S1, S2, S3, S4, S5, S6, S7

Tables S1-6

References (34-45)

Movie S1



B

Nanobody 8780

QVQLQESGGGLVQPGGSLRLSCAPSGPFSPNSMGWYRQAPGKQRELVAVM
TIDGRTNYQDSVKGRFTISRDYVKNTAYLQMNNLKPDDTAVYICNAETRGFM
HWGQGTQVTVSSHHHHHH

Fig. S1. The Vps34-recognizing nanobody. **(A)** Nanobody enabling complex II crystallization binds to the helical domain of Vps34. The interaction is mainly mediated by the CDR1 (green) and CDR3 (magenta) with no significant contribution from CDR2 (yellow). The epitope on Vps34 (red) is consistent with the peptide identified by HDX-MS (Table S4). No other interaction is observed with the rest of the complex. The nanobody has limited additional contacts with other molecules in the crystal. It is possible that these additional contacts facilitated crystallization. **(B)** The sequence of Nanobody 8780 with CDRs highlighted.

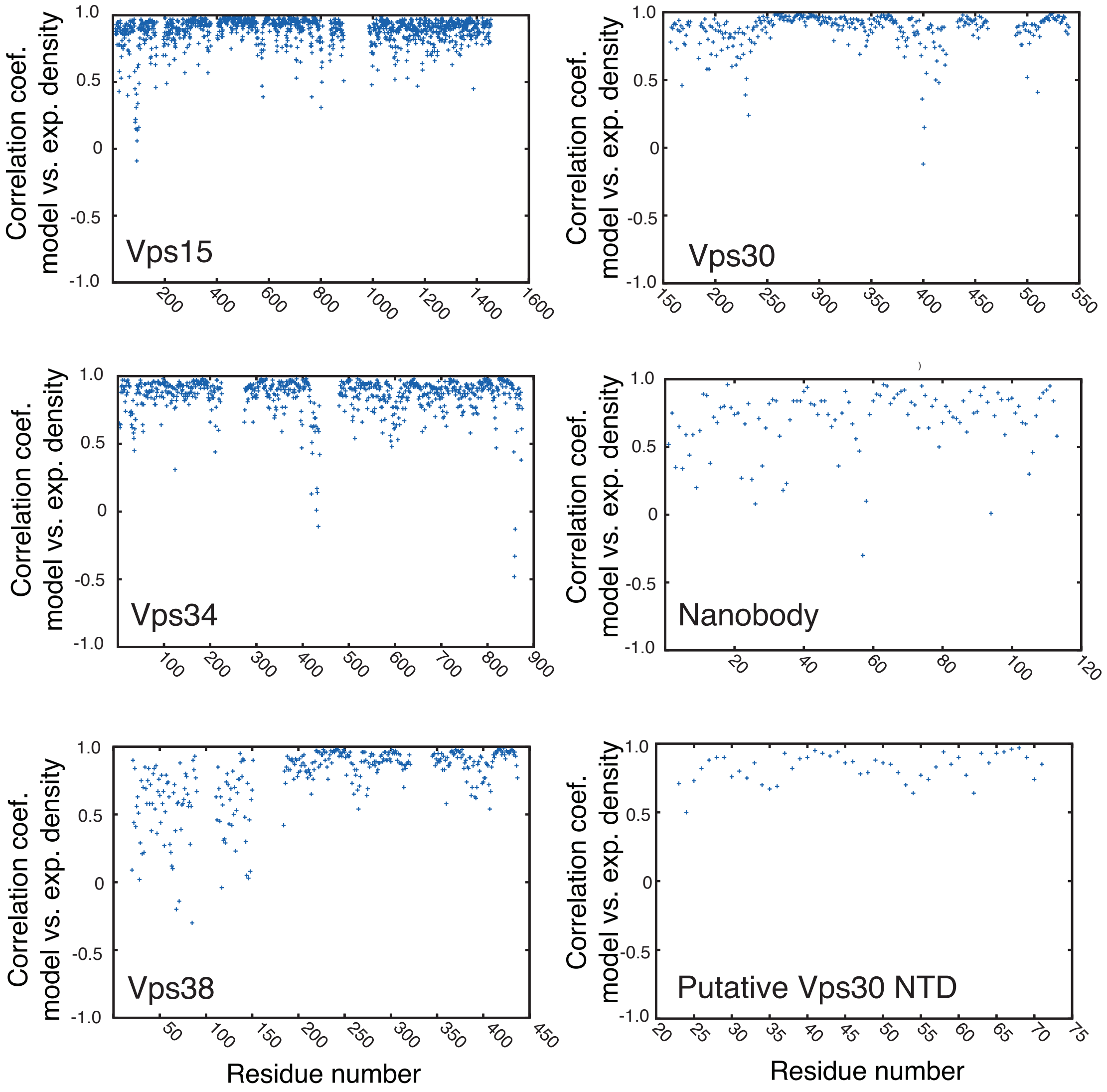


Fig. S2A. Real-space correlation of the model electron density with the experimental (MIRAS) electron electron density map after modification with SOLOMON.

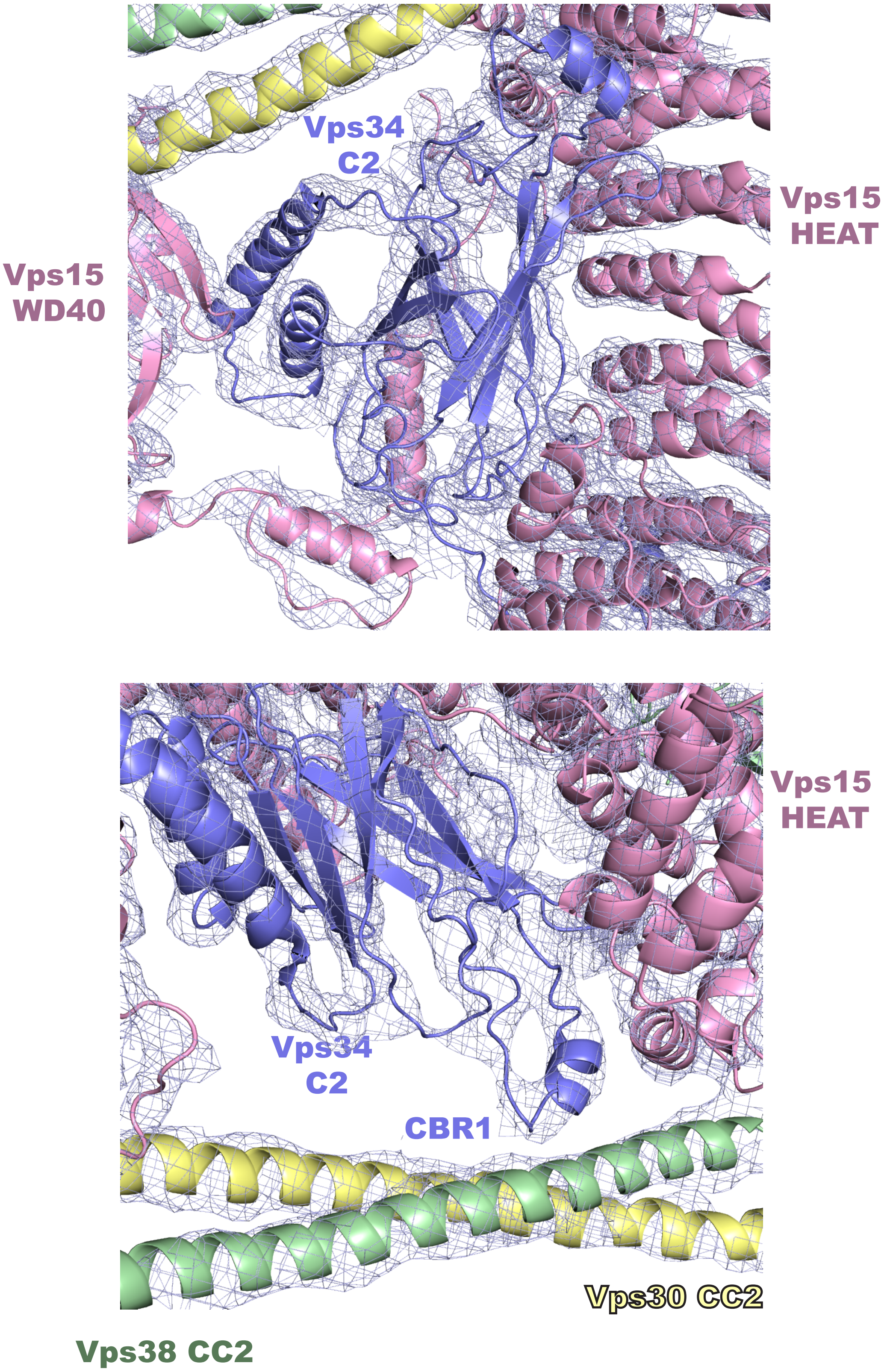


Fig. S2B. The experimental (MIRAS) electron density map after modification with SOLOMON. The map is displayed at a 1.1σ contour level. The Vps34 C2 and its interfaces are shown.

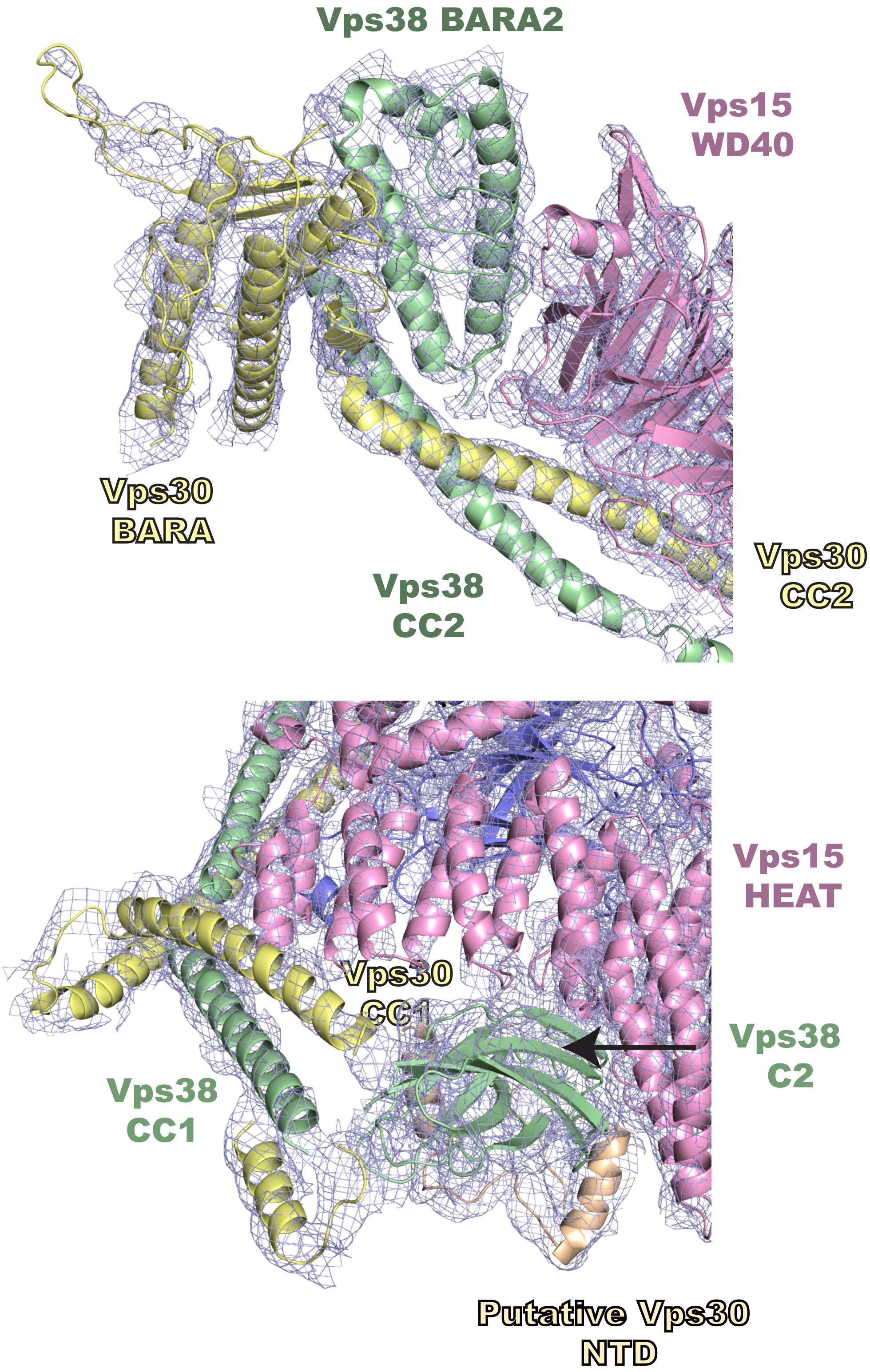


Fig. S2C. The experimental (MIRAS) electron density map after modification with SOLOMON. The map is displayed at a 1.1σ contour level. The Vps38 C2 and the CC1 domains are shown.

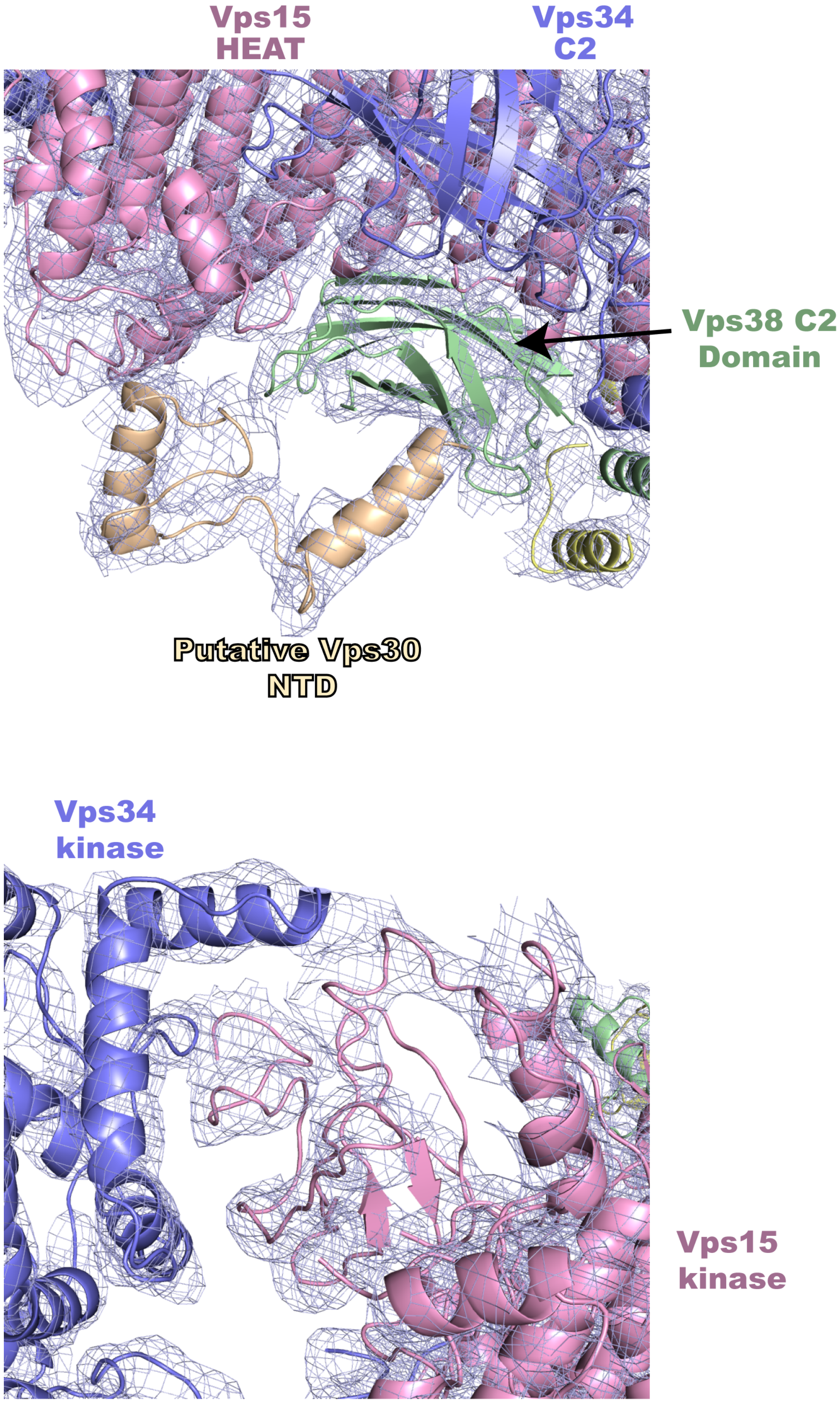


Fig. S2D. The experimental (MIRAS) electron density map after modification with SOLOMON. The map is displayed at a 1.1σ contour level. The putative Vps30 NTD (upper) and the Vps15 kinase/Vps34 kinase domain interface (lower) are shown.

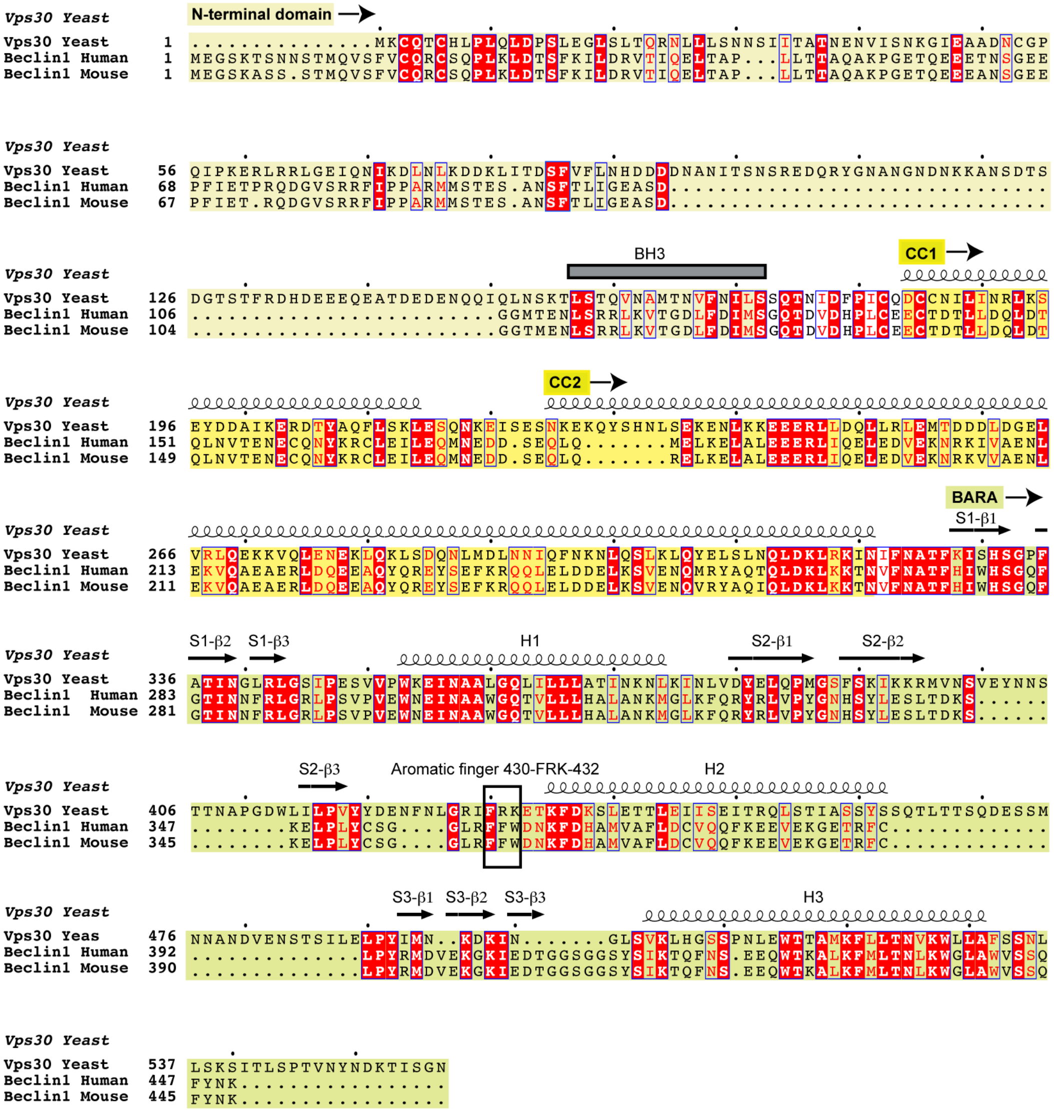


Fig. S3 (A-D). Structure-based sequence alignments for Vps15, Vps34, Vps30 and Vps38. The sequence is shaded with different colours for each domain. Secondary structure elements are asigned based on complex II structure. Completely conserved regions among the species are coloured bright red and predominatly conserved regions are enclosed in blue boxes.

(A) Structure-based sequence alignment of *S. cerevisiae*, *H. sapiens* and *M. musculus* Vps30. Secondary structure prediction is shown for BH3 helix, that cannot be confidently assigned from complex II structure. Residues 430-FRK-432, involved in membrane binding in yeast complex II, align to the previously described “aromatic finger” in Beclin 1 (359-FFW-361). The previous suggestion that the “aromatic finger” is not conserved in yeast was based on the observation that no phenotype arose from mutating residues 418-VYY-420 in yeast Vps30 that were assumed (before the yeast structure was available) to correspond to the human “aromatic finger” (*34*).

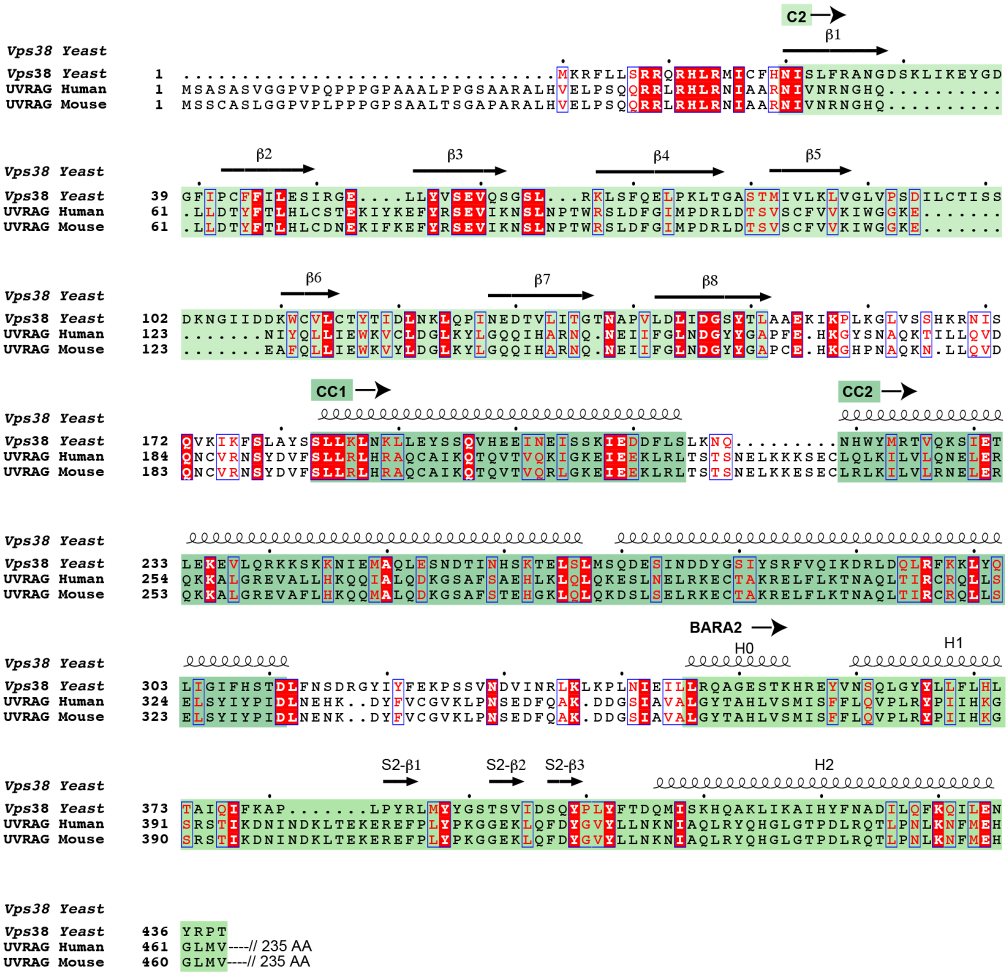
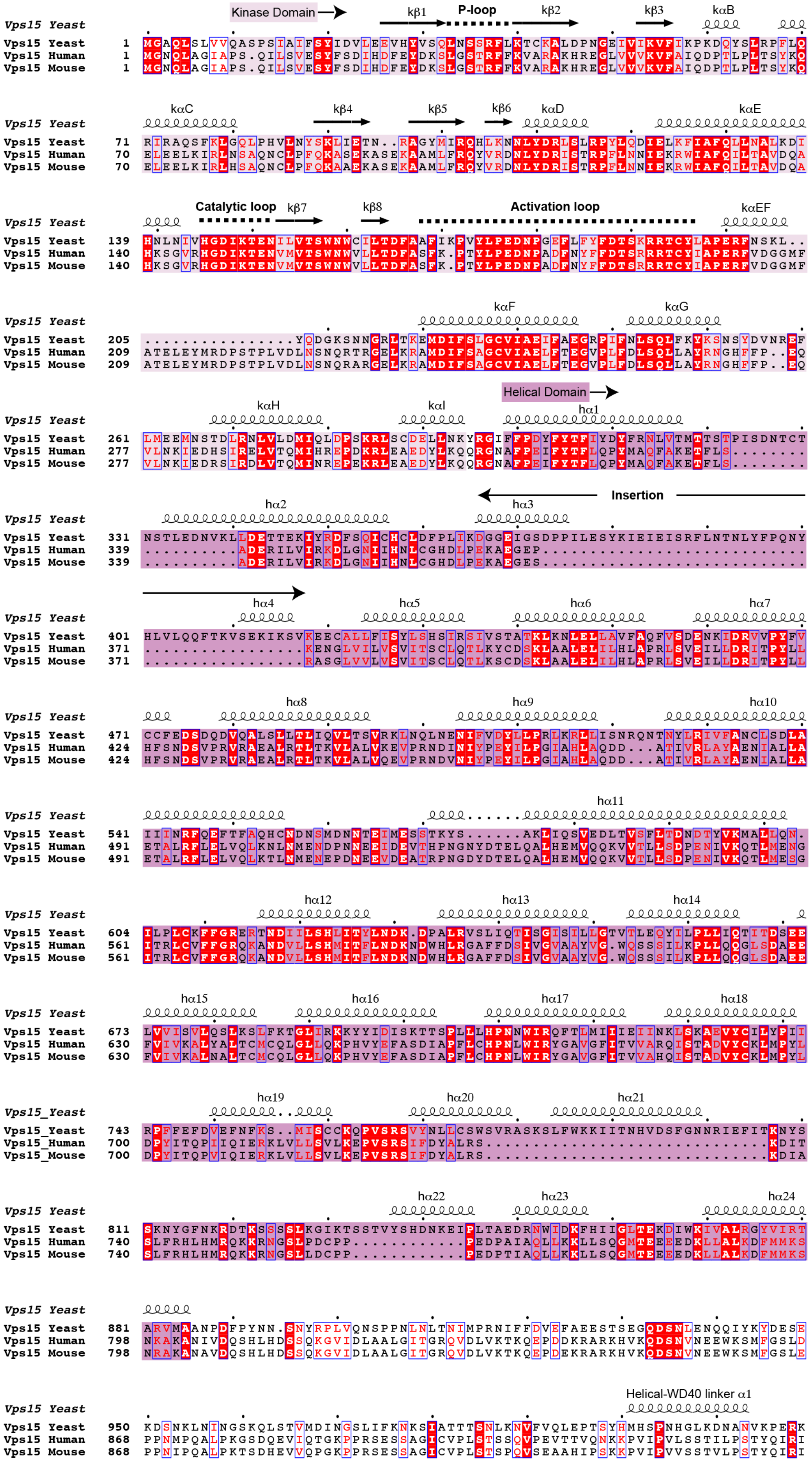


Fig. S3B. Structure-based sequence alignment of *S. cerevisiae*, *H. sapiens* and *M. musculus* Vps38. The sequence register for the strands of the C2 domain is less clear due to weak density, and our interpretation is shown above the sequence.


Kinase Domain
kβ1
P-loop
kβ2
kβ3
kαB
Vps15 Yeast
Vps15 Yeast 1 MGAQLSLVVQASPSIAIFSYIDVLEEVHYVSQLNSSRFLKTCKALDPNGEIVIKVFIKPKDQYSLRPFLQ
Vps15 Human 1 MGNQLAGIAPS.QILSVESYFSDIHDFEYDKSLGSTRFFKVARAKHREGLVVVKVFAIQDPTLPLTSYKQ
Vps15 Mouse 1 MGNQLAGIAPS.QILSVESYFSDIHDFEYDKSLGSTRFFKVARAKHREGLVVVKVFAIQDPTLPLTSYKQ
kαC
kβ4
kβ5
kβ6
kαD
kαE
Vps15 Yeast
Vps15 Yeast 71 RIRAQSFKLGQLPHVLNYSKLIETN..RAGYMIRQHLKNNLYDRLSLRPYLQDIELKFIAFQLLNALKDI
Vps15 Human 70 ELEELKIRLNSAQNCLPFQKASEKASEKAAMLFRQYVRDNLYDRISTRPFLNNIEKRWIAFQILTAVDQA
Vps15 Mouse 70 ELEELKIRLHSAQNCLPFQKAAEKASEKAAMLFRQYVRDNLYDRISTRPFLNNIEKRWIAFQILTAVDQA
Catalytic loop
kβ7
kβ8
Activation loop
kαEF
Vps15 Yeast
Vps15 Yeast 139 HNLNIVHGDIKTENILVTSWNWCILTDFAAFIKPVYLPEDNPGEFLFYFDTSKRRTCYLAPERFNSKL..
Vps15 Human 140 HKSGVRHGDIKTENVMVTSWNWVLLTDFASFK.PTYLPEDNPADFNYFFDTSRRRTCYIAPERFVDGGMF
Vps15 Mouse 140 HKSGVRHGDIKTENVMVTSWNWVLLTDFASFK.PTYLPEDNPADFNYFFDTSRRRTCYIAPERFVDGGMF
kαF
kαG
Vps15 Yeast
Vps15 Yeast 205 ................YQDGKSNNGRLTKEMDIFSLGCVIAEIFAEGRPIFNLSQLFKYKSNSYDVNREF
Vps15 Human 209 ATELEYMRDPSTPLVDLNSNQRTRGELKRAMDIFSAGCVIAELFTEGVPLFDLSQLLAYRNGHFFP..EQ
Vps15 Mouse 209 ATELEYMRDPSTPLVDLNSNQRARGELKRAMDIFSAGCVIAELFTEGVPLFDLSQLLAYRNGHFFP..EQ
Helical Domain
kαH
kαI
hα1
Vps15 Yeast
Vps15 Yeast 261 LMEEMNSTDLRNLVLDMIQLDPSKRLSCDELLNKYRGIFFPDYFYTFIYDYFRNLVTMTTSTPISDNTCT
Vps15 Human 277 VLNKIEDHSIRELVTQMIHREPDKRLEAEDYLKQQRGNAFPEIFYTFLQPYMAQFAKETFLS........
Vps15 Mouse 277 VLNKIEDRSIRDLVTQMINREPEKRLEAEDYLKQQRGNAFPEIFYTFLQPYMAQFAKETFLS........
Insertion
hα2
hα3
Vps15 Yeast
Vps15 Yeast 331 NSTLEDNVKLLDETTEKIYRDFSQICHCLDFPLIKDGGEIGSDPPILESYKIEIEISRFLNTNLYFPQNY
Vps15 Human 339 ..........ADERILVIRKDLGNIIHNLCGHDLPEKAEGEP............................
Vps15 Mouse 339 ..........ADERILVIRKDLGNIIHNLCGHDLPEKAEGES............................
hα4
hα5
hα6
hα7
Vps15 Yeast
Vps15 Yeast 401 HLVLQQFTKVSEKIKSVKEECALLFISYLSHSIRSIVSTATKLKNLELLAVFAQFVSDENKIDRVVPYFV
Vps15 Human 371 .................KENGLVILVSVITSCLQTLKYCDSKLAALELILHLAPRLSVEILLDRITPYLL
Vps15 Mouse 371 .................RASGLVVLVSVITSCLQTLKSCDSKLAALELILHLAPRLSVEILLDRITPYLL
hα8
hα9
hα10
Vps15 Yeast
Vps15 Yeast 471 CCFEDSDQDVQALSLLTLIQVLTSVRKLNQLNENIFVDYLLPRLKRLLISNRQNTNYLRIVFANCLSDLA
Vps15 Human 424 HFSNDSVPRVRAEALRTLTKVLALVKEVPRNDINIYPEYILPGIAHLAQDD...ATIVRLAYAENIALLA
Vps15 Mouse 424 HFSNDSVPRVRAEALRTLTKVLALVQEVPRNDVNIYPEYILPGIAHLAQDD...ATIVRLAYAENIALLA
hα11
Vps15 Yeast
Vps15 Yeast 541 IIINRFQEFTFAQHCNDNSMDNNTEIMESSTKYS......AKLIQSVEDLTVSFLTDNDTYVKMALLQN.
Vps15 Human 491 ETALRFLELVQLKNLNMENDPNNEEIDEVTHPNGNYDTELQALHEMVQQKVVTLLSDPENIVKQTLMENG
Vps15 Mouse 491 ETALRFLELVQLKTLNMENEPDNEEVDEATRPNGDYDTELQALHEMVQQKVVTLLSDPENIVKQTLMESG
hα12
hα13
hα14
Vps15 Yeast
Vps15 Yeast 604 ILPLCKFFGRERTNDIILSHLITYLNDK.DPALRVSLIQTISGISILLGTVTLEQYILPLLIQTITDSEE
Vps15 Human 561 ITRLCVFFGRQKANDVLLSHMITFLNDKNDWHLRGAFFDSIVGVAAYVG.WQSSSILKPLLQQGLSDAEE
Vps15 Mouse 561 ITRLCVFFGRQKANDVLLSHMITFLNDKNDWHLRGAFFDSIVGVAAYVG.WQSSSILKPLLQQGLSDAEE
hα15
hα16
hα17
hα18
Vps15 Yeast
Vps15 Yeast 673 LVVISVLQSLKSLFKTGLIRKKYYIDISKTTSPLLLHPNNWIRQFTLMIIIEIINKLSKAEVYCILYPII
Vps15 Human 630 FVIVKALYALTCMCQLGLLQKPHVYEFASDIAPFLCHPNLWIRYGAVGFITVVARQISTADVYCKLMPYL
Vps15 Mouse 630 FVIVKALNALTCMCQLGLLQKPHVYEFASDIAPFLCHPNLWIRYGAVGFITVVAHQISTADVYCKLMPYL
hα19
hα20
hα21
Vps15_Yeast
Vps15_Yeast 743 RPFFEFDVEFNFKS..MISCCKQPVSRSVYNLLCSWSVRASKSLFWKKIITNHVDSFGNNRIEFITKNYS
Vps15_Human 700 DPYITQPIIQIERKLVLLSVLKEPVSRSIFDYALRS..............................KDIT
Vps15_Mouse 700 DPYITQPVIQIERKLVLLSVLKEPVSRSIFDYALRS..............................KDIA
hα22
hα23
hα24
Vps15 Yeast
Vps15 Yeast 811 SKNYGFNKRDTKSSSSLKGIKTSSTVYSHDNKEIPLTAEDRNWIDKFHIIGLTEKDIWKIVALRGYVIRT
Vps15 Human 740 SLFRHLHMRQKKRNGSLPDCPP............PEDPAIAQLLKKLLSQGMTEEEEDKLLALKDFMMKS
Vps15 Mouse 740 SLFRHLHMRQKKRNGSLLDCPP............PEDPTIAQLLKKLLSQGMTEEEEDKLLALKDFMMKS
Vps15 Yeast
Vps15 Yeast 881 ARVMAANPDFPYNN.SNYRPLVQNSPPNLNLTNIMPRNIFFDVEFAEESTSEGQDSNLENQQIYKYDESE
Vps15 Human 798 NKAKANIVDQSHLHDSSQKGVIDLAALGITGRQVDLVKTKQEPDDKRARKHVKQDSNVNEEWKSMFGSLD
Vps15 Mouse 798 NRAKANAVDQSHLHDSSQKGVIDLAALGITGRQVDLVKTKQEPDEKRARKHVKQDSNVNEEWKSMFGSLE
Helical-WD40 linker α1
Vps15 Yeast
Vps15 Yeast 950 KDSNKLNINGSKQLSTVMDINGSLIFKNKSIATTTSNLKNVFVQLEPTSYHMHSPNHGLKDNANVKPERK
Vps15 Human 868 PPNMPQALPKGSDQEVIQTGKPPRSESSAGICVPLSTSSQVPEVTTVQNKKPVIPVLSSTILPSTYQIRI
Vps15 Mouse 868 PPNIPQALPKTSDHEVVQPGKPPRSESSAGICVPLSTSPQVSEAAHIPSKKPVIPVVSSTVLPSTYQIRI

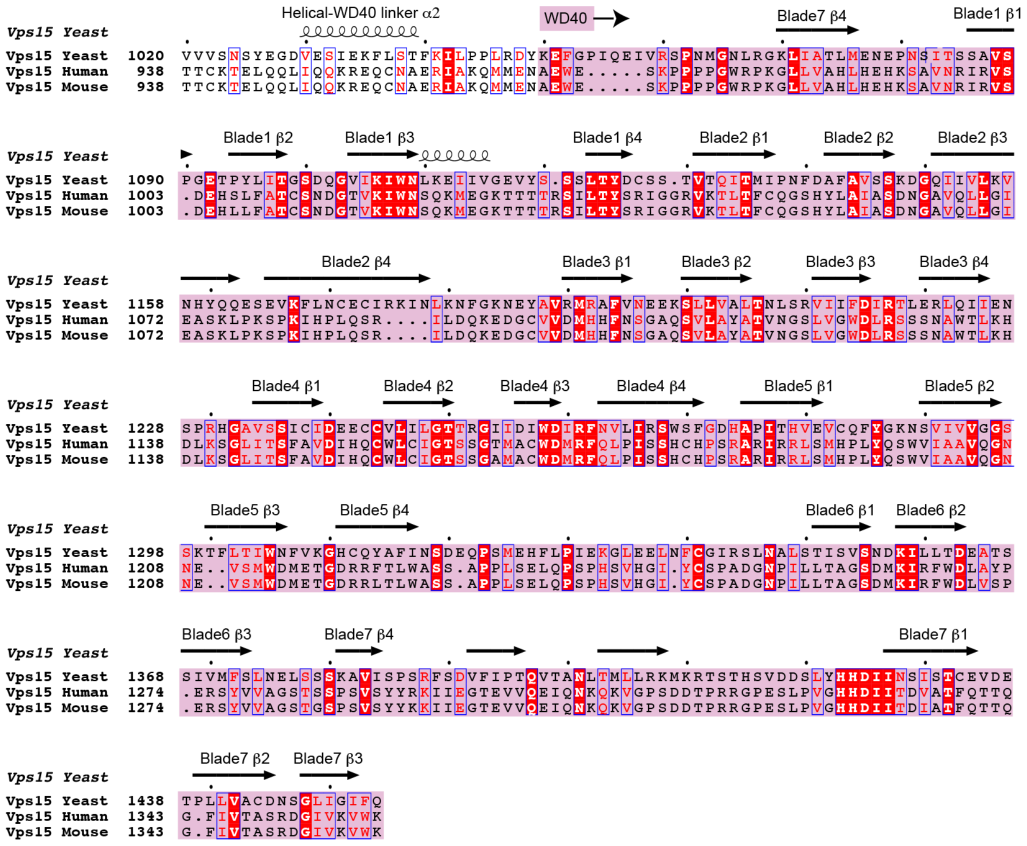


Fig. S3C. Structure-based sequence alignment of *S. cerevisiae*, *H. sapiens* and *M. musculus* Vps15.

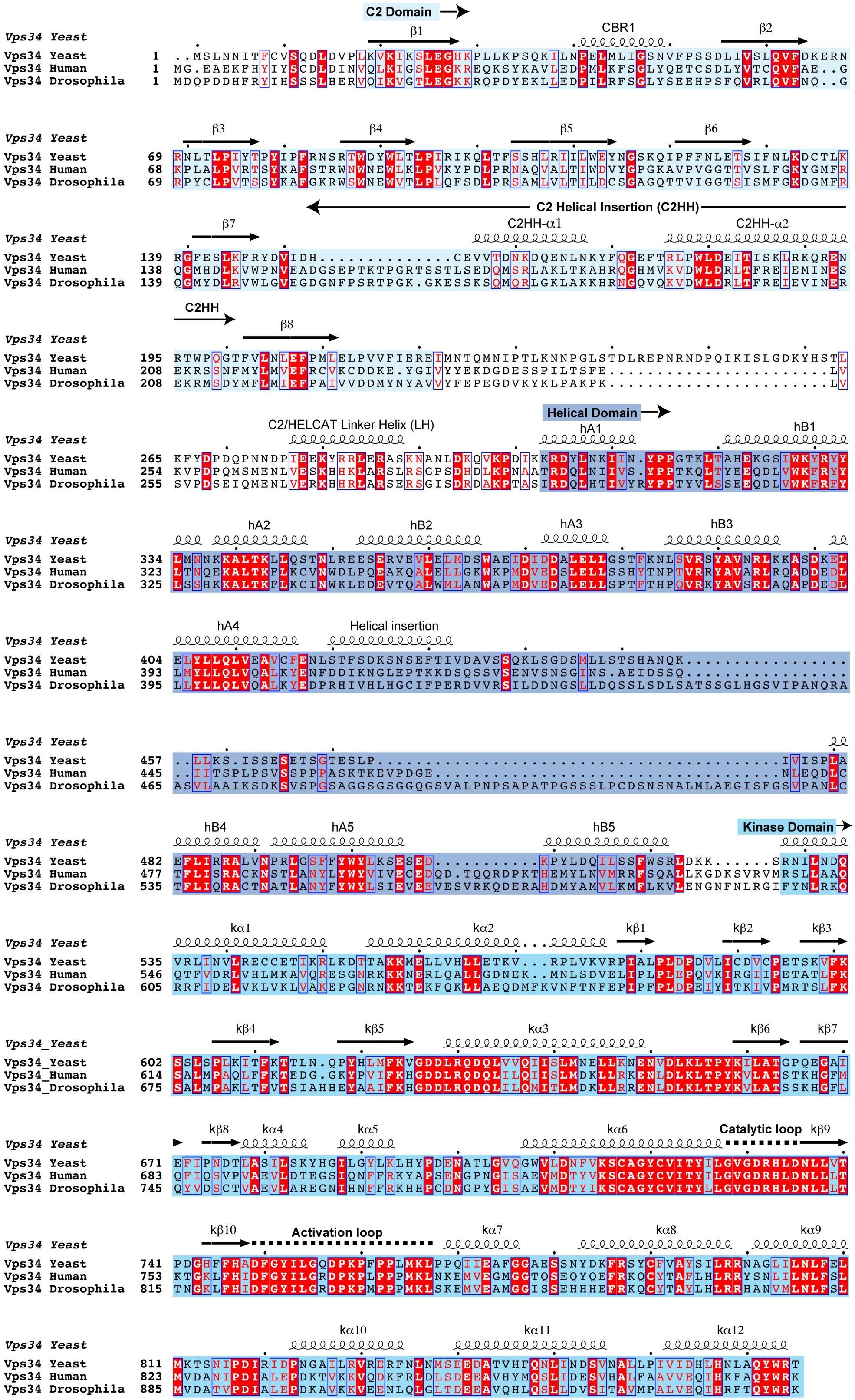
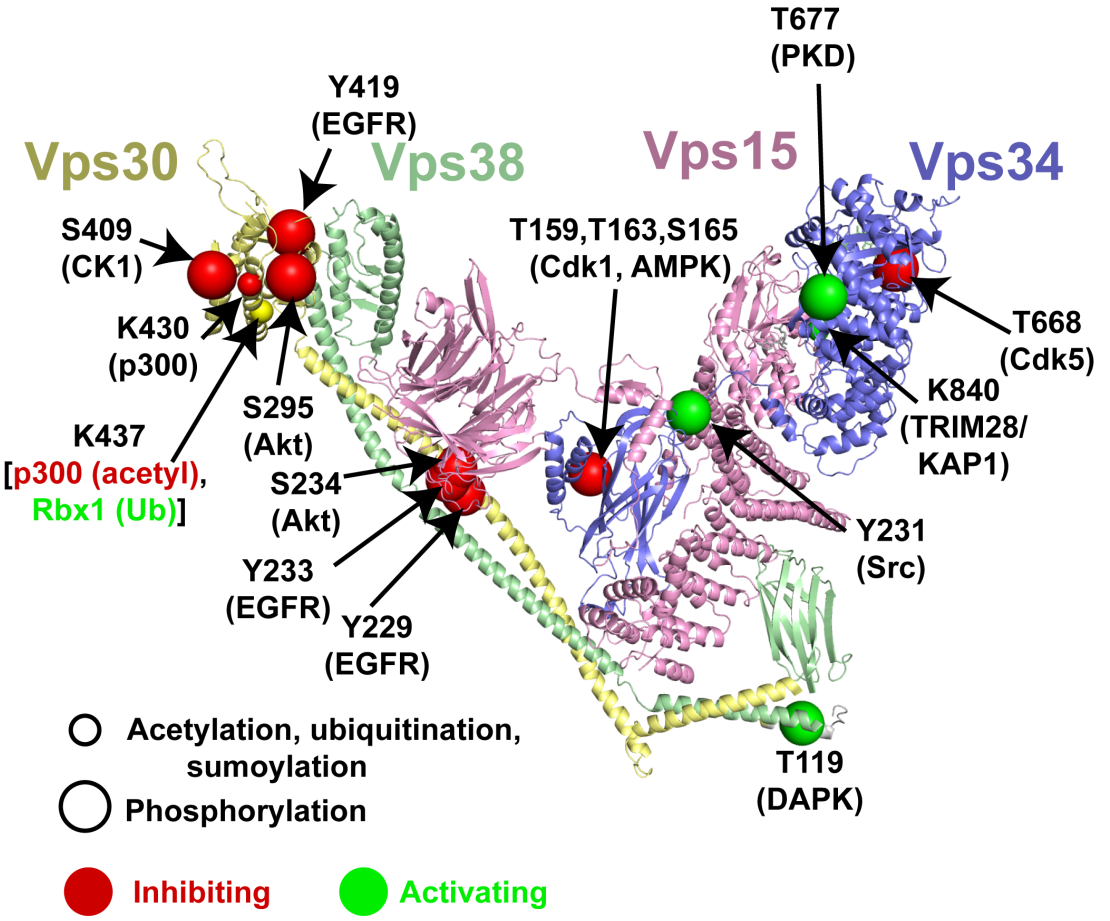


Fig. S3D. Structure-based sequence alignment of *S. cerevisiae*, *H. sapiens* and *D. melanogaster* Vps34.



Post translational modification sites of complex I or II (human numbering)

Fig. S4A. Sites of post-translational modifications (PTMs) of complex II subunits mapped onto the structure of yeast complex II. In almost all cases, the residue type is not conserved in yeast, however, the structures of yeast and human complex II are likely to be closely related, and the illustration portrays where the PTM would be located in the human complex. Details of the sites are listed in fig. S4B.

Target subunit	Modifying enzyme	Site of PTM (human numbering)	Effect of PTM	Region of structure	Reference
Beclin 1	DAPK (death-associated protein kinase 1) (S/T kinase)	T119	Stimulates autophagy by dissociating Beclin 1 from its inhibitors Bcl-2/ Bcl-XL	BH3 region of Beclin 1	(*53*)
Beclin 1	p38 Map kinase	S90	Stimulates autophagy	NTD of Beclin 1	(*54*)
Beclin 1	EGFR (Tyr kinase)	Y229,Y23, Y352	Inhibits autophagy. Promotes formation of homodimers unable to bind Vps34	Hinge of CC2 in contact with Vps15 WD40	(*55*)
Beclin 1	Ulk1, Ulk2 (S/T kinase)	S15	Induces autophagy	NTD of Beclin 1	(*56*)
Beclin 1	AMPK (S/T kinase)	S93, S96	Induces Atg14L complex and activates autophagy	NTD of Beclin 1	(*31*)
Beclin 1	P300 (acetyl-transferase)	K430, K437	Inhibits autophagosome maturation and endocytic trafficking by promoting the recruitment of Rubicon	BARA domain of Beclin 1	(*57*)
Beclin 1	CK1 (S/T kinase)	S409	Required for the subsequent p300 binding and Beclin 1 acetylation	BARA domain of Beclin 1	(*57*)
Beclin 1	Akt (S/T kinase)	S234, S295	Suppresses autophagy	Hinge of CC2, Bara domain of Beclin 1	(*58*)
Beclin 1	Traf6 E3 ligase (K63 chains)	K117	Induces autophagy	NTD of Beclin 1	(*59*)
Beclin 1	Ambra-1-DDB1-Cul4-Rbx1 E3 ligase (k63 chains)	K437	Induces autophagy	BARA domain of Beclin 1	(*60*)
UVRAG	mTOR (S/T kinase)	S550, S571	Loss of these phosphorylation sites reduces VPS34 lipid kinase activity and results in an increase in number and length of lysosomal tubules	Part of C-terminal extension in UVRAG (No Vps38 counterpart)	(*10*)
Vps34	PKD (S/T kinase)	T677	Stimulates autophagy	N-lobe of Vps34 kinase domain	(*61*)
Vps34	AMPK (S/T kinase)	T163, S165	Inhibits non-autophagic complex (i.e., without Atg14L)	Helical hairpin insertion in C2 domain	(*31*, *62*)
Vps34	Src	Y231	Insulin-mediated	C2-Helical linker	(*63*)

	(Tyr kinase)		activation of S6 kinase 1		
Vps34	Cdk1 (S/T kinase)	T159	Inhibits its interaction with Beclin 1	Helical hairpin insertion in C2 domain	(*32*)
Vps34	Cdk5	T668	Eliminates Vps34 activity	N-lobe of Vps34 kinase domain	(*32*)
Vps34	TRIM28/KAP1	K840	Induces complex assembly	C-lobe of Vps34 kinase domain	(*27*)

Fig. S4B. Post-translational modifications reported for the complex II subunits. Numbering of the sites is for the human proteins. The sites are illustrated in the structure of the yeast complex II in fig. S6A. These modifications occur in three clusters: The C-terminal BARA domain of Beclin 1, the Beclin 1 CC2, the N-terminal (NTD) domain of Beclin 1, and the C2 and kinase domains of Vps34. The BARA domain binds membranes in both human and yeast complexes, so PTMs in this domain may interfere with this interaction. In the CC2 region of Vps30, there is a short break in the helical structure, and several of the PTM's are at residues in this region. This region is part of the contact with the WD40 domain of Vps15, so phosphorylation of these residues might cause Vps30 to dissociate from the Vps15/Vps34 dimer. Modifications of the Beclin 1 NTD and BH3 are likely to prevent Beclin 1 from being sequestered in non-catalytic complexes such as the Bcl-2/ Bcl-XL. The C2 domain and its helical insertion are critical to the integrity of the complex, as shown by temperature-sensitive mutations of these regions (Fig. 3B).

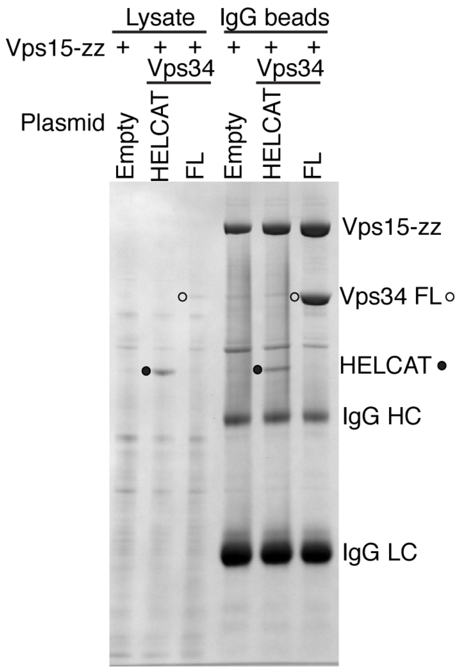


Fig. S5. Effect of Vps34-C2 deletion on interaction with Vps15. Protein A-tagged (zz) Vps15 was coexpressed with an empty vector (Empty), HELCAT, or full-length Vps34 (FL) in yeast and pulled down with IgG beads. White circle, full-length Vps34; black circle, HELCAT; IgG HC, IgG heavy-chain; IgG LC, IgG light-chain.

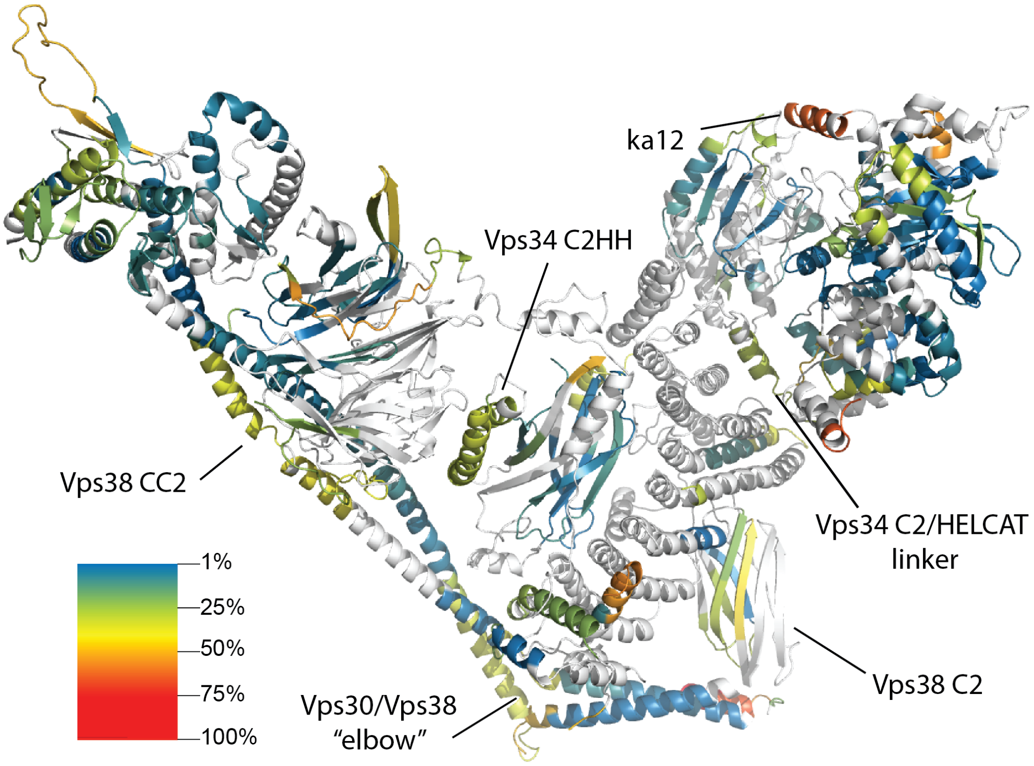


Fig. S6. Global HDX at the earliest time point (0.3 s on ice) mapped onto complex II structure. The warmer the colour, the higher is the exchange rate of the corresponding peptide. Regions with no coverage are coloured in grey. Regions exchanging at a higher rate may indicate flexible regions in the complex. These regions include Vps38 CC2 (near the break), the "elbow" between CC1 and CC2 of Vps30 and Vps38, Vps34 C2HH, Vps34 kα12 helix, part of the Vps38 C2 and the C2/HELCAT linker.

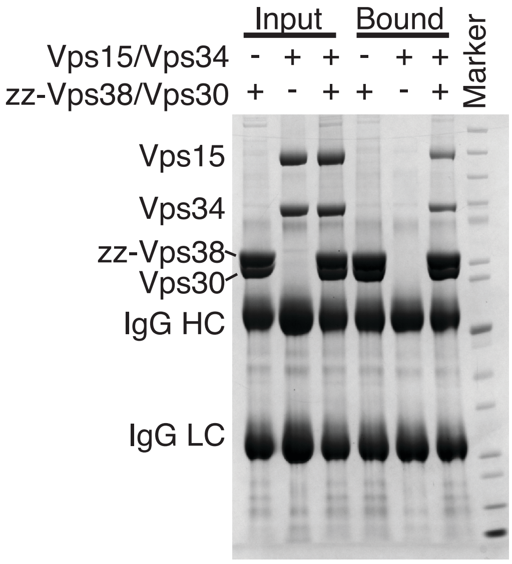


Fig. S7. Heterotetrameric Complex II can assemble from separately purified Vps15/Vps34 and Vps38/Vps30 heterodimers. Protein A tagged (zz) Vps38/Vps30 heterodimer was captured on IgG resin. Purified Vps15/Vps34 heterodimer was then captured onto the Vps38/Vps30 heterodimer on the resin. IgG HC: IgG heavy chain, IgG LC: IgG light chain.

Table S1 Data collection, phasing and refinement statistics for **MIRAS**

	Native (7crystals)	Crystal 1 KR166 (Ta_6Br_{12})		Crystal 2 KR168 (Ta_6Br_{12})
Data collection				
Space group	$P2_12_12$	$P2_12_12$		$P2_12_12$
Cell dimensions				
a, b, c (Å)	215.18 226.84 127.21	215.15 226.52 127.03		216.14 226.13 127.29
		Peak	*Inflection*	*Peak*
Wavelength		1.254380	1.254860	1.254380
Resolution (Å)	49.92 – 4.4 (4.58 - 4.4) *	60.20 - 6.2 (6.93- 6.2) *	60.00 – 6.8 (7.62- 6.8) *	58.94-8.8 (6.48-5.8) *
No. of unique reflections	40224	14659	10925	17907
R_{sym} or R_{merge}	0.317 (4.82)	0.117 (1.91)	0.118 (1.17)	0.123 (2.14)
R_{pim}	0.077 (0.67)	0.063 (1.02)	0.048 (0.48)	0.055 (0.96)
$I/ʃI$	21.0 (1.0)	9.4 (1.0)	13.9 (2.1)	11.9 (1.2)
Completeness (%)	99.8 (98.8)	99.9 (100.0)	98.9 (96.8)	99.9 (100.0)
Redundancy	32.2 (15.8)	8.1 (8.3)	12.7 (12.6)	11.1 (11.6)
CC 1/2	0.999 (0.30)	0.999 (0.522)	0.999 (0.794)	0.999 (0.626)
Refinement				
Resolution (Å)	49.92 – 4.40			
No. reflections	40211			
R_{work}/ R_{free}	0.369/0.376			
No. atoms	14160			
Protein	14160			
Ligand/ion	0			
Water	0			
B-factors (Å^2)				
Protein	259			
R.m.s deviations				
Bond lengths (Å)	0.002			
Bond angles (º)	0.70			
Ramachandran values				
Preferred regions	2652 (94.5%)			
Allowed regions	155 (5.5%)			
Outliers	0			

*Highest resolution shell is shown in parenthesis.

Table S2A

Vps30						CompII				ScVps30		
Start	*End*	*CS*	*#D*	*RT*	*0.3*	*3*	*30*	*300*	*0.3*	*3*	*30*	*300*
4	16	2	9	15.54-15.58	18%	17%	24%	33%	39%	43%	51%	50%
17	27	2	9	14.10-14.28	42%	66%	72%	71%	74%	73%	76%	73%
17	31	2	13	13.62-13.85	42%	63%	73%	70%	74%	72%	74%	71%
19	27	2	7	11.33-11.43	4%	9%	15%	32%	69%	76%	76%	75%
32	39	1	6	10.10-10.11	84%	87%	88%	85%	80%	81%	82%	81%
40	62	3	19	11.82-11.85	63%	67%	68%	69%	69%	69%	70%	68%
40	76	5	33	13.33-13.45	43%	50%	53%	58%	60%	60%	62%	59%
42	62	3	17	11.78-11.85	63%	67%	68%	70%	70%	70%	71%	70%
49	76	4	24	13.21-13.29	31%	39%	43%	52%	55%	54%	57%	54%
63	76	3	12	12.68-12.93	32%	40%	47%	59%	62%	62%	63%	61%
77	85	2	7	11.04-11.06	17%	34%	57%	64%	63%	62%	64%	63%
82	86	1	3	12.10-12.16	9%	28%	53%	67%	66%	66%	67%	65%
86	97	2	10	13.49-13.54	14%	16%	21%	27%	27%	27%	28%	27%
87	97	2	9	9.86-9.92	18%	19%	21%	21%	22%	22%	23%	22%
87	130	5	42	9.07-9.12	39%	41%	41%	39%	39%	40%	41%	40%
98	130	4	31	6.47-6.64	48%	48%	49%	46%	46%	47%	48%	50%
131	140	2	8	6.11-6.26	32%	33%	31%	30%	29%	30%	32%	32%
131	148	3	16	7.58-7.61	47%	47%	47%	45%	45%	46%	46%	48%
147	152	1	4	12.21-12.27	81%	85%	84%	86%	90%	91%	92%	92%
153	163	2	9	7.96-8.02	80%	80%	80%	79%	79%	80%	80%	81%
153	168	2	14	14.82-14.98	68%	73%	78%	78%	79%	79%	82%	79%
153	171	2	17	17.94-18.03	54%	63%	70%	74%	82%	81%	81%	78%
169	175	1	5	10.57-10.60	21%	33%	48%	68%	83%	84%	85%	84%
188	200	2	11	11.73-11.97	2%	3%	6%	17%	49%	60%	65%	67%
188	210	4	21	14.28-14.59	1%	3%	5%	14%	64%	70%	73%	71%
190	210	4	19	12.93-12.97	2%	4%	7%	17%	66%	71%	72%	69%
194	210	3	15	12.22-12.28	1%	3%	4%	11%	61%	63%	64%	62%
201	210	2	8	10.88-10.93	2%	4%	6%	18%	54%	54%	55%	54%
211	220	2	8	5.99-6.04	49%	52%	61%	58%	59%	59%	60%	62%
211	247	6	35	9.39-9.44	35%	41%	43%	39%	41%	43%	44%	43%
211	251	6	39	12.66-12.70	29%	37%	40%	39%	45%	46%	48%	45%
215	247	6	31	9.31-9.36	36%	40%	43%	39%	42%	43%	44%	43%
215	251	5	35	12.99-13.03	29%	36%	40%	40%	45%	47%	48%	46%
252	265	2	12	15.19-15.20	5%	10%	22%	32%	48%	52%	53%	51%
266	277	3	10	7.95-7.98	9%	24%	53%	64%	64%	71%	71%	72%
266	278	3	11	8.30-8.34	8%	24%	49%	57%	59%	63%	65%	65%
266	280	4	13	9.51-9.55	6%	18%	43%	57%	57%	60%	61%	61%
266	288	4	21	10.52-10.74	8%	21%	43%	55%	56%	59%	60%	59%
289	305	3	15	15.76-15.81	5%	10%	27%	43%	64%	66%	68%	65%
297	305	2	7	11.33-11.43	4%	11%	14%	33%	69%	76%	76%	76%
297	327	5	29	16.80-16.97	7%	11%	15%	33%	72%	77%	79%	76%
306	311	1	4	15.07-15.13	7%	9%	9%	19%	69%	83%	83%	82%
310	327	3	16	16.19-16.26	12%	15%	21%	33%	59%	65%	67%	64%
312	327	3	14	15.88-16.05	15%	21%	27%	42%	67%	75%	77%	74%
318	327	2	8	14.85-14.92	20%	28%	33%	47%	67%	76%	77%	76%
327	357	4	26	16.21-16.25	10%	13%	18%	24%	12%	16%	23%	35%
328	357	4	25	15.74-15.89	10%	13%	17%	24%	12%	16%	23%	36%
341	363	3	19	17.95-18.04	9%	13%	18%	26%	11%	15%	22%	31%
358	363	1	4	12.35-12.41	3%	5%	3%	4%	1%	0%	0%	0%
366	378	3	11	14.00-14.04	19%	23%	26%	38%	17%	22%	31%	42%
367	378	2	10	12.90-12.95	21%	24%	28%	40%	17%	22%	32%	44%
368	378	2	9	12.05-12.11	25%	27%	33%	47%	20%	26%	37%	50%
369	378	2	8	12.86-12.89	25%	28%	33%	48%	20%	26%	36%	49%
385	389	1	3	16.21-16.39	13%	20%	29%	32%	26%	29%	38%	50%
390	399	3	8	4.10-4.36	33%	36%	39%	45%	32%	36%	36%	46%
390	401	3	10	5.50-5.54	41%	45%	46%	48%	40%	43%	44%	51%
390	412	3	20	7.52-7.56	49%	52%	52%	52%	49%	51%	52%	56%
390	413	3	21	10.40-10.59	45%	49%	49%	48%	45%	46%	48%	49%
414	419	1	3	16.25-16.29	8%	11%	10%	18%	4%	3%	3%	4%
424	443	4	18	13.00-13.08	31%	37%	35%	37%	31%	34%	37%	38%
425	444	4	18	12.49-12.53	25%	32%	31%	33%	25%	28%	31%	33%
427	444	3	16	10.80-10.98	13%	17%	19%	20%	12%	14%	17%	21%
445	449	1	3	9.52-9.56	4%	5%	4%	7%	1%	1%	2%	4%
450	456	2	5	7.14-7.16	25%	34%	40%	49%	17%	26%	36%	43%
451	456	1	4	7.14-7.18	30%	38%	46%	57%	20%	28%	40%	48%
451	457	2	5	7.14-7.16	26%	34%	40%	49%	17%	25%	36%	43%
487	502	3	13	16.01-16.07	21%	28%	33%	35%	22%	29%	35%	36%
491	502	3	10	11.88-12.02	20%	27%	34%	37%	23%	32%	40%	42%
503	513	2	8	8.81-8.88	22%	25%	27%	30%	21%	22%	25%	34%
503	518	2	13	12.24-12.41	21%	24%	25%	31%	20%	20%	23%	30%
503	521	3	16	14.80-14.84	11%	11%	13%	18%	12%	13%	15%	22%
514	521	2	6	15.01-15.05	5%	4%	5%	7%	1%	1%	1%	1%
522	531	2	8	17.28-17.40	1%	1%	3%	9%	1%	4%	10%	11%
522	532	2	9	18.27-18.32	0%	2%	4%	12%	2%	5%	14%	21%

Table S2B

Vps15					CompII					ScVps34-15				
Start	*End*	*CS*	*#D*	*RT*	*0.3*	*3*	*30*	*300*	*3000*	*0.3*	*3*	*30*	*300*	*3000*
34	50	3	14	11.52-11.55	3%	6%	8%	12%	15%	6%	9%	11%	16%	20%
39	50	2	9	8.87-8.90	2%	4%	4%	4%	8%	3%	5%	4%	7%	10%
56	69	3	10	14.23-14.37	27%	33%	37%	43%	49%	29%	35%	40%	46%	53%
70	77	2	6	8.84-8.88	6%	10%	20%	41%	61%	2%	8%	21%	42%	65%
70	91	5	19	13.43-13.47	7%	11%	15%	25%	31%	8%	13%	19%	29%	39%
77	88	2	9	15.10-15.14	10%	13%	19%	33%	40%	11%	16%	25%	37%	46%
78	88	2	8	13.22-13.30	10%	13%	21%	36%	39%	11%	16%	25%	38%	42%
78	91	3	11	12.90-12.94	10%	15%	19%	28%	31%	13%	18%	23%	32%	40%
92	109	3	16	11.22-11.30	3%	7%	9%	12%	16%	3%	6%	9%	14%	19%
100	109	3	8	7.59-7.94	3%	7%	9%	14%	15%	3%	7%	11%	17%	19%
184	196	3	11	13.20-13.30	11%	20%	28%	38%	34%	9%	14%	18%	24%	28%
233	243	2	8	16.67-16.84	4%	10%	14%	23%	37%	7%	12%	16%	30%	45%
233	246	2	11	17.42-17.55	6%	15%	18%	34%	44%	9%	16%	20%	37%	52%
234	246	2	10	16.96-17.10	11%	19%	24%	37%	46%	12%	21%	26%	40%	55%
247	261	3	13	12.62-12.66	13%	17%	27%	34%	39%	14%	21%	31%	38%	43%
262	272	2	9	9.85-9.89	23%	23%	31%	33%	47%	23%	26%	34%	39%	52%
359	377	2	14	17.43-17.51	36%	37%	43%	45%	49%	37%	39%	45%	49%	53%
540	551	2	10	17.47-17.60	10%	18%	25%	32%	41%	21%	27%	33%	38%	52%
552	565	2	12	6.38-6.70	39%	37%	39%	38%	36%	39%	40%	42%	41%	41%
601	610	2	7	17.24-17.33	32%	31%	37%	48%	52%	24%	29%	32%	43%	51%
634	640	1	4	15.68-15.79	1%	1%	0%	1%	1%	1%	0%	0%	-1%	1%
761	773	2	10	13.89-13.97	17%	24%	26%	26%	26%	19%	24%	27%	28%	30%
776	785	2	8	13.64-13.75	20%	27%	26%	31%	42%	22%	24%	26%	34%	47%
777	785	2	7	11.08-11.16	26%	33%	34%	40%	47%	26%	29%	32%	39%	49%
786	800	2	13	10.59-10.64	22%	34%	42%	43%	43%	24%	36%	44%	45%	46%
805	816	2	10	11.18-11.21	49%	51%	51%	47%	46%	50%	51%	53%	51%	52%
850	857	2	6	14.71-14.75	2%	4%	5%	9%	15%	2%	3%	4%	9%	17%
858	862	1	3	12.10-12.43	21%	26%	29%	41%	60%	22%	23%	28%	46%	61%
858	873	3	14	17.35-17.39	15%	21%	25%	33%	41%	16%	22%	26%	35%	44%
863	873	2	9	16.33-16.36	9%	16%	17%	25%	29%	5%	10%	15%	22%	29%
863	875	3	11	14.25-14.33	9%	13%	17%	23%	31%	10%	14%	18%	24%	33%
876	896	3	17	12.26-12.31	53%	58%	60%	59%	59%	53%	59%	60%	62%	67%
881	910	3	23	14.35-14.45	57%	62%	59%	58%	56%	56%	62%	60%	60%	65%
897	910	2	9	13.42-13.52	73%	75%	75%	73%	69%	75%	77%	76%	75%	77%
909	920	2	9	17.89-18.13	30%	44%	58%	71%	73%	45%	59%	65%	72%	78%
911	920	2	7	17.33-17.43	23%	37%	57%	73%	75%	41%	59%	65%	73%	81%
1040	1057	3	13	14.40-14.46	14%	26%	36%	51%	59%	25%	46%	55%	63%	68%
1049	1057	2	6	12.24-12.42	27%	37%	46%	63%	74%	35%	50%	59%	70%	73%
1058	1071	3	11	10.73-10.76	53%	61%	63%	60%	59%	54%	58%	62%	62%	63%
1058	1075	4	15	13.55-13.60	33%	41%	43%	47%	53%	41%	51%	58%	62%	66%
1059	1071	3	10	10.52-10.68	52%	59%	62%	59%	59%	51%	58%	61%	61%	62%
1076	1096	2	16	14.28-14.64	9%	16%	22%	27%	31%	23%	34%	40%	45%	55%
1097	1110	2	12	17.04-17.09	8%	11%	12%	17%	25%	13%	17%	24%	35%	50%
1143	1155	2	11	15.06-15.10	2%	3%	3%	9%	17%	2%	4%	5%	11%	22%
1156	1164	2	7	4.95-5.00	45%	46%	51%	52%	52%	44%	50%	55%	57%	55%
1156	1165	2	8	5.47-5.50	45%	46%	52%	50%	51%	44%	49%	55%	56%	55%
1156	1168	3	11	10.10-10.14	34%	42%	48%	46%	47%	37%	43%	49%	48%	49%
1156	1171	3	14	12.22-12.29	28%	36%	45%	45%	44%	33%	40%	45%	46%	46%
1315	1338	3	20	17.59-17.64	24%	34%	36%	36%	38%	39%	44%	46%	47%	52%
1327	1338	2	9	15.47-15.51	39%	56%	58%	64%	59%	60%	68%	67%	74%	72%
1352	1364	2	11	12.99-13.07	12%	14%	20%	23%	26%	35%	38%	43%	48%	54%
1352	1368	2	15	12.52-12.64	8%	13%	20%	27%	34%	42%	42%	49%	55%	60%
1352	1371	2	18	15.40-15.44	5%	11%	18%	27%	36%	34%	35%	44%	53%	55%
1406	1421	3	14	6.89-7.01	23%	25%	25%	23%	22%	23%	25%	29%	29%	27%
1422	1429	2	6	7.78-8.09	2%	4%	4%	7%	20%	40%	41%	43%	45%	45%
1442	1449	1	6	9.88-9.99	4%	4%	4%	6%	15%	47%	47%	54%	57%	60%

Table S2C

Vps34					CompII					ScVps34-15				
Start	*End*	*CS*	*#D*	*RT*	*0.3*	*3*	*30*	*300*	*3000*	*0.3*	*3*	*30*	*300*	*3000*
2	8	1	5	14.97-14.98	23%	31%	39%	53%	56%	40%	56%	61%	67%	74%
56	60	1	3	15.00-15.18	2%	2%	1%	2%	1%	2%	1%	1%	0%	2%
61	73	3	11	10.68-10.75	2%	5%	10%	19%	32%	3%	6%	13%	21%	34%
63	73	3	9	9.66-9.84	12%	12%	13%	22%	30%	2%	6%	13%	21%	32%
74	87	3	10	13.77-13.80	11%	13%	13%	24%	41%	12%	13%	14%	27%	48%
74	91	3	14	17.72-17.80	5%	9%	12%	19%	33%	7%	10%	13%	22%	40%
92	107	3	13	14.91-15.01	3%	6%	8%	11%	14%	4%	6%	8%	12%	17%
94	107	3	12	14.96-15.00	3%	5%	8%	11%	14%	4%	6%	8%	12%	17%
112	123	2	9	17.45-17.57	2%	7%	16%	18%	18%	3%	9%	17%	19%	26%
112	125	2	11	17.89-18.07	2%	6%	16%	20%	20%	3%	9%	18%	21%	28%
128	144	3	15	15.80-15.90	10%	14%	19%	24%	27%	12%	19%	24%	28%	33%
131	144	3	12	13.18-13.24	7%	11%	18%	25%	27%	11%	19%	27%	31%	37%
138	144	2	5	10.34-10.37	2%	6%	14%	25%	25%	7%	15%	23%	27%	30%
138	154	4	15	12.47-12.51	3%	7%	12%	21%	27%	5%	12%	20%	26%	29%
145	154	3	8	11.54-11.58	6%	11%	20%	30%	34%	9%	19%	28%	35%	39%
147	154	2	6	10.33-10.36	10%	17%	28%	34%	34%	13%	23%	33%	35%	36%
155	166	2	10	9.12-9.19	30%	39%	51%	56%	54%	45%	54%	59%	60%	59%
155	174	3	18	14.88-15.09	15%	23%	42%	55%	56%	37%	48%	56%	57%	60%
174	182	2	6	17.01-17.05	22%	28%	33%	51%	54%	34%	44%	48%	53%	57%
175	180	2	3	16.49-16.53	36%	42%	50%	80%	87%	54%	71%	79%	90%	95%
181	193	3	11	8.94-8.97	28%	36%	44%	52%	64%	37%	53%	63%	69%	70%
181	201	4	18	10.06-10.14	32%	39%	44%	50%	57%	41%	50%	58%	61%	64%
181	202	4	19	11.71-11.75	28%	35%	39%	44%	49%	34%	43%	49%	53%	56%
183	201	4	16	8.75-8.78	31%	37%	39%	41%	46%	35%	42%	48%	49%	50%
209	213	1	3	16.16-16.24	21%	33%	43%	75%	84%	37%	55%	68%	82%	89%
218	223	2	4	10.23-10.27	30%	44%	66%	72%	64%	41%	58%	71%	72%	72%
218	227	2	8	11.62-11.76	50%	63%	71%	75%	75%	60%	71%	78%	81%	81%
224	241	2	14	12.28-12.33	47%	57%	68%	66%	64%	53%	65%	69%	67%	69%
228	241	2	10	10.89-11.02	55%	66%	75%	75%	71%	60%	71%	74%	74%	75%
242	264	5	19	11.24-11.26	24%	32%	38%	40%	42%	28%	35%	41%	44%	48%
265	279	2	10	11.74-12.03	14%	23%	32%	43%	39%	17%	26%	35%	45%	47%
265	293	4	24	11.85-11.96	20%	26%	34%	41%	49%	22%	30%	39%	49%	59%
280	293	3	12	6.29-6.34	31%	34%	38%	39%	41%	31%	37%	44%	46%	50%
294	307	4	11	8.27-8.34	32%	43%	50%	49%	53%	35%	46%	52%	54%	58%
294	319	5	21	11.20-11.29	19%	27%	32%	35%	42%	22%	31%	34%	41%	49%
294	332	6	34	11.32-11.58	9%	13%	16%	17%	24%	11%	15%	18%	22%	29%
320	332	4	11	10.82-10.86	1%	2%	2%	4%	6%	1%	2%	2%	4%	10%
333	344	3	10	10.31-10.34	1%	5%	10%	22%	31%	2%	5%	12%	26%	37%
345	350	1	4	9.67-9.74	48%	52%	71%	72%	77%	52%	60%	79%	79%	80%
376	387	2	10	15.31-15.36	35%	42%	51%	47%	50%	38%	44%	50%	49%	53%
376	405	4	28	13.65-13.83	11%	17%	19%	22%	28%	13%	18%	22%	25%	34%
386	405	4	18	11.18-11.22	8%	13%	18%	22%	30%	10%	16%	21%	27%	36%
388	402	4	13	6.14-6.36	10%	11%	16%	16%	25%	11%	13%	18%	22%	33%
388	405	4	16	9.95-10.10	6%	9%	10%	12%	19%	7%	9%	12%	15%	23%
431	447	2	15	13.96-14.04	65%	65%	65%	63%	65%	65%	67%	66%	65%	68%
435	447	2	11	11.82-11.95	47%	47%	47%	45%	49%	53%	55%	55%	54%	55%
448	464	3	15	10.26-10.31	49%	52%	51%	49%	49%	50%	50%	54%	50%	52%
448	473	3	24	11.59-11.66	52%	53%	52%	49%	50%	50%	52%	54%	52%	53%
448	483	4	32	16.66-16.74	40%	45%	45%	44%	46%	41%	46%	46%	46%	49%
449	464	3	14	10.25-10.28	57%	57%	57%	54%	53%	56%	57%	59%	57%	58%
474	483	2	7	18.07-18.13	34%	47%	50%	57%	67%	39%	48%	50%	58%	72%
483	497	3	12	13.54-13.60	1%	4%	8%	15%	21%	2%	4%	9%	16%	23%
484	497	3	11	11.99-12.08	1%	3%	9%	16%	22%	2%	4%	10%	18%	25%
484	498	3	12	13.54-13.59	2%	4%	8%	15%	20%	2%	4%	9%	16%	23%
485	497	3	10	11.60-11.64	1%	3%	9%	17%	25%	2%	5%	11%	19%	27%
501	512	2	9	10.95-11.00	13%	18%	24%	30%	33%	14%	18%	27%	35%	36%
503	512	2	7	9.81-9.85	17%	23%	32%	43%	42%	18%	22%	33%	44%	43%
503	516	2	11	14.87-14.88	13%	15%	25%	38%	40%	16%	18%	32%	41%	46%
520	533	3	12	9.67-9.83	7%	11%	15%	20%	27%	8%	11%	17%	24%	33%
546	561	4	14	9.54-9.60	31%	41%	50%	55%	58%	32%	42%	57%	59%	63%
547	561	4	13	8.51-8.64	28%	38%	48%	51%	53%	28%	39%	53%	55%	58%
548	561	3	12	7.96-7.99	23%	32%	41%	44%	43%	25%	34%	46%	49%	48%
562	581	4	16	12.64-12.70	2%	5%	9%	14%	25%	3%	6%	11%	16%	30%
562	588	4	21	14.62-14.82	2%	4%	7%	11%	21%	2%	5%	9%	14%	27%
582	588	1	4	14.12-14.29	7%	10%	8%	26%	31%	3%	3%	3%	16%	34%
589	611	3	19	15.88-15.98	22%	27%	33%	42%	50%	23%	29%	37%	47%	54%
600	611	2	9	15.19-15.27	18%	20%	22%	36%	44%	17%	19%	25%	37%	46%
612	634	4	20	14.42-14.54	5%	11%	18%	27%	29%	7%	13%	22%	27%	30%
624	634	2	9	10.45-10.51	4%	11%	22%	35%	37%	7%	15%	29%	38%	40%
642	653	2	10	13.48-13.53	3%	5%	6%	11%	19%	4%	5%	6%	13%	25%
654	671	2	14	12.37-12.66	3%	4%	8%	14%	16%	2%	5%	9%	13%	17%
654	672	2	15	15.01-15.06	2%	4%	7%	10%	13%	2%	6%	8%	12%	15%
654	678	3	20	15.74-15.88	2%	5%	10%	15%	19%	3%	7%	12%	17%	22%
679	690	2	10	13.53-13.64	16%	34%	44%	43%	46%	23%	39%	46%	46%	52%
679	703	4	22	16.02-16.07	14%	21%	26%	26%	29%	16%	24%	27%	28%	34%
708	712	1	3	13.36-13.54	2%	6%	2%	4%	6%	1%	1%	1%	1%	9%
715	719	1	3	6.40-6.65	1%	3%	2%	4%	4%	1%	1%	1%	1%	1%
738	750	2	10	14.88-14.92	1%	3%	3%	7%	11%	2%	3%	5%	10%	14%
740	750	2	9	14.89-14.95	1%	1%	2%	5%	10%	1%	1%	3%	8%	12%
751	773	3	15	16.34-16.38	14%	24%	37%	49%	58%	22%	31%	42%	52%	65%
751	774	3	16	16.51-16.68	13%	23%	36%	47%	59%	21%	30%	40%	50%	66%
775	782	1	6	6.74-6.87	55%	56%	60%	57%	56%	55%	59%	63%	62%	62%
775	786	2	10	12.53-12.57	20%	29%	36%	40%	41%	20%	30%	37%	41%	46%
775	791	3	15	15.37-15.44	5%	10%	14%	18%	25%	6%	11%	14%	18%	27%
792	807	3	14	16.52-16.57	2%	6%	7%	11%	18%	3%	7%	8%	12%	20%
793	803	2	9	12.11-12.16	4%	10%	13%	16%	25%	6%	10%	12%	17%	29%
798	807	2	8	14.26-14.37	6%	11%	13%	16%	24%	5%	7%	10%	16%	25%
812	828	2	13	14.22-14.26	3%	10%	13%	23%	42%	4%	9%	14%	28%	48%
829	838	3	8	11.11-11.30	23%	28%	38%	44%	54%	23%	30%	40%	47%	59%
839	850	2	10	13.27-13.33	5%	7%	13%	30%	49%	5%	7%	16%	36%	55%
839	853	2	13	14.06-14.16	3%	5%	12%	27%	43%	3%	7%	15%	32%	48%
851	856	1	4	6.78-6.82	19%	31%	61%	77%	77%	27%	41%	72%	79%	79%
857	872	2	13	17.33-17.38	64%	70%	71%	72%	72%	64%	70%	73%	72%	73%
860	872	2	11	13.20-13.29	52%	54%	54%	52%	52%	53%	53%	53%	52%	54%

Table S2D

Vps38								CompII		
Start	*End*	*CS*	*#D*	*RT*	*0.3*	*0.3*	*3*	*30*	*300*	*3000*
24	32	2	7	7.97-8.05	8%	45%	54%	54%	52%	50%
33	44	2	9	17.68-17.77	18%	17%	26%	27%	33%	33%
33	45	2	10	18.30-18.37	18%	15%	23%	24%	29%	29%
45	55	2	9	17.29-17.34	17%	29%	35%	42%	47%	46%
58	70	2	11	13.13-13.35	13%	13%	27%	36%	42%	42%
66	82	3	14	12.84-13.02	13%	22%	32%	34%	39%	42%
71	82	2	9	11.84-12.03	12%	31%	44%	48%	53%	58%
83	98	2	13	17.88-17.93	18%	2%	4%	9%	19%	25%
86	96	2	8	16.16-16.20	16%	3%	5%	7%	13%	22%
99	111	2	11	12.68-12.78	13%	25%	29%	28%	29%	31%
117	129	2	10	13.94-14.06	14%	17%	21%	28%	33%	40%
152	172	3	18	7.57-7.67	8%	44%	48%	48%	46%	44%
185	203	3	17	13.48-13.53	13%	2%	5%	11%	25%	43%
191	203	2	11	10.70-10.71	11%	3%	10%	18%	35%	53%
191	212	3	20	17.74-17.76	18%	4%	11%	17%	36%	58%
204	212	2	7	12.94-12.96	13%	9%	22%	31%	39%	48%
213	221	2	7	10.94-11.01	11%	40%	50%	48%	50%	45%
222	231	2	8	8.25-8.28	8%	3%	4%	7%	23%	41%
222	233	2	10	11.88-11.95	12%	2%	3%	5%	24%	41%
253	265	2	11	8.54-8.59	9%	42%	43%	44%	41%	40%
268	285	2	16	15.65-15.72	16%	38%	45%	52%	58%	60%
286	295	2	8	11.92-11.99	12%	2%	3%	3%	11%	20%
286	297	3	10	13.51-13.67	14%	2%	4%	3%	6%	14%
304	312	2	7	14.45-14.57	14%	4%	7%	7%	9%	14%
313	322	2	8	16.11-16.16	16%	16%	24%	33%	47%	54%
343	365	3	21	10.88-11.09	11%	6%	11%	17%	21%	27%
346	365	3	18	9.15-9.19	9%	5%	8%	12%	14%	15%
371	386	3	12	15.41-15.45	15%	12%	17%	23%	32%	47%
373	386	3	10	15.56-15.60	16%	14%	19%	27%	36%	50%
375	386	3	8	15.24-15.30	15%	17%	24%	32%	41%	51%
375	388	3	10	16.26-16.43	16%	17%	25%	34%	42%	52%
387	402	2	13	16.46-16.50	16%	8%	10%	13%	14%	20%

Table S2. Relative percentage of global deuteration level of Vps30 **(A)**, Vps15 **(B)**, Vps34 **(C)**, and Vps38 **(D)**. Column labeling: S, starting amino acid; E, ending amino acid; CS, charge state, #D, number of deuterons; RT, retention time. Time course: 0.3 (3 s on ice); 3, 30 and 300 are in seconds.

Table S3A

Vps34						%HDX exchange apo								%HDX exchange + liposomes							
S	E	z	#D	rt	i	0.3	SD	3	SD	30	SD	300	SD	0.3	SD	3	SD	30	SD	300	SD
56	60	1	3	13.3	58	3	1	3	0	3	1	4	1	3	0	3	0	4	1	4	1
57	73	3	15	11.9	65	5	0	7	1	9	0	16	0	4	1	6	0	10	1	16	0
74	87	3	10	12.1	81	18	1	18	1	22	1	38	1	19	1	20	0	24	2	38	1
74	89	3	12	13.4	82	15	5	11	1	16	1	27	1	13	1	13	1	18	2	29	1
74	90	3	13	13.9	82	11	1	10	1	14	0	26	1	12	1	12	0	17	2	28	1
74	91	3	14	15.9	83	14	1	15	1	20	1	29	1	13	1	15	1	20	1	28	1
92	107	4	13	13.0	100	7	0	9	0	14	0	20	1	7	0	9	1	14	1	21	1
94	107	3	12	13.0	101	7	0	9	1	13	0	19	1	7	0	9	1	13	1	19	1
95	107	3	11	13.0	101	7	0	9	0	13	0	19	1	7	0	9	0	13	1	19	1
112	123	2	9	15.4	118	7	1	12	1	23	1	26	2	7	1	13	2	25	2	28	1
112	125	2	11	16.3	119	6	0	10	1	25	0	28	2	5	0	11	1	23	1	29	1
138	144	2	5	9.2	141	8	1	10	0	21	1	30	1	6	1	9	1	21	1	30	0
138	154	4	15	10.9	146	6	1	9	1	17	1	27	1	6	0	9	1	17	2	27	1
145	154	3	8	10.1	150	11	1	16	0	26	0	38	0	11	0	16	2	27	1	41	1
155	166	2	10	8.6	161	69	1	72	1	81	1	85	2	69	0	72	2	83	1	87	1
155	174	3	18	12.9	165	28	2	32	1	56	1	65	1	26	1	34	3	56	3	67	1
174	180	2	4	15.7	177	44	1	44	1	59	0	83	1	42	1	46	1	58	1	84	1
174	182	2	6	14.1	178	51	1	53	1	68	2	69	1	51	0	53	2	66	1	71	2
181	201	4	18	8.9	191	44	1	46	1	55	1	66	1	42	1	47	1	55	2	67	1
209	213	1	3	14.3	211	31	2	34	1	54	1	82	1	29	0	35	2	52	1	83	2
218	223	1	4	9.1	221	53	2	54	1	72	2	78	2	51	1	57	3	72	3	81	3
218	241	3	20	11.5	230	52	1	56	1	70	3	70	3	52	2	60	1	70	3	73	1
224	241	2	14	10.7	233	59	1	62	1	75	3	75	2	58	1	65	2	76	2	78	2
228	241	2	10	9.6	235	61	2	64	1	74	3	74	3	59	1	65	2	75	2	74	2
242	264	5	19	9.9	253	33	2	35	1	44	1	48	1	32	1	36	1	43	2	48	1
265	279	2	10	10.3	272	24	1	27	1	39	3	50	1	23	1	29	2	41	0	51	0
265	293	5	24	10.3	279	29	1	32	0	41	1	51	1	28	0	33	1	42	1	53	1
267	293	4	22	10.3	280	32	0	35	1	45	1	53	1	31	0	37	2	45	1	55	1
280	293	3	12	5.0	287	39	0	42	0	47	0	50	1	39	1	43	1	48	2	53	1
294	307	3	11	7.0	301	46	3	51	0	56	1	61	1	44	0	52	4	57	1	62	1
294	307	4	11	6.6	301	45	3	51	1	56	2	62	1	44	1	53	1	59	2	63	2
294	319	4	21	9.8	307	31	1	34	0	40	1	48	1	29	0	35	1	41	1	50	0
294	332	6	34	10.0	313	18	1	20	1	24	1	29	1	17	0	20	1	24	1	30	0
333	344	3	10	9.1	339	6	0	8	1	19	0	34	1	7	1	11	2	20	1	35	1
345	350	1	4	8.5	348	52	1	56	1	78	1	82	1	50	1	58	2	76	2	81	1
351	359	2	7	6.7	355	18	1	23	0	42	1	64	0	18	1	24	3	42	0	65	1
376	385	2	8	13.2	381	47	2	48	1	55	2	59	0	45	1	48	1	56	2	58	1
376	389	3	12	11.1	383	35	1	36	1	45	1	58	2	35	1	38	2	47	0	60	1
388	405	4	16	8.9	397	12	0	13	0	15	0	21	0	13	0	15	1	17	0	23	1
408	412	1	3	10.7	410	2	1	1	1	2	1	4	1	4	2	2	1	3	1	4	2
435	447	2	11	10.5	441	57	1	55	1	58	2	57	1	57	0	57	1	58	2	59	1
448	464	3	15	9.1	456	59	1	58	0	61	1	61	0	59	1	60	1	62	1	61	1
449	464	3	14	8.9	457	64	1	63	0	65	1	64	1	63	1	64	1	65	1	65	1
474	483	2	7	16.4	479	43	2	44	1	51	1	57	0	38	2	46	2	52	1	58	1
483	497	3	12	12.8	490	3	0	4	1	10	1	19	1	3	1	5	0	10	1	19	1
484	497	3	11	10.6	491	5	0	7	0	14	0	22	1	4	1	6	1	13	1	22	0
484	498	3	12	12.0	491	5	2	6	2	11	2	21	2	4	2	6	0	12	2	21	1
485	497	3	10	10.3	491	6	0	8	1	15	1	25	1	5	1	7	0	15	1	25	1
501	512	2	9	9.7	507	21	1	23	1	35	1	46	1	19	0	22	1	35	1	47	0
503	512	2	7	8.6	508	24	1	28	0	41	0	53	1	23	0	26	1	39	1	53	2
520	533	3	12	8.6	527	15	1	17	0	24	0	33	1	13	0	17	1	24	1	33	1
524	533	2	8	6.7	529	18	1	21	1	31	1	44	1	15	0	20	4	29	1	43	1
546	561	4	14	8.5	554	40	2	45	2	60	1	66	1	40	1	47	2	60	1	65	2
547	561	4	13	7.4	554	41	2	47	0	61	1	67	0	41	1	47	2	61	2	67	1
548	561	3	12	6.4	555	36	2	41	0	54	1	60	1	36	0	43	2	55	2	61	1
562	588	3	21	12.9	575	6	0	7	0	11	0	17	1	5	1	7	1	12	1	18	1
566	581	3	12	9.9	574	7	1	8	1	15	1	21	1	5	0	8	2	16	1	23	1
569	588	3	14	12.4	579	8	1	10	0	15	1	23	1	7	0	9	1	16	2	24	1
582	588	1	4	12.6	585	2	2	2	1	4	0	19	2	2	1	2	0	5	3	18	2
589	599	2	8	9.7	594	38	2	41	1	54	1	66	3	38	1	43	1	53	1	67	1
589	611	3	19	13.6	600	30	1	31	0	41	1	53	1	29	1	31	1	41	2	53	1
591	599	2	6	7.4	595	30	1	31	1	41	1	45	1	31	3	29	2	42	3	49	4
591	611	3	17	13.5	601	31	1	32	1	44	1	53	2	30	0	34	1	44	2	54	1
600	611	2	9	13.3	606	21	1	22	2	28	1	43	1	22	2	22	1	30	2	44	0
612	623	2	9	13.4	618	21	1	22	1	27	1	29	2	19	0	23	1	28	2	31	1
624	634	2	9	9.1	629	14	2	16	0	30	0	43	1	12	2	17	4	31	2	44	2
635	641	1	5	13.8	638	1	0	1	0	1	0	2	1	1	0	1	0	2	1	2	1
642	653	2	10	11.8	648	6	1	6	2	8	0	17	0	5	0	6	0	7	1	17	0
654	671	2	14	11.0	663	9	1	10	1	15	0	21	2	8	1	11	1	16	2	22	2
654	672	2	15	13.1	663	6	0	7	0	12	0	16	2	5	1	9	2	12	2	17	2
654	678	3	20	13.7	666	8	1	10	0	16	0	23	2	8	1	10	1	18	1	24	2
679	690	2	10	12.0	685	34	5	43	2	55	1	55	1	29	1	45	2	56	2	57	1
680	702	5	20	9.3	691	23	3	28	0	53	1	63	1	16	1	29	4	53	1	65	0
691	703	2	10	11.5	697	31	1	30	0	33	1	36	1	29	1	31	1	34	1	39	1
721	737	3	15	15.6	729	8	0	9	0	12	0	19	1	9	1	11	1	14	1	22	1
723	737	2	13	15.6	730	9	0	9	1	14	1	21	1	11	1	11	0	16	3	24	2
725	737	2	11	11.9	731	8	1	7	2	10	0	18	2	8	2	7	0	11	1	20	1
727	737	2	9	11.9	732	8	1	8	1	12	0	20	1	8	0	9	0	13	1	23	1
738	750	2	10	13.1	744	4	1	4	0	5	1	12	1	4	0	4	1	6	1	12	0
740	750	2	9	13.0	745	2	0	2	0	3	0	9	1	2	0	2	0	4	1	11	1
740	751	2	10	12.6	746	3	1	3	0	7	0	15	1	3	0	4	0	7	1	16	0
741	750	2	8	13.1	746	3	1	3	0	4	0	10	0	3	0	2	0	4	1	10	0
751	773	3	15	14.2	762	24	2	28	1	42	1	53	0	23	1	30	3	44	2	60	1
751	774	3	16	14.3	763	24	2	28	1	41	0	52	1	22	1	30	2	43	1	59	1
775	782	1	6	5.4	779	63	1	62	1	66	2	62	2	62	1	64	0	65	4	66	3
808	828	3	17	14.0	818	10	0	11	1	19	1	31	2	9	1	12	1	21	2	36	1
811	828	2	14	12.6	820	11	1	11	0	18	1	33	2	10	0	12	1	21	1	38	2
812	828	2	13	12.7	820	9	1	10	1	17	0	30	1	9	1	11	1	19	1	35	1
829	838	3	8	9.9	834	27	1	30	0	42	0	50	0	25	1	30	1	41	1	50	1
839	850	2	10	11.6	845	8	1	9	0	19	1	39	1	8	1	10	1	19	1	42	1
851	856	1	4	5.2	854	33	4	40	1	74	1	82	2	32	1	47	5	77	4	85	3
851	871	3	18	16.1	861	66	1	66	1	73	0	75	1	65	1	67	2	75	1	77	0
856	872	2	14	16.3	864	78	2	76	1	82	1	82	1	76	1	79	2	81	1	85	2
857	872	2	13	15.4	865	70	1	69	2	74	1	74	0	69	1	70	3	74	1	74	1
859	872	2	12	15.3	866	72	2	67	1	74	3	75	2	67	1	71	0	75	2	75	2
860	872	3	11	11.7	866	53	1	54	1	53	1	53	1	54	2	55	2	57	1	55	0

Table S3B

Vps38						%HDX exchange apo								%HDX exchange + liposomes							
S	E	z	#D	rt	i	0.3	SD	3	SD	30	SD	300	SD	0.3	SD	3	SD	30	SD	300	SD
24	32	2	7	6.5	28	58	2	60	0	63	1	63	1	56	1	61	0	63	2	64	2
33	44	2	9	15.8	38.5	26	1	27	1	33	1	39	1	25	0	29	0	34	2	41	1
33	45	2	10	17.6	39	23	1	24	1	30	1	36	1	22	0	26	0	30	1	37	1
45	54	2	8	14.9	49.5	21	1	22	0	30	1	44	1	21	0	23	1	30	1	45	0
45	55	2	9	15.2	50	34	2	34	0	43	1	45	1	34	1	36	1	42	1	45	2
46	55	2	8	13.0	50.5	39	1	41	1	49	1	51	1	38	1	41	1	49	1	51	1
55	59	1	3	8.7	57	2	1	3	0	3	0	6	1	3	1	3	0	4	1	7	2
71	82	2	9	10.5	76.5	45	2	47	1	54	2	60	2	42	0	49	1	57	2	65	1
86	96	2	8	14.1	91	6	0	6	0	12	0	20	1	6	0	7	1	13	1	23	1
99	111	3	11	11.2	105	29	1	27	1	32	2	37	2	29	0	30	0	35	3	41	3
100	111	2	10	11.2	105.5	30	1	29	1	33	1	34	1	31	1	31	0	34	1	38	0
185	192	2	6	10.7	188.5	3	0	4	0	5	1	17	1	5	1	7	0	10	0	24	1
191	203	2	11	9.4	197	8	1	11	0	26	1	49	1	7	1	12	1	26	1	51	1
204	212	2	7	11.4	208	21	2	28	1	38	1	50	1	22	0	30	1	39	1	52	1
212	221	2	8	9.2	216.5	13	2	17	0	33	0	47	0	12	0	18	3	33	1	48	0
222	231	2	8	6.7	226.5	5	0	6	1	13	1	33	1	3	0	5	1	12	2	31	2
222	233	2	10	10.5	227.5	5	2	6	1	9	2	32	2	5	0	6	1	9	0	29	1
253	265	2	11	7.3	259	49	1	47	0	50	1	50	1	49	1	49	1	50	1	50	0
253	267	2	13	10.0	260	51	1	50	0	52	1	52	1	50	0	50	1	52	1	52	1
286	292	2	5	8.3	289	7	0	10	2	17	1	34	1	7	1	9	1	20	2	37	0
286	295	2	8	10.5	290.5	6	1	8	1	10	3	19	2	8	0	10	1	13	3	24	1
304	312	2	7	12.8	308	8	1	11	2	11	0	16	1	7	0	10	1	12	1	18	0
313	322	2	8	14.2	317.5	29	1	31	1	45	0	59	1	31	1	35	2	49	2	63	1
346	365	3	18	8.0	355.5	14	0	16	0	22	1	25	1	12	1	15	1	23	1	27	1
373	386	3	10	13.6	379.5	23	1	25	0	36	0	50	1	21	1	26	1	36	1	52	0
373	388	3	12	14.5	380.5	25	2	28	1	39	0	50	1	22	1	28	1	39	1	51	1
375	386	2	8	13.3	380.5	27	1	31	0	42	0	55	1	26	0	31	1	43	1	56	0
375	388	3	10	14.2	381.5	29	2	32	0	43	1	54	1	26	0	33	2	43	1	55	1
377	386	2	6	13.2	381.5	34	1	37	0	47	1	60	0	33	0	38	1	49	1	62	0
387	402	2	13	14.4	394.5	16	0	16	1	20	1	23	1	15	1	16	1	21	1	24	2
403	415	3	11	8.8	409	43	2	43	1	51	2	52	1	43	0	46	2	51	1	55	1

Table S3C

Vps15						%HDX exchange apo								%HDX exchange + liposomes							
S	E	z	#D	rt	i	0.3	SD	3	SD	30	SD	300	SD	0.3	SD	3	SD	30	SD	300	SD
56	69	3	10	12.5	62.5	37	2	37	1	46	0	52	1	33	3	35	2	42	4	50	3
77	88	2	9	13.1	82.5	14	0	16	1	26	1	40	1	14	1	15	0	25	2	41	1
100	109	2	8	5.9	104.5	8	1	11	1	14	0	21	1	7	1	12	2	15	2	22	1
233	243	2	8	14.8	238	12	1	15	1	22	2	38	1	9	2	15	1	20	1	36	1
262	272	2	9	8.6	267	30	1	31	1	40	1	43	2	29	1	33	2	38	1	45	2
359	377	2	14	15.4	368	38	3	38	2	49	3	51	4	34	3	34	1	41	3	47	5
634	640	1	5	13.8	637	1	0	1	0	1	0	1	0	1	0	2	2	1	1	2	1
761	773	2	10	12.3	767	28	2	29	1	35	1	34	1	25	0	28	1	34	1	38	1
786	800	2	13	9.3	793	34	2	38	1	48	1	51	1	30	2	37	1	46	2	50	2
858	873	3	14	15.2	865.5	23	1	26	0	34	0	44	1	23	1	27	1	37	1	52	1
863	873	2	9	14.2	868	10	1	11	1	18	1	25	0	6	3	12	1	21	1	32	2
863	875	3	11	12.4	869	15	1	17	0	22	0	30	1	13	1	18	1	26	1	40	1
881	910	3	23	12.5	895.5	64	0	62	0	67	3	67	3	63	1	62	2	66	3	68	3
897	910	2	9	11.8	903.5	77	2	75	1	79	2	77	3	78	2	75	1	77	2	78	4
909	920	2	9	16.4	914.5	42	2	49	1	66	1	78	1	41	1	49	3	65	2	81	2
911	920	2	7	15.3	915.5	34	2	44	0	64	1	78	1	33	1	44	2	64	2	83	2
961	973	2	11	8.5	967	32	1	34	1	43	1	46	1	32	0	35	2	44	1	47	0
974	987	2	12	9.2	980.5	65	1	64	1	67	1	67	1	64	3	63	0	65	1	68	2
1040	1048	2	5	11.0	1044	23	0	30	1	52	2	73	1	21	2	33	4	51	2	75	4
1049	1057	2	6	10.8	1053	39	1	41	1	56	1	69	1	36	2	42	1	55	1	68	2
1058	1071	3	11	9.4	1064.5	68	2	68	2	71	2	71	1	64	2	66	2	70	2	72	1
1059	1071	3	10	9.4	1065	65	2	65	0	71	2	70	2	63	2	65	1	70	3	71	1
1076	1096	2	16	12.6	1086	20	1	22	1	32	1	40	2	19	1	23	1	33	2	41	1
1097	1110	2	12	14.9	1103.5	12	1	13	0	17	0	22	0	12	0	13	1	16	1	22	1
1156	1165	2	8	4.8	1160.5	50	2	52	1	58	2	60	2	49	3	52	2	55	2	58	2
1305	1314	2	8	14.2	1309.5	28	1	31	1	46	2	58	0	30	1	35	1	48	2	61	2
1341	1351	2	9	10.9	1346	21	1	22	0	30	0	39	1	21	0	24	1	31	2	40	1
1352	1368	2	15	11.0	1360	16	1	20	2	30	1	39	2	17	1	24	1	31	1	39	2
1406	1421	3	14	5.1	1413.5	34	0	33	1	36	1	36	2	33	2	34	1	35	2	37	3
1422	1429	2	6	6.2	1425.5	8	0	9	1	10	2	17	4	8	2	9	0	12	1	21	2
1442	1449	1	6	8.7	1445.5	12	2	14	2	15	2	19	3	9	1	12	3	13	5	19	2

Table S3D

Vps30						%HDX exchange apo								%HDX exchange + liposomes							
S	E	z	#D	rt	i	0.3	SD	3	SD	30	SD	300	SD	0.3	SD	3	SD	30	SD	300	SD
6	16	2	7	13.5	11	23	4	25	1	29	1	40	0	21	1	25	1	34	3	44	2
17	27	2	9	12.5	22	59	4	68	1	77	2	77	2	58	1	71	1	78	2	79	1
19	27	2	7	10.1	23	17	2	19	2	32	1	47	1	19	5	28	2	41	1	58	0
32	39	1	6	8.9	35.5	87	2	86	0	89	1	86	2	85	0	86	1	85	2	84	2
40	62	3	19	10.2	51	69	0	68	1	73	2	75	1	68	0	70	1	74	2	76	1
40	76	5	33	11.6	58	51	1	52	0	60	2	67	1	51	0	54	1	62	2	69	1
42	62	3	17	10.2	52	69	1	68	0	74	2	76	1	69	0	71	1	75	2	78	1
43	62	3	16	10.3	52.5	72	2	71	0	77	1	79	1	72	0	74	0	78	3	81	2
49	76	4	24	11.3	62.5	40	1	42	0	52	1	62	1	40	1	45	1	55	2	64	1
51	76	4	22	11.5	63.5	41	1	42	1	53	1	65	1	40	0	45	1	55	2	66	1
63	69	2	5	8.3	66	10	2	12	1	17	1	33	1	8	2	11	3	20	3	37	1
63	73	3	9	7.5	68	40	1	43	1	54	1	69	1	40	1	46	2	57	1	73	2
63	76	3	12	11.2	69.5	41	1	43	1	55	1	67	1	41	0	46	1	57	2	71	1
77	81	1	3	8.5	79	43	4	54	0	63	2	62	1	43	0	56	3	63	1	64	1
82	86	1	3	10.6	84	20	3	37	0	63	2	71	1	19	1	41	6	64	2	71	1
86	97	2	10	11.8	91.5	17	0	18	0	26	1	32	1	18	1	20	1	27	1	33	1
87	97	2	9	8.7	92	22	0	22	1	27	1	28	2	22	1	23	1	27	1	29	1
98	130	4	31	5.0	114	60	0	59	0	61	3	60	1	60	0	61	1	62	1	62	1
131	138	2	6	5.0	135	23	0	24	6	24	1	25	4	24	3	24	3	24	5	27	4
131	139	2	7	4.9	135	32	1	31	1	34	1	32	2	33	1	32	0	32	3	34	1
153	163	2	9	6.0	158	85	2	83	1	85	1	85	1	84	1	84	0	85	1	86	1
153	168	2	14	12.8	161	74	0	76	1	82	1	82	2	73	3	79	0	83	3	83	2
164	168	1	3	12.3	166	53	6	65	2	87	1	90	2	52	1	70	2	88	3	91	1
188	210	4	21	12.6	199	4	1	5	0	9	1	23	1	4	1	5	0	9	1	22	1
194	210	2	15	10.6	202	4	1	4	0	7	1	18	2	4	1	5	0	8	1	24	4
194	210	3	15	10.6	202	4	1	5	0	7	1	21	2	4	1	5	0	8	1	24	0
201	210	2	8	9.7	206	3	1	4	0	9	1	24	1	4	0	5	0	10	2	27	1
211	223	2	11	6.2	217	59	1	60	1	68	1	69	2	56	0	62	3	68	3	70	1
211	247	5	35	8.2	229	51	1	53	1	56	1	57	1	51	0	54	1	58	1	59	0
211	251	6	39	11.1	231	43	1	46	1	53	1	55	1	44	1	49	1	54	1	59	1
215	247	6	31	8.1	231	50	1	53	1	55	1	55	1	51	1	54	1	56	1	57	1
215	251	5	35	11.3	233	43	1	46	1	53	1	55	1	44	0	48	1	54	0	57	0
266	288	3	21	9.4	277	24	2	33	1	57	1	70	1	22	1	34	4	56	1	72	1
289	293	1	3	6.9	291	27	2	34	1	52	2	70	1	29	2	41	3	58	0	73	1
297	327	5	29	14.7	312	11	1	14	0	22	1	44	3	10	1	14	1	22	1	43	1
312	327	3	14	13.8	320	22	2	26	1	37	1	57	0	23	2	30	4	44	0	59	1
318	327	2	8	11.5	323	44	0	43	0	45	1	47	1	44	0	44	1	46	1	47	1
327	340	2	11	11.5	334	18	1	21	5	24	3	32	1	18	1	19	0	23	1	33	1
327	357	4	26	14.1	342	17	1	19	1	25	0	36	1	17	0	19	1	24	2	34	1
328	357	4	25	13.6	343	17	0	18	0	25	1	35	1	16	0	18	1	25	1	35	1
367	378	2	10	11.3	373	25	1	25	0	33	0	50	1	24	1	27	1	34	1	52	0
368	378	2	9	11.3	373	27	1	28	0	35	1	53	1	27	0	29	1	37	1	55	1
369	378	2	8	11.1	374	30	1	31	0	39	1	58	1	30	0	32	0	40	1	60	1
385	389	1	3	14.2	387	26	1	28	1	36	1	41	0	23	2	30	2	37	2	42	1
390	401	2	10	4.8	396	51	1	51	1	54	1	59	0	49	0	51	3	54	0	57	2
390	413	3	21	9.2	402	54	0	52	1	56	1	58	1	53	0	54	0	57	1	59	1
414	419	1	3	14.4	417	4	1	4	0	4	1	5	2	5	1	5	0	8	2	11	1
424	443	4	18	11.3	434	41	1	41	1	46	1	51	1	31	1	35	2	46	1	50	1
425	444	4	18	10.9	435	34	1	34	1	39	1	42	1	27	0	31	1	37	1	43	0
427	441	4	13	7.7	434	30	0	30	1	36	0	41	1	23	1	27	1	33	1	40	2
427	443	4	15	7.8	435	25	0	26	1	31	1	36	1	19	1	23	1	28	1	35	1
427	444	4	16	9.6	436	21	1	22	1	28	0	32	1	17	0	20	2	26	1	33	2
445	449	1	3	8.4	447	2	0	2	1	3	0	7	1	2	0	3	1	5	1	10	0
450	456	1	5	5.3	453	34	1	37	0	43	0	58	0	32	0	38	1	45	1	59	1
451	456	1	4	5.2	454	38	2	42	1	50	2	64	0	35	1	45	3	50	4	66	2
451	457	2	5	5.2	454	36	2	39	1	45	2	59	1	33	1	41	1	46	3	62	2
485	490	1	4	14.2	488	77	2	77	0	80	2	81	1	71	1	78	2	82	0	84	1
487	502	3	13	14.0	495	31	1	33	0	40	1	42	2	28	0	34	2	41	2	46	1
491	502	3	10	10.5	497	31	2	34	0	43	0	47	0	29	0	35	1	44	1	48	1
491	503	2	11	9.6	497	34	2	37	1	45	1	48	1	31	0	37	2	45	1	49	1
503	513	2	8	7.7	508	28	1	29	1	33	1	40	1	27	1	29	2	33	1	38	1
504	513	2	7	7.4	509	30	1	31	1	36	1	44	0	28	0	31	2	35	0	39	1
522	531	2	8	15.3	527	1	0	2	0	5	0	11	0	1	0	2	0	6	0	13	0
522	532	2	9	17.6	527	2	1	3	1	8	1	16	2	2	0	4	1	7	0	17	0
532	536	1	3	10.1	534	58	3	67	3	82	1	86	2	59	3	69	1	85	2	88	2

Table S3. Relative percentage of global deuteration level of Vps34 **(A)**, Vps38 **(B)**, Vps15 **(C)**, and Vps30 **(D)** in presence and in absence of liposomes. Column labels as in Table S2.

Table S4A

ScVps34						%HDX exchange apo		%HDX exchange +NB 8780	
S	E	z	#D	rt	i	0.3	SD	0.3	SD
2	8	1	5	12.9	5	27	1	29	2
44	55	2	9	12.8	49.5	10	0	11	1
61	73	3	11	9.0	67	3	0	5	0
63	73	3	9	10.1	68	30	0	30	1
74	87	3	10	11.6	80.5	16	1	18	2
74	89	3	12	13.1	81.5	9	0	11	2
74	90	3	13	13.8	82	8	0	9	1
74	91	3	14	15.5	82.5	11	1	11	1
92	107	4	13	12.8	99.5	5	0	5	1
112	123	2	9	15.0	117.5	3	0	4	1
112	125	2	11	15.9	118.5	3	1	4	1
135	144	2	8	14.4	139.5	23	0	24	1
138	144	2	5	8.8	141	4	0	6	0
138	154	4	15	10.5	146	5	0	5	1
145	154	3	8	9.7	149.5	8	0	9	1
155	166	2	10	7.7	160.5	39	0	40	2
155	173	3	17	10.6	164	22	0	23	2
155	174	3	18	12.7	164.5	21	1	22	2
174	180	2	4	15.3	177	38	1	37	0
174	182	2	6	14.6	178	25	1	25	2
175	180	2	3	14.2	177.5	34	1	34	0
181	201	4	18	8.5	191	38	0	41	2
183	201	4	16	7.3	192	40	0	41	1
209	213	1	3	13.9	211	22	1	23	1
218	223	2	4	8.7	220.5	37	2	37	1
218	225	2	6	8.0	221.5	50	1	50	1
224	241	2	14	10.3	232.5	54	0	55	2
228	241	2	10	9.1	234.5	60	1	61	1
242	264	5	19	9.4	253	31	1	31	1
265	293	4	24	10.0	279	24	0	26	1
280	293	3	12	5.2	286.5	37	0	38	1
294	307	3	11	7.0	300.5	38	2	39	1
294	307	4	11	7.0	300.5	38	2	38	2
294	319	4	21	9.4	306.5	24	0	25	1
294	319	5	21	9.4	306.5	24	0	25	2
294	332	6	34	9.6	313	13	1	14	1
320	332	4	11	9.1	326	1	0	2	0
333	344	3	10	8.7	338.5	6	0	7	0
345	350	1	4	8.3	347.5	47	1	47	1
351	359	2	7	6.8	355	14	0	14	0
376	385	2	8	12.9	380.5	40	0	39	1
376	387	2	10	13.1	381.5	40	0	44	6
376	389	2	12	11.3	382.5	37	0	36	0
386	405	4	18	9.6	395.5	12	0	4	0
388	405	4	16	8.5	396.5	9	0	2	0
391	406	4	14	8.8	398.5	11	0	2	0
408	412	1	3	10.3	410	0	0	1	0
417	421	1	3	5.9	419	86	0	83	1
420	430	2	9	9.6	425	58	0	59	1
431	447	2	15	11.8	439	69	0	70	2
435	447	2	11	10.0	441	59	1	61	1
448	464	2	15	8.5	456	60	0	60	2
448	473	4	24	9.7	460.5	58	0	59	2
449	464	3	14	8.6	456.5	62	0	62	1
474	483	2	7	16.0	478.5	36	1	33	1
474	484	2	8	17.6	479	31	1	28	1
485	497	3	10	9.8	491	2	0	3	1
501	512	2	9	9.2	506.5	18	0	18	1
503	512	2	7	8.3	507.5	22	1	22	1
503	516	2	11	12.7	509.5	15	0	15	1
520	533	3	12	8.2	526.5	12	0	12	0
534	540	2	5	10.7	537	5	0	5	1
546	561	4	14	8.0	553.5	37	1	36	1
547	561	4	13	7.1	554	36	2	35	1
548	561	4	12	6.6	554.5	33	1	32	0
562	581	4	16	10.7	571.5	4	0	5	0
562	588	3	21	12.7	575	2	0	3	1
566	581	4	12	9.6	573.5	4	0	6	2
569	588	3	14	12.2	578.5	4	0	5	1
582	588	1	4	12.1	585	2	1	3	1
589	599	2	8	9.3	594	35	0	35	1
589	611	3	19	13.6	600	26	0	25	2
591	611	3	17	13.3	601	27	0	27	2
600	611	2	9	13.1	605.5	19	0	20	1
612	623	2	9	13.0	617.5	16	0	17	1
624	634	2	9	8.8	629	7	0	8	1
624	634	3	9	8.7	629	9	1	9	2
635	641	1	5	13.5	638	0	0	1	0
642	653	2	10	11.4	647.5	5	1	5	1
646	653	2	6	8.6	649.5	10	0	9	1
654	671	2	14	10.7	662.5	5	0	7	1
654	678	3	20	13.5	666	4	0	6	0
679	689	2	9	11.8	684	22	1	22	0
679	690	2	10	11.5	684.5	22	2	22	2
721	737	3	15	15.4	729	6	0	7	0
727	737	2	9	11.6	732	7	0	7	1
738	750	3	10	12.9	744	1	0	2	0
751	773	3	15	13.9	762	18	0	18	1
751	774	3	16	14.2	762.5	17	0	18	1
775	782	1	6	5.8	778.5	61	0	60	2
792	803	3	10	10.3	797.5	5	0	7	0
792	807	3	14	14.3	799.5	4	1	5	1
811	828	2	14	12.3	819.5	5	0	6	1
812	828	2	13	12.1	820	5	0	6	1
829	838	3	8	9.5	833.5	24	0	25	0
839	850	2	10	11.2	844.5	6	0	7	0
851	856	1	4	5.7	853.5	24	1	24	0
851	871	3	18	15.8	861	61	2	61	2
857	872	2	13	15.0	864.5	68	0	68	2
860	872	3	11	11.1	866	54	0	53	2

Table S4B

ScVps38			%HDX exchange apo					%HDX exchange +NB8780	
S	E	z	#D	rt	i	0.3	SD	0.3	SD
24	32	2	7	6.7	28	55	0	55	2
33	44	2	9	15.3	39	20	1	21	2
33	45	2	10	17.1	39	18	0	18	1
45	54	2	8	13.8	50	36	2	34	1
45	55	2	9	15.0	50	31	1	30	1
46	55	2	8	12.7	51	32	0	33	0
55	59	1	3	8.4	57	2	0	3	0
71	81	2	8	8.8	76	44	1	45	1
71	82	2	9	10.1	77	37	1	38	1
86	96	2	8	13.8	91	4	0	5	1
99	111	3	11	10.8	105	30	0	31	1
117	129	2	10	11.9	123	19	1	20	1
185	192	2	6	10.2	189	1	0	2	0
185	203	3	17	11.4	194	4	0	5	0
191	203	2	11	9.0	197	4	0	4	0
193	203	2	9	7.5	198	3	0	4	0
204	212	2	7	10.9	208	12	1	12	1
222	231	2	8	6.8	227	4	0	5	1
222	233	2	10	10.1	228	2	0	3	0
253	265	2	11	7.2	259	49	0	49	1
253	267	2	13	9.6	260	52	0	52	1
286	292	2	5	7.9	289	4	0	5	0
286	295	2	8	10.1	291	3	0	4	0
304	312	2	7	12.4	308	6	0	7	1
346	365	3	18	7.6	356	10	0	11	1
373	386	3	10	13.4	380	17	0	18	1
375	386	3	8	13.1	381	20	1	22	1
375	388	3	10	14.0	382	22	2	22	1
387	402	2	13	14.1	395	11	0	12	1
403	415	3	11	8.4	409	40	0	42	0

Table S4C

Vps15						%HDX exchange apo		%HDX exchange + NB 8780	
S	E	z	#D	rt	i	0.3	SD	0.3	SD
25	33	2	7	10.1	29	16	1	16	0
39	50	2	9	7.4	44.5	3	0	6	1
56	69	3	10	12.2	62.5	31	0	31	1
77	88	2	9	12.8	82.5	11	0	11	1
78	88	2	8	11.2	83	12	0	13	1
92	99	2	6	6.5	95.5	28	0	30	1
100	109	3	8	6.5	105	3	0	5	1
233	243	2	8	14.4	238	7	0	8	2
233	246	2	11	15.1	240	7	0	8	1
247	261	3	13	10.7	254	14	0	14	1
359	377	2	14	14.9	368	36	1	35	1
540	551	2	10	15.1	546	11	1	10	1
568	577	2	8	7.2	573	61	3	63	3
601	610	2	7	14.8	606	24	1	24	1
634	640	1	5	13.5	637	0	0	1	0
761	773	2	10	11.9	767	22	0	23	1
786	800	2	13	8.9	793	29	0	28	1
858	873	3	14	14.9	866	18	1	18	1
858	875	3	16	13.6	867	21	1	21	0
909	920	2	9	15.9	915	33	0	33	2
911	920	2	7	15.0	916	24	0	25	1
961	973	2	11	8.1	967	28	1	28	1
974	987	2	12	8.7	981	66	0	67	1
1040	1048	2	5	10.7	1044	10	0	11	1
1049	1057	2	6	10.4	1053	27	1	28	1
1058	1071	3	11	8.8	1065	64	1	67	1
1059	1071	3	10	9.0	1065	60	0	60	2
1076	1096	2	16	12.2	1086	13	0	14	1
1097	1110	2	12	14.6	1104	10	0	11	1
1144	1155	2	10	11.5	1150	3	1	4	1
1156	1164	2	7	6.8	1160	14	0	14	1
1156	1165	2	8	4.6	1161	47	2	48	1
1316	1338	3	19	15.0	1327	27	1	26	2
1341	1351	2	9	10.1	1346	58	1	59	2
1352	1368	2	15	10.6	1360	11	0	12	1
1375	1389	3	12	9.9	1382	45	0	45	1
1406	1421	3	14	5.9	1414	33	1	34	1
1422	1429	2	6	6.6	1426	4	0	5	0
1435	1441	1	4	12.1	1438	8	1	9	1
1442	1449	1	6	8.4	1446	6	1	7	1

Table S4D

ScVps30					%HDX exchange apo			%HDX exchange +NB 8780	
S	E	z	#D	rt	i	0.3	SD	0.3	SD
2	16	2	11	12.6	9	11	0	13	1
6	16	2	7	13.2	11	17	0	18	1
17	27	2	9	12.1	22	46	2	47	2
20	27	2	6	8.6	23.5	10	0	9	0
32	39	1	6	8.5	35.5	84	1	84	2
40	62	3	19	9.8	51	66	0	65	2
40	76	5	33	11.2	58	47	1	46	2
49	62	3	10	9.1	55.5	55	0	56	1
49	76	4	24	11.0	62.5	35	0	35	2
63	69	2	5	7.9	66	4	0	5	0
63	73	3	9	7.3	68	34	0	34	1
63	76	2	12	10.8	69.5	36	0	37	1
77	81	1	3	8.0	79	38	1	38	0
82	86	1	3	10.3	84	9	1	11	0
86	97	2	10	11.3	91.5	17	0	18	1
87	97	2	9	8.4	92	23	0	24	2
87	130	5	42	7.4	108.5	46	0	47	2
98	130	4	31	5.2	114	61	1	62	1
147	152	1	4	10.3	149.5	78	2	78	1
153	163	2	9	6.6	158	83	1	83	2
153	168	2	14	12.7	160.5	69	2	69	1
172	179	1	6	10.9	175.5	20	1	22	2
176	187	2	9	14.1	181.5	6	0	7	1
188	193	2	4	10.9	190.5	3	0	4	1
188	200	2	11	10.0	194	2	0	3	1
188	210	4	21	12.5	199	2	0	3	0
189	201	3	11	10.0	195	2	0	3	0
190	210	3	19	10.8	200	2	0	2	0
194	210	3	15	10.2	202	2	0	3	0
201	210	2	8	9.3	205.5	2	0	3	0
211	247	5	35	7.8	229	45	1	45	2
211	247	6	35	7.8	229	45	1	45	2
211	251	6	39	10.7	231	37	1	36	2
215	247	6	31	7.7	231	46	1	46	2
266	278	3	11	6.9	272	9	1	9	1
266	280	4	13	7.9	273	8	0	7	1
266	288	3	21	9.0	277	10	1	11	1
289	293	1	3	7.0	291	21	0	21	1
289	296	2	6	13.9	292.5	22	1	21	1
297	305	2	7	11.6	301	46	1	48	1
297	311	2	13	12.2	304	4	0	4	0
312	327	3	14	13.6	319.5	17	0	18	1
315	326	2	10	10.3	320.5	23	0	23	1
315	327	3	11	13.0	321	19	1	19	1
318	327	2	8	12.8	322.5	25	1	27	1
327	340	2	11	11.1	333.5	15	0	18	0
328	357	4	25	13.5	342.5	13	0	14	1
328	360	4	28	14.7	344	12	0	13	1
341	360	3	16	14.5	350.5	16	1	16	1
358	363	1	4	10.5	360.5	2	1	3	2
366	378	2	11	11.9	372	21	0	22	1
366	378	3	11	11.9	372	22	0	21	0
367	378	3	10	10.8	372.5	24	0	23	1
367	380	2	12	10.8	373.5	32	0	32	1
368	378	2	9	10.2	373	26	0	26	1
369	378	2	8	10.8	373.5	27	0	27	1
385	389	1	3	13.9	387	16	0	18	1
390	401	2	10	4.6	395.5	47	0	47	1
390	413	3	21	8.8	401.5	51	0	52	2
390	413	4	21	8.8	401.5	51	0	51	2
414	419	1	3	14.0	416.5	2	1	3	0
414	423	2	7	13.6	418.5	19	0	22	1
425	444	4	18	10.6	434.5	30	1	30	2
427	441	4	13	7.9	434	7	1	7	0
427	443	4	15	7.4	435	23	1	23	0
445	449	1	3	8.0	447	1	1	1	1
450	456	1	5	5.9	453	30	0	29	1
450	456	2	5	6.0	453	30	1	29	1
451	456	1	4	5.9	453.5	35	1	33	1
451	457	2	5	6.0	454	30	1	29	1
487	502	3	13	13.7	494.5	24	1	25	2
491	502	2	10	8.1	496.5	31	1	31	1
491	502	3	10	10.0	496.5	27	0	27	1
491	503	2	11	9.3	497	29	1	30	1
503	513	2	8	7.4	508	26	0	27	0
504	513	2	7	7.2	508.5	29	0	30	1
514	521	2	6	12.9	517.5	2	0	3	0
522	531	2	8	14.9	526.5	1	0	1	0
522	532	2	9	17.1	527	1	0	1	0
532	536	1	3	9.6	534	53	1	55	2

Table S4. Relative percentage of global deuteration level of Vps34 **(A)**, Vps38 **(B)**, Vps15 **(C)**, and Vps30 **(D)** in presence and in absence of nanobody 8780. Peptides showing decreased HDX on nanobody binding (in red) suggest a binding epitope. Column labels as in Table S2.

ID	Construct	Backbone	Use
pRS426	empty		Pull down assay, transformed with pYO225
pYO69	ScVPS34	pRS424	Vps34/Vps15 heterodimer coexpression for HDX-MS, pull-down, GUV, and kinase assays, transformed with pYO225
pYO225	ScVPS15-3xTEV-ZZ-ADH1t	pRS426	Vps34/Vps15 heterodimer coexpression for HDX-MS, pull-down, GUV, and kinase assays, transformed with pYO069, pYO290, or pYO361
pYO290	ScVPS34 D731N	pRS424	Vps34/Vps15 heterodimer coexpression for kinase assay, transformed with pYO225
pYO361	Scvps34 HELCAT (Δ267)	pRS424	Vps34/Vps15 heterodimer coexpression for pull-down, transformed with pYO225
pYO227	ZZ-3xTEV-ScVPS30	pRS426	Vps30 single expression for HDX-MS
pYO359	ScVPS34+ScVPS15-3xTEV-ZZ-ADH1t	pRS424	Complex II crystallization, transformed with pYO539
pYO379	ZZ-3xTEV-ScVPS30+ScVPS34+ScVPS15-3xTEV-ZZ-ADH1t	pRS426	Complex I expression for GUV and kinase assays, transformed with pYO411
pYO391	ScVPS38+ZZ-3xTEV-ScVPS30+ScVPS34+ScVPS15-3xTEV-ZZ-ADH1t	pRS426	Complex II expression for HDX-MS, MALLS, and crystallization
pYO411	3xTEV-ScATG14	pRS424	Complex I expression for GUV and kinase assays, transformed with pYO379
pYO539	ScVPS30+ScVPS38	pRS426	Complex II crystallization, transformed with pYO359
pYO734	ScVPS30+ZZ-3xTEV-ScVPS38	pRS426	In vitro reconstitution
pYO831	ZZ-3xTEV-Scvps38 (1-212F)	pRS424	Vps38 C2 domain/Vps30 NTD coexpression for MALLS, transformed with pYO832
pYO832	Scvps30 (1-215E)	pRS426	Vps38 C2 domain/Vps30 NTD coexpression for MALLS, transformed with pYO831
pYO979	ScVPS38+Scvps30 (FRK to DDD at 430-432)	pRS426	Complex II with "aromatic finger" mutant vps30 expression for GUV and kinase assays, transformed with pYO359
pRS416	empty		Cell biology
pYO478	pVPS34-ScVPS34 FL (1-899)-3xFLAG-ADH1t	pRS416	Cell biology
pYO759	pVPS38-ScVPS38 FL (1-440)-3xFLAG-ADH1t	pRS416	Cell biology
pYO760	pVPS38-Scvps38 ΔC2 (155-440)-3xFLAG-ADH1t	pRS416	Cell biology
pYO761	pVPS38-Scvps38 ΔBARA2 (1-319)-3xFLAG-ADH1t	pRS416	Cell biology
pYO764	pVPS38-ScVPS38 FL (1-440)-EGFP-ADH1t	pRS416	Cell biology
pYO765	pVPS38-Scvps38 ΔC2 (155-440)-EGFP-ADH1t	pRS416	Cell biology
pYO766	pVPS38-Scvps38 ΔBARA2 (1-319)-EGFP-ADH1t	pRS416	Cell biology
pYO932	pVPS34-Scvps34 HELCAT (Δ267)-3x FLAG-ADH1t	pRS416	Cell biology
pYO933	pVPS34-Scvps34 ΔC2HH (154-202)-3xFLAG-ADH1t	pRS416	Cell biology
pYO934	pVPS34-Scvps34 ΔCBR1(31-55)-3xFLAG-ADH1t	pRS416	Cell biology
CA8780	nanobody against complex II	pMESy4	HDX-MS mapping and crystallization with complexII

Table S5. Plasmid list. All protein expression plasmids are under the GAL1 promoter (Somatogen Inc.) and the MF (ALPHA)1 terminator, unless noted otherwise. pVPS38: VPS38 promoter from -934 bp to -1 bp. pVPS34: VPS34 promoter from -214 to -1 bp. ADH1t: ADH1 terminator. Nanobody expression plasmid is C-terminally tagged with 6xHIS and the CaptureSelect C-terminal EPEA tag.

ID	Genotype	Source
BCY123	Matα *pep4*::HIS3 *prb*::LEU2 *bar1*:HISG *lys2*::GAL1/10-GAL4 *can1 ade2 ura3 leu2–3,112 trp1*	(64)
YOY193	BCY123 *TRX1-ADE2::ade2Δ*	This study
BY4741	*Mata his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*	Open Biosystems
vps38Δ	BY4741 *vps38Δ*::KnamMX	Open Biosystems
SEY6210	*MATα leu2-3,112 ura3-52 his3-Δ200 trp1-Δ901 suc2-Δ9 lys2-801; GAL*	(65)
vps34Δ	SEY6210 vps34Δ::HIS5	This study

Table S6. Yeast strains used in this study.

Movie S1. Movie depicting the first non-trivial normal mode of complex II, as computed using the Bio3D Package (*66*).